Feeding Behavior of Lycorma delicatula (Hemiptera: Fulgoridae) and Response on Feeding Stimulants of Some Plants

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Lycorma delicatula was not an insect that was widely known to people a few years ago. However, it started to draw attention when a big population of Lycorma delicatula appeared in Seoul and the Gyeonggi area in 2006. After 2006, its population steadily grew, causing damage all around the nation (KFRI, 2007; Han et al., 2008; Park et al., 2009). Lycorma delicatula horrifies people because it lives as a group. Lycorma delicatula also sucks the sap out of branches, leaving honeydew on leaves, hindering plants from photosynthesizing, and causing sooty mold disease. When sooty mold disease is serious, plants wither and die. Lycorma delicatula was first discovered in Korea by Doi in 1932. Lycorma delicatula usually lives in sub-tropical areas like China and Southeastern Asia, but it started to live in South Korea in 2006, after coming into Korea through China (Han et al., 2008). 41 plant species, including 38 species of trees like tree of heaven, chinaberry tree, picramnia tree, parthenocissus tricuspidata, wild korean berry, grapevine, and 3 species of herb plants are reported as a host plants of Lycorma delicatula (Park et al., 2009). Control of Lycorma delicatula is needed because of recent damage caused by major outbreaks of Lycorma delicatula.

In order to find which plants that Lycorma delicatula can feed on, and to find what plants can be potentially be harmed by Lycorma delicatula, research on feeding behavior of Lycorma delicatula is needed. Observation of the feeding behavior of juice sucking insects is usually done with EPG (Electrical Penetration Graph)(McLean and Kinsey, 1967; Kim et al., 2005). EPG was first used to research aphid feeding behavior (McLean and Kinsey, 1967; Tjallingii, 1988). It was later used to research jassid feeding behavior (Khan and Saxena, 1985) and on other leafhoppers (Yong and Chang, 1993). However feeding behavior research on Lycorma delicatula hasn't been done yet.

Chemical and physical factors are very important in determining which plants herbivore insects will pick as their host. Lepidoptera senses certain chemical materials in the plant, and is also affected by plant-insect interactions (Renwick and chew, 1994; Honda et al., 1997). A lot of insects need carbohydrates for energy and it is also known as an insect feeding stimulant (Bernays and Simpson, 1982). Chlorogenic acid and sucrose are known feeding stimulant for insects that feed on solanaceous plants, like the colorado potato beetle (Hsiao and Franekel, 1968). Endo et al.(2004) says that methyl linolenate, fructose, and glucose stimulate the feeding behavior of Epilahna vigintioctomaculata. Sugar acts as feeding stimulant but also as a necessary nutrition for insects. Therefore, it is necessary to research stimulants of Lycorma delicatula, and how the sugar content of plants effect how Lycorma delicatula picks its host.

In this research paper, the team observed the differences in the feeding behavior of Lycorma delicatula between 7 species of plants, as well as the effect of sugar content of plant on host plant choosing process of Lycorma delicatula. The purpose of these

experiments were to provide basic information for the population control of Lycorma delicatula.

Materials and Methods

Plants and Insects Used for Experiments

2nd to 4th stage Lycorma delicatula nymphs were collected from trees of heaven at the Chung-buk university and te area around the Chung-buk university. Collected nymphs were raised in 25 ∓ 2℃, 50 - 60% relative humidity, and 16L:8D light conditions. They fed on tree of heaven in a cage sized 27 x 30 x 46 cm. Only 3rd stage nymphs that entered the 3rd developmental stage within 24 hours and female imagoes were used in the experiments. Both female and male nymphs were used, but only female imagoes were used in experiment. The team differentiated female from male Lycorma delicatula by checking whether they had red ovipositors at the end of their abdomens. Tree of heaven, grapevine, red pine tree, rose of sharon, apple tree, pear tree, and preach tree were used in the experiment (Table 1). All these trees were harvested organically, without pesticides, from Chung-buk university farm and farms near Chung-buk university. Only healthy branches and fruits were used in the experiment.

Preference and Survival time

Each of the 7 types of plant branches (tree of heaven, grapevine, red pine tree, rose of sharon, apple tree, pear tree, and preach tree) were cut into 20 cm branches and placed in a jug with water. All of the jugs were placed in cage sized 40 x 40 x 40 cm with a distance between each jug. After that, nymphs and imagoes were placed in the cage, and the cage was placed in a room with 25 ∓ 2℃, 50 - 60% relative humidity, and 16L:8D light condition. The number of Lycorma delicatula in each plant was recorded during each time

period. A survival time experiment was done by cutting each of the 7 types of plant branches into 20 cm segments and putting them in jugs.There were 3 cages size 28 x 28 x28 cm, each with a different type of fruit. In each cage, there were 20 fruits, as well as some branches from the fruit trees and Lycorma delicatula imagoes and 3rd stage nymphs. The survival time of nymphs and imagoes was then recorded. The molting rate was also checked in the case of nymphs. The condition of the cages was 25 ∓ 2℃, 50 - 60% relative humidity, and 16L:8D light condition. Water was provided in jug everyday, and branches were replaced before they dried out. Every experiment was repeated 3 times.

EPG Record and Data Analysis

In order to observe feeding and probing activity, the team recorded feeding waveforms by using Tjallingii's (1998) DC system. Using silver conductive paint (RS, 101-5621, UK), a gold thread (Goodfellow, UK) with diameter of 100μm and length of 5cm was attached to the back of the thorax. The attached thread was connected to Giga-8 DC EPG amplifier. Lycorma delicatula was placed on a plant's branch. Recording was done for 6 hours. The EPG signal was recorded in a PC and analyzed using STYLET 3.8 (Tjallingii and Mayoral, 1992). Every experiment was done inside a Faraday cage sealed with copper in order to denoise other wave. The types of waveform and the length of time was recorded based on the electrical signal. The experiment was repeated 10 times for each species of plant. In every experiment, data was collected during a six hour window of time. Afterwards, The different feeding behaviors of Lycorma delicatula depending on species of plants were tested through Turkey's Studentized Range Test (SAS Institute, 2003).

Preparing and Analyzing Sample for Sugar Content Analysis

1kg of tree of heaven, grapevine, apple tree, pear tree, and rose of sharon branches were prepared by soaking in methanol for 3 days and extracted. Then it was concentrated with a concentrator. 20 g of branch extraction was resolved in 800 ml of distilled water, then placed in a separatory funnel with 800 ml of hexane. After mixing it with hexane, the hexane layer was separated. Using the same method, leftover extraction was separated from the chloroform layer and the ethyl acetate layer. Final extraction went through decompression concentration using a rotational vacuum concentrator (Heidolph, Germany) at 45℃. After these processes, the sample was used in sugar content analysis. Sugar analysis was done using HPLC (Agilent Technologies 1200 series, Santa Clara, CA; pump : Quaternary pump, column : Zorbax carbohydrate (4.6 mm × 250 mm, 5 μm), solvent : acetonitrile + water (8:2), flow rate : 2 ml/min, detector : RID, injection : 1 μℓ).

The standard sugar sample was resolved in acetonitrile : water (8:2) solution to make 4mg/ml concentration solution. Later it was diluted to make a standard sample. A concentration checking graph was made by putting 1μℓ of standard sample in HPLC. 3g of samples were mixed with 3g of distilled water in a 1 to 1 ratio then went through an ADVANTEC cellulose acetate 0.45μm hydrophilic filter. The sample was diluted again with preservation liquid by ratio of preservation liquid : sample = 10 : 1 before analyzing with HPLC. Using the result of the sample, the peak retention time, and the standard sample's retention time, the sugar content was analyzed. Using the area of the peak, each plant's sugar content ratio was analyzed. Standard sugar samples including rhamnose, fructose, glucose, sucrose, maltose used in experiment were products of

Sigma (St. Louis, MO, USA). Acetonitrile for HPLC was product of Burdick & Jackson (USA).

Research on Survival time Depending on Sugar Content.

The HPLC analyzed standard sugar sample was diluted with distilled water to make 5%, 1%, and 0.1% solution. Sugars were also mixed in a plant's HPLC analyzed sugar ratio and diluted with distilled water to make 10% and 5% plant sugar solution. Parafilm membrane (parafilm M, USA) was used to test solution on live specimens (Mittler and dadd, 1962). Using the method that Mitsuhashi and Koyamar (1969) used, parafilm membrane was stretched by hand to certain constant thickness to cover the petri dish (Ø : 9cm, h: 0.6mm) filled with solution. The solution filled petridish was then placed diagonally in a live specimen testing jar (Ø : 11 cm, h: 8 cm), so that with a parafilm membrane stretched over the top of the jar. The empty space was filled with cotton so that Lycorma delicatula wouldn't get stuck. After placing nymphs and imagoes, the condition was set to 25 ∓ 2℃, 50 - 60% relative humidity, and 16L:8D light condition. The survival time was checked. Every experiment was repeated 3 times and the results were analyzed using Tukey's studentized range test (SAS institute, 2003).

Result and Contemplation

Preference on Plants

Results show among tree of heaven, grapevine, apple tree, pear tree, red pine tree, rose of sharon, and peach tree, Lycorma delicatula nymphs and imagoes prefer tree of heaven the most, followed by grapevine. There wasn't much preference in trees other than tree of heaven and grapevine (Fig. 1). Nymphs demonstrated different preference towards tree of heaven versus grapevine. Nymphs did not prefer the other plants. Imagoes showed similar preference to both tree of heaven and grapevine. Imago liked the red pine tree the least. It is known that a lot of insects look for their host plant using the host plant's scent (Visser, 1986). Xiao et al. (2002) said that female Helicoverpa armigera find their non-host plant, Pterocarya stenopter, by sensing substances that come from the wet leaf. Like Helicoverpa armigera, many insects find their host by sensing the plant's volatile material. According to the research of Hori et al. (2006), his team used volatile materials from both host plants and non-host plants of Galerucella vittaticollis to attract Galerucella vittaticollis. Galerucella vittaticollis was only attracted to cis-3-hexenyl acetate which is one of the main materials inside the host plants. On the other hand, there was little to no cis-3-hexenyl acetate in non-host plants. It is considered that Lycorma delicatula also has sensed some type of volatile material in order to find its' host plant. There must be a difference in the volatile materials between host plants and non-host plants. There hasn't been any research on the reaction of Lycorma delicatula to volatile materials, but volatile materials likely play a big part in how Lycorma delicatula differentiates host and non-host plants. Research should be done on the reaction of Lycorma delicatula to volatile materials.

Difference of Survival Time depending on Plant.

The results of observing the molting rate and survival time of Lycorma delicatula nymphs and female imagoes on 7 plants and 3 fruits are shown in Table 2. The survival time of nymphs that became 3rd stage within 24 hour was 15 days in tree of heaven and 15.4 days in grapevine, the longest out of all the plants. The survival times of nymphs that became 3rd stage within 24 hour among apple tree, pear tree, rose of sharon, and peach tree were all under 6 days. The survival time of nymphs on red pine tree was under 5 days. Nymphs survived less than 2 days on all 3 types of fruits. The survival time of imagoes on trees of heaven was 6.8 days, which was longest. The survival time of imagoes on grapevines was 6.1 days. On every other plant, they survived less than 2 days. There was only a small difference in survival times between tree of heaven and grapevine when tested with both nymph and imago. There wasn't much of a difference in survival times among apple tree, pear tree, red pine tree, rose of sharon, and peach tree, but they all differed significantly from grapevine and tree of heaven. Jung and Im (2005) said that rice leafhoppers survived longer on sensitive rice plant compared to resistant rice plant. (Translator's note : Resistant rice plant are genetically modified to be resistant to pest while sensitive rice plant is not.) Yang et al. (2009) did research on the growth time and survival rate of Bemisia tabaci on B-biotype and Q-biotype pepper. On B-biotype pepper, nymphs couldn't survive and the hatching rate was only 5.8%. Like in these cases, the plant's impact on insects are significant and insects can't survive on plants other than host plants. Lycorma delicatula survived a long time on its host plants, tree of heaven and grapevine, but didn't last long on other plants. The molting rate of 3rd stage Lycorma delicatula nymphs was high on tree of heaven and grapevine. It was 63.3% on tree of heaven and 63% on grapevine. The

molting rate on apple tree was 17.7%, 9.3% on pear tree, 7.8% on rose of sharon, 5.9% on red pine tree, 0% on peachtree, and 0% on apple, pear, and peach fruit. No Lycorma delicatula molted into an imago. When the survival rate was low, the molting rate was also low. An insect's choice of plant has a big impact on the growth and survival of that insect. Insects pick the right host plants in order to survive. Campbell et al. (1982) said a plant's resistance to being a host depends on if it has feeding depressants or low levels of feeding stimulants. Miller and Miller (1986) said that the nutrition condition of the host plant's leaf as well as its physical and chemical defense materials plays a major part when insects are judging whether it is suitable as a host plant. It seems that non-host plants either don't have feeding stimulants or have feeding depressant materials that keep insects from absorbing nutrition, making them unable to survive or molt properly.

EPG Pattern Analysis and Comparison of Feeding Behavior

As far as the author, prior to this study, there was no research on the feeding waveforms of Lycorma delicatula. The waveforms of Lycorma delicatula was divided using the following method. Lycorma delicatula fed on the phloem and tracheal tube of a plant. Then, the team categorized common waveforms of nymphs and imagoes on each plant. They categorized non-penetration waveform and pathway phase waveform by observing the penetrating activity of Lycorma delicatula by eye observation. Observing the waveforms of nymphs, waveforms that indicated phloem and tracheal tube feeding were only observed on tree of heaven and grapevine therefore these waveforms were assumed to be feeding type. Since this waveform is also observed among imagoes only on grapevine and tree of heaven, the team thought this waveform was important waveform in feeding behavior. After comparing this waveform with other feeding

waveforms of true bug, the team judged that this waveform was the phloem feeding waveform and another waveform was the tracheal tube feeding waveform. 4 types of waveforms are shown in Fig.2. The 1st waveform shows 1 hour of whole waveform record. The 3rd waveform shows the pathway phase where Lycorma delicatula pierces the plant with its mouth to check the plant (note: the original was not clear what it is checking for). The 4th waveform is considered the xylem phase, where Lycorma delicatula feeds on the tracheal tube. The 5th waveform is called the phloem phase, where Lycorma delicatula feeds on phloem. The 2nd waveform is the non-penetration waveform. It appears when Lycorma delicatula is not using its mouth part. The marked area of the 2nd waveform is when Lycorma delicatula used its mouth part to pierce but not suck on the plant. Lack of voltage change indicated no feeding activity. An Aphid shows 5 types of waveforms when analyzed with EPG (Tjallingii, 1978, 1985; Kimmins et al., 1987), when analyzing leafhopper, there are 6 types of waveforms (Kim et al., 2005; Youn and Chang, 1993), Bemisia tabaci shows 7 types of waveform (Lei *et al*., 1996; Lei *et al.*, 1999; Jiang *et al.,* 1999; Yang *et al*., 2009). In this experiment, the team used this data to categorize the 4 waveforms of Lycorma delicatula to check whether Lycorma delicatula was feeding on a plant or not.

After analyzing the feeding behavior of imagoes and nymphs of Lycorma delicatula on 7 plants, there were significant differences in the non-penetration waveform. On the tree of heaven, nymphs had 242 minutes of non-penetration waveform while imagos had 223.2 minutes of non-penetration waveform. On grapevine, nymph had 228.7 minutes of non-penetration waveform while imago had 245.6 minutes. These 2 plants showed shorter non-penetration times compared to 5 other plants. There

were also big differences in phloem feeding time compared to other plants. On the tree of heaven, nymph had 45.7 minutes of phloem phase waveform while imago had 52.8 minutes of phloem phase waveform. On grapevine nymph had 23.7 minutes of phloem phase waveform while imago had 29.0 minutes of waveform. These 2 plants showed over 20 minutes of phloem phase waveform. On the other 5 plants including apple tree, pear tree, red pine tree, rose of sharon, and peach tree, there wasn't any phloem phase. Fulgoridae are known to feed on phloem of the plants (Naskrecki, and Nishida). Therefore, whether Lycorma delicatula feeds on phloem or not is important factor when it comes to picking a host plants and very important in the research on the feeding behavior of Lycorma delicatula.

On tree of heaven, grapevine, and peach tree, both nymphs and imagoes showed showed a period of tracheal tube feeding that was 20 minutes or longer. On apple tree, pear tree, and rose of sharon, nymphs showed 9 minute or less tracheal tube feeding waveform but imagoes showed showed a period of tracheal tube feeding that was 15 minutes or longer. On red pine tree, both nymphs and imagoes didn't show any tracheal tube feeding waveform (Table 3). Other than red pine tree, Lycorma delicatula imagoes always show a tracheal tube feeding waveform. However, in order for phloem feeding insects to get energy, they have to feed on phloem. Alate aphid is a phloem feeding insect and they can't survive unless they feed on phloem (Ward 1991). Davis and Radcliffe (2008) said that a phloem feeding insect, the aphid, shows tracheal tubing feeding activity in 4 different types of plant but they can only survive by feeding on phloem and whether they feed on phloem or not is an important factor when it comes to picking a host plants. Therefore, tracheal tube feeding is not sufficient to determine

whether or not a plant can be a host plant of Lycorma delicatula. It can only be conclusively considered a host plant when there is is both tracheal tube and phloem feeding waveforms. Using EPG analyzation, the team found out whether or not Lycorma delicatula actually fed on 7 different plants. Looking at the overall preference and survival time, it was concluded that tree of heaven and grapevine are host plants of Lycorma delicatula, while pear tree, apple tree, rose of sharon, peach tree, and red pine tree are not.

Sugar Content Analysis and survival time Comparison.

Previous research showed that Lycorma delicatula prefers tree of heaven and grapevine. It also showed that Lycorma delicatula can survive and feed on tree of heaven and grapevine. There are probably a lot of factors that make grapevine and tree of heaven ideal hosts for Lycorma delicatula. Sugar inside plants is essential for survival of Lycorma delicatula. It seems that grapevine and tree of heaven have the sugar that Lycorma delicatula needs for its survival, and other plants have little or none of the sugar that Lycorma delicatula needs. The results of analyzing the sugar content of each plant are shown in Fig.3. The sugars in the tree of heaven were mostly sucrose, followed by fructose, followed by glucose. In grapevine, the proportion of sugar was the following: glucose, fructose, maltose, sucrose, rhamnose, from highest to lowest. In apple tree, the proportion of sugar was the following: glucose, fructose, from highest to lowest. In pear tree, the sugar proportion was the following: glucose, unknown, fructose, from highest to lowest. In rose of sharon, the sugar proportion was the following: sucrose, glucose, from highest to lowest (Table 4). It was observed that plants that Lycorma delicatula prefers plants like grapevine and tree of heaven that contain sucrose

and fructose at the same time. On grapevine there were also sugars other than fructose and sucrose. On the other hand, apple tree, pear tree, and rose of sharon had a high percentage of glucose. In the case of rose of sharon, it had high percentage of sucrose but it also contained glucose, and unlike host plants of Lycorma delicatula, it didn't contain fructose. The effect of the proportion and combination of sugar on the survival of Lycorma delicatula nymphs and imagoes is shown in Fig.4. Nymphs survived 13.1 days in 5% sucrose solution, 11.4 days in 5% fructose solution, 7.8 days in rhamnose solution, 6.1 days in maltose solution, and 6.0 days in glucose solution (Translator's note: does not mention what percentage of rhamnose and maltose solution). Sucrose solution showed a survival time longer than 10 days even in 0.1% and 1% sucrose solution. Fructose solution showed survival time longer than 10 days until 1% fructose solution. Overall, as the solution's concentration decreased, the survival time of Lycorma delicatula also decreased. 3rd stage Lycorma delicatula nymphs that fed on 5% sucrose solution showed a molting rate of 73.3% which was the highest of all the other solutions. 3rd stage Lycorma delicatula nymphs that fed on 5% fructose showed a molting rate of 66.6%. Other 3rd stage Lycorma delicatula nymphs that fed on other sugar solutions didn't survive long enough to molt. Similar tendencies were seen when the experiment was done on female Lycorma delicatula imagoes (Fig 4). Tree of heaven is mostly sucrose, but also contains glucose. Grapevine has high proportion of fructose and also contains sucrose. Looking at the results, it appears that sucrose and fructose have a significant effect on the survival and feeding stimulation of Lycorma delicatula. According to the research of Onagbola et al. (2007), *pteromalus cerealellae* survived the longest when it was fed with 25% sucrose solution. Luo et al. (2009) did experiment

on sugar's effect on *Microplitis mediator.* They also survived longest in sucrose. After sucrose, they survived longest in fructose, and glucose. In mannose, galactose, and raffinose penthhydrate, *Microplitis mediator* survived only for a short time. Lycorma delicatula also survived longest in sucrose solution. Fructose solution showed the longest survival time after sucrose, and glucose solution showed a short survival time. This is because each insect needs a different type of nutrition, and because sugar has an effect on the lifespan of an insect. Sugar is already known as a feeding stimulant for a lot of insects (Bernays and Simpson, 1982). Endo et al. (2004) found the feeding stimulants of Epilachna vigintiocto maculata from a methanol extraction of potato leaf, and they were methyl linolenate, fructose and glucose. Sucrose and fructose are essential for the survival of Lycorma delicatula, and they act as feeding stimulants of Lycorma delicatula.

In previous experiments, testing on live specimens was done with only pure types of sugars, but in order to find the effects of mixed sugar, the team conducted experiments using sugar mixed to the proportion of each plant (Table 4). Lycorma delicatula nymphs survived 13.1 days in 10 % F+G+S (tree of heaven), 14.2 days in 5% F+G+S (tree of heaven), 14 days in 10% R+F+G+S+M (grapevine), and 13.1 days in 5% R+F+G+S+M (grapevine). The sugar combination of grapevine and tree of heaven showed the longest survival time. The sugar combination of apple tree, pear tree, and rose of sharon showed a survival time of 6 to 8 days, a big difference from tree of heaven and grapevine (Table 5). Lycorma delicatula survived 4.8 days when only water was provided. The molting rate of Lycorma delicatula nymphs was 60.0% in 10 % F+G+S (tree of heaven), 66.7% in 5% F+G+S (tree of heaven), 66.7% in 10%

R+F+G+S+M (grapevine), and 60.0% in 5% R+F+G+S+M (grapevine). In other sugar combinations, the molting rate was 0%. When the survival time was longer, the molting rate was higher. Female Lycorma delicatula imagoes survived the longest in sugar combinations of F+G+S (tree of heaven), and R+F+G+S+M (grapevine). Female Lycorma delicatula imagoes didn't live for longer than 3 days in every other sugar combination (Table 5). Imagoes survived 2.6 days when only water was provided. As shown in Fig. 3, tree of heaven and grapevine contain both sucrose and fructose. Looking at the overall results and previous experiments, Lycorma delicatula probably chooses tree of heaven and grapevine as host plants because of their sugar content. Lycorma delicatula can't survive on pear tree or apple tree because they have a high proportion of glucose. Rose of sharon contains glucose but also sucrose. However when this sugar combination was used in a live specimen test, it had the shortest survival time, even though it contained sucrose. This appeared to have happened because of the interaction between the sugars when they were mixed. Mitsuhashi and Koyama (1969) experimented on the effect of mixed sugar on the survival time of rice leafhoppers. The survival time of rice leafhoppers decreased when both glucose and fructose were used, compared to when only glucose was used. When sucrose and maltose was mixed, the survival time differed depending on the combination and proportion of the sugar.

In this experiment, (Translator's note : this report as a whole not the experiment of Mitsuhashi and Koyama) the team mainly studied sugar as a feeding stimulant, but it is also necessary to research possible stimulants other than sugar.