

0001

Host-associated genetic differentiation in the cotton-melon aphid, *Aphis gossypii* Glover

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Many polyphagous herbivore insects considered as a single species are indeed comprised of genetically differentiated host related races that utilize several unrelated plant families. *Aphis gossypii* Glover, one of the extremely polyphagous aphid species, has more than 100 host plants distributed worldwide. In Korea, it takes holocyclic life cycle, with a number of primary hosts, which is unusual for other aphid species. However, former population genetic studies on *A. gossypii* have dealt with the populations from only a few agricultural host plants. In this study, we hypothesized that different primary host utilization may be restricted for certain host races and this may play a key role of the genetic differentiation in this species. We collected the cotton aphid populations from 36 different host plants, 5 primary and 31 secondary hosts including various wild and non-cultivated plants. To determine population genetic structure, we analyzed genetic differentiation among those populations using 9 microsatellite loci. As results, four genetically distinct clusters were verified. Of the five primary host populations, there were significant gaps in genetic differences between *Rhamnus*-associated group and *Hibiscus*-associated group, and the other populations were affinitive with the *Hibiscus*-associated group. Overall, primary host populations have a higher level of genetic diversity than secondary host populations. Several populations associated with secondary hosts such as spiderwort, madder, and Korean lettuce were considerably differentiated in the result of principle component analysis, which nearly showed the species level difference similar to *Aphis glycines* Matsumura and *Aphis* sp. ex *Rhamnus*. Thus, there could be reproductively isolated by the loss of primary hosts, and might be already speciated from *A. gossypii*.

Key words: host races, microsatellite, population genetics, ecological speciation, *Aphis gossypii*

Larval Gall-Making moth, *Borboryctis euryae* Kumata et Kuroko (Lepidoptera, Gracillariidae) New to Korea

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Mine and gall makers among Lepidoptera are found in the family Gracillariidae. The genus *Borboryctis* Kumata et Kuroko, 1988, have 2 species *B. euryae* Kumata et Kuroko and *B. triplaca* (Mayrick). *B. euryae* is known a gall maker of *Eurya* spp.

In this study, *Borboryctis euryae* Kumata et Kuroko, 1988 feeding on *Eurya japonica* Thunb. and *E. emarginata* Makino (Theaceae), is reported for the first time from Is. Jeju and Geoje, Korea. The host plant, *Eurya* spp. an evergreen tree, is widely distributed in East Asia including Korea.

The early instar larva makes linear mine near the surface of the leaves and mature larva makes gall on the leaves of the host plant. Larval frass ejected behaviour were not observed. The mature larva makes a light brown spindle-shape cocoon on the somewhere of the host plant, mainly edge of the leaf. The descriptions and figures of the male and female genitalia, mine, gall, larvae and pupae in immature stages, are provided.

Key words: *Borboryctis euryae*, gall maker, leaf miner, Gracillariidae, *Eurya japonica*, *E. emarginata*, unrecorded species, Korea

0003

Contribution to the hymenopteran fauna of Cambodia and its adjacent countries: Two new species and three new records of Bethylidae (Hymenoptera: Chrysidoidea)

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Members of Bethylidae are widely distributed from the Tropic to the Subarctic regions around the world. The family contains about 2,216 species worldwide, and the majority of recorded species are found from the tropical regions. From the Oriental region, 368 species of 46 genera in four subfamilies has been recorded up to date.

Two species are recognized as new to science and three to the fauna of Cambodia and its adjacent countries in the present study. We provide microphotographs and diagnoses of each species with a general introduction of Cambodian bethylids.

Key words: Bethylidae, Cambodia, new record, new species, Oriental region

0004

Phylogenetic relationships of the tribe Macrosiphini (Hemiptera: Aphididae: Aphidinae): Habitat and morphological characters based on molecular data

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Macrosiphini is the most largest group in aphids, comprising near half of the described aphid species. The existence of Macrosiphini could be related to host alternation and various life cycle. Since the phylogenetic relationship of the tribe Macrosiphini has been still controversial, we analyzed Bayesian phylogeny (BP) and Maximum-likelihood (ML) based on molecular data (cytochrome oxidase I, tRNA-leucine+cytochrome oxidase II, 16S ribosomal RNA and elongation factor-1 alpha gene), and compared with the Shaposhnikov (1998) 's subtribal division: Macrosiphina, Myzian, Anuraphidina and Liosomatinae. Analyses for host range association with the morphological characters (e.g. Siphunculus and antennal tubercle on head) correspond to the tendency of host range radiation in the tribe Macrosiphini.

Key words: Aphididae, Macrosiphini, COI, tRNA+COII, 16S rRNA, EF1 α .

0005

Biodiversity of Subfamily Phylinae (Heteroptera: Miridae) in Cambodia

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The landmass of Cambodia is located in the Southern portion of Indochina Peninsula in Southeast Asia which landscape is characterized by a low-lying central plain, eastern highlands, southwestern upland blocks and northern plain of sandstones. The low lands and small mountain clefs of Cambodia is known for deep tropical rain forest which flourish wide range of plants and animals that are mostly concentrated in the "Tonle Sap" range. On the evidence of our investigation it suggests that the evergreen forest of Cambodia suit common fauna with tropical Africa, Indo-pacific region and its adjacent neighbouring countries (Distant, 1904; 1911; Schuh, 1979, 1984; Yasunaga, 2010; Duwal et al., 2010). From the survey data 2010- 2012 (April) more than 30 species of subfamily Phylinae of genera: *Acrorrhinium* Noualhier, 1895; *Alloeomimus* Reuter, 1910; *Atractotomus* Fieber, 1858; *Biliranoides* Schuh, 1984; *Campylomma* Reuter, 1878; *Decomia* Poppius, 1915; *Hallodapus* Fieber, 1858; *Lasiolabops* Poppius, 1914; *Malaysiamiroides* Schuh, 1984; *Papuamimus* Schuh, 1984, *Pilophorus* Hahn, 1826; *Sthenaridea* Reuter, 1885; *Tytthus* Fieber, 1864, etc. with some new species are found. Therefore it is suggested that Cambodian climate and flora shelter members of Oirental fauna included in most tribes of the subfamily Phylinae; Auricillocorini, Hallodapini, Leucophoropterini, Phylini and Pilophorini. Of course this study is not enough for to clarify the phylinae fauna of Cambodia, but it's a preliminary data for further accomplishment in the study of phylinae bugs from this region which is expected to be distributed for about 250-300 species.

Key words: Biodiversity, Hemiptera, Heteroptera, Miridae, Phylinae, Cambodia.

DNA Barcoding from Old Insect Specimens

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DNA barcoding is a strong species identification tool for all animal taxa, and can easily be conducted when materials are under DNA friendly conditions. In contract, a full-length (659 bp) sequencing has been limited for the degraded DNAs extracted from old museum specimens. The initial challenges to retrieve the authentic DNA fragments from old museum specimens were attempted by obtaining short sequences (<300 bp) with the cloning process after PCR, making it both expensive and time-consuming. In this study, we employed a modified method to analyze the full-length DNA barcoding regions in 31~52 year-old butterfly specimens (301 dried specimens of 39 species) using direct sequencing after PCR with two different methods: 1) the successful PCR rates of 0 to 5.6% using four universal primer sets were too low to obtain authentic sequences and the cross-contamination was detected in almost all successful amplicons; 2) the success rates of PCR using specie-specific overlapping primer sets were distinctly high, reaching up to 75% with 98% authentic and 2% non-specific sequences. Thus, the result showed the method that using species-specific primer set per species yields the most effective success rates of both PCR and sequencing from degraded DNA without incorrect sequences.

Key words: taxonom, old insect specimens, DNA barcodes, direct PCR sequencing, Korea.

DNA barcode reference library for the Korean butterfly for the reliable identification

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We tested the identification ability of DNA barcodes comparing with morphological data using the Korean butterflies. The 921 samples (4.6 samples per species) for 202 resident Korean species except migratory species were used. The obtained samples were morphologically identified based on wing patterns. In a result, genetic divergence to the nearest-neighbouring taxon varied from 0 to 28.2%, with an average of 13.4 per cent. The neighbour joining (NJ) tree profile showed that sequence data for 185 of the 202 species formed distinct barcode clusters. Thus, our results indicated that 91.6 percent of the species were possible to allow the reliable identification using DNA barcoding. The rest 17 species (8.4%) consist of following four cases: clustering separated from each species by less than 1% branch length (two species pairs), paraphyletic clustering (two species pairs and one triple species pair), polyphyletic clustering with sharing barcodes (three species pairs), and clustering separated from existing species by the deep branch divergence (four clusters). However, it was not easy to interpret these ambiguous cases only using our current taxonomic evidences. Therefore, we are performing integrative taxonomy on these cases using other additional evidences such as examination on male genitalia and analysis of other gene regions.

Key words: DNA barcoding, DNA barcode library, COI, Butterfly, South Korea

Lepidopteran insect pests on grape, *Vitis vinifera* L., from Korea

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Until now, seven species, *Acosmeryx nega*, *Herpetogramma luctuosalis*, *Ostrinia furnacalis*, *Endoclyta excrescens*, *Spodoptera litura*, *Paranthrene regalis*, and *Nippoptilia vitis*, have been known as lepidopteran grape pests in Korea (Woo, 1980; Lee, 1981; Kim, 1991; NIAST, 2002; Korea grape community, 2009).

We discovered thirteen additional species belonging to eight families from Gwangwon and Chungbuk provinces, Korea. Additionally, we corrected the previous records of *Deuterocopus albipunctatus* and *Nippoptilia vitis*. They were identified by adult external or genitalic characters first, and also confirmed by the COI marker, compared with two public databases, NCBI and BOLD.

Key words: lepidopteran grape pest, identification, COI sequences, morphological study

Correlated evolution of feeding habits and morphological characters in the dark-winged fungus gnats (Diptera: Sciaridae)

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Relationship with fungi is one of the habitat adaptation of Sciaridae. While the earlier studies suggested that many fungus gnats are playing as the important vectors of various fungus diseases on plants and mushrooms, evidences have not been well reported. Based on a multi-gene phylogeny, by the Bayesian framework, we propose the correlation of four morphological characters of adults and habitat adaptation from saproxylic to phytophagous in the Sciaridae. Our results suggest that the evolution of habitat transition from dead plant litter to the live plant is related to habitat adaptation to the phytophagy and the morphological characters of fore tibia apex is also related to the functional linkage between these traits. We performed Bayes factor-based tests, referred with evolutionary pathway test (EPT), to decide the correlated traits gained the during evolutionary processes. The EPTs strongly suggest that fore tibia structures appeared first and followed by plant-feeding adaptation. The divergence time estimations of Sciaridae are also largely congruent with the fossil records. The members of subfamily Megalosphyinae have radiated explosively and contemporaneously since the Oligocene, with the expansion of modern grasslands and the increase of herbivores. Consequently, we suggest that the evolutionary benefit of tibial structure may be closely related with the fungal carrying in Sciaridae.

Key words: Sciaridae, correlated evolution, divergence time estimations.

Tracing the invasion route of *Metcalfa pruinosa* (Hemiptera: Flatidae)

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Metcalfa pruinosa (Say), native to North Korea, is spreading rapidly in the Korean Peninsula, causing serious damages on many deciduous forest trees, ornamental trees, and agricultural crops. Before the first report from Korea in 2005, *M. pruinosa* has not been reported from any other countries in the Eastern Palaearctic, while it has been record in Italy in 1979, and rapidly spreading into many European countries. To trace the invasion route of this species, we analyzed haplotype analysis of the mitochondrial cytochrome *c* oxidase subunit-I (mtCOI, 577bp), and developed microsatellite markers. In the haplotype analysis, a total of fourteen haplotypes were found from 69 individuals in 18 populations: 12 haplotypes (native region), 4 haplotypes (European region) and 2 haplotypes (Korea). Interestingly, Korean populations were clustered with some European populations. Eight polymorphic microsatellite loci were developed, and population structures were analyzed from 145 individuals in 8 populations. The origin and invasion route of *M. pruinosa* are under investigation.

Key words: *Metcalfa pruinosa*, Flatidae, Hemiptera, invasion, microsatellite, population genetics

Identification of the *Diadegma fenestrale* ichnovirus (DfIV) and analysis results of genome draft

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The *Diadegma fenestrale* was known as parasitoid on potato tuber moth, *Phthorimaea operculella* and diamondback moth, *Plutella xylostella*. This species, genus *Diadegma* are first reported from Korea. DfIV showed typical ichnovirus shape which two membranes surround virus capsids. The genome contents of DfIV consist about sixteen double-stranded DNA segments ranging 2 to 6 kb. To identify DfIV genes, whole genome sequencing based on GS-FLX was conducted using purified total viral DNA extracted from *D. fenestrale* calyx. About sixty ORFs were analyzed and several typical polydnavirus gene family detected such as cys-motif, rep, vinnexin and vankyrin. This is the first report of DfIV and these lepidopteran host immune suppression genes will be deeply identified.

Key words: *Diadegma fenestrale*, ichnovirus, DfIV, *Phthorimaea operculella*, *Plutella xylostella*

Characterization of molecular and enzymatic properties of three cholinesterases from *Cimex lectularius*

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We analyzed molecular and enzymatic properties of three cholinesterases (ChEs; ClAChE1, ClAChE2 and ClSChE) from *Cimex lectularius*. The ClAChE1 and ClAChE2 were generally present as a membrane-anchored dimeric insoluble form in the brain and ganglia. In the case of ClSChE, monomeric and dimeric soluble forms were observed. To investigate enzymatic properties, three ChEs were functionally expressed using baculovirus expression system. ClAChE1 revealed a significantly higher activity than ClAChE2 to acetylthiocholine iodide (ATChI) substrate. Kinetic analysis using two choline substrates (ATChI and butyrylthiocholine iodide) demonstrated that ClAChE2 had higher catalytic efficiency but lower substrate specificity than ClAChE1. Inhibition assay was conducted by using three inhibitors (BW284C51, eserine, Iso-OMPA) and two insecticides (chlorpyrifos-methyl and carbaryl). Two ClAChEs revealed high sensitivities to BW284C51, eserine, chlorpyrifos-methyl and carbaryl, but were not sensitive to Iso-OMPA. This inhibition profile confirmed that both ClAChEs are categorized as ChEs. Interestingly, the salivary specific cholinesterase did not show any measurable activities to choline substrates, confirming its non-synaptic function in *C. lectularius*

Key words: Cholinesterase, *Cimex lectularius*, Baculovirus expression, Kinetics, Molecular from.

0013

Inhibitory effect of a polydnavirus, *Cotesia plutellae* bracovirus, on larva-to-pupa metamorphosis of *Plutella xylostella*

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Parasitization by an endoparasitoid wasp, *Cotesia plutellae*, extends a larval period of *Plutella xylostella* and inhibits a larva-to-pupa metamorphosis. To determine antimetamorphic parasitic factor(s) in this host-parasitoid interaction, an effect of its symbiotic polydnavirus, *Cotesia plutellae* bracovirus (CpBV), was investigated by injecting purified virus particles to nonparasitized larvae of *P. xylostella*. Larvae injected with CpBV exhibited antimetamorphosis in a viral dose-dependent manner. Also, the susceptibility to the viral injection was increased at young larval stages. Parasitized or virus-injected larvae showed significant decrease in cell size of prothoracic gland and reduction in expression of ecdysone receptor (EcR) gene. However, they increased and maintained expression of insulin receptor (InR) gene. Twenty four CpBV segments were individually injected to nonparasitized larvae. Only two segments (S22 and S27) had significant antimetamorphic effect. Subsequent RNA interference using double stranded RNA (dsRNA) was performed in each of encoded genes in each segment. Protein tyrosine phosphatase, ELP, and three hypothetical genes were determined to be antimetamorphic factors.

Key words: Polydnavirus, larva-to-pupa metamorphosis, RNAi, transient expression, *Cotesia plutellae*, *Plutella xylostella*

Physiological disruption of stress sounds on immature development and adult reproduction in *Spodoptera exigua*

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Sound treatments have been considered as a non-chemical insect pest control technique. Different frequency and intensity sounds were applied to immune and adult stages to screen any stress sounds to alter physiological processes. At 95 dB, 5,000 Hz and 30,000 Hz were selected to be stress sounds in audible and inaudible sound ranges, respectively. Both stress sounds significantly inhibited larval and pupal development. In biochemical analyses, lipid and sugar levels in plasma significantly increased in response to the stress sound treatments. Moreover, a digestive phospholipase A2 enzyme activity in midgut was significantly reduced. In adult stage, ultrasound treatment significantly inhibited mating behavior, which resulted in a reduced fecundity. These stress sounds altered gene expressions of stress-related genes, such as heat-shock proteins and apolipophorin III. This study suggests that extreme sounds play a role in physiological stress factors in *S. exigua* by altering developmental and reproductive processes.

Key words: Stress sound, phospholipase A₂, heat-shock protein, apolipophorin III, *Spodoptera exigua*

0015

The mitochondrial genetic population structure of the ant species, *Vollenhovia emeryi* (Hymenoptera: Myrmicinae) in Korea

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The ant species, *Vollenhovia emeryi*, is distributed in Far East. The species can be divided into two major groups by their wing morphology of reproductives: short-winged and long-winged. A nationwide survey of the species was conducted for analyzing the mitochondrial haplotype diversity and genetic population structure. We collected 91 samples from 40 locations. A total of the 1239 bp partial COI (cytochrome C oxidase 1) region was used for the analyses. We found the total of 21 haplotypes. The mitochondrial haplotypes may correspond to the wing morphology. The genetic population structure examined potential geographic barriers of gene flow such as distance, mountains, rivers and plains which are non-mountain areas to prevent dispersal through mountain range. The result implied that no barriers considered in this study affected differently gene flow. Therefore, the behavioral characteristics of the ant may be the causal constraint of its genetic exchange.

Key words: *Vollenhovia emeryi*, wing morphology, population structure, mt-COI haplotype diversity, gene flow barrier

The genetic relatedness assay through the nuclear gene analysis in *Vollenhovia emeryi* (Hymenoptera: Myrmicinae)

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Vollenhovia emeryi ant is distinguished by its wing morphology; short winged (SW) and long winged (LW). Its reproduction shows a bizarre genetic caste system distinct from other social hymenopteran insects. Unfertilized eggs undergo genome duplication and develop into clonal gynes. Fertilized eggs develop either into workers or males. The fate of the fertilized eggs is determined whether maternal genome loss (MGL) takes place after fertilization. Eggs with MGL become haploid males with only paternal half of the genome. Without MGL, the eggs become workers with maternal and paternal half of the genome.

In this research, we analysed 5 nuclear genes of SW and LW individual ants. Among them, two genes from an SW male are identical to those of LW, and one gene from the SW male seems a variant of LW. The result indicates that SW males are derived from LW colonies. From the genetic relatedness point of view individuals in the same castes are genetically identical. On the other hand, between workers and two reproductives, the relatedness is asymmetrical and there is even no gene sharing between gynes and males. The conventional genetic relatedness by Hamilton is revised under this condition.

Key words: genetic caste system, wing morphology, genetic relatedness

0017

Genomic imprinting and Sex determination in *Vollenhovia emeryi* (Hymenoptera: Myrmicinae)

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In haplodiploid sex determination, females are sexually reproduced from fertilized diploid eggs, and males from unfertilized haploid eggs. Haplodiploid sex determination seems simple in that sex depends simply on the ploid level. However, the underlying genetic mechanisms are thought to be much more complicated than expected. Among them, a powerful proposed mechanism is genomic imprinting. All epigenetic on-off systems require target genes, unless the systems target histone proteins on chromosomes. For Hymenoptera, a good candidate target gene in terms of sex determination is known either as feminizer (*fem*) or transformer (*tra*) in many insects. These two genes are essential for expressing femaleness. In most Hymenopteran insects, the maternal *tra* seems to be methylated and consequently not expressed, while the paternally derived *tra* gene is not methylated. Therefore, a fertilized egg with the paternally derived active *tra* gene will develop into a functional female.

Like all Hymenoptera, ants (Formicidae) have haplodiploid sex determination. In *Vollenhovia emeryi*, however, queens are produced clonally while workers derive from fertilized eggs. Males are haploid, likewise deriving from fertilized eggs, but only after selective elimination of their maternal genome. Under the conventional genomic imprinting model, we would have expected that the opposite pattern of what is observed in others.

Here we present extraordinary sex determination and suggest our hypothesis about genomic imprinting pattern in *V. emeryi*

Key words: *Vollenhovia emeryi*, genomic imprinting, Hymenoptera, haplodiploid, sex determinaton, epigenetics, formicidae, myrmicinae

Viral Coat Protein Analysis of a Polydnavirus, *Cotesia plutellae* Bracovirus, by a Tandem Mass MALDI-TOF

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A polydnavirus, *Cotesia plutellae* bracovirus (CpBV), is symbiotic to an endoparasitoid wasp, *C.plutellae*, which specifically parasitizes young larvae of the diamondback moth, *Plutella xylostella*. A recent study on CpBV replication by analysis of ovary transcriptome of *C.Plutellae* suggests several candidate coat protein genes. This study was conducted to confirm the coat protein genes by analyzing coat proteins of CpBV viral particles by a tandem mass MALDI-TOF. Immunoprecipitation of ovary protein extract with a polyclonal CpBV antibody captured three proteins named as p35, p60, and p70. More number of coat proteins were resolved in a protein extract directly from viral particles. All candidate coat proteins are analyzed in amino acid sequences by MALDI-TOF. A comprehensive analysis of viral proteomics and ovary transcriptome determined novel viral coat proteins from CpBV.

Key words :- CpBV, Polydnavirus, replication, MALDI-TOF, Proteomics

0019

Promoter analysis of CpBV15α and its host translation inhibitory activity

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An endoparasitoid wasp, *Cotesia plutellae* parasitized young larvae of diamondback moth, *Plutella xylostella*. Parasitized larvae exhibit significant immunosuppression and fail to metamorphose to pupal stage. Especially, during last instar of parasitized *P.xylostella*, massive nutrients divert from host to wasp development. CpBV15α, a host translation inhibitory factors encoded in *C. Plutella* bracovirus(CpBV), plays a crucial role in suppressing host usage of amino acids. Its promoter analysis shows that CpBV15α specifically inhibit host development in late larval period. To understand its inhibitory target, its specific expression was performed in non-parasitized *P. xylostella* by in vivo transient expression technique. Total plasma proteins were analyzed by 2D gel electrophoresis and determined target genes inhibited by CpBV15α. Immunoprecipitation of cellular extract with CpBV15α antibody captured eIF2B. CpBV15α shares sequence homology with eIF5, especially at its eIF2B-binding region. Our results suggest that CpBV15α may sequester eIF2B, which result in malfunctioning of eIF2 cycling to form a translation initiation complex.

Key words: HTIFs, *Cotesia plutellae*, bracovirus, translation control

Functional analysis of teratocytes originated from *Cotesia plutellae* on host larva ldevelopment and pupal metamorphosis

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Teratocytes are originated from embryonic serosal membrane of some endoparasitoid wasps. *Cotesia plutellae* eggs release teratocytes in parasitoid host hemocoel at hatch in about 150 cells per egg. Teratocytes of *C. plutellae* were cultured in an insect culture medium for at least 14 days. Teratocytes cultured *in vitro* showed no increase in cell numbers but increased in cell size. Similarly, teratocytes in parasitized larvae did not increase cell numbers, but increased their cell size. Microinjection of *invitro* cultured teratocytes in to third instar larvae of nonparasitized *Plutella xylostella* showed a dose-dependently inhibitory effect on development and larval-pupal metamorphosis. In addition, teratocytes prolonged the immature developmental period and reduced the pupation rate, in which young aged host larvae were more sensitive to teratocytes treatment than old larvae. These results suggest that teratocytes play a crucial role in successful parasitization of *C. plutellae* by altering host developmental program.

Key words: Teratocytes; *Cotesia plutellae*; *Plutella xylostella*; Development; Metamorphosis.

0021

Eicosanoids modulate hemocyte population in response to immune challenge in *Spodoptera exigua*

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As the immune reactions in human white blood cells of certain substances from insects to defend it when invaded by immune blood cells is increased. We experiment with changes in the total number of blood cells through the blood cells which increases and decreases, as well as to observe whether the immune response through any route is to evaluate what happens. Hemocyte population was analyzed in the last instar larvae of *Spodoptera exigua*. Granulocyte and plasmatocyte were predominant (>75%) types of hemocytes, whereas spherulocyte, prohemocyte, and oenocytoid hemocytes were observed in small densities (5~10%). Total hemocyte counts (THCs) were varied among different ages (day1-day5) of the last instar, in which day 3 larvae (L5D3) had the maximal density. Upon bacterial challenge to L5D3 larvae, THC was further enhanced within 2 h and then decreased to background level. This rapid THC increase in response to bacterial challenge was inhibited by injection with dexamethasone (1 μ g per larva). However, the addition of arachidonic acid reversed the inhibitory activity of dexamethasone and allowed the larvae to increase THC. This THC increase was mediated by cyclooxygenase products, but not by lipoxygenase products.

Key words: *Spodoptera exigua*, eicosanoid, hemocyte, dexamethasone, prostaglandin

Cytotoxicity of bacterial metabolites produced by *Xenorhabdus nematophila* and *Photorhabdus temperata* subsp *temperata*

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An entomopathogenic bacteria, *Xenorhabdus nematophila* (Xn) and *Photorhabdus temperata* subsp *temperata* (Ptt), suppresses insect immune responses and facilitates its symbiotic nematode development in target insect. Benzylideneacetone (BZA), PY, cPY, Ac-FGV, indole, 2-oxindole and 3-(4-hydroxyphenylpropionic) acid (PHPP) were compounds derived from the bacterial. Their immunosuppressive activities have been induced by inhibitory activity against eicosanoid biosynthesis and used to develop an additive to enhance control efficacy of other commercial microbial insecticides. This study investigated any cytotoxicity of their culture broth and bacterial metabolites on *Spodoptera exigua* hemocyte. When Xn or Ptt (<100 cells per larva) were injected to larval of *S. exigua*, the bacteria increased in density with incubation time, while the insect hemocyte numbers significantly and the resulting culture broths were sampled for analysis of their cytotoxicity against *S. exigua* hemocytes. In addition, the sequential culture broth samples were analyzed in active component chemicals using a reverse phase HPLC. Finally, seven bacterial metabolites were analyzed in relative cytotoxicity against *S. exigua*. These results suggest that BZA is a major cytotoxic compound.

Key words: *Xenorhabdus nematophila*, *Photorhabdus temperata* subsp *temperata*, Cytotoxicity, benzylideneacetone, HPLC

0023

Different types of fruit damages of three internal apple feeders diagnosed with molecular markers derived from mitochondrial DNA

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Three tortricid pests, *Grapholita dimorpha* (Komai), *G. molesta* (Busck), and *Carposina sasakii* (Matsumura) are known as internal apple feeders in Korea. For identify young larvae which occurring serious damage in fruits, the molecular maker was developed from their mitochondrial DNA (mtDNA) sequences. To develop of PCR-RFLP marker, ND4 locus was digested with *Swa* I. ND4-*Swa* I digests showed two bands (396, 292 bp), one band (700 bp), and three bands (408, 178, and 103 bp) of *G. dimorpha*, *G. molesta*, and *C. sasakii*, respectively. Species-specific diagnostic PCR primers were developed in the ND4 locus and gave species-specific PCR products. Finally, these markers were applied to diagnose larvae infesting apples and showed species-specific fruit damage patterns, in which most feeders of *G. dimorpha*, *G. molesta*, and *C. sasakii* showed major feedings in apex, surface, and core of apple fruits, respectively.

Key words: *Grapholita dimorpha*, *Grapholita molesta*, PCR-RFLP, diagnostic primer, diagnosis

Comparative transcriptome analysis of two sibling *Grapholita* species and their pheromone biosynthesis machineries

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The oriental fruit moth (*Grapholita molesta*) and the plum fruit moth (*G. dimorpha*) are internal feeders of apples. Their sympatric and similar sex pheromone compositions suggest their recent divergence in speciation. This study aims to determine genetic factors in this speciation by comparing transcriptomes associated in sex pheromone biosynthesis in these sibling species. Total RNAs were collected from two female abdominal tips and read by a short read deep sequencing technology using an Illumina HiSeq. Almost 3-4 Gb reads were de novo assembled and resulted in 76,361 contigs of *G. dimorpha* and 104,463 contigs of *G. molesta*. More than 70% of these contigs were annotated and classified by a typical GO analysis. Transcriptomes related with sex pheromone biosynthesis were selected and grouped into fatty acid synthase, fatty acid oxidation. These analyses identified sex pheromone biosynthesis machineries, which showed significant differential expressions between two sibling species.

Key words: sympatric speciation, next generation sequencing, transcriptome, sex pheromone, *Grapholita*

0025

Influence of host foods on female reproduction and aggregation pheromone production of males of *Riptortus pedestris*

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Male pheromone production and female reproduction of *R. pedestris* were evaluated on two different kinds of foods; sweet (non-astringent) persimmon and soybean. Male adults fed on soybean produced all the four pheromone components, (*E*)-2-hexenyl (*Z*)-3-hexenoate, (*E*)-2-hexenyl (*E*)-2-hexenoate, tetradecyl isobutyrate (C14iBu), octadecyl isobutyrate (C18iBu), whereas those fed on sweet persimmon did not produce C14iBu which is a key component in the function of the pheromone, and C18iBu. Female adults fed on soybean produced eggs, however, those fed on sweet persimmon did not at all. From these results, we concluded that host resource greatly affects the chemical communication and reproduction of both male and female of *R. pedestris*, and that sweet persimmon is not a proper food for its completion of life cycle.

Key words: Host resource, *Riptortus pedestris*, tetradecyl isobutyrate, sweet persimmon, soybean

사과원에서 날개매미충(*Pochazia* sp.)의 발생과 이의 친환경적 방제

조신혜, 박민우, 김세희, 권혜리, 서미자, 유용만, 윤영남

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날개매미충(*Pochazia* sp.)의 기주로는 사과, 감, 밤, 오미자, 블루베리, 복분자 등이 알려져 있다. 날개매미충은 5월 말부터 부화하여 7월 중·하순에 약충, 9월 초에 성충의 발생최성기를 보였다. 날개매미충에 의한 사과에서의 피해는 약충과 성충의 섭식에 의해서보다는 성충의 산란에 의한 피해가 심각하다. 사과원 주변 잡목에서 성충들이 과수원으로 날아 들어와 1년생 가지마다 산란을 하여 피해를 입히기 때문이다. 이는 암컷성충이 산란관을 이용하여 1년생 사과나무 가지의 껍질을 벗겨내고 산란을 하기 때문이다. 이 시기는 사과 수확기와 맞물려 있어 화학농약을 이용한 방제를 할 수가 없기 때문에 약제잔류에 문제가 없는 친환경방제제의 선발이 필요하다. 친환경방제제의 선발은 식물추출물들을 사용하였다. 멀구슬추출물(toosendanin), 괴각추출물(oxymathrin), 정향나무추출물(eugenol), 백합과추출물(veratrine), 데리스추출물(rotenone), 고삼추출물(matrine), neem나무추출물(neem oil), 결명자추출물(emodin), 담배잎차추출물 등이 실험에 사용되었다.

각각의 유효성분은 0.3%비중의 원제를 사용하였고, 9개의 식물추출물을 각각 500배, 1000배, 2000배 희석하여 spray tower를 이용해 날개매미충 2~3령충에 직접살포 하였다. 처리구당 10마리의 날개매미충 2~3령충을 처리하여 그 사충수를 조사하였다. 식물추출물 처리 결과, 고삼추출물과 데리스추출물이 비교적 높은 살충효과를 보였다.

검색어 : 날개매미충, *Ricania* sp., 생활사, 식물추출물, 친환경

DYMEX[®]를 이용한 애멸구 (*Laodelphax striatellus* Fallen) 개체군 밀도 변동 모델 구축 및 평가

박창균, 이시우, 박홍현, 김광호, 이상계

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애멸구(*Laodelphax striatellus* Fallen)는 국내에서 월동 가능한 해충으로 벼의 줄무늬잎마름병 (RSV)을 매개하여 피해를 발생시킨다. 최근에는 5월하-6월 상순경에 중국으로부터 대량으로 비래하여 서해안 지역의 벼 줄무늬잎마름병 발병에 관여하고 있는 것으로 추정하고 있다. 본 연구는 수리적 모형과 상용 프로그램을 이용하여 보다 정확한 애멸구 개체군의 발육 및 밀도 변동을 예측하기 위하여 수행되었다.

예측 모델은 상용프로그램인 DYMEX[®] (Maywald *et. al.*, 2007)를 이용하여 구축하였으며, Lifecycle 모듈을 포함한 8개의 모듈로 구성하였다. Lifecycle 모듈은 알, 1, 2, 3, 4, 5령, 성충의 7개 발육 단계로 구성되었으며, 각 영기의 발육을 계산에 사용된 비선형 모형은 변형된 Sharpe & DeMichele 함수를 사용하였다. 발육완료 함수는 Logistic 함수 (Neter & Wasserman, 1974)를 사용하였으며, 성충 산란모델은 총산란수함수, 사망률함수, 누적산란율 함수로 구성하였고, 성충의 성비는 0.5로 가정하였다.

모델의 평가를 위해 2011년 7월 6일 벼 포트에 성충 3쌍을 접종하여 증식시키며 4회 전수 밀도 조사한 결과 7월 21일, 8월 5일, 8월 12일 조사에서는 육안조사 밀도가 모델을 이용한 예측 밀도보다 16~112마리 많았으나 8월 19일에는 20마리 가량 적었다. 조사 시기별 개체군내 발육태별 상대적 비율 변화를 분석한 결과, 모델을 사용하여 영기 진전을 예측하였을 경우 실측 조사 영기보다 1~2령 정도 느리게 발육하는 것으로 추정되었다. 본 연구 결과 예측치와 실측치간의 절대 밀도와 영기 비율의 부분적인 불일치는 사망률 정보의 추가와 적절한 온도 자료의 제공을 통해 정확도를 높일 수 있을 것으로 판단되었다.

검색어 : 애멸구, 개체군 밀도 변동 예측, DYMEX[®], 모델, 평가

Growth of the box tree pyralid, *Glyphodes perspectalis*, reared on an artificial diet and field test of sex pheromone (Part II)

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The box tree pyralid, *Glyphodes perspectalis*, was reared on an artificial diet based on dried powder of box tree leaves, *Buxus microphylla* var. *koreana*, and INSECTA F-II (Nihon Nosan Co., Ltd) at a ratio of 3:7 (w:w) and analyzed in their developmental characteristics from 1st to 4th generation. The developmental period of larva reared on artificial diet was longer than that of larva reared on host plant. Significant difference in larval period was also observed between 1st and other generation groups. Pupation rate was the highest in 4th generation followed by 3rd, 2nd, 1st and host plant group. Significant difference in pupal period was noted between 1st and 3rd generation group. Emergence rate was the highest in 4th generation group followed by 3rd, host plant, 1st and 2nd generation group. There is a significant difference in male and female size between 1st and 4th generation group. Our result indicated that stable rearing of box tree pyralid through the year is possible by artificial diet, and the best adaptation to artificial diet was achieved at 4th generation. The pheromone components of this moth have been identified as (Z)-11-hexadecenal (Z11-16:Ald), (E)-11-hexadecenal (E11-16:Ald), and (Z)-11-hexadecenol (Z-11-16:OH). We did several field tests, and found that there was a difference between Korea and Japan population.

Key words: box tree pyralid, artificial diet, sex pheromone, field test

꼬마배나무이(*Cacopsylla pyricola*)의 섭식행동과 몇 가지 약제에 대한 반응

박민우, 조신혁, 김세희, 권혜리, 서미자, 유용만, 윤영남

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꼬마배나무이(*Cacopsylla pyricola*)는 최근 들어 배과원에 발생량이 증가하면서 그 피해가 점차 증가하는 경향을 보이고 있다. 이에 따라 꼬마배나무이를 방제하기 위한 살충제들이 많이 등록되어 사용되고 있으며, 그 방제 효과 또한 우수한 것으로 평가 되고 있다. 본 실험은 배나무 앞에서 흡즙을 하는 꼬마배나무이의 섭식행동을 관찰하고, 살충제 처리에 따른 섭식행동 변화를 관찰하고 분석하였다. 약제를 처리하지 않은 정상적인 상태에서 꼬마배나무이가 배나무 잎을 흡즙할 때에 나타나는 EPG파형은 PA, PB, PC1, PC2, PD, PE1, PE2, PG 등으로 구분할 수 있었다. 일상적인 상태에서 꼬마배나무이의 섭식양상을 EPG패턴에 따라 분석한 결과, 체관부를 섭식하는 패턴이 가장 많이 나타났다. 약제를 처리하였을 경우 약제별로 EPG패턴의 시간적인 양상과 출현빈도가 다르게 나타났다. 일반적으로 약제를 처리하였을 경우에는 섭식을 하지 않는 파형인 Np 시간이 길어지게 되는데 가장 길게 나타난 것은 benfuracarb를 처리하였을 때이다. 뿐만 아니라 식물체에 구침이 침투할 때에 나타나는 파형인 PA가 달라지는데 flonicamid를 처리하였을 때 가장 오랜 시간이 경과되었다. 한편, 체관부 섭식형인 꼬마배나무이가 물관부에서 섭식하는 시간이 길어지는 경우를 볼 수 있는데, 이 때에 나타나는 EPG파형인 PG에 소요된 시간이 처리약제별로 차이가 있었으며, deltamethrin과 fenitrothion을 처리하였을 때 물관부 섭식에 가장 많은 시간을 소비하였다. 이는 침투성 약제의 침투이동경로로 생각되는 체관부를 피하여 섭식하는 행동으로 추론할 수 있다.

검색어 : 꼬마배나무이(*Cacopsylla pyricola*), 흡즙행동, EPG 패턴, 살충제

현지 내 (In situ) 생물다양성 보존을 위한 유기농경지 활용 방안

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생물다양성은 국제적 규범이자 국제 쟁점이다. 1994년 우리나라도 비준한 생물다양성협약(CBD, Convention of Biological Diversity) 뿐만 아니라 식량농업식물유전자원국제조약(ITPGRFA, International Treaty on Plant Genetic Resources for Food and Agriculture), FAO 지구행동계획(Global Plan of Action) 등 다양한 국제 규범과 정책이 생물다양성의 현지 외(Ex situ), 현지 내(In situ) 및 농장에서의(On Farm) 보존에 대해 강조하고 있다. 그럼에도 불구하고 대부분의 농경지에서 생물다양성에 대한 구체적인 보존 정책과 기술이 제시되지 못하고, 종자의 상업적 활용에 국제 쟁점이 치우친 나머지 현지 외, 즉 유전자원센터에서 종자의 보관 등에만 공공 연구 자원의 대부분이 사용되고 있는 실정이다. 본 연구에서는 2011년 유기농경지와 관행농경지의 논, 밭, 사과 과수원에서 절지동물 및 수서생물 다양성 비교 연구를 수행하면서 확보된 생물 종 인벤토리 자료와 경험을 바탕으로 농경지에서 현지내 생물다양성 보존을 위한 가능성을 검토하고자 하였다. 거의 대부분의 비교 사례에서 유기농경지가 관행농경지보다 높은 생물다양성을 보였으나, 잘못 관리된 유기농경지에서는 관행보다 절지동물의 발생 종수가 적은 경우도 발견되었다. 따라서 유기농경지에서 생물다양성을 보존하기 위해서는 체계적인 경관관리와 식생관리가 필요한 것으로 확인되었다. 이와 더불어 곤충 등 식물 종자 이외의 생물자원에 대해서는 현지 외 유전자원 보존 및 관리 체계의 정비도 시급히 해결해야 할 과제이다. 본 연구에서는 현지 내 절지동물 등의 생물다양성 보존을 위하여 유기농경지 활용 방안을 제시하고자 한다.

검색어 : 생물다양성, 국가생물다양성센터, 유기농경지, 현지 내 보존

성페로몬을 이용한 자두 과수원에서 복숭아순나방, 복숭아순나방붙이, 복숭아심식나방의 발생 패턴

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경북은 자두의 주요 생산지이며, 자두재배면적은 김천, 의성, 경산 순이다. 최근 자두 생산이 증가하는 추세이나, 자두 해충에 대한 기존 연구는 매우 미흡하여, 농가 해충 관리는 경험에 의존한 화학약제 살포가 주를 이룬다. 이에 의성과 경산지역에서 자두의 1차 해충인 복숭아순나방, 복숭아순나방붙이, 복숭아심식나방의 발생 패턴을 성페로몬 트랩을 이용하여 2010-2012년에 조사하여 보고한다. 연간 발생량 비는 의성의 경우 복숭아순나방:복숭아순나방붙이:복숭아심식나방이 50:30-36:13-18%로 나타났으나, 경산의 경우 51-70:18-38:10-11%로 다르게 나타났다. 발생량은 지역 간 큰 차이 없었다. 발생 시기는 경산지역이 의성보다 조금 빠른 것으로 나타났다. 경산에서 복숭아순나방 초발생은 4월 초순이며, 세대별 최성기는 4월 하순, 6월 중순, 3세대는 7월 하순, 9월 중순이었다. 복숭아순나방붙이는 복숭아순나방보다 약 1주일 정도 늦게 발생하였다. 복숭아심식나방은 5월 말에 발생을 시작하여 9월 중순까지 포획되었는데, 역시 경산에서 마지막 포획이 1주일 정도 빨랐다. 복숭아심식나방의 경우 2010년은 전형적인 2회 최성기를 보였으나 2011년에는 3회 최성기를 보였다. 세 해충 모두 과수원의 중앙에 설치한 트랩과 외곽에 설치한 트랩에서 포획량 차이는 없었다.

심식나방류들의 발생패턴을 년도와 지역에 따라 달라짐을 알 수 있고, 지역 내 경관 요소에 대한 연구가 보충된다면 발생 패턴에 대한 이해가 높아질 것이다.

검색어 : 복숭아순나방, 복숭아순나방붙이, 복숭아심식나방, 성페로몬트랩

Development of overwintering oriental fruit moth larvae, *Grapholita molesta*(Busck) even under the lower developmental threshold temperature

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Many insects overwinter in diapause in the temperate region. Winter diapause often terminates far yearly than our thought, eg. Many in December. After diapause termination, insect becomes in quiescence. The lower developmental threshold of overwintering Oriental fruit moth larvae is in the range of 7.5-8.4°C depending on the geographic location. We have determined the developmental period of overwintering OFM larvae from the time series interval collection. Collections were made from mid Jan to late March at 10d interval. Collected larvae were held in a cold chamber at 6°C for 5 days, and then subjected at 20°C with 75% R.H., 16:8(L:D).hr. The developmental period from overwintering cocooned larvae to adults was longest in mid. January collections, became shorter after 31 Jan. collection, then gradually decreased until mid March. Cocooned larvae developmental period became shorter after 31 Jan. while that of pupae became shorter after 20 Feb. During this study, female ratio was not different to 0.5. Mortality of winter collected larvae to adults was 45% including 30% parasitism. Partial explanation of the insect development under the lower developmental threshold was discussed; limitation of linear model estimation, temperature data quality and physiological metabolism after diapause termination. From January to February, daily average temperature fluctuated but under the lower developmental threshold. However, daily maximum temperature became noticeably higher than the lower developmental threshold after 20 Jan. However, this result indicates that after late Jan, physiological state of OFM is altered.

Key words: Diapause termination, lower developmental threshold, developmental period, Sex ratio, mortality, parasitism

0033

Development of the seasonal phenology model of *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) in pear orchards and application to other fruit orchards

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The study was conducted to develop the full seasonal phenology model of *G. molesta* in pear orchard. *G. molesta* is a multi-voltine insect with four to five generations per year depending on temperature, food resources and geographic location. For precise model construction, information on temperature-dependent development and the distribution of developmental completion of overwintering and summer *G. molesta* population was used. The performance of model was based on single cohort population of *G. molesta*. The validation of model system was performed with the male moth catches in sex pheromone-baited traps of pear orchards in four pear production regions (Anseong, Icheon, Naju and Ulju), three apple production regions (Andong, Chungju and Geochang), and four plum orchards (Uiseong) of Korea in 2010 and 2011, respectively. The observed phenology of seasonal population of *G. molesta* was explained by our model system. The predicted dates for the cumulative 50% male moth catches per each generation were within seven days variation per each generation. The precise predictive model of *G. molesta* adult occurrence could help decision making and enhancing control efficacy.

Key words: *Grapholita molesta*, apple, pear, phenology, plum, temperature dependent development, Weibull distribution

Effect of Essential Oils and Paraffin Oil on Growth and Mortality of Black Cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae) in Perennial Ryegrass, *Lolium perenne*

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The black cutworm, *Agrotis ipsilon* (Hufnagel), damages various cultivated crops and is also occasionally a serious pest of turfgrass, especially on golf courses. Essential oils have potential as alternative control agents for insect pests. Sixteen essential oils (anise, camphor, cinnamon, citronella, clove, fennel, geranium, lavender, lemongrass, linseed, neem, peppermint, pine, thyme, turpentine and tea saponin) and paraffin oil were assessed in the laboratory and the green house for their efficacy against black cutworm larvae. Treatment of potted perennial ryegrass with anise, cinnamon, neem, paraffin or turpentine reduced black cutworm damage in a greenhouse trial. Neem oil (2000 ppm) reduced growth of black cutworms feeding on treated clippings within 3 and 5 days. Treatment of perennial ryegrass in pots resulted in 100, 100 and 46% mortality of black cutworm at 4000, 2000 and 1000 ppm, respectively. Weight of survivors at the 1000 ppm rate was 5 fold less than weight of comparably-aged controls.

Key words: *Agrotis ipsilon*, environmental friendly control, essential oil, neem oil, turfgrass

Enhanced toxicity of binary mixtures of *Bacillus thuringiensis* and three essential oil major constituents against wild *Anopheles sinensis* and *Aedes albopictus*.

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An assessment was made of the toxicity of 12 insecticides, three essential oils and *Bacillus thuringiensis* var. *israelensis* (*Bti*) alone or in combination with the oil major constituents, (*E*)-anethole (AN), (*E*)-cinnamaldehyde (CA) and eugenol (EU), to third instars from bamboo forest collected *Aedes albopictus* and rice paddy field collected *Anopheles sinensis* resistant to various groups of insecticides. The toxicity of the test insecticides, essential oils and binary mixtures of *Bti* and the oil constituents (1:1 ratio) was evaluated using a direct-contact mortality bioassay. Binary mixtures of *B.t.i.* and CA, AN or EU were significantly more toxic against *Ae. albopictus* larvae (LC₅₀, 0.0084, 0.0134 and 0.0237 mg/l) and *An. sinensis* larvae (0.0159, 0.0388 and 0.0541 mg/l) than either *B.t.i.* (1.7884 and 2.1681 mg/l) or CA (11.46 and 19.43 mg/l), AN (16.66 and 25.11 mg/l) or EU (24.60 and 32.14 mg/l) alone. Based on the co-toxicity coefficient (CC) and synergistic factor (SF), the three binary mixtures operated in a synergy pattern (CC, 140.7–368.3 and SF, 76–213 for *Ae. albopictus* CC, 75.1–245.3 and SF, 40–136 for *An. sinensis*). The binary mixtures of *Bti* and essential oil constituents described, particularly (*E*)-cinnamaldehyde, merit further study as potential mosquito larvicides for the control of malaria vector mosquito populations in light of global efforts to reduce the level of highly toxic synthetic insecticides in the aquatic environment.

Key words: *Bti*, essential oils, insecticide, *Aedes albopictus*, *Anopheles sinensis*

파밤나방에 활성 있는 *Bacillus thuringiensis* 균주와 tannic acid와의 기질 특이성

진나영, 곽창순, 오산나, 이유경, 전미진, 서미자, 윤영남, 유용만

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나비목유충 소화액의 다양한 Protease는 *Bacillus thuringiensis*(이하 *B. thuringiensis*)가 생성한 protoxin의 활성을 결정하는데 가장 중요한 소화효소로 알려져 있다. 나비목유충의 소화효소 중 Serine protease인 trypsin은 단백질 가수분해과정에 주요한 역할을 하는 것으로 알려져 있다. 그러나 Protease의 지속적인 가수분해결과 독소 단백질의 불활성화를 초래하여 *B. thuringiensis*의 살충활성에 부정적인 영향을 초래할 수 있다. 이전 실험에서도 *Bacillus thuringiensis* subsp. *kurstaki* KB100과 protease inhibitor중의 하나인 tannic acid를 혼합하여 증장액에 처리하였을 때, 파밤나방에 대한 살충활성이 높아진 원인으로 protease activity의 감소를 예상할 수 있었다. 따라서 본 실험은 다양한 Protease가 있는 증장액에 protease 특이적기질로 tannic acid가 어떤 종류의 protease activity를 낮추는지 확인하고자 하였다.

파밤나방 증장액과 농도별(10, 20, 40, 80mM) Tannic acid와의 protease activity를 측정 한 결과 tannic acid의 농도가 높아질수록 protease activity(%control)는 각각 83.1 ± 2.1 , 77.6 ± 1.6 , 68.0 ± 0.4 , 40.1 ± 2.2 로 감소됨을 확인 하였다. 파밤나방 증장액과 serine(azocasein), trypsin(BApNA, BPVApNA), chymotrypsin(BTpNA, SAAPPpNA, AAVApNA), elastase(SAAApNA, SAAPLpNA)와의 기질 반응을 분석 한 결과 trypsin 기질에서 protease activity가 높음을 확인 하였다. 추후 연구에서 파밤나방 증장액과 Tannic acid를 반응 시킨 후 기질과의 protease activity를 측정 하고 zymogram을 통해 protease 활성 부분을 연구 할 것이다.

검색어 : *Bacillus thuringiensis*, 파밤나방, protease, Tannic acid, 기질

0037

Screening of lethal genes for feeding RNA interference by leaf disc-mediated systematic delivery in *Tetranychus urticae*

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The screening of effective lethal genes was conducted via the systemic delivery of dsRNA for the RNA interference (RNAi)-based management of *Tetranychus urticae*. Four candidate genes (coatamer protein complex, T-COP; M1 metalloprotease, T-M1MP; Ribosomal protein S4, T-RPS4; A subunit of V-ATPase, T-VATPase) with a reference genes (EGFP) were tested for the experiment. Translocated dsRNA to the leaf disc (ca. 30 mm diameter) was detected at 12 h post-treatment, indicating that dsRNA could move through leaf veins. In the evaluation of mortality, the T-COP, T-RPS4 and T-VATPase exerted moderate levels of toxicity at 72 h post-treatment among four dsRNA tested. Knockdown of the target genes were confirmed by quantitative PCR, suggesting that RNAi of target genes was accomplished by feeding dsRNA. In summary, the dsRNA delivery via leaf disc was effective in screening lethal genes, and some genes, such as T-COP, T-M1MP, T-RPS4 and T-VATPase, can be applicable for establishing a RNAi-based control system against *T. urticae*.

Key words: RNAi, dsRNA, Systemic delivery, *Tetranychus urticae*

Monitoring of carbamate and organophosphate resistance levels in *Nilaparvata lugens* based on bioassay and quantitative sequencing

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The resistance levels to carbamate (CB) and organophosphate (OP) insecticides were determined by topical application in 14 field strains of *Nilaparvata lugens*. The resistance levels to CB and OP were 1.3~47.5-fold and 1.4~14.4-fold higher than a susceptible strain, respectively. A quantitative sequencing (QS) protocol was established to determine the allele frequencies of four acetylcholinesterase point mutations putatively associated with CB and OP resistance. The allele frequencies of the four mutations (G119A, F/Y330S, F331H and I332L) in field strains ranged from ca. 0.0~51.7%, 0.0~88.9%, 2.5~47.7%, 5.1~56.0% and 6.7~57.3%, respectively. The F331H and I332L were tightly linked each other, suggesting these mutations may occur simultaneously. In the correlation analysis, G119A was not well correlated with actual resistance levels ($r^2 = < 0.232$), whereas F331H and I332L showed a better correlation with the resistance levels of benzofuranyl methylcarbamates ($r^2 = 0.595$). This finding indicates that F331H and I332L mutation frequencies may be used as molecular markers for detecting carbamate resistance in *N. lugens*. A QS protocol detecting the F331H and I332L mutation frequencies would be employed as a supportive tool for rapid monitoring of CB insecticide resistance levels in *N. lugens*.

Key words: Quantitative sequencing, *Nilaparvata lugens*, Acetylcholinesterase, Point mutations, Carbamate

0039

시설국화에서 트랩식물과 천적의 동시활용성 및 국화에 등록된 약제의 천적에 대한 독성평가

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총채벌레의 유충과 성충은 잎, 열매, 꽃 등에 몸을 숨기기 좋아하며 번데기는 땅에 떨어져 토양속에서 지내기 때문에 약제에 노출이 어려워 화학약제만으로는 방제가 어려운 실정이다. 따라서 본 연구는 시설 국화에서 초기발생 예측이 어려운 꽃노랑총채벌레(*Frankliniella occidentalis* Pergande)를 생육초반에 방제할 수 있는 ‘트랩식물 적용기술’과 천적을 동시에 활용하기 위해 1차 선발된 트랩식물(가지)과 총채벌레 천적(*Orius laevigatus* Fieber)의 동시 활용성과 국화에 등록된 약제에 대한 천적의 독성평가를 수행하였다. 트랩식물인 가지와 대조식물인 채송화에서 미끌애꽃노린재의 산란수를 비교한 결과 가지에서 16.3 ± 7.0 개, 채송화에서 22 ± 7.9 개이었다. 독성평가 결과는 약충 접촉실험에서 아세퀴노실 15% 액상수화제 처리 7일째 $28.9 \pm 4.6\%$, 성충 섭식실험에서 처리 15일째 아세퀴노실 15% 액상수화제에서 $-14.8 \pm 25.9\%$, 테부코나졸 25% 수화제에서 $-84.7 \pm 16.6\%$, 트리플록시스트로빈 50% 입상수화제에서 $10.1 \pm 51.9\%$, 피라클로스트로빈 22% 유제에서 $-14.8 \pm 38.3\%$, 잔류독성실험에서 처리 1일째 아세퀴노실 15% 액상수화제에서 $20.8 \pm 10.6\%$, 테부코나졸 25% 수화제에서 $4.6 \pm 13.8\%$, 트리플록시스트로빈 50% 입상수화제에서 $25.0 \pm 12.1\%$, 피라클로스트로빈 22% 유제에서 $10.4 \pm 12.5\%$ 의 보정사충율을 나타내었다.

검색어 : 트랩식물, 천적, 총채벌레, 독성평가, 국화

Sulfoxaflor, a Novel Sulfoximine Insecticide

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Sulfoxaflor, a novel sulfoximine insecticide, discovered by and proprietary to Dow AgroSciences is being developed globally. Sulfoxaflor will be used in all major crop groups, including fruits, vegetables, rice, cereals, soybean, and many other crops. Sulfoxaflor provides excellent efficacy at low use rates through contact and ingestion against important and difficult-to-control sap-feeding insect pests, including certain species of aphids, scales, plant bugs, whiteflies, planthoppers and other sap feeders.

Sulfoxaflor's mode of action is via agonism of the insect nicotinic acetylcholine receptor (nAChR), which is the same target site of neo-nicotinoid insecticides. However, relative to most neonicotinoids, sulfoxaflor is a high efficacy agonist of the nAChR with relatively low affinity for the imidacloprid binding site. Furthermore, sulfoxaflor is less susceptible to metabolism by monooxygenase enzymes and displays a broad lack of metabolism-based cross-resistance with neonicotinoids. This difference as well as its novel chemical structure led to sulfoxaflor being designated as the sole member of Group 4, Subgroup C of the IRAC Mode of Action Classification Scheme. Because of broad lack of cross-resistance, sulfoxaflor will be a useful rotational partner with other insecticide chemistries, enhancing Insect Resistance Management strategies.

In Korea, sulfoxaflor is being developed for use in fruit and leafy and fruiting vegetable by several companies. Results from several laboratory and field trials revealed that sulfoxaflor at use rate of 35 ppm (7% product, X2,000 dilution rate) provided excellent performance against economically important aphids in several crops compared with current sap-feeding pest control agents. Sulfoxaflor was registered in October 2011 as brandname TRANSFORM® (Sulfoxaflor 7% SC which is produced by Dongbu Hannong) will be launched for apple, pear and red-pepper in 2012.

Key words: Sulfoxaflor, TRANSFORM, Sulfoximine, Sap feeding, IRM, IRAC

The Development of the Very Efficient Mosquito Traps, Mos-hole and DMS

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We developed two kinds of selective mosquito traps. The first selective mosquito trap called Mos-hole was developed with emitting carbon dioxide. The principle was that CO₂ gas was obtained from burning liquid naphtha. The process principle was very unique that some heat and moisture which cannot be obtained by using dry ice could be generated additionally. It is the main cause to bring more mosquitoes. The trap is consisted of the acidic and CO₂-baited suction system. CO₂ was so powerful mosquito attractant gas, and the color and shape of a suction trap was also very important factors for improving the mosquito capturing efficiency. The trap burned naphtha in a rate of 1.3g/hr~3.0g/hr (CO₂:50ml/min-110ml/min) and the efficiency of the trap was higher at the burning rate with the high number of female mosquitoes. The second mosquito trap called DMS (Digital Mosquito Monitoring System) is developed for reporting the number of captured mosquito automatically every day. This automatic reporting device called DMS uses CO₂ gas as a mosquito attractant and it has a IR array sensor for counting mosquito. We established a sensor network with several DMSs and one server. The server collected the data of each DMS through Internet or CDMA RF communication system. This data were analyzed in the GIS pest prevention information system and were sometimes used as a reference for the next pest control activities. The DMS systems emit CO₂ about 300cc/min. CO₂ was very effective for attracting mosquito. DMS systems were very effective to count the number of mosquitoes at the certain areas. By using two kinds of mosquito traps together, we could determine the mosquito population size increase and decrease at certain areas

Key words: Mosquito, CO₂, Digital System, Efficient Mosquito Traps

Dispersal pattern of egg parasitoids of *Riptortus pedestris* in sesame field where aggregation pheromone traps were installed with non-viable host eggs

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Aggregation pheromone of *Riptortus pedestris* (Fabricius) (Hemiptera: Alydidae) has kairomonal effect on its egg parasitoids. Thus, inclusion of non-viable host eggs of *R. pedestris* to aggregation pheromone trap was suggested to propagate parasitoids attracted in the trap. In this study, spatial and temporal distribution of egg parasitoids were investigated in sesame fields in Songcheon, Andong where aggregation pheromone traps were installed with non-viable host eggs. Parasitism changes were assessed after collecting non-viable host eggs released at distances of 8, 16, 24, and 32 m from the trap. Higher egg parasitism and adult emergence was found up to 16 m from the point of release. Parasitism increased over time, but decreased with distance. For the comparison of change in temporal parasitism, traps were installed without host eggs in another sesame field. Parasitism rate in sesame field without host eggs increased by 13%, whereas, in the field with host eggs, the parasitism increased up to 44%. These findings suggest that host eggs attached to the pheromone traps help the parasitoids propagate and disperse.

Key words: *Ooencyrtus nezarae*, *Gryon japonicum*, cold storage, conservational biological control, egg parasitism

0043

저온 저장한 톱다리개미허리노린재의 알을 부착한 페로몬 트랩의 밀도별 기생률 변화

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톱다리개미허리노린재(*Riptortus pedestris* [Fabricius] [Hemiptera: Alydidae])는 콩 포장에서 주요 해충이며, 기생천적으로는 노린재깡충좀벌(*Ooencyrtus nezarae* Ishii [Hymenoptera: Encyrtidae])과 노린재검정알벌(*Gryon japonicum* (Ashmead) [Hymenoptera: Scelionidae])이 있다. 저온 저장시켜 불활성화된 노린재 알을 집합페로몬 트랩에 부착하면 기생률이 증가한다고 보고되었다. 본 연구에서는 위 기술을 이용하여 불활성화된 노린재 알의 밀도에 따른 기생률 향상 효과를 조사하였다. 경북 의성군 옥산면에 위치한 콩 포장(100×140m)을 12개의 시험구로 나누고 집합페로몬 트랩을 각각 4개씩 설치하였다. 시험구에는 3개의 처리구와 1개의 대조구를 두었으며, 처리구에는 1, 2, 4개의 집합페로몬 트랩에 트랩당 60개의 알을 부착하였다. 그 결과 처리구에서 노린재깡충좀벌에 의한 기생률은 23~51%이었고, 대조구에서는 25%이었다. 노린재검정알벌의 경우 처리구에서 19~42%의 기생률을 보여 주었고, 대조구의 기생률은 22%이었다. 240개의 알을 부착한 시험구에서 노린재깡충좀벌과 노린재검정알벌에 의한 기생률이 최대 51, 42%이었고, 노린재 알을 설치하지 않은 대조구의 기생률 보다 각각 2.1, 1.9배가 높았지만 톱다리개미허리노린재의 밀도는 차이가 없었다.

검색어 : 기생률, 페로몬트랩, 노린재검정알벌(*Gryon japonicum*), 노린재깡충좀벌(*Ooencyrtus nezarae*)

Demonstration of interspecific competition between two sympatric egg parasitoids of bean bug to explain coexistence in temporal scales

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Ooencyrtus nezarae Ishii and *Gryon japonicum* (Ashmead), egg parasitoids of *Riptortus pedestris* (Fabricius), coexist despite direct competition for host eggs. As asymmetrical pattern of seasonal occurrence, i.e., more *G. japonicum* during spring-summer and more *O. nezarae* during summer-fall, has been reported, host resource partitioning may occur in temporal scales. To test this hypothesis, we demonstrated the interspecific competition between the two species by measuring parasitism in nine combinations of host densities and exposure times. To reflect gregarious-solitary dichotomy, three *O. nezarae* and one *G. japonicum* mated females were used in each experiment. *O. nezarae* was better competitor when exposure time was longer than 1 day irrespective of host densities. Parasitism rate and progeny emergence of *O. nezarae* was 1.6-2.8 and 4.7-7.3 times higher than *G. japonicum*. *O. nezarae* has higher potential rate of increase than *G. japonicum* due to gregariousness, and be more successful in larval competition inside multiparasitized host egg as it acts as a facultative hyperparasitoid. Although *G. japonicum* was more effective in host finding (as they showed relatively higher per capita parasitism and progeny emergence), their progeny suffered high mortality from the larval competition with *O. nezarae*. These results may explain the asymmetrical occurrence pattern in the field.

Key words: encyrtid; scelionid; alydid; *Riptortus pedestris*; host resource partitioning

R81T mutation in nAChR associated with imidacloprid resistance in the cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae)

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The cotton aphid, *Aphis gossypii* (Glover), is one of the most serious pest in seed potato and various vegetable cultivation. The imidacloprid-resistant strain (IR) was over 300-fold more resistant to imidacloprid compared to a susceptible strain (S) as judged by LC₅₀ values. A highly imidacloprid-resistant local field population (L) was collected from cucumber at Gangwha island in 4th August 2011. Even though neonicotinoid insecticides especially imidacloprid were sprayed six times during June and July, aphid density was too high to be counted. To identify differentially expressed genes in IR or L, comparative transcriptome analyses based on GS-FLX were conducted using total RNAs extracted from IR, L and S strains. Furthermore, to search the resistance associated proteins in IR or L, comparative proteome analyses based on 2DE were conducted using total proteins extracted from IR, L and S strains. Few common candidate genes detected among IR and L such as ABC genes. Comparison of the nucleotide sequence of six nicotinic acetylcholine receptor (nAChR) subunit (alpha 1-5, beta 1) genes from IR, L and S strain revealed a point mutation in the loop D region of the nAChR beta 1 subunit of the IR, causing an arginine to threonine substitution (R81T). These mechanisms also reported in *Myzus persicae* and this amino acid change confers a vertebrate-like character to the insect nAChR and results in reduced sensitivity to neonicotinoids.

Key words: *Aphis gossypii*, insecticide resistance, nicotinic acetylcholine receptor