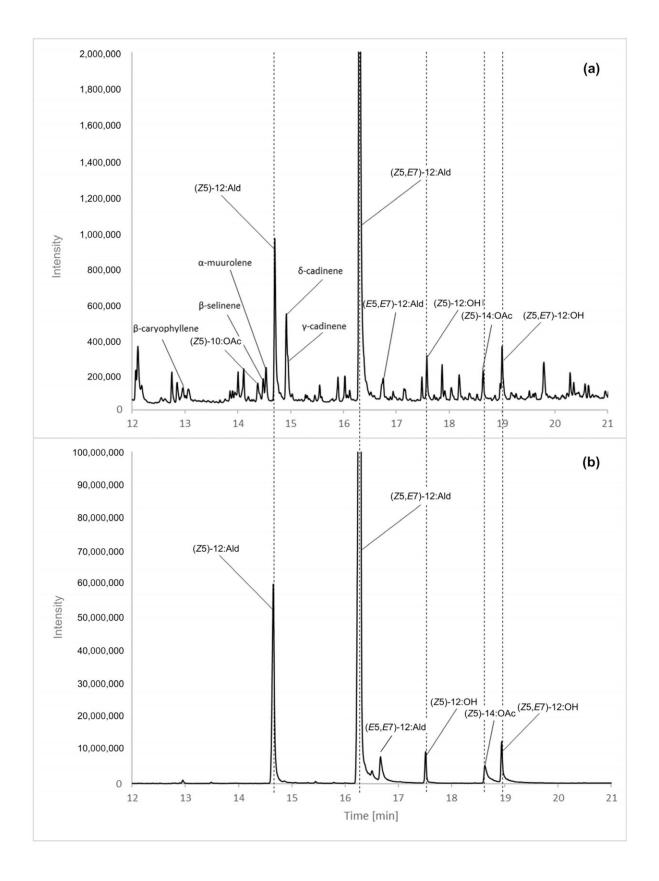


Newly discovered components of Dendrolimus pini sex pheromone

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Total Ion Current (TIC) chromatograms of volatiles emitted by a calling D. pini female sampled with a PDMS SPME fibers (a), and the mixture of authentic compounds composed like MD17 lure in Table 1 (b). Chromatograms were obtained using a polar ZBWAX column. Credit: *Insects* (2022). DOI: 10.3390/insects13111063

Beyond the seven mountains and forests, a hungry beast was stealing away. Does this sound like a fairy tale? In reality, such a beast does exist. It is the caterpillar of the nocturnal pine-tree lappet moth (Dendrolimus pini, L.), which feeds on pine needles and wreaks havoc in the forests on a massive scale.

However, words of love between these night butterflies may be the key to preventing outbreaks of their terrifying hunger. Monitoring the population of D. pini and its control has been possible thanks to the recent discovery of a team of scientists from the Institute of Physical Chemistry of the Polish Academy of Sciences (IPC PAS) led by Prof. Rafał Szmigielski.

They have deciphered the chemical hieroglyphs of the moth's language by which females attract males, finding a way to deal with the voracious beast and save the <u>pine forests</u>.

The voracious caterpillars of the pine-tree lappet Dendrolimus pini can destroy tens of thousands of hectares of coniferous forests. Despite the lack of ecological solutions to effectively combat this insect, we will not be saying goodbye to our <u>pine trees</u>. Scientists from the Institute of Physical Chemistry of the Polish Academy of Sciences (IPC PAS) have found a way towards effective protection against the lappet moth. They have deciphered the chemical 'love words' by which female Dendrolimus pini moths attract their males.



A new pheromone blend based, among others, on newly discovered biocomponents of the sex pheromone of the voracious insect developed by scientists from the IPC PAS may serve as an effective tool for monitoring and, in the long term, also for controlling the abundance of pine-tree lappet moth in our forests.

The pine-tree lappet moth Dendrolimus pini, a moth with a stocky body and gray-brown coloration, is one of Poland's most giant nocturnal butterflies. After overwintering in the mulch, the caterpillars of this butterfly climb into the crowns of pine trees, where they busily devour the needles. As up to several hundred perpetually hungry caterpillars can feed on a single tree, a rapid increase in the Dendrolimus pini population can lead to the extermination of large forest areas.

Without ecological and efficient tools for controlling the moth's population, the data on their population growth is crucial. However, synthetic pheromone traps used by foresters to attract Dendrolimus pini males were found to be insufficient. In the project carried out by scientists from the IPC PAS in collaboration with the Institute of Forestry Research (IBL), a novel and far more effective sex pheromone lure for the pine-tree moth has been developed.

The research into the pine-tree lappet moth's chemical love language proved more difficult than scientists had anticipated for entirely surprising reasons. The study began by the identifying the pheromones' components secreted by young Dendrolimus pini females. To develop a preparation to attract the voracious males, all the chemical compounds that are components of the sexual pheromone were put under the microscope.

Interestingly, in addition to the previously known chemical compounds, the secreted 'pheromone perfume' was found to contain other components, including—compounds derived from Scot's pine needles



and other organic compounds hitherto undiscovered, either significantly influencing the behavior of the caterpillars. The captured substances were chromatographically separated into single components and subjected to a detailed chemical analysis using tandem mass spectrometry.

The comparison of the molecular structures of the identified Dendrolimus pini sex pheromone components with the composition of a commercially available product strengthened the researchers' conviction that one of the most important reasons for the pine-tree lappet moths' poor response to the synthetic pheromone was its oversimplified chemical composition and the lack of key bioactive components.

"We aimed to identify all the substances in the pheromones secreted by the Dendrolimus pini moth and to create a unique formulation to divert the lappet moth's attention from the conifers, including the Scot's pine trees. And all this to save the forests from Dendrolimus pini hunger," says Prof. Rafał Szmigielski.

Based on the results of detailed analyses, chemists from the IPC PAS designed new formulations of the pheromone mixtures, which were subjected to numerous tests in a laboratory framework using modern bioanalytical techniques, such as wind tunnel, olfactometry, and electroanthenography, and then in the field measurements.

Dendrolimus pini moths were placed in wind tunnels exposed to specific chemicals and self-built terraria to test behavior in the natural environment. Hundreds of experiments were carried out with female and male butterflies in this way, exposing them to various compounds. The scientists looked at (Z5)-dodecanal, abbreviated as (Z5)-12:Ald, which occurs naturally in pine needles and attracts caterpillars, and (Z5)-decen-1-yl acetate, known as (Z5)-10:OAc, which in turn discourages them.



Studies have shown the critical role of these two compounds on barnacle behavior.

"The mixture of <u>chemical compounds</u> we have identified and patented can—naturally with appropriate conventionality—be treated as certain words of non-trivial structure, spoken in the chemical language of the moth. The main accent here is on two 'sounds,' one of which people pronounce differently from the other. For this chemical 'I love you' to sound convincing, additional words are needed to increase the carrying capacity of the main message, such as plant-derived compounds," explains Prof. Rafał Szmigielski.

Previous attempts attracted a maximum of a few dozen pine barnacle males per trap over several weeks to pheromone traps. Meanwhile, chemical calling using a mixture of substances developed by a group from the IPC PAS increased this number to as high as 160 males per trap in just 3-4 days. Foresters unanimously considered such an increase in insect interest to be a huge success, bringing us closer to saving the pine forests from the appetite of these voracious insects.

Identifying new components of the sex pheromone emitted by the female moth Dendrolimus pini and their correlation with the specific behavior of this tiny insect is a step forward toward the effective protection of pine forests. The scientists' research into creating the synthetic pheromone lures is also more than just monitoring the moth's behavior. It is, first and foremost, the <u>interdisciplinary work</u>, a love of nature, and the fascinating the micro-world.

The findings are published in the journal *Insects*.

More information: Krzysztof J. Rudziński et al, Newly Discovered Components of Dendrolimus pini Sex Pheromone, *Insects* (2022). <u>DOI:</u> 10.3390/insects13111063



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