

Restoring a natural root signal helps to fight a major corn pest

August 3 2009



The Western corn rootworm (insert) is a devastating pest of maize roots. By emitting the volatile compound E-beta-caryophyllene in response to rootworm feeding, maize plants naturally attract insect-killing nematodes. Using genetic transformation, the signal was restored in an American line and in field tests it was shown that this dramatically increased the protection that the plants received from the nematodes. Credit: Matthias Held and Sergio Rasmann, University of Neuchâtel, Switzerland

A longstanding and fruitful collaboration between researchers at the Max Planck Institute for Chemical Ecology and the University of Neuchâtel in Switzerland, together with contributions from colleagues in Munich and the US, has produced another first: the successful manipulation of a crop plant to emit a signal that attracts beneficial organisms.



Genetic transformation of <u>maize</u> plants resulted in the release of the naturally active substance (E)-beta-caryophyllene from their roots. The substance attracts nematodes that attack and kill larvae of the Western corn rootworm, a voracious root pest. In field tests, the enhanced nematode attraction resulted in reduced root damage and considerably fewer surviving rootworms. Further fine-tuning of this natural defense strategy will allow for an environmentally friendly growing of maize with minimized use of synthetic insecticides. The project was carried out within the framework of the Swiss National Centre of Competence in Research (NCCR Plant Survival).

The Western corn rootworm (Diabrotica virgifera virgifera) is the most damaging maize pest in the US and is responsible for enormous financial losses. Current methods to control the rootworm pest include insecticides, crop rotation and transgenic Bt maize lines that are not yet approved in Europe. After first invading the Balkans, the pest has since 2007 also been found in southern Germany. The corn rootworm larvae feed on root hairs and bore themselves into the maize roots. The results are devastating: The plants take up less water and nutrients, and with the root mass severely reduced the plants lodge and collapse. In areas in Germany where the corn rootworm is a potential threat, the Federal Office of Consumer Protection and Food Safety (BVL) establishes safety zones and enacts the use of the insecticide chlothianidine. In spring 2008 this insecticide was directly applied on the seeds, but during sowing it was unintentionally emitted as dust from abraded seeds, contaminated flowers, and poisoned 330 million honey-bees.

"Instead of using insecticides, the use of natural enemies of the corn rootworm could be much more environmentally friendly," says Jörg Degenhardt, who was recently appointed professor at the University of Halle. While working in the group of Jonathan Gershenzon at the Max Planck Institute for Chemical Ecology in Jena he had already contributed to a key discovery four years ago by Sergio Rasmann in the group of Ted



Turlings at the University of Neuchâtel. They found that maize roots attacked by rootworm attract nematodes by releasing (E)-betacaryophyllene (E β C). One striking finding was that, after decades of breeding, most North American maize varieties no longer emitted E β C and had lost the ability to attract protective nematodes.

Therefore the research group in Jena and Neuchâtel teamed up again in an attempt to restore the E β C signal in a variety that normally does not emit the substance. Jörg Degenhardt, with the help of Monika Frey at the Technical University of Munich, transformed a non-emitting maize line with a gene that encodes an E β C generating enzyme, resulting in continuous emissions of E β C. Next, the Turlings group in Neuchâtel sent Ivan Hiltpold to Missouri, where, under the guidance of Bruce Hibbard of the United States Department of Agriculture, the transformed plants were tested in the field.

"Our study showed that the re-established natural $E\beta C$ signal greatly enhanced the effectiveness of nematodes in controlling Western corn rootworm", Hiltpold reports. In rows with $E\beta C$ -producing maize plants root damage was greatly reduced; 60% fewer Diabrotica beetles emerged as compared to rows with non-transformed maize plants. This control efficiency approaches that of conventional synthetic insecticides used to fight Diabrotica. Subsequent laboratory studies confirmed that transgenic plants attracted significantly more nematodes than the nontransformed equivalents.

"The use of this indirect defense is an attractive strategy to increase plant resistance against herbivores and to reduce the use of chemical pesticides," Degenhardt says. "The transgenic corn plants used in these experiments have no commercial value and the experiments simply served a 'proof of principle' that the E β C emission helps to protect the plants against underground infestation." The E β C trait is present in other, mainly European, corn varieties as well as in the maize ancestor



species. The trait could be reintroduced into deficient plants by conventional breeding. On the other hand, generating $E\beta C$ emitting maize varieties by means of gene technology may have advantages: it is faster and prevents the loss of other important traits.

In further experiments the researchers want to determine the most effective way the nematodes and their response to the $E\beta C$ can be applied. Moreover, the diffusing properties of caryophyllene make it an ideal belowground signal that could also serve to protect other crop plants. A patent for this approach has been filed. [JWK, TT]

<u>More information</u>: Jörg Degenhardt, Ivan Hiltpold, Tobias G. Köllner, Monika Frey, Alfons Gierl, Jonathan Gershenzon, Bruce E. Hibbard, Mark R. Ellersieck and Ted C. J. Turlings: Restoring a maize root signal that attracts insect-killing nematodes to control a major pest. Proc. Natl. Acad. Sci. USA, Early Edition, August 3???, 2009<u>DOI</u>: <u>10.1073/pnas.0906365106</u>

Source: Max Planck Institute for Chemical Ecology

Citation: Restoring a natural root signal helps to fight a major corn pest (2009, August 3) retrieved 6 May 2024 from <u>https://phys.org/news/2009-08-natural-root-major-corn-pest.html</u>

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