Original Article

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Effect of Mixed Species with *Apis mellifera* and *Apis cerana* for Maintaining Temperature and Moisture in Same Hive

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Abstract

Beekeeping is an important industry in Korea. Sacbrood virus is the cause of sacbrood disease, the most important infectious disease in honeybee *Apis cerana*. Sacbrood disease can cause a collapse of a bee colony and even the whole apiary. Sacbrood disease was first reported in Korea in 2009. The occurrence of sacbrood disease symptom can be caused by several reasons such as genetic (immune system), climate change, weak colony etc. We have many experiences about the relation with climate conditions and sacbrood disease. The occurrence of sacbrood disease was defendant of humidity and temperature. Weak honeybee colony cannot resist the disease. In this study, we investigated the effects of mixing honeybee colonies of *A. mellifera* and *A. cerana* vis-à-vis occurrence of sacbrood disease at different humidity and temperature. The mixed colony was consisted super hive (Top hive: *A. cerana* and bottom hive: *A. mellifera*). Our results of temperature and humidity variable in mixed colony were: am 9:00-35.3°C and 57.3%, pm 12:00-35.6°C and 52.3%, pm 15:00-35.8°C and 50.3%, pm 18:00-35.7 and 50.5%, respectably. Single species colonies were destroyed by scabrood virus (SBV). So, Heat stress and humidity variable, *A. cerana* colonies are more sensitive and initiate fanning of hive earlier than after mixing them with *A. mellifera* mixed colonies. Our data show a strong impact of environment on the development of colonies. The results further suggest that in *A. mellifera* colony can be helped benefited by *A. cerana* colony which controls hive temperature and humidity.

Key words: Honeybee, *Apis cerana*, Temperature, Humidity, Sacbrood virus

INTRODUCTION

Among honeybees, there has been a report of research conducted on mixed species with *Apis mellifera* and *Apis cerana* by construction of the comb and nursing in same colony (Yang *et al*., 2010). Various pheromones were known to be complicatedly applied in the course when honeybees combs (Wilson, 1971; Belic *et al*., 1986). However, several researches provided an opinion that very simple rules are applied to build combs according to physical and chemical features (Camazine *et al*., 1990; Camazine, 1991; Jenkins *et al*., 1992). However, due to the difference in chemical ingredients and sizes of hives of work bees between *A. mellifera* and *A. cerana* (Winston, 1987; Ruttner, 1988), comb with different size of hives is required to build combs for raring the broods. However,

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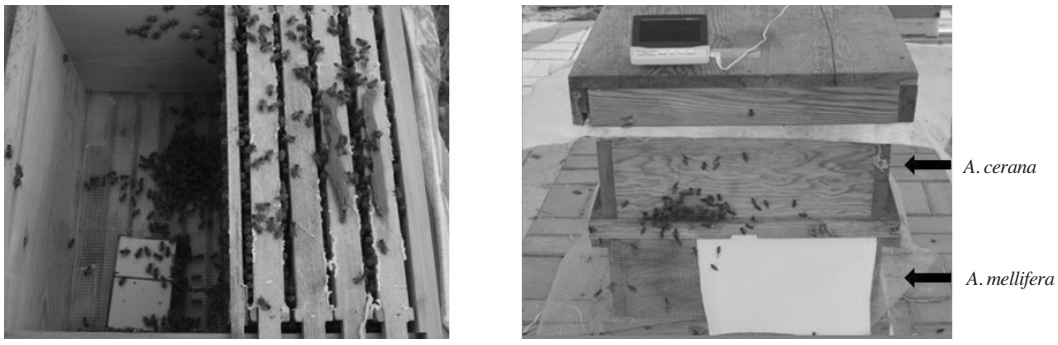

Fig. 1. Construction of mixed colony.

there has not been a case measuring the bee colony environment by separating the single hive and double hive where *A. mellifera* and *A. cerana* build bee colonies based on each queen bee. Therefore, *A. mellifera* colony was located in the down hive, and *A. cerana* colony was located in the top hive. When management of colony in these conditions, it seems that a research is required to identify the effect from mixed colony. Especially, Sacbrood virus firstly occurred in Korea in 2009 (Choi *et al*., 2010) caused the decrease in bee colony of *A. cerana*. Sacbrood virus was firstly diagnosed in USA in 1913 followed by report in various countries in all over the world (Allen and Ball 1996; Tentcheva *et al*., 2004).

Sacbrood virus occurred in Korea turned out to have similar sequence listing with *A. cerana* occurred in China and Japan (Choe, *et al*., 2012). There has recently been a report that disease from Sacbrood virus occurred due to an increase of stress from expression of immune protein of *A. cerana* by infection of Sacbrood virus (Liu Shan *et al*., 2017). Such Sacbrood virus has widely occurred in Asian countries to *A. cerana*. According to opinion suggested by beekeepers on the period of occurrence of Sacbrood virus in Korea, it has been known that there was a high chance for such virus to occur during rainy season with high daily temperature range or high humidity.

However, there has not been a case of researching the degree of Sacbrood virus from the temperature of bee colony such as humidity or daily temperature range or growth of bee colony. Therefore, this study has conducted a research to clarify the effect of suppressing the occurrence of Sacbrood virus and the growth of bee colony when management of *A. mellifera* and *A. cerana* in the separated form of down hive and up hive as a part of management techniques for reducing the occurrence of Sacbrood virus.

MATERIALS AND METHODS

Construction of Mix colony

A. cerana and *A. mellifera* bee colonies used in this study have been constituted including eight coms of *A. mellifera* in experimental apiary in National Institute of Agricultural Sciences on the bottom hive. On the top hive, three combs of *A. cerana* were placed in the bee colony. Each of the bee colonies (*A. mellifera* and *A. cerana*) built hives based on queen bees, and exit was in different directions from entrance so that worker bees are not mixed. In order to build such mix hive, propolis collecting net was covered on the down hive with *A. mellifera* with additional cloth cover. And then, nine days after the bees completely recognize in their hives each other, a 30% of cover was taken off every 3 days that two different species (*A. mellifera* and *A. cerana*) shared the smell of each bee colony. Nine days later, cloth cover was completely taken off preventing queen bees and work bees on the down hive and up hive from interacting with each other. After that, the thermo-hygrometer was installed in the *A. cerana* colony to investigate temperature and humidity (Fig. 1).

Management of temperature and humidity in the bee colony

Quick Start for the HOBO U0 series Data Logger was

used for measuring temperature and humidity in the test of bee colony in each time period.

Separation and appropriate amount of virus

In order to check whether Sacbrood virus in the test bee colony and the reduction of growth of bee colony from occurrence, larvae with symptoms of Sacbrood virus were collected, and ten of them was pulverized and 1ml sterilized water was added and centrifuged with 4,000rpm for five minutes to separate supernatant that contained virus. Separated virus was prepared with RNA by using total RNA extraction kit (Promrga), and used as a template for quantification of virus by separating RNA. Quantification of virus was done by using Quantitative Real Time PCR, and Forward primer 5'-GAC CAA GAA GGG AAT CAGC-3', Reverse primer 5'-CAT CTT CTT TAG CAC CAG TAT CCA-3' as a Sacbrood virus (SBV) specific primer were used to setup the total volume of qPCR as 25μl by mixing 10 pmol primer, 1×SYBR Green (Applied Biosystems), and 1st strand cDNA 1μl. With analysis of Ct value of final results of reaction, virus was quantified (Applied Biosystems, user bulletin #2). The primer set was deigned on GenBank No. NC-002066 on NCBI.

Investigation of growth of bee colony

In early March, after artificially infecting each of the test bee colonies with Sacbrood virus in the concentration of 2×10^6/ml, certain periods were chosen from March to July to investigate the number of worker bees in each bee colony, pupa, and larva to compare the status of their growth.

SBV diagnosis

In order to investigate the occurrence of SBV of test bee colony, ten worker bees of *A. cerana* were collected and separated with total RNA by using Trizol. Using liquid nitrogen and pulverized then mixed 200μl chloroform and Trizol 1ml with 100mg samples, vortexing for 15 seconds, and reacting in the room temperature for 2~3 minutes. After the reaction, samples were centrifuged in conditions of 12,000 x g and in 4°C for 15 minutes while separating supernatant and mixing it with 500μl isopropanol and reacting in the room temperature for 10 minutes. Then, they were centrifuged in conditions of 12,000xg and in 4°C for 15 minutes, and pellet was washed by using 80% EtOH. Final pellet was melted with DEPC water and used for cDNA synthesis.

cDNA synthesis was made in 42°C for 60 minutes after cooling down in ice for five minutes in 65°C with PrimeScript II 1st strand cDNA Synthesis kit by using oligo dT primer with extracted total RNA as a mold.

Synthesized cDNA was used for amplifying the Sacbrood virus (SBV) gene by PCR, and SBV gene was repeated in 33 cycles in conditions of pre-denature 94°C for 5 minutes, denature 94°C for 1 minute, annealing 50°C for 1 minute, and extension 72°C for 1 minute by using Forward primer 5'-GAC CAA GAA GGG AAT CAGC-3', Reverse primer 5'-CAT CTT CTT TAG CAC CAG TAT CCA-3', while performing final extension in 72°C for five minutes. The primer set was deigned on GenBank No. NC-002066 on NCBI. PCR outcome was 1% agarose gel containing 0.5ug/ml ethidium bromide and confirmed with UV-light by proceeding electrophoresis.

RESULTS AND DISCUSSION

Comparison of temperature and humidity in the test bee colony

The temperature and humidity of the *A. cerana* in the colony, which is mixture of *A. cerana* and *A. mellifera*, was compared with that of a single species of *A. cerana*. Changes in temperature and humidity were measured at 9:00, 12:00, 15:00 and 18:00 hours daily. In the single sepsis of *A. cerana* in single hive, there were changes in temperature of 34.3°C, 35.6°C, 36.1°C, and 35.9°C. In the single sepsis of *A. cerana* in double hive were changes in temperature by 33.9°C, 34.7°C, 35.5°C, and 35.6°C. When mix with *A. mellifera* and *A. cerana*, there were changes in temperature of 35.3°C, 35.6°C, 35.8°C, 35.7°C as the temperature of bee colonies mixed species colony (Fig. 2A). Therefore, it was confirmed that stable temperature was maintained in mixed species colony. The mixed

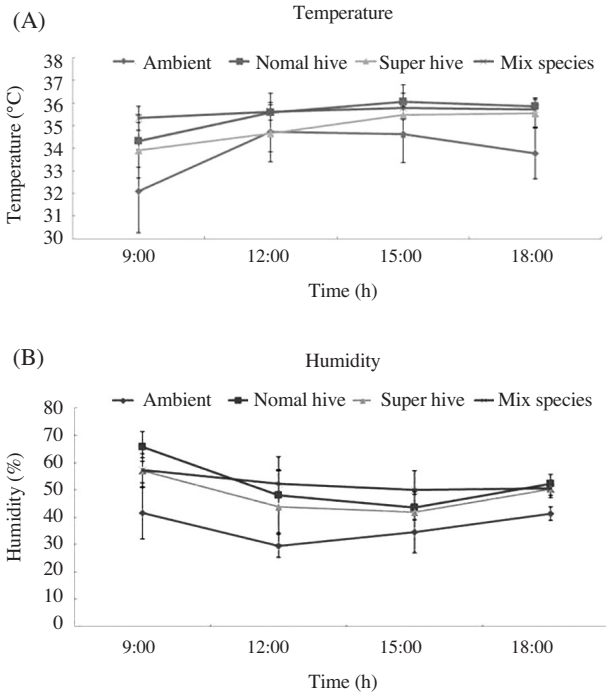

Fig. 2. The temperature and humidity changes in colonies. The normal and Super hive are *A. cerana* colonies. The mix species was mix with *A. mellifera* and *A. cerana*. Temperature and humidity are checked by Quick Start for the HOBO U0 Series DATA Logger at 9:00, 12:00, 15:00, and 18:00.

colonies were found to maintain a very stable temperature compared to other colonies with temperature changes, so that the probability of sacbrood virus occurring is expected to be low.

As a result of observing the change in the internal humidity of the colony used in the experiment, the humidity around the beehive was 41.7%, 29.5%, 34.7%, 41.3%. The variation of the humidity of the colony management for *A. cerana* in single hive was found to be 65.8%, 48.2%, 43.7%, and 52.2%. In the single sepsis of *A. cerana* in double hive were changes in humidity by 57.3%, 52.3%, 50.3%, and 50.5%. When mix with *A. mellifera* and *A. cerana*, there were changes in humidity of 57.3%, 52.3%, 50.3%, 50.5% (Fig. 2B). Therefore, it was confirmed that stable temperature and humidity was maintained in mixed species colony very stably. The mixed colonies were found to maintain a very stable temperature and humidity was compared to other colonies with temperature changes, so that the probability of sacbrood virus occurring is expected to be low.

In 2009, about 90% of the beehives were killed after sacbrood virus infected with *A. cerana* colony in Korea. Sacbrood virus infection has been chromized for about 1 year. The characteristics observed in the apiary, which manages the *A. cerana* in Korea for 10 years, show a tendency of sacbrood in the high seasonal fluctuations of spring and fall or in the rainy season with high humidity. Therefore, it is expected that mixed colony can prevent the onset of sacbrood virus because it can maintain temperature and humidity stably.

Comparison of growth in bee colony

In order to confirm the influence of mixed species on the growth of bee colonies, investigation was made on the growth of test bee colony. Test bee colonies were artificially infected with Sacbrood virus on early March 2016 by supplying the feed soaked with 2×10^6/ml viruses on the liquid. In generally, Number of sacbrood virus particles for occurring sacbrood disease symptom is over 2×10^6/ml viruses per bee. Investigation on the growth of bee colony has been made by measuring and comparing the number of worker bees, pupa, and larvae form March to July 2016. According to the results of investigation on the growth of bee colonies managed after mix the *A. mellifera* and *A. cerana* into down hive and up hive, the number of worker bees was 3,819.2 on March in early spring and 3,992.1 on July 2016. Therefore, it was confirmed that the number of worker bees was consistently maintained before contamination of Sacbrood virus. However, in case of single species for *A. cerana*, the number of worker bees was 3,185.2 on March that ended up decreasing to 722.4 on June. Therefore, single species colonies were collapsed due to Sacbrood viruses on July (Fig. 3A).

In case of growth on pupa of test colonies, the number of pupas in mixed colonies was 1,006.4 on March that ended up decreasing to 523.7 on July. However, in case of single species, the number of them was 1,201.1 on March that ended up decreasing to 108 on June, and all died on July (Fig. 3B). As a result of growth on larvae, the number of

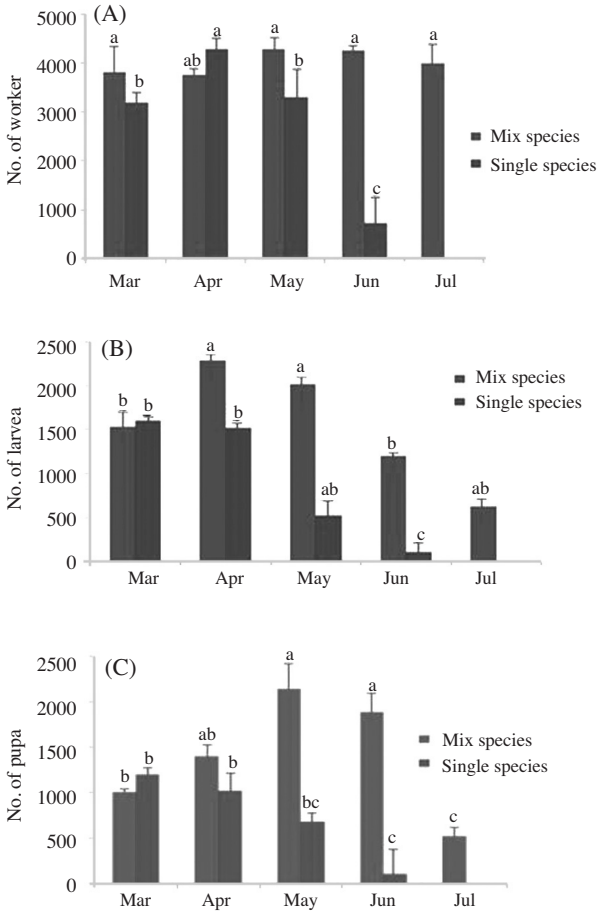

Fig. 3. Comparison with development of colonies in hives (mixed species and single species). Adult worker bee, Pupa, and larva was counted for comparison of colony developments by temperature and humidity variable.

larvae was 1,530.6 in case of mixed species that ended up decreasing to 623.2 on July. However, in case of single species, the number of larvae was 1,601.4 on March that ended up decreasing to 109.3 on June, and all colonies were collapsed on July (Fig. 3C).

Therefore, as for mixed species, it was possible to maintain the growth in the colonies that Sacbrood virus occurred, and collapse rate was lower than single species. However, according to characteristics of Sacbrood virus, once the disease occurs, there is a time difference that makes colonies collapses. However, most of the cases were that bees went out of the colonies or collapsed. It seems that the most important assignment to pursue in the future is to develop medicine for preventing and treating such Sacbrood virus or selecting and breeding systems that are outstanding in resistance against Sacbrood virus.

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