First results in the detection of four important forest pests using ion mobility spectrometry to recognize their specific odour molecules

Maja Bentele [1], Christine Rachow [2] & Michael Müller [1]

[1] Technische Universität Dresden, Professur für Waldschutz

[2] Georg-August-Universität Göttingen, Büsgen-Institut, Abteilung Forstzoologie und Waldschutz

Zusammenfassung: Insekten nutzen Duftstoffe zur inter- und intraspezifischen Kommunikation. Diese können mittels Ionenmobilitätsspektrometrie als Molekülmuster erkannt werden. Im Fokus der Untersuchung standen die Arten *Ips typographus* (L.), *Pityogenes chalcographus* (L.), *Lymantria monacha* (L.) und *Thaumetopoea processionea* (L.). Im vergangenen Jahr wurden frisch geschnittene Fichtenstamm-Abschnitte mit lebend gefangenen *I. typographus* im Labor zwangsbesiedelt. 116 Stunden nach dem Einbohren konnte die Besiedlung durch *I. typographus* mittels Ionenmobilitätsspektrometrie nachgewiesen werden. Eigelege aus Befallsgebieten von *L. monacha* und *T. processionea*, Bohrmehl von *I. typographus* und Imagines von *P. chalcographus* wurden ebenfalls im Labor untersucht und es zeigten sich erste Hinweise, dass diese voneinander unterschieden werden können. Zusätzlich wurden Eigelege von *L. monacha* mittels GC-MS auf artspezifische Duftstoffe untersucht.

Abstract: Insects use volatile organic compounds for inter- and intraspecific communication. Ion mobility spectrometry has the ability to recognize specific molecule mixtures as patterns. In this paper, experiments are presented focusing on *Ips typographus* (L.), *Pityogenes chalcographus* (L.), *Lymantria monacha* (L.) and *Thaumetopoea processionea* (L.). In the laboratory, freshly cut logs of *Picea abies* (L.) H. Karst. were analysed for molecule patterns with and without artificial infestation with *I. typographus* in 2018. 116 hours after artificial infestation, the results show a change in the molecule pattern. In addition, a comparison was done measuring egg masses of *T. processionea* and *L. monacha,* as well as imagines of *P. chalcographus* and frass of *I. typographus.* It was found that the molecule patterns of these were unique compared to other species examined. GC-MS was used to look for specific odour compounds in egg masses of *L. monacha*.

Keywords: *Ips typographus*, *Lymantria monacha*, *Thaumetopoea processionea*, *Pityogenes chalcographus*, bark beetles, insect pheromone, volatile organic compounds, ion mobility spectrometry, GC-MS

Maja Bentele, Technische Universität Dresden, Professur für Waldschutz, Pienner Straße 8, 01737 Tharandt, Deutschland; E-Mail: maja.bentele@tu-dresden.de

The RiMIS(Wald) project

RiMIS(Wald), funded by the European Union (EFRE), is a joint project of TU Dresden, Ostdeutsche Gesellschaft für Forstplanung mbH (OGF) and IFU GmbH Privates Institut für Analytik. One aim of the project is the development of a risk assessment tool, which examines inventory data for predisposition traits that support or suppress a number of biotic and abiotic risk factors in forest stands. Another aim is the detection of volatile organic compounds (VOCs) originating from egg masses and bark beetle galleries using ion mobility spectrometry. The work focuses on the four species *Ips typographus* (L.) (spruce bark beetle), *Pityogenes chalcographus* (L.) (spruce wood engraver), *Thaumetopoea processionea* (L.) (oak processionary moth) and *Lymantria monacha* (L.) (nun moth).

Ion mobility spectrometry

Ion mobility spectrometry (IMS) is based on the separation of molecules depending on their size and their charge after ionization. The device used in this work was the Smellmaster 2 provided by IFU GmbH (Frankenberg, Germany). It contains a radioactive source, which consists of tritium, offering a soft ionization without massive fragmentation of molecules. For analysis, ambient air is pumped into the system. The molecules in the air travel past the radioactive source and become ionized. Then they are sent into the drift tube passing through an electric field towards the detector plate. The time each molecule needs from entering the drift tube to arriving at the detector is measured and used to generate a spectrum. Every peak of the spectrum indicates that a high number of molecules arrived at the same time, either being alike in size, mass or structure (or sometimes being alike in more than one feature). Every molecule mix shows a specific peak pattern which can be recognized. For this, it is not necessary to know the chemical identity of every single compound. The device rather learns the pattern of the peaks that are specific for this molecule mix and recognizes this pattern. The generated spectra are analysed using cluster analysis with the algorithms k-means (MacQueen, 1967), Expectation Maximization (Dempster & al., 1977) and DBSCAN (Ester & al., 1996). One advantage of this technology is that ambient air and pressure are used, which makes the device handy, transportable and easy to use in the forest stand. Teepe & al. showed in 2001 that it is possible to miniaturize an IMS so that it has the size of a common cellular phone. The system is also able to perform measurements in the forest stand on a long time run with low maintenance, sending the data via cellular radio to the office for analysis.

***Ips typographus* (L.)**

Recently, forest stands consisting mainly of spruce have suffered severely from storms such as Herwart (2017) and Friederike (2018), with the drought in the summer of 2018 adding to it. Weakened by these events, they were susceptible to infestation with *I. typographus*, leading to mass outbreaks and three generations in most regions in 2018 (Lemme & Lobinger, 2018).

In the early summer of 2018 *I. typographus* adults were captured alive in the Tharandter Wald using Theysohn® slot-traps (Flügel GmbH, Osterode am Harz) baited with Pheroprax® lure (BASF, Ludwigshafen am Rhein). Fresh *Picea abies* (L.) H. Karst. tree trunks were cut into logs of 40 cm for experimental infestation with *I. typographus*. The logs were kept in glass boxes at the IFU GmbH laboratory. VOCs emitted from these spruce logs were measured as control sample, generating 50 spectra, before adding *I. typographus* adults and taking another 250 measurements over a period of 145 hours.

Figure 1 shows a levelplot of all generated spectra of two *P. abies* logs originating from 145 hours of IMS measurement(s), analysed by cluster analysis using DBSCAN algorithm. Spectra 0 to 350 and 501 to 850 show some alikeness, which was expected given that both logs originate from the same spruce tree. Spectra 351 to 500 and spectra 851 to 1000 show the same traits with all points plotted in the same trait number 3. That means that IMS was able to detect the infestation with *I. typographus* on both logs. Furthermore, in both cases the trait identified was the same, making the results comparable and leading to the assumption that infestations can be detected with IMS. Interestingly the molecule pattern did not change immediately after adding *I. typographus* adults to the logs (spectra 251 and 751, respectively) but remained stable for some time before the changes took place. This change in the trait of the molecule pattern is presumably caused by the active production of aggregation pheromones by the bark beetles. It is known that *I. typographus* males produce (S)-cis-verbenol to attract other adults (Bakke, 1970; Vité & al., 1972). The exact time of the change cannot be stated, since there was a disruption in measurement between 48 to 116 hours due to technical problems. Further experiments are required for the exact determination.

Comparison of species

The extreme drought in the summer of 2018 promoted a mass outbreak of *L. monacha* in a young *Pinus silvestris* (L.) forest stand near Borkheide (Dippmannsdorf), where female moths were collected to obtain egg masses for IMS experiments. LELF Brandenburg kindly provided egg masses of *T. processionea* collected in Hangelsberg. Frass of *I. typographus* was collected at an infestation site in the Tharandter Wald. Adults of *P. chalcographus* were captured alive in the Tharandter Wald using

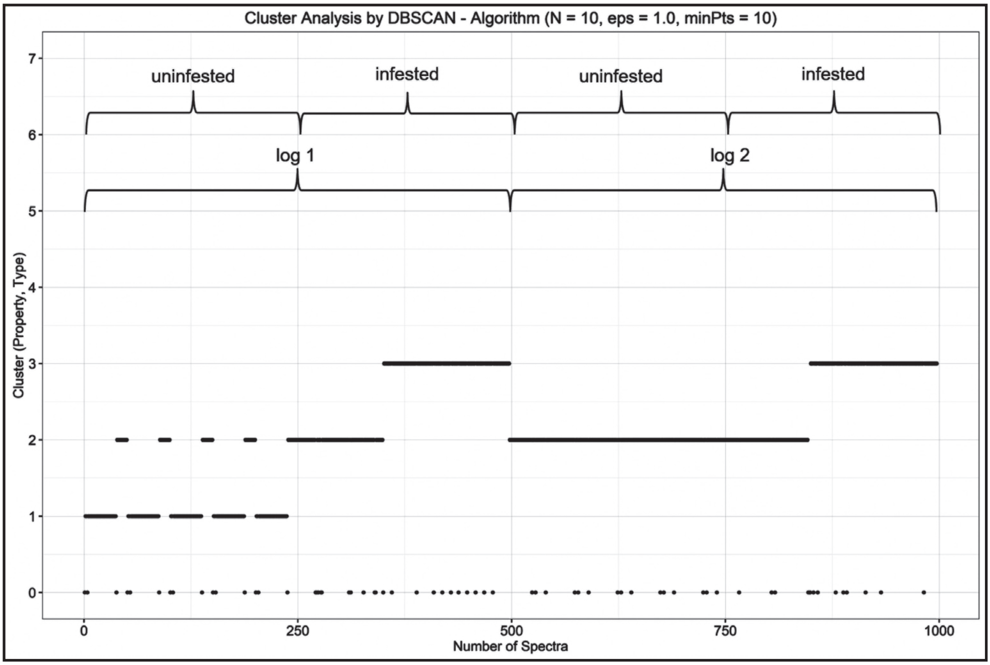

Fig. 1: Plot of cluster analysis using DBSCAN algorithm. The y-axis represents various traits, with every number being one trait that is significantly different from the others. The x-axis represents the index of every spectrum. Every point represents one spectrum according to its traits. The assignment of traits to numbers one to three in the plot was done randomly. Level numbers four to six were not assigned. Level zero represents noise. The left side displays 500 spectra gained from log no. 1, consisting of 250 spectra (50 spectra fivefold periodically arranged) of the uninfested log and 250 spectra of the log with *I. typographus* adults added. The right side has the according display for log no. 2. (Diagram provided by IFU, 2018)

Theysohn® slot-traps (Flügel GmbH, Osterode am Harz) baited with Chalcoprax® lure (BASF GmbH, Ludwigshafen am Rhein).

The spectra analyses show that the VOC patterns of the lepidopteran species differ significantly from each other, whereas both bark beetle species had some spectra in the same traits (traits three and four) (Figure 2). Overall, the bark beetle spectra were significantly different from the lepidopteran ones.

The spectra of the four species form four distinct cluster plots (Figure 3). The two clusters of the bark beetles species in the middle do not touch but lie very close to each other, meaning they are similar to some extent but can still be distinguished by cluster analysis. The similarity of their spectra can be explained by their similar host species and pheromonal chemistry. Both *I. typographus* and *P. chalcographus* have the same main host (*P. abies*) and use pinene as a precursor for their specific pheromones, which are mainly cyclic hydrocarbons. It can be assumed that these pheromones are a major part of the IMS spectra.

However, it is not known which VOCs are detected by IMS when measuring egg masses of the two lepidopteran species. It may be specific adhesives covering the eggs or female pheromones transferred to the egg masses during oviposition. The structure of the sexual pheromones used to lure males is different for each species: *L. monacha* produces pheromones made up of carbon chains with 18 to 19 carbon molecules (Gries & al., 1996), while the pheromones of *T. processionea* consist of 16 to 18 carbon molecules building a chain (Quero & al., 2003). It is also likely that the VOCs of the host trees of these moths, *Pinus sylvestris* for *L. monacha* and *Quercus* spec. for *T. processionea*, play a role in the detection signals. Overall, these results are a first evidence that IMS is able to distinguish different species from each other in the laboratory. The accurate detection of forest pest species in the laboratory is necessary to monitor them in a forest stand.

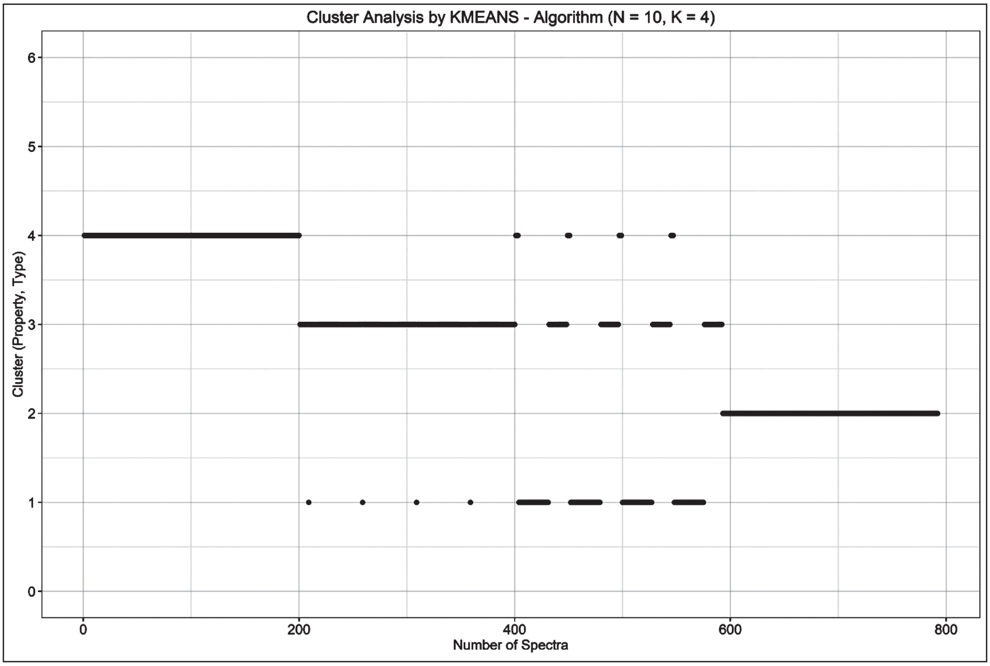

Fig 2: Plot of cluster analysis of the spectra of *L. monacha* (egg masses), *T. processionea* (egg masses), *I. typographus* (frass) and *P. chalcographus* (adults) using k-means algorithm. Every point represents one spectrum with a total of 200 spectra plotted for each species (see x-axis). If spectra are considered alike by cluster analysis, they are plotted in the same level, meaning they share the same traits. Overall, four traits were found (see y-axis). For both bark beetles, 50 spectra were fourfold periodically arranged for reasons of analysis. (Diagram provided by IFU, 2018)

GC-MS of *Lymantria monacha* egg masses

Due to the lack of information on the volatiles of nun moth egg masses, an examination using gas chromatography coupled with mass spectrometry (GC-MS) was carried out with egg masses collected in November 2018 and February 2019 in previously mentioned pine stand near Borkheide. Different solvents (hexane, pentane, dichloromethane/methanol 2 : 1) were used for the extraction of VOCs from the egg masses, of which 50 eggs were covered with the solvent in small beakers for 30 minutes. After that, the eggs were removed from the beakers and the residue was left to concentrate under the fume hood before 150 µl of dichloromethane/methanol were added. Gas chromatography was performed on a Agilent Technologies (Santa Clara, USA) model 6890 N (split/splitless injector, Agilent 19091S-433, polar HP-5MS capillary column [0.20 mm i. d., 30 m], helium as carrier gas) using the following program: 2.5 minutes isothermal at 40 °C, then increasing with 6.2 °C per second to 250 °C. Mass spectrometry was carried out using the MS Agilent Technologies model 5973N (EI ion source, 70 eV). Identification was done using NIST Mass Spectral Search program version 2.0, comparing spectra with data published in literature and the NIST database. Each extraction (except pentane due to no results in the first run) was repeated with 750 eggs. As a result, triacetin was found as a VOC in all samples extracted with hexane and dichloromethane/methanol, but not in the control (pure dichloromethane/methanol) (Figure 4).

However, the function of the ester triacetin for *L. monacha* is unknown. The substance is widely used as humectant (Kale & al., 2015), as additive in PVC (Lambertini & al., 2016) and in bio diesel (Elias & al., 2016). It is known to be attractive for *Helicoverpa zea* (Lepidoptera: Noctuidae) during oviposition (Jones & al., 1970). Another substance found was palmitate ester (extracted with hexane), which is known to be emitted from egg masses of *Lobesia botrana* (Lepidoptera: Tortricidae) (Thiéry & al., 1992) and *Cydia pomonella* (Lepidoptera: Tortricidae) (Thiéry & al., 1995). It also attracts *Lymantria dispar* in

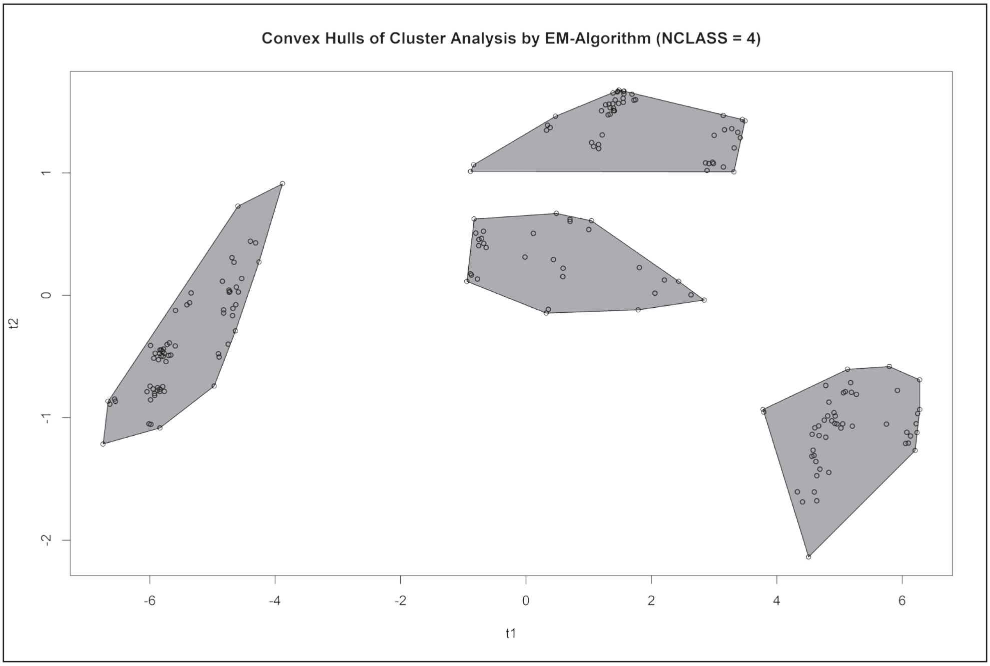

Fig 3: Plot of convex hulls of clusters after analysis of 200 spectra for each of the four species *L. monacha* (egg masses), *T. processionea* (egg masses), *I. typographus* (frass) and *P. chalcographus* (adults) using (the) Expectation Maximization algorithm (left to right). (Diagram provided by IFU, 2018)

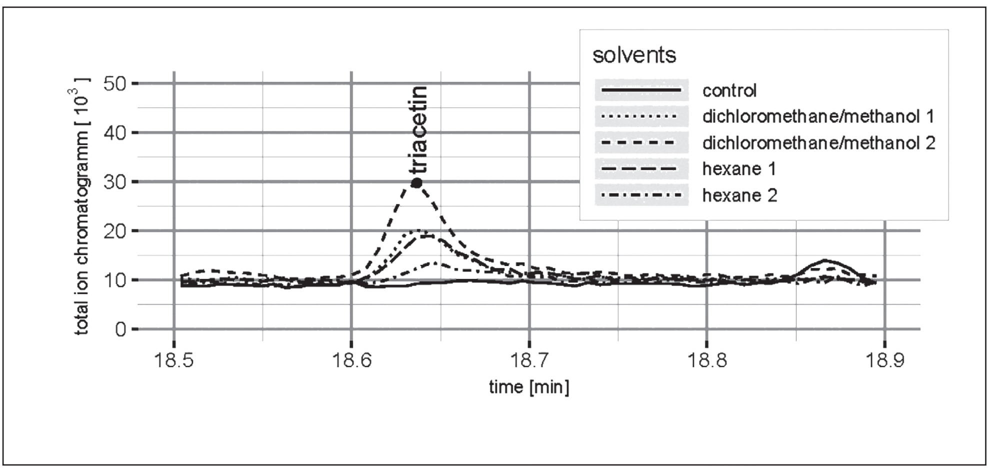

Fig 4: Details of combined chromatograms of VOC extractions using hexane and dichloromethane/methanol as solvents compared to control (dichloromethane/methanol alone) from minute 18.5 to 18.9. Numbers 1 and 2 after the solvent name refer to extraction 1 and its repetition, extraction 2.

natural flight (Beroza & al., 1971). Triacetin was found to be antifungal (Knight, 1957; Peng & Don, 2013) and could protect the eggs with its activity against fungi. In addition, testing the effect of these substances on parasitoid antennae using electroantennography (EAG) could help identify their relevance in the host-parasitoid interactions.

Summary

The results presented have been a first step towards a new approach in the detection of forest pests. Even though detection of the four species was possible in the laboratory, trials performed within the forest stand measuring spruce trees infested with *I. typographus* have not yet been successful, due to the IMS being very sensitive to sources of interfering molecules. It will be necessary to increase the selectivity of the IMS by finding a dopant molecule or developing a prior sample accumulation. Looking at the application of handy IMS devices e. g. for the detection of explosives in airports (Eiceman & Stone, 2004) it seems possible to develop a similar device to examine pine trees for nun moth egg masses without having to remove bark scales or to detect bark beetle infestation in spruce forest stands earlier than with common monitoring methods. Another possibility provided by IMS is the continuous unsupervised measuring on site providing data in real time via cellular radio. For this, a new measuring method needs to be developed. This would include the advantages of automation, long-term stability, data transfer from the forest stand into the office and low maintenance.

References

Bakke, A. (1970): Evidence of a population aggregating pheromone in *Ips typographus* (Coleoptera: Scolytidae). – Symposium on population attractants held at Freiburg University, Freiburg im Breisgau, Germany: 309-310.

Beroza, M., B.A. Bierl, J.G.R. Tardif, D.A. Cook & E.C. Paszek (1971): Activity and persistence of synthetic and natural sex attractants of the gypsy moth in laboratory and field trials. – Journal of Economic Entomology **64**(6): 1499-1508, DOI:10.1093/jee/64.6.1499.

Dempster, A.P., N.M. Laird & D.B. Rubin (1977): Maximum likelihood from incomplete data via the EM algorithm. – Journal of the Royal Statistical Society: Series B (Methodological): **39**(1), 1-22, DOI:10.1111/j.2517-6161.1977.tb01600.x.

Eiceman, G.A. & J.A. Stone (2004): Ion mobility spectrometry in national defense. – Analytical Chemistry: 390-397.

Elias, R.C., M. Senra & L. Soh (2016): Cold flow properties of fatty acid methyl ester blends with and without triacetin. – Energy Fuels **30**(9): 7400-7409, DOI:10.1021/acs.energyfuels.6b01334.

Ester, M., H.-P. Kriegel, J. Sander & X. Xu (1996): A density-based algorithm for discovering clusters in large spatial databases with noise. – The Second International Conference on Knowledge Discovery and Data Mining, Portland, Oregon.

Gries, G., R. Gries, G. Khaskin, K.N. Slessor, G.G. Grant, J. Liška & P. Kapitola (1996): Specificity of nun and gypsy moth sexual communication through multiple-component pheromone blends. – Naturwissenschaften **83**(8), 382-385, DOI:10.1007/BF01142006.

Jones, R.L., R.L. Burton, M.C. Bowman & M. Beroza (1970): Chemical inducers of oviposition for the corn earworm, *Heliothis zea* (Boddie). – Science **168** (3933): 856-857, DOI:10.1126/science.168.3933.856.

Kale, S., S.B. Umbarkar, M.K. Dongare, R. Eckelt, U. Armbruster & A. Martin (2015): Selective formation of triacetin by glycerol acetylation using acidic ion-exchange resins as catalyst and toluene as an entrainer. – Applied Catalysis A: General **490**: 10-16, DOI:10.1016/j.apcata.2014.10.059.

Knight, S.G. (1957): The in vitro antifungal activity of triacetin. – Antibiotics & Chemotherapy **7**(4).

Lambertini, F., D. Catellani, M. Vindigni & M. Suman (2016): Multiresidual LC-MS analysis of plasticizers used in PVC gaskets of lids and assessment of their migration into food sauces. – Journal of Mass Spectrometry **51**(9): 805-813, DOI:10.1002/jms.3815.

Lemme, H. & G. Lobinger (2018): Nach wie vor starker Befall durch Fichtenborkenkäfer – Blickpunkt Waldschutz 12/2018: http://www.lwf.bayern.de/waldschutz/monitoring/198384/index.php, Zugriff: 19.02.19.

MacQueen, J. (1967): Some methods for classification and analysis of multivariate observations. – Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability, Vol. **1**: Statistics, University of California Press, Berkeley, California: 281-297.

Peng, T.Y. & M.M. Don (2013): Antifungal activity of in-vitro grown *Earliella scabrosa*, a malaysian fungus on selected wood-degrading fungi of rubberwood. – Journal of Physical Science **24**(2): 21-33.

Quero, C., J. Bau, A. Guerrero, M. Breuer, A. de Loof, H.-G. Kontzog & F. Camps (2003): Sex pheromone of the oak processionary moth *Thaumetopoea processionea*. Identification and biological activity. – Journal of agricultural and food chemistry **51**(10): 2987-2991, DOI:10.1021/jf021035g.

Teepe, M., J.I. Baumbach, A. Neyer, H. Schmidt & P. Pilzecker (2001): Miniaturized ion mobility spectrometer. – International Journal of Interdisciplinary and Multidisciplinary Studies (IJIMS) **4**(1): 60-64.

Thiéry, D., B. Gabel, P. Farkas & M. Jarry (1995): Egg dispersion in codling moth: Influence of egg extract and of its fatty acid constituents. – Journal of Chemical Ecology **21**(12): 2015-2026, DOI:10.1007/BF02033859.

Thiéry, D., B. Gabel, P. Farkas & V. Pronier (1992): Identification of an oviposition-regulating pheromone in the european grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae). – Experientia **48**(7): 697-699, DOI:10.1007/BF02118323.

Vité, J.P., A. Bakke & J.A.A. Renwick (1972): Pheromones in *Ips* (Coleoptera: Scolytidae): occurence and production. – The Canadian Entomologist **104**(12): 1967-1975, DOI:10.4039/Ent1041967-12.