Buzz pollination: https://upload.wikimedia.org/wikipedia/commons/8/8b/Bumblebee_buzzpollinating_Solanum_dulcamara.webm

FIELD WORK SUMMER 2022 - BSc or MSc Level - Erasmus Grant can be requested

The ecological consequences of chemodiversity in *Solanum dulcamara*

Level: BSc or MSc internship
Start: June 2022 (start of the field work, preparation of project can start earlier in Nijmegen)
Duration: 12 to 36 ec
Location: Leipzig and Bad Lauchstädt, Germany; THUS ERASMUS GRANT CAN BE REQUESTED
Project form: Field work with the option to extend this with lab work (see details below)
Supervision: Adam Anaia and Janny Peters
Contact: adam.anaia@idiv.de and janny.peters@ru.nl

We propose to observe buzz pollinators on flowers and fruit predators (Hemiptera) on berries, to learn more about the ecological and evolutionary consequences of steroidal glycoalkaloids (SGA) chemodiversity in *Solanum dulcamara*. **The field work is expected to start in June 2022**. In case of a master thesis (>30 EC), the field work can be extended and combined laboratory work. In our lab, we are able to measure floral bouquets with gas chromatography coupled to mass spectrometry (GC-MS) based metabolomics.

Examples of research questions: **1)** Do buzz pollinators prefer certain plots or plants of certain leaf SGA chemotypes? **2)** Are there qualitative and/or quantitative differences in flower visitation by buzz pollinators? **3)** Do stink bugs prefer berries of plants in certain plots and do they prefer any chemotypes? **4)** Are there differences in volatile blends between plants of different leaf SGA chemotypes?

We are looking for a motivated bachelor or master student with a background in ecology, biology, molecular life sciences or chemistry. Experience with (ecological) modelling and the possession of driver's license B is preferred.

You will..

- .. explore chemical ecology research: study the ecological consequences and molecular mechanisms of chemodiversity in *Solanum dulcamara*
- .. learn how to use mass spectrometry as a tool for ecological and/or evolutionary inference (don't worry, you don't have to be a star in chemistry already)
- .. gain experience conducting scientific research in both field and laboratory work
- .. integrate previous results with results from your own project
- .. communicate your work verbally in a presentation and written in a report

Interested student are encouraged to contact Adam Anaia (adam.anaia@idiv.de) or Janny Peters (janny.peters@ru.nl) with a short motivation (half A4) and a concise curriculum vitae. This work will be a collaborative effort between Molecular-Interaction-Ecology (FSU Jena, iDiv, Leipzig, Germany) and the Plant Ecology and Physiology (Radboud University Nijmegen). The work is intended to be conducted in Leipzig, Germany, from which we will travel 2-3 days per week to the field site in Bad Lauchstädt by car.

Relevant Background Information

After rooting, plants are largely immobile. Therefore, plants have to defend themselves against pathogens, herbivores and other threats. Plant specialized metabolites (PSMs) are produced to survive in an ever-changing (a)biotic environment. The nightshade family (Solanaceae) consists of many economically important species. The genus *Solanum* - to which crop-species like potato, tomato and eggplant belong - is characterized by the production of glycosylated steroidal compounds (GSCs) including steroidal glycoalkaloids (SGAs), which are cholesterol-derived [1] nitrogen-containing steroidal (C27) glycosides. SGAs are associated with plant defense against herbivores and pathogens; different mechanisms of action were proposed including its action as deterrent (saponins are soap-forming molecules). Recently, the genes responsible for the biosynthesis of SGAs have been elucidated in crop species [2,3]. GAME25, a 3β-hydroxysteroid dehydrogenase/Δ5,4 isomerase was shown to catalyze the reaction from unsaturated SGAs to saturated SGAs in potato [4,5]. This is predicted to happen in concert with a reductase from primary metabolism [4,5].

Solanum dulcamara (known as the bittersweet nightshade[1]), is a wild, woody perennial native to Eurasia that thrives in contrasting hydrological conditions, ranging from wetlands to dry coastal dunes[6]. High structural diversity of SGAs is detected in bittersweet and compounds with different saturation levels are hypothesized to exists. Intraspecific variation of SGAs in *S. dulcamara* has been associated with differences in preferences by generalist gastropods: 1. Gastropods preferred plants with saturated SGAs over plants producing unsaturated SGAs and 2. Gastropods preferred plants with non-nitrogenous GSCs over plants producing SGAs [7]. For specialized flea beetles, the opposite trend in preference was observed in a common garden experiment [8].

Genes known for SGA biosynthesis in *Solanum* crop species have been used to query *S. dulcamara* transcriptomes for candidate-genes based on homology. The expression of a gene-of-interest (GOI), a putative *SdGAME25*, is associated with the presence or absence of saturated compounds in *S. dulcamara*. Recently, a fitness-effect has been found in a common garden experiment in which the effect of chemotype (individual-level) and phytochemical diversity (AKA 'chemodiversity'; plot-level) on fitness measures and herbivory were assessed. Plants with unsaturated SGAs ('mono' chemotype) produced more berries than plants with 'mixed' chemotypes [producing predominantly saturated SGAs, but also unsaturated SGAs], however, the opposite trend was observed for seeds). Interestingly, mono-chemotype plants produced more berries in plots that were heterogenous (e.g. containing multiple chemotypes) compared to monogenous plots (e.g. containing only one chemotype). Again, the opposite trend is true for the seeds: plants in monogenous plots produce more seeds than plants in heterogeneous plots. (Anaia, in preparation).

1. Sonawane, P. D. *et al.* Plant cholesterol biosynthetic pathway overlaps with phytosterol metabolism. *Nature Plants* **3**, 16205 (2017).
2. Cárdenas, P. D. *et al.* The bitter side of the nightshades: Genomics drives discovery in Solanaceae steroidal alkaloid metabolism. *Phytochemistry* **113**, 24–32 (2015).
3. Itkin, M. Biosynthesis of Antinutritional Alkaloids in Solanaceous Crops Is Mediated by Clustered Genes. (2013) doi:10.1126/science.1240230.
4. Lee, H. J. *et al.* Identification of a 3β-Hydroxysteroid Dehydrogenase/ 3-Ketosteroid Reductase Involved in α-Tomatine Biosynthesis in Tomato. *Plant and Cell Physiology* **60**, 1304–1315 (2019).
5. Sonawane, P. D. *et al.* Short-chain dehydrogenase/reductase governs steroidal specialized metabolites structural diversity and toxicity in the genus Solanum. *Proceedings of the National Academy of Sciences of the United States of America* **115**, E5419–E5428 (2018).
6. Zhang, Q., Peters, J. L., Visser, E. J. W., de Kroon, H. & Huber, H. Hydrologically contrasting environments induce genetic but not phenotypic differentiation in Solanum dulcamara. *Journal of Ecology* **104**, 1649–1661 (2016).
7. Calf, O. W. *et al.* Gastropods and Insects Prefer Different Solanum dulcamara Chemotypes. *J Chem Ecol* **45**, 146–161 (2019).
8. Calf, O. W. *et al.* Gastropods and Insects Prefer Different Solanum dulcamara Chemotypes. *Journal of Chemical Ecology* **45**, 146–161 (2019).

[1] Among many other names, which might hint into the ancient ethnobotanical/traditional uses of *Solanum dulcamara*