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**Poster presentation - 화분매개 및 밀원자원****Analysis of regional climate and production of acacia honey in 2023****You-Young Jo, Yong-soo Choi, Su-bae Kim, Bo Sun Park and Daegeun Oh**

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현대사회에서 기후변화는 지구 생태계와 인간 사회에 많은 영향을 미치고 있다. 폭염, 강우, 홍수 등 극단적인 기후의 변화에 따른 강도와 빈도 또한 최근 매우 심각하다. 양봉산물의 대부분을 차지하고 있는 벌꿀의 생산 역시 밀원수의 생육 및 개화와 관련하여 기후변화의 영향을 받고 있으며, 이에 따라 벌꿀의 생산량 또한 좌우된다. 아까시 나무는 국내 벌꿀 생산량의 70%를 차지하는 중요한 밀원으로 양봉농가의 소득과 밀접하다. 따라서, 농촌진흥청에서는 2018년부터 기후변화에 따른 전국 지역별 아까시나무의 개화시기, 꿀벌상태, 꿀 생산량 등을 현지 조사하고 아까시 개화기의 기상조건을 지역별로 분석하였다. 아까시 나무의 개화기간은 2023년 11.7일로 2022년 12.2일 보다 줄어들었으며, 특히 남부지역은 개화초기 비바람의 영향을 받아 최저기온과 최고기온이 낮아 지고 평균강수량이 증가하였다. 하지만 중북부 지역의 개화기간 이상기상(강우, 강풍, 저온) 현상이 없어 전체 아카시아 꿀 생산량은 봉군당 평균 생산량 26.8Kg으로 평년(2017) 대비 1.5배 증가하였다.

검색어: 아카시아꿀, 지역, 기후분석, 2023년

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**Poster presentation - 화분매개 및 밀원자원****Design and Demonstration of an Application for Analyzing the Honeybee Colony Environment and Activities****Jea-Chul Kim, Chihyeon Ahn, Hyeon Seung Shin and Jin-Hwan Jeong**

AirTech Inc

Currently, about 70% of domestic honey production comes from the Robinia pseudo-acacia, but recent changes in the flowering season of the Robinia pseudo-acacia have reduced the productivity of migratory beekeeping. Changes in the flowering season in local areas are leading to improper beekeeping management and pest outbreaks by beekeepers. To prevent these damages and to ensure stable beekeeping activities, it is important to accurately analyze the beehive environment and identify the flowering period of honey plants according to local weather conditions. In this study, we designed a service that uses spatial information to determine the distribution of pollen plants and the possibility of bee activity according to weather conditions, and to establish a pollination schedule according to weather forecasts. The service developed as an application allows users to register their interest areas to receive information on the flowering time of pollinator plants and various pollination activities.

**Key words:** Migratory beekeeping, Climate change, Honey plants, Weather forecast, Appplication

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## Poster presentation - 화분매개 및 밀원자원

## Effects of carbohydrate feeding types on honey bee survival rate

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꿀의 근원 식물종이 꿀벌의 생존율에 미치는 영향을 분석하였다. 본 연구를 수행하기 위해 양봉꿀벌(*Apis mellifera*)을 대상으로 선정하였다. 실험에 사용된 꿀은 피나무, 밤나무, 아까시나무, 잡화로 하였다. 대상 꿀이 실제로 해당 식물에 기원하였는지 평가하기 위해 DNA metabarcoding 분석을 수행하였다. 또한, 자당용액 50%, 25%, 5% 처리군을 추가하여 각각의 생존율을 비교하였다. 생존수는 100시간까지 측정하였다. 그 결과 자당용액 50%가 가장 높은 생존율을 나타냈고, 잡화꿀이 두 번째로 높은 생존율을 나타냈다. 생존율의 결과에 가장 큰 요인은 수분량인 것으로 보이며, 추가적인 실험을 통해 결과를 보충할 예정이다.

**Key words:** Monofloral honey, Multifloral honey, Amur linden, Black locust, chestnut

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## Poster presentation - 육종 및 생리생태

Comparison of Pollination Activities Between Honeybee (*Apis mellifera* L) A Bred in the watermelon Green House

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우리나라 시설과채류의 서양종꿀벌 사용률은 2011년 45.8%에서 2016년 64.8%, 2020년 92.7%로 증가하였다('21, 농과원). 기존 꿀벌 신품종을 화분매개용으로 이용가능성 모색을 위하여 수박에 서양종꿀벌 수밀력 우수 A품종과 일반벌의 화분매개 특성조사를 하였다. 그 결과 평균 소문 출입 총활동 수는 A품종 46.8±31.5, 일반벌 44.8±30.1, 평균 방화활동비율 A품종 43.2±8.9, 일반벌 40.3±9.5, 평균 천장 및 비행활동수 130.3±85.2, 일반벌 63.3±83.0으로 A품종의 활동성이 높은 경향은 있었으나, 통계적으로 유의미한 차이가 없었다. 시간대별 평균 방화활동 수는 A품종과 일반벌 모두 오전 9-11시가 높고, 오후 1시 이후로 낮아짐을 알 수 있었다. 평균방화활동시간(second)은 장원벌 7.4±4.1, 일반벌 7.1±3.6으로 통계적 차이는 없었다. A품종과 일반벌의 수박 착과율은 88.3±9.9, 91.7±4.1로 일반벌이 높았으나 통계적으로 유의미한 차이가 없었다. 이를 통해 A품종은 단기사용 화분매개작물 이용에 일반벌과 차이가 없음을 알 수 있었다.

검색어: 화분매개, 서양종꿀벌, 수박, 시설재배

사사: 본 연구는 농촌진흥청 연구과제 PJ0158704의 연구비로 지원된 결과이다.

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## Poster presentation - 육종 및 생리생태

**Initial results for selecting breeding lines of *Apis cerana koreana* with low level of swarm**

**Peter Njukang Akongte<sup>1,2</sup>, Hlaing Min Oo<sup>1</sup>, Bo-Sun Park<sup>1</sup>, Dong-won Kim<sup>1</sup>, Ogweng Geoffrey Ebuu<sup>1</sup>,  
Minwoong Son<sup>1</sup>, Dae-Geun Oh<sup>1</sup>, You-Young Jo<sup>1</sup>, Su-bae Kim<sup>1</sup> and Yong-Soo Choi<sup>1\*</sup>**

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Swarm behavior in honeybees is another form of reproduction though it creates an interruption in the brood cycle of the original colony which may lead to colony collapse. In the history of beekeeping, beekeepers are faced with colony lost as a result of swarm. Selecting and maintaining colonies with low levels of swarm is key in mitigating the effects of colony lost. Twelve colonies (three colonies per breeding line) were placed on the same condition and the number of queen cells, drone cells, clusters, and swarm were recorded weekly. Pure bred lines X and R had the highest number of queen cells and the lowest number of drone cells compared to unknown bred lines (mixed breeding). Though the weekly number of queen cells fluctuates, it was found to be more stable in mixed bred lines. Body morphological traits were evaluated, and the results demonstrated that bred lines with large size workers' abdomen showed more ability of constructing queen cells and consequently high probability of swarm compared to bred lines with small size workers' abdomen. Generally, pure bred line X showed more swarm characteristics compared to other lines while this behavior was more stable in the mixed bred line.

**Key words:** *Apis cerana Koreana*, swarm behavior, breeding lines, morphological traits.

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## Poster presentation - 육종 및 생리생태

**Effects of Different Beebread on Performances of Queen Honeybee (*Apis mellifera*)  
(Hymenoptera: Apidae)**

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The European honeybee (*Apis mellifera*) is an important pollinator of wild plants and agricultural crops. Therefore, honeybee health is of paramount importance to the apiculture and agriculture industries. Nutritional value of bee diets has a direct impact on the health and strength of colonies. Dietary problems have been linked to declining honeybee health and population growth. For these reasons, beekeepers feed artificial diets (beebread) to their bees during off-seasons to provide energy and biochemical components required for brood rearing, thermoregulation, foraging, and to stimulate immune responses to disease. We have developed three novel formulations of beebread (D1, D2, and D3) and compared their efficacy with commercial beebread under field conditions. Mustard pollen, clove powder, and turmeric powder were the major constituents of new formulations. During this study, the egg laying capacity of bee queens and the colony growth of honeybees were evaluated at the apiary of National Pingtung University of Sciences and Technology. In comparison with the other bee colonies fed with different beebreads, the colonies that received beebread D1 showed a higher egg count, a sealed brood area, a higher bee strength, and a higher pupae survival rate. According to the results of this study, D1 beebread has the potential to be a useful alternative to commercial beebread when natural pollen is in short supply or unavailable.

**Key words:** Bee, beebread, clove, mustard, pollen, turmeric

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## Poster presentation - 육종 및 생리생태

Colony development of *Bombus terrestris* by various stable period after artificial insemination

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In this particular experiment, the focus was on enhancing the artificial insemination success rate for rear *B. terrestris* queen bees and ensuring the retention of superior traits. We achieved this by varying the stable period to 2, 4, 7, and 10 days within the existing artificial insemination method. Several key parameters, such as the oviposition rate, colony development rate, new queen or worker bee emergence rates, were investigated. Remarkably, the results of the experiment demonstrated a insemination succeed rate exceeding 99% across all experimental groups. Additionally, the oviposition rate was confirmed to be over 80% in all cases. The emergence rate of worker bees reached its peak at 22.8% on the second day of stability, while the emergence rate of queen bees significantly peaked at 6.1%. Furthermore, it was observed that worker bees appeared the fastest at 25.9 days on the 7th day of stability, while queen bees appeared the fastest at 33 days on the 4th day of stability. These findings indicate significant progress in optimizing the artificial insemination process for rear queen bees and ultimately contribute to the preservation of the species.

**Key word:** *Bombus terrestris*, artificial insemination, stabilizing days, colony development

**Acknowledgment:** This work was supported by a grant from the National Institute of Agricultural Sciences, Rural Development Administration (Project No.: PJ01587802)

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## Poster presentation - 육종 및 생리생태

## 3종의 화분매개곤충에서 형광 가루를 이용한 비벡터링 작동

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과수화상병은 세균성 병해로 전염성이 강하고 과수를 고사시키기 때문에 세계적으로 문제가 되고 있다. 하지만, 아직 치료 방법이 알려지지 않았으며 발병하기 전 예방이 중요하며, 과수화상병 병원균이 증식하기 시작하는 개화기는 중요한 방제 시기이다. 많은 과수원에서 과수 수분을 위해 서양꿀벌(*Apis mellifera*)과 서양뒤영벌(*Bombus terrestris*)이 사용되고 있고, 벌이 꽃을 방문하는 과정을 이용하는 비벡터링은 개화기에 과수의 수분과 동시에 방제를 가능하게 한다.

본 연구는 비벡터링을 활용하여 과수화상병 방제기술을 개발하고자 수행되었다. 현장에서 육안으로 확인하기 어려운 미생물제제를 형광 가루와 혼합해 사용하여, 3종의 화분매개곤충(토종꿀벌(*A. cerana*), 서양꿀벌(*A. mellifera*) 그리고 서양뒤영벌(*B. terrestris*))에서 비벡터링 작동을 검증하고 비교하였다. 3종이 각각 투입된 격리된 과수원의 샘플링된 사과꽃 모두에서 형광가루가 확인되었다. 종에 따라 전달되는 정도에서 약간의 차이는 있었지만, 3종 모두에서 비벡터링을 통해 매우 높은 확률로 작물에 전달됨을 알 수 있었다. 추가적으로 외에도 형광가루를 확인할 수 있었고, 적절한 미생물제제를 선택하면 벌을 통해 수분하는 작물들에서 화상병뿐 아니라 다양한 병해충 방제에 활용될 수 있을 것으로 기대된다.

검색어: 화분매개, 비벡터링, 과수화상병

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## Poster presentation - 육종 및 생리생태

## Assessing the impact of bee vaccines on social immunity in honeybee hives

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Transgenerational immune priming in honeybees (*Apis mellifera*) has been attracting increasing attention as a promising strategy to protect colonies from infectious diseases. This study investigated whether feeding honeybees with bee vaccines containing heat-killed pathogens can provide them with transgenerational immunity to these pathogens. Here, we produced bee vaccines containing heat-killed *Ascosphaera apis* (A) and/or *Paenibacillus larvae* (P) supplemented with or without peppermint oil (PO). For the hive experiments, we supplied bee vaccines, such as A + P cocktail (heat-killed *A. apis* (10<sup>5</sup> spores/ml) and *P. larvae* (10<sup>4</sup> cells/ml) in a 1:0.5 ratio), A + P + PO cocktail (A + P cocktail supplemented with 0.05% PO), P (heat-killed *P. larvae* at the concentration of 10<sup>4</sup> cells/ml), and P + PO cocktail (P supplemented with 0.05% PO), to the hives. Control hives were supplied 40% honey with or without 0.05% PO. We found that *vitellogenin* (*Vg*), *defensin-1*, *insulin-like peptide 2* (*ILP2*), and body weight were significantly increased in nurse bees upon feeding them with bee vaccines. In the drone bees of the hives supplied with bee vaccines, *defensin-1*, *ILP2*, and body weight were also increased. Additionally, *defensin-1* was upregulated in the young worker larvae in these hives. In correlation, the young worker larvae showed high pathogen resistance to *A. apis* and *P. larvae* infection. Our results demonstrate that introduction of bee vaccines into hives is an efficient method for the protection of honeybee colonies from infections.

**Key words:** *Apis mellifera*, Honeybee, Immune priming, Bee vaccine, Defensin**Acknowledgement:** This work was performed with the support of the “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ0157552021)” of the Rural Development Administration, Korea

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## Poster presentation - 육종 및 생리생태

## Diversity of the pollinator communities in agroecosystem across Korea

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농업생태계에서 흔히 관찰되는 수분매개자 중 나비와 나방은 꿀벌이나 파리목 곤충보다는 중요성이 덜 하지만 비교적 많은 부분을 차지하고 있다. 이 연구는 우리나라 농업생태계에 서식하는 나비 군집의 다양성을 알아보려고 하였다. 우리는 2020년 이후 2022년까지 전국 13개 지점을 3개 권역으로 나누어 1-2주 간격으로 선조사법을 실시하여 나비 군집 다양성을 살펴보았다. 그 결과 91종 10,986개체가 확인되었다. 종 다양성이 가장 높은 지역은 서북부이었고 개체수가 가장 많은 곳은 남부지역이었다. 각 조사권역에서 채집된 나비의 군집분석 결과 3개의 독립된 군집 구조를 나타내었다. 현재 진행되고 있는 기후변화가 이들 권역별 나비 군집 다양성에 어떠한 영향을 미치고 있는가에 대한 지속적인 조사가 필요하다.

검색어: 나비, 군집분석, 농업생태계, 화분매개, 기후변화

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## Poster presentation - 육종 및 생리생태

## Generation of Bee Immune Enhancer using honeybee gut microbiota

Hyunchan Song and Ki-Young Kim

Kyung Hee University

105 gut microbiota of honeybee were isolated according to its phenotype and 33 microorganisms were related to honeybee immunity. They were incubated individually and were filtered to eliminate left microorganisms. For in vitro experiments, All filtered solutions were treated on *T. ni* cells. qRT-PCR of immunoprotein transcripts revealed 6 microorganisms had immune enhancing effect. They were fed to honeybees and *A. kunkeei*, *W. anomalus* enhanced immunity of honeybees. They had their best synergy when ratio of *A. kunkeei*, *W. anomalus* were 60%, 40% respectively. Also 49 chemicals were screened for immune-boosting effect in insect cells and 2'-Methoxyflavone was the most effective and 5ug/uL, it had its best effect. As a product, mixture of *A. kunkeei*, *W. anomalus* in ratio of 60%, 40% respectively with 5ug/uL of 2'-Methoxyflavone had great effect on immunity of honeybees and could be used as a cure for honeybee disease.

**Key words:** *Apis mellifera*, Immune, microbita

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## Poster presentation - 육종 및 생리생태

## Anti prostate cancer effect caused by Trp-Arg rich region peptide of bee

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Antimicrobial and anticancer effects of Trp-Arg rich peptides such as tritrypticin, lactoferricin, and indolicidin are well known, and indolicidin. Most anticancer drugs target the inside of the cell, so drugs must be carried into the cell. It easily makes drug-resistant cancer cells. But anticancer peptides target cell membranes without being carried into the cell. We found some Trp-Arg rich regions in the cDNA of various bees and figured out conserved sequences for the synthesis of anticancer-targeting peptides. Synthesized peptides have anti prostate cancer effects in 2D and 3D cultures. We predicted the peptide has an anticancer effect through cancer cell apoptosis. Indeed, upon microscopic examination subsequent to peptide treatment, the observed anti prostate cancer effect was postulated to be a result of apoptosis. To find the target of the peptide, qRT-PCR, and western blot was performed. The western blot result indicates anticancer peptides reduce JNK and p53. Therefore, our study revealed that the Trp-Arg rich region peptide exhibits an anti-prostate cancer effect caused by apoptosis.

**Key words:** anticancer, trp-arg rich peptide, apoptosis

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## Poster presentation - 육종 및 생리생태

## A Comparative Investigation of Various Pollen Substitute Diets and Their Impact on Honeybee Colony Performance in the Early Spring Season

**Hyun Jee Kim<sup>1</sup>, Frunze Olga<sup>1</sup>, Myeong Lyeol Lee<sup>1</sup> and Hyung Wook Kwon<sup>1,2</sup>**<sup>1</sup>Convergence Research Center for Insect Vectors, Incheon National University<sup>2</sup>Department of Life Sciences, College of Life Science and Bioengineering, Incheon National University

이른 봄의 영양은 꿀벌 봉군의 건강과 세력에 영향을 준다. 사료별로 봉군 세력에 미치는 영향을 평가하기 위하여 후보화분대체사료 3가지와 대조군 (Megabee)을 준비하여 이른 봄 강릉에서 봉개 면적, 봉군 밀도, 섭취량, 봉군 무게, 꿀생산량, 위생행동, 그리고 Vitellogenin (Vg) 발현을 평가하였다. 봉개 면적, 봉군 밀도, 섭취량, 봉군무게, 꿀생산과 Vg 발현량이 한가지 후보사료에서 통계적으로 유의하게 가장 높게 나타났다. 종합적으로 개발중인 후보화분대체사료가 이른 봄 봉군 유지와 세력 강화와 꿀생산량에 효과적인 것으로 나타났다. 이는 현재 꿀벌 개체수 감소에 대응하여 건강한 봉군 성장에 이바지할 수 있을 것으로 기대된다.

검색어: 화분대체사료, 꿀벌, 봉개 면적, 봉군 밀도, 섭취량, 꿀생산량

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## Poster presentation - 육종 및 생리생태

Seasonal monitoring of honey bee (*Apis mellifera*) hive growth**Bo-Sun Park, Minwoong Son, Daegeun Oh, Akongte Peter Njukang, Dongwon Kim, Su-Bae Kim and Yong-Soo Choi**

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최근 이상기후 및 농약의 사용은 꿀벌류를 대상으로 큰 위협이 되고 있다. 안전한 꿀벌 봉군 관리와 피해 방지 대책을 위한 기초 정보 자료가 요구되고 있다. 그래서 본 연구는 양봉꿀벌 봉군을 대상으로 연중 봉군 발육 모니터링을 수행하였다. 시험 대상 봉군은 단상과 계상이 섞인 평균 소비 6매 규모 봉군으로, 총 10개의 봉군을 선정하였다. 2020년 4월부터 2022년 8월까지 봉군 발육도를 조사하였다. 기상데이터는 백엽상을 이용하여 평균 온습도, 최고, 최저 온습도 등을 기록하였다. 월동 시기를 제외한 연중 외부 온습도에 따른 일벌, 봉판, 유충 및 산란의 비율을 모두 확인한 결과 6월에는 봉군 발육도가 양호한 반면에 고온 다습 기온이 지속되는 8월에는 매년 일벌수, 봉판수, 유충의 수가 전체적으로 감소하는 경향을 보였다. 본 연구의 결과를 통해 외부 기상에 따른 양봉꿀벌 봉군 발육 추정에 필요한 기초 자료로 사용될 수 있을 것으로 사료된다.

검색어: 생물계절학, 이상기온, 봉세, 온도 의존성, 산란율

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## Poster presentation - 육종 및 생리생태

**Using image deep learning to measure flight speeds honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) near their hives****Kyeong Yong Lee, Young Bo Lee, Sankkar Kathanan, Lee Su Jin and Kwak Kyu-Won**

Department of Agricultural Biology, The National Institute of Agricultural Science, RDA

Bee traffic can be used as an important indicator of foraging activity. We investigated the flight speed of honeybees and bumblebees near their hives as a basis for calculating bee traffic using image deep learning. The flying speed of bumblebees ( $0.48 \pm 0.36$  m/s) near the hive was 1.4 times faster than that of honeybees ( $0.35 \pm 0.21$  m/s). The flight speed of honeybee leaving the hive ( $0.54 \pm 0.33$  m/s) was 1.7 times faster than that when entering the beehive ( $0.32 \pm 0.18$  m/s). Distance from the hive and flight speed showed a positive correlation (honeybee  $r=0.600$ , bumblebee  $0.659$ ), and a significant linear regression model was derived (honey  $R^2=0.516$ , bumblebee  $=0.433$ ). Therefore, our results can help determine the ideal frame rate for effectively capturing and recognizing the flying image of bees when calculating bee traffic.

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**Key words:** hive insulation, strawberry, pollination, honeybee

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## Poster presentation - 육종 및 생리생태

**Effect on hive lifespan by thermal insulation of *Apis mellifera* L. hive in strawberry greenhouse****Kyeong Yong Lee, Young Bo Lee, Sankkar Kathanan, Lee Su Jin and Kwak Kyu-Won**

Department of Agricultural Biology, The National Institute of Agricultural Science, RDA

Bee pollination is imperative for strawberry production during winter in Korea. We examined the effect of thermal insulation of the hive on strawberry pollination. In terms of hive lifespan, the average temperature inside the insulated hive bee box was  $4.6$  °C higher than ordinary bee box, and the time taken for the bees to completely vanish from the hive was 20 days longer. Therefore, it is thought that thermal insulation of the hive is essential for pollination of strawberries in winter because the heat-insulating hive can improve the internal temperature of the hive, increase the activity of bees and the lifespan of the hive.

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**Key words:** hive insulation, strawberry, pollination, honeybee

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## Poster presentation - 육종 및 생리생태

**Evaluation of bees' ability to build a hive according to frame materials and color****You-Young Jo<sup>1</sup>, Bosun Park<sup>1</sup>, Changhoon Lee<sup>1</sup>, Jin Won Park<sup>2</sup>, Jong Hee Kim<sup>3</sup> and Seung Goo Lee<sup>2</sup>**<sup>1</sup>Apiculture Division, Department of Agricultural biology, National Institute of Agricultural Science, RDA<sup>2</sup>Department of Organic Applied Materials Engineering, Chungnam National University, Daejeon, Korea<sup>3</sup>Research Institute for Applied Chemistry and Biological Engineering, Daejeon, Korea

꿀벌이 집을 짓는데 기초가 되는 소초는 밀랍을 주원료로 사용하고 있다. 하지만, 벌꿀을 수차례 생산하면서 소비는 사용이 곤란해지며, 밀랍에는 각종질병, 인자, 농약, 등이 축적될 수 있으나 세척이 불가능해 지속적으로 사용하기는 어려운 실정이다. 또한 적당한 폐기 방법이 없으므로 정해지지 않은 곳에서 소각되고 있다. 이러한 문제를 해결하기 위하여 반영구적으로 사용할 수 있는 재질의 소초를 제작하고 이들 소초의 재료구성과 색상에 따른 조소능력을 비교 분석하였다. 주 원료로는 PLA(poly lactic acid), PP(polypropylene), Beeswax, Talc, wood flour, compatilizer 등을 사용하여 재료의 첨가에 따라 다양한 소비를 3D 프린팅을 이용해 제작하였다. 꿀벌의 친화력을 위해 약간의 Beeswax를 혼합하였으며 제작된 소비의 강도를 조절하기 위해 다양한 화합물을 사용하였다. 또한 검정, 노랑, 빨강색의 소비를 제작하여 색상에 따른 조소 능력을 비교하였다.

검색어: 벌집조성물, 색상, 조소능력

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## Poster presentation - 육종 및 생리생태

**Validation of Reference Genes and Expression analysis in the Bumblebee (*Bombus terrestris*) and its Three Seasoning relatives by Quantitative real-time RT-PCR****Kathannan Sankar, Kyu-Won Kwak, Kyeong Yong Lee, Young Bo Lee and Su Jin Lee**

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As post genomics studies on bumblebees (*Bombus terrestris*) increase exponentially it is particularly important to normalize quantitative real-time RT-PCR (qRT-PCR) data with appropriate reference genes. For this aim, a software-based approaches (Ref-Finder) to evaluate the suitability of five candidate reference genes (actin, arginine kinase, phospholipase A2, ribosomal protein s18, and elongation factor 1-alpha) and used of these genes as biological endogenous controls. Their expression was examined during rearing condition and different environment seasoning by bumblebee development, in different tissues. Furthermore, the importance of choosing an appropriate reference gene was investigated for two fundamental genes of pathway; Endocrine regulatory target genes. The results led us to consider all candidate genes as suitable genes for normalization in *B. terrestris*. However, each the conditions evaluated in this study revealed a specific set of genes that were most appropriate for normalization in *B. terrestris*.

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**Key words:** reference gene; qRT-PCR; gene expression; *Bombus terrestris*; Ref-Finder; gene validation