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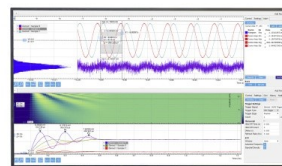
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Application of Eco-metabolomics in Biological Science

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Abstract. Secondary metabolites provide a tremendous potential for natural crop protection. This is especially important due to the rapid spread of agricultural pests worldwide. At the same time international law regulations restrict the use of synthetic pesticides. Therefore, we developed the eco-metabolomic approach to identify candidate compounds related to host plant resistance using NMR (Nuclear Magnetic Resonance Spectroscopy). We classify resistant and susceptible plants using *in-vivo* bioassays. Subsequently, we compare their metabolomic profiles by multivariate statistics to identify metabolites involved in host plant resistance. The negative effect of the candidate compounds is validated with *in-vitro* bioassays. As a proof of principle we used western flower thrips (*Frankliniella occidentalis*) a key pest worldwide. We applied the approach in different host systems including Senecio as a wild plant, chrysanthemum as an ornamental and tomato and carrot as vegetables. In all systems the metabolomic profiles of resistant and susceptible plants were significantly different leading to a range of different metabolites involved in thrips resistance. The majority of these compounds were phenylpropanoids and flavanoids. Interestingly, these did not only show a negative effect on thrips, but as anti-oxidants, were also linked to positive human health effects such as prevention of cancer and cardio-vascular diseases. As such these compounds do not only contribute to pest control but also to human health improvement. Therefore, our approach is of great relevance for application and implementation in sustainable crop protection programmes. Currently we are busy developing genetic and chemical markers of secondary metabolites to be incorporated in host plant resistance breeding. Besides we investigate the incorporation of secondary plant metabolites into seed treatments and the enhancement of plant secondary metabolite concentrations through the use of elicitors such as phytohormones, UV-light and bacteria.

Keywords: Eco-metabolomics, host plant resistance, NMR, thrips.

INTRODUCTION

Mankind faces the need of feeding an increasing world population. This challenge comprises three components: increasing yield, sustainability of food production and promotion of health by means of food. Plant breeding plays an essential role in reaching these ultimate goals. While traditional breeding mainly focussed on high yield it is now clear that health and environmental issues become increasingly important. In both issues plant secondary metabolites play a critical role.

Plants synthesize an immense number of secondary compounds. This vast metabolic diversity is explained by a stepwise and reciprocal process of adaptation and counter adaptation between plants and their abiotic and biotic environment, moulded by mutual selection. As such plant secondary metabolites provide a huge reservoir for the generation of both plant defence and human health compounds.

Plant defence against pests and pathogens based on secondary metabolites has been weakened through selection of yield and palatability traits. Crops are, therefore, in general, vulnerable to pest and pathogen attacks leading to an increasing demand of pesticides. Excessive use of pesticides has led to resistance of pests and pathogens, toxicity to

non-target organisms, contamination of the environment and residue problems on marketable crops. This gained special importance due to new regulation regarding pesticide registration and application resulting in a decreased availability of pesticides.

In the framework of integrated pest management (IPM) we, therefore, need to develop and integrate resistant cultivars targeting a proper combination of secondary compounds. Up to now the study of host plant resistance has, for technical reasons, been restricted to the identification of single compounds applying specific chemical analyses adapted to the compound in question. In biological processes however, usually more than one compound is involved. A metabolomic approach allows the simultaneous detection of a wide range of compounds, providing an immediate image of the metabolome of a plant. In this framework we have developed the eco-metabolomic approach to study constitutive chemical host plant resistance using western flower thrips (*Frankliniella occidentalis*) as a model [1].

Western Flower Thrips (*Frankliniella occidentalis*)

Over the last few decades, western flower thrips (*Frankliniella occidentalis*) has spread worldwide to become a key insect pest of agricultural and horticultural crops resulting in substantial losses of millions of Euros. There has been a massive increase in the international movement of plant material leading to the accidental transport of thrips. Thrips have many traits that predispose them to be successful invaders: small size, affinity for enclosed spaces and high reproductive potential. In addition western flower thrips is highly polypagous infesting a wide range of about 200 wild and cultivated host species. Thrips have piercing-sucking mouthparts, which enable them to ingest whole plant cells. They usually prefer to feed on older leaves. Western flower thrips causes considerable damage through feeding, oviposition and transmission of tospoviruses. Feeding and oviposition cause direct damage on leaves, flowers and fruits. Feeding on actively growing tissue leads to distortion, reduction in plant growth and eventually yield loss, while feeding on expanding tissue results in the characteristic silver leaf scars, which affect product appearance and reduce market quality.

Metabolomics

The metabolome comprises all compounds present in a cell, tissue or organism. Type and quantity of metabolites are dynamic and vary within a plant. Metabolites, therefore, offer a great opportunity to understand plant physiology and adaptation. Two quite distinctive hardware platforms can be distinguished in metabolomics, (i) those principally based on gas or liquid chromatography coupled to mass Spectrometry (GC- or LC-MS) and (ii) those based on Nuclear Magnetic Resonance (NMR). NMR has a lower sensitivity in detecting metabolites compared to MS but provides simultaneous access to both qualitative and quantitative information and allows unequivocal determination of metabolite structure, the main bottleneck of MS. The Natural Products Laboratory at Leiden University has build up a NMR platform with an extensive database of NMR spectra.

NMR spectroscopy measures the resonances of magnetic nuclei such as ^1H , ^{13}C and ^{15}N that interact with an external magnetic field. NMR spectra are unique and specific for each single compound and can be used to identify metabolites of biological origin of which no *a-priori* knowledge is needed. The latter makes the need for authentic standards, often a major barrier to structure determination, unnecessary. NMR provides simultaneous access to both qualitative and quantitative information since the signal intensity is directly proportional to the molar concentration. NMR requires minimal sample preparation, is highly reproducible and allows a high sample throughput. So far, very few studies have been using NMR to investigate plant-host interactions. We applied NMR to study chemical host plant resistance to western flower thrips.

Eco-Metabolomic Approach

To investigate the metabolomic basis of thrips resistance we first identify thrips-resistant and susceptible plants, on which subsequently NMR is applied. Thrips resistance is assessed in an *in-vivo* thrips bioassay. Individual vegetative plants are placed in thrips proof cages to which ten adults western flower thrips are added and left for a week. Thereafter, silver damage, expressed as leaf area damaged in square millimeter is visually scored for each leaf. The most resistant and the most susceptible plants are then used for NMR metabolomics. We usually choose an older leaf of each plant for analysis, since thrips prefer feeding on these in contrast to younger leaves. Each sample is freeze-dried, ground and usually extracted with methanol. NMR spectra are recorded on a 600 MHz Bruker spectrometer. NMR signals of thrips resistant and susceptible plants are compared by multivariate analysis to

identify the metabolites involved in thrips resistance. Once identified the concentrations of the metabolites in question are measured and statistically analysed. The effect of the metabolites identified is then confirmed by a thrips *in-vitro* bioassay. The metabolite in question is added to a liquid medium for which the survival of first instar thrips larvae is recorded.

We successfully applied this approach on different host systems including *Senecio* as a wild plant [2, 3], chrysanthemum [4] and gladiolus as ornamentals and tomato [5] and carrot [6] as vegetables. In all host systems the metabolomic profiles of thrips resistant and susceptible plants were significantly different, leading to different metabolites involved in thrips resistance. In *Senecio* the resistant plants contained higher amounts of pyrrolizidine alkaloids (PAs). PAs are regarded as constitutive defense against general herbivores. They deter chewing insects like caterpillars, locusts and beetles. In chrysanthemum the two phenylpropanoids chlorogenic acid and feruloyl quinic acid were involved in thrips resistance. These phenols reduce the bioavailability of amino acids and decrease the digestibility of dietary proteins. As such they have been recorded in the literature as antifeedants of caterpillars, leaf beetles, leafhoppers and aphids. Moreover, chlorogenic acid also shows a negative effect on fungi, bacteria and virus. But most interesting, chlorogenic acid is the most widespread natural plant dietary anti-oxidant and as such it is thought to prevent development of cancer and cardiovascular diseases in humans. In gladiolus two triterpenoids seem to be involved in thrips resistance: amyirin and lupeol. Comparing wild and cultivated tomatoes for thrips resistance it appeared that the wild tomatoes were more resistant to thrips and especially *Lycopersicon pennellii* and *L. hirsutum* showed very little damage. Both showed high amounts of acylsugars, which are produced in glandular leaf hairs and which are known for their negative effects on caterpillars, leafminers, whiteflies and aphids. In carrot the flavonoid luteolin known to deter caterpillars, weevils and leafminers, the phenylpropanoid sinapic acid and the amino acid β -alanine were involved in thrips resistance. Interestingly, all three compounds are positively linked to human health either through prevention of cancer or as in the case of β -alanine as part of vitamin B₅.

APPLICATIONS

Next to the identification of secondary metabolites involved in host plant resistance the eco-metabolomic approach opens a way to study the spatial and temporal dynamics of these compounds in the plant and their consequences on resistance. We investigated the spatial distribution of thrips defence related compounds over different leaf tissues in *Senecio* observing that pyrrolizidine alkaloids (PAs) were concentrated in the mesophyll while chlorogenic acid was concentrated in the epidermis [7]. Exploring the reason for this tissue differentiation of compounds it became evident that combination of PAs and chlorogenic acid led to a drastic decrease of PA toxicity. Likewise, significant temporal differences in PA mesophyll concentrations during the day were observed. PA accumulated in the morning, the time thrips is most active in host finding, in contrast to the afternoon and evening. Investigating the role of plant development stages no changes in the leaf concentration of triterpenoids in vegetative, bud and flower stages of gladiolus were observed. In all plant stages plants were equally thrips resistant. Likewise, no changes in triterpenoid leaves and ensuing thrips resistance were observed in gladiolus grown in different environments, i.e. field vs. climate room.

In contrast to the combination of PAs and chlorogenic acid combinations of different compounds related to thrips resistance may confer additive or synergistic effects on thrips mortality or oviposition. We therefore, formed two- and three way mixtures of luteolin, sinapic acid and β -alanine at different concentrations and offered these to thrips in *in-vitro* mortality and oviposition assays. To our surprise none of the mixtures was more effective than any of its single compounds.

Next to thrips the identified compounds may confer resistance to other pests as well. Indeed, we were able to show that thrips resistance in chrysanthemum was correlated with resistance to leafminer (*Lyriomiza trifolii*) [8] and that this cross-resistance was based on the same phenylpropanoid compound.

Finally, the results of the eco-metabolomic approach, next to improvement of resistance breeding can be used for development of external control. We, recently, started investigations into the incorporation of thrips resistance related compounds into seed coats and dippings of cuttings.

CONCLUSION

Applying the eco-metabolomic approach as a tool to study herbivore resistance is a significant advance in the study of plant-insect relationships providing key information for herbivore resistance breeding in crops. Being an universal tool it can not only be applied for plant pests, but is also usable for the study of resistance to pathogens and weeds. This approach opens the way for the challenge to breed resistant crops with additive human health benefits in a sustainable way.

REFERENCES

1. K. Leiss, Y. Choi, R. Verpoorte and P. Klinkhamer, [Phytochemistry Reviews](#) **10**, 205–216 (2010).
2. T. Nuringtyas, R. Verpoorte, P. Klinkhamer, M. van Oers and K. Leiss, [Journal of Chemical Ecology](#) **40**, 609–616 (2014).
3. K. Leiss, Y. Choi, I. Abdel-Farid, R. Verpoorte and P. Klinkhamer, [Journal of Chemical Ecology](#) **35**, 219–229 (2009).
4. K. Leiss, F. Maltese, Y. Choi, R. Verpoorte and P. Klinkhamer, [Plant Physiology](#) **150**, 1567–1575 (2009).
5. M. Mirnezhad, R. Romero-González, K. Leiss, Y. Choi, R. Verpoorte and P. Klinkhamer, [Photochemical Analysis](#) **21**, 10–117 (2010).
6. K. Leiss, G. Cristofori, R. van Steenis, R. Verpoorte and P. G. L. Klinkhamer, [Phytochemistry](#) **93**, 63–70 (2013).
7. T. Nuringtyas, Y. Choi, R. Verpoorte, P. Klinkhamer and K. Leiss, [Phytochemistry](#) **78**, 89–97 (2012).
8. S. Kos, P. Klinkhamer and K. Leiss, [Entomologia Experimentalis et Applicata](#) **151**, 198–208 (2014).