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The NCCR Plant Survival at the University of Neuchâtel# – The Role of Chemistry in an Interdisciplinary Swiss Research Network

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Abstract: The survival of plants is of fundamental importance to guarantee the biodiversity in natural areas and a sustainable agriculture. The National Centre of Competence in Research 'Plant Survival in Natural and Agricultural Ecosystems' devotes its research efforts to the understanding of mechanisms of plant survival. The mechanisms that plants employ to adapt to their biotic and abiotic environment and to cope with important stress factors are investigated. This is achieved by interdisciplinary interaction of disciplines within the NCCR. The particular role played by natural products and analytical chemistry in seven different projects is summarized.

Keywords: Fungal pathogens · Pheromones · Phytotoxins · Plants volatiles · Secondary metabolites

Introduction

Plant Survival: How it Works

Plants are the primary producers of organic matter on land and central to almost all ecosystems. The survival and performance of plants is therefore of fundamental importance to both the preservation of biodiversity and sustainable agriculture. The National Centre of Competence in Research (NCCR) 'Plant Survival in Natural and Agricultural Ecosystems' investigates the mechanisms that plants employ to adapt to their biotic and abiotic environment and to cope with important stress factors. The active participation of federal research institutes as well as the interdisciplinary interaction of NCCR scientists and external partners contribute to the application of obtained knowledge towards the protection of plants in natural and agricultural ecosystems.

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A Broad Range of Research Projects

In the field of plant physiology, studies focus on the interactions between fungal pathogens, crop and model plants, the function of plastids, and new genetic plant model species. On the ecosystem level, research covers a wide range of topics such as the reactions of plants and their associated microflora to nutrient stress, the effect of long-term copper fungicide applications, the dynamics of pasture woodlands, and the interaction of plants, insect herbivores, and their natural enemies. The projects with a high application potential form a key link between fundamental research and practice, including biodiversity in agro-ecosystems, management of vineyard pests and diseases, as well as risk assessment of genetically modified organisms and advanced statistical methods.

The Role of Chemistry

Throughout the covered domains, about half of all NCCR projects undertake research in chemistry. This involves the study of the genetic basis and the biochemistry involved in resistance of grapevine to two major fungal pathogens. Other groups investigate the role of plant volatiles with respect to Petunia as a new genetic model species, tritrophic interactions between crop plants, insects, and natural enemies as well as improved pest control using pheromones. Finally, the efficiency of transfer mechanisms of nutrients and toxic metals from different types of rock substrates to the soil and plants is examined.

Selected Projects

Project 1a, J.-M. Neuhaus: Identification of Genes Involved in Resistance of Grape to Grey Mould (Botrytis cinerea) *and Downy Mildew* (Plasmopara viticola)

Fungal, bacterial, and viral diseases are the causes of major yield losses in agriculture and therefore fought by pesticides. Major advances have been accomplished in the understanding of the plant's own defence reactions against pathogens. New technical developments allow the analysis of the expression pattern of many genes potentially involved in successful resistance to a pathogen. The major fruit crop world-wide and an important crop in Switzerland, grapevine, is affected by several fungal diseases. Two pathogens in particular cause serious problems in Swiss vineyards. The grey mould *Botrytis cinerea* colonises the berries at an early stage but only develops at fruit maturation. It can be controlled chemically but is known to readily develop fungicide resistance. By comparing two related cultivars, Gamay and Gamaret, which differ in susceptibility to *B. cinerea*, we intend to identify protective factors with a great potential for future developments.

The other major fungal disease of grapevine in Switzerland is downy mildew (*Plasmopara viticola*), which infests plants at various stages of development and is the major reason for repeated massive use of fungicides including copper salts, which are even used in organic agriculture. The cultivar Freiburger 250-75 is more resistant to this disease than Chasselas, but unfortunately not at the same time resistant to *B. cinerea*. A better understanding of both diseases will improve their management and contribute to a reduction of the negative environmental effects of viticulture.

The wild grapevine population of Switzerland has been subjected to a massive reduction in numbers since the arrival of several diseases and parasites in Europe. This substantial selection pressure may have selected genotypes conferring a better resistance to some of these agents. We will thus also include wild grapevine isolates into our evaluation, taking advantage of the characterisation of the Swiss *Vitis* populations by the laboratory of Plant Ecology and the current multiplication of wild *Vitis* cuttings by the laboratory of Plant Systematics.

The integration of this project into a larger programme devoted in particular to grapevine and its problems will ensure an efficient transfer of knowledge between molecular biologists, biochemist, phytopathologists, entomologists, and plant breeders. This will promote the development of methods connected with agronomic practices.

Project 2, C. Kuhlemeier: **Petunia** *as a Genetic Model Species for Plant–Pollinator Interaction*

Isolation of genes from *Petunia* inbredlines that are important for the attraction of *Petunia* pollinators such as bees and nocturnal moths will permit us to better understand the origin of angiosperm species. In fact, it is believed that different *Petunia* species arose after a mutation in an ancestral species that was visited after mutation by a new pollinator. These mutated genes were probably responsible for the colour, odour, nectar production and morphology of the flowers (characters that are important for the attraction of pollinators, called pollination syndromes). We already have a large *Petunia*-inbredlines collection that contains lines that differ in colour, odour, morphology, and nectar production.

Behavioural experiments by the use of the bumblebee *Bombus terrestris* and the nocturnal sphinx *Manduca sexta* are carried out in a greenhouse to determine what flower character is important to attract these pollinators. We use wild *Petunia* species and inbredlines for this purpose. Some results indicate that for both pollinators flower colour is very important for short distance attraction.

Odour collections of different *Petunia* wild species and ecotypes were carried out in the laboratory of Chemical Ecology (Dr. T. Turlings) at the University of Neuchâtel. We detected qualitative and quantitative variations of the volatiles emitted between species and among ecotypes of the same subspecies. The bee-pollinated species emit very low amounts of almost exclusively benzaldehyde and the different *P. axillaris* subspecies and ecotypes produce high amounts of phenolpropanoids/benzenoids. We showed that a circadian rhythm of odour release exists in *P. axillaris axillaris* flowers and that higher odour amounts are emitted during the night (period in which the flowers are pollinated by nocturnal sphingids) than during the day.

Electroantennogram studies carried out in collaboration with Dr. P. Guerin at the University of Neuchâtel indicate high *Manduca sexta* antenna responsiveness to the three major compounds emitted by *Petunia* flowers: benzaldehyde, benzylalcohol, and methylbenzoate.

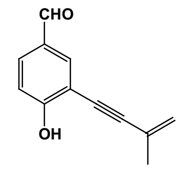
In November 2002, we carried out some odour collections of flowers of natural *Petunia* populations in different geographic regions in Uruguay. We collected the seeds of these populations and we now have Uruguay populations growing in the greenhouse. We are using these populations to carry out self-compatibility experiments to confirm differences in flower-odour blends among *Petunia* species and subspecies. The first trip to Uruguay allowed us to detect populations that will be used during the next years to observe/trap pollinators and to carry out choice experiments in the *Petunia* natural habitat. It also gave us the opportunity to initiate a collaboration with scientists (in particular Dr. V. Balbi) from the University of Montevideo (Institute of Agronomy).

Project 3a, R. Tabacchi, E. Martinoia: Plant Defence Mechanisms Against Biotic Toxins

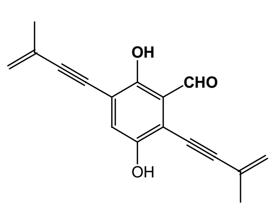
Fungal diseases remain a serious factor limiting crop productivity. The cost of chemicals used for crop protection and the impact on the environment and human health associated with their use are serious problems. Many fungi produce toxins harmful to plants. However, in most plant pathogen systems studied so far, the chemical nature, the mode of action of toxins, and the enzymatic steps detoxifying these toxins are still largely unknown. We propose to identify and purify several biotic toxins from pathogens of grapevine. Using the purified toxins, we will investigate whether plants or microorganisms are able to metabolise the toxins to non-toxic products and identify the enzymes involved in the detoxification process.

Eutypiose and esca are two severe grapevine wood diseases. They are responsible for considerable yield losses and are one of the main causes of shortened production life of vineyards. In order to find an alternative control method, which is as efficient as the highly toxic sodium arsenite treatment, the search for the pathogenically active secondary metabolites is needed.

The ascomycete *Eutypa lata* is the causal agent of eutypiosis (also known as 'dying-arm disease') and it occurs in esca disease as well. From the culture medium of this fungus, the main phytotoxic compound eutypine, was isolated in our laboratory [1]. *Stereum hirsutum* is a basidiomycete also involved in the last stage of esca disease. The chemical structure of sterehirsutinal, the main isolated toxin, is very close to eutypine [2]. *Phaeomoniella chlamydospora* and *Phaeocremonium aleophylum* are two mitosporic fungi involved in young esca decline and the first stage of esca disease. Several naphtalenones have been isolated from these two fungi and their activity assigned [3].



Eutypine



Sterehirsutinal

In order to reach our objectives, different approaches are used:

In a first approach using complementary strategies, we will elucidate the metabo-

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lism of a number of toxins in order to develop strategies that will improve the resistance of the plants against biotic toxins and pathogens (R. Tabacchi).

We will use *Arabidopsis* as a model plant in order to identify enzymes involved in biotic toxin detoxification and to elucidate the principles involved in this process (E. Martinoia).

In a second approach, we will take advantage of the fact that microorganisms might be more efficient in metabolising biotic toxins compared with plants. Hence, we will screen microorganisms for degradation of toxins from grapevine pathogens and identify the genes involved in the degradation process. Coinoculation of these beneficial microorganisms with the pathogens will allow us to understand if the toxins are key factors in disease development (G. Défago).

The aim of the *Analytical Organic Chemistry* project within the NCCR is to give chemical solutions to questions posed by plant pathologists and physiologists. Host selective toxins, a group of structurally complex and chemically diverse metabolites produced by plant pathogenic strains of certain fungal species function as essential determinants of pathogenicity or virulence.

Three main research areas are developed:

Natural products chemistry and analytical chemistry, with two questions often raised: What are these compounds and how much is present? The interaction between these two disciplines is constant. New products are identified continually, which have to be quantified. Phytotoxins and several biogenetically related analogues are frequently present at very low levels in the culture medium of the fungus. In order to establish the toxicity of the different secondary metabolites and for the investigation of the detoxification process by specific enzymes or microorganisms, we resort to synthesis, which is the third area of research within the group. *Synthesis* of toxins is sometimes more rapid and efficient than isolating them from fungal cultures.

Project 4, K. Föllmi: Plant Nutrition Under Stress Conditions

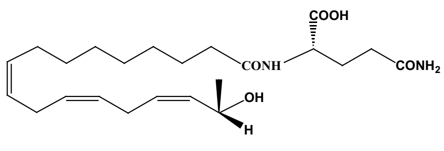
Flavonoids are secondary metabolites that are present at high levels in most plant seeds and grains. These compounds appear to play a vital role in defence against pathogens and predators and contribute to physiological functions such as seed maturation and dormancy.

White lupine is a non-mycorrhizal leguminous plant, which is able to grow on soils with phosphate deficiency by producing cluster roots. These cluster roots exudate large amounts of organic acids, mainly citrate and malate, and secondary phenolics causing fast and considerable changes in their proximity. These compounds are very likely to influence the surrounding microflora, with respect to its diversity as well as to its potential in promoting a deleterious effect on root growth and nutrition. A less-well known aspect of cluster root physiology is the excretion of phenolics and the interaction with bacterial microflora.

Analyses of the excretion profiles of flavonoids in developing cluster roots and their correlation with the molecular fingerprints of the associated bacterial communities should be considered from a plant growth promoting perspective. We will assess the importance of the suppression of host-defence responses for successful establishment of both rhizobial and mycorrhizal symbioses. For this, we use analytical (LC-ESI-MS/MS) methods for the identification of isoflavonoids and their glucosides obtained from lupine root extracts.

Project 7, T. Turlings: Plant-mediated Interactions in Tritrophic Ecological Systems – When Plants Cry for Help with Chemical Signals

Plants have to deal with a multitude of antagonists such as herbivores and pathogens that use plant tissues to acquire essential nutrients. They are not just passive victims to these attackers; many plants are able to mobilise defensive substances that can be toxic to their enemies. These toxins are either constitutively present in a plant or are only then produced when a plant is under attack. The latter form is referred to as inducible defence, and, as it directly affects the performance of the antagonist, it is considered a *direct defence*. Our research, however, concerns the phenomenon of inducible *indirect defences*, whereby plants under attack send out chemical signals that attract the natural enemies of their attackers. Thus, the plants are capable of protecting themselves by attracting the enemies of their enemies. We have been studying the behaviour of parasitic wasps that lay their eggs in various caterpillars. Many of these caterpillars are pests of important crops and parasitic wasps find them with the use of odours emitted by plants under caterpillar attack. Maize (*Zea mays* L.) and the larvae of various *Spodoptera* species (Lepidoptera: Noctuidae) have served as the respective plant and herbivore models. Healthy maize plants release very small amounts of volatiles, but after an attack by caterpillars, a dramatic change in their odour profile can be detected within hours. The emission is systemic and takes place in both damaged and undamaged leaves of an attacked plant [4]. We have found that specific elicitors in the oral secretions of the caterpillars are responsible for this reaction and one such elicitor, which we named volicitin, was identified as N-(17-hydroxylinolenoloyl)-L-glutamine [5][6].



Volicitin

When plants are incubated in this conjugate of 17-hydroxy linolenic acid and glutamine they emit a blend of volatiles from all their leaves and become highly attractive to parasitic wasps. In the context of the NCCR Plant Survival we continue to combine behavioural and chemical studies in order to develop novel strategies by which we can exploit the plant-produced signals to enhance the biological control of pest insects.

Project 9, P. Guerin: Development of Attract-and-Kill Methods for Control of Grape Moths Based on Their Sex Pheromones and Host Plant Attractants

Despite the reliance on insecticides for efficient control of insect pests of crops, biotechnical control methods are making strong inroads into this sector. In the case of moth pests, sex attractants are now used to control key pests on crops over large continental areas, such as for pome fruit protection in Australia and the Americas, and on cotton in North Africa and North America. This so-called confusion method relies on dispensers charged with pheromone to keep male moths in search of a mate on a false trail and so avoid fertilisation of the females. Within Europe, Switzerland stands as a pioneer as mating disruption based on the sex pheromones of grape moth pests has been adopted by one third of Swiss vineyards [7]. More recently, moth sex pheromones have also been incorporated in pastes with insecticides to provide an attract-and-kill product. Here the pest insect is selectively attracted to the pheromone lure to be exposed to a lethal dose of insecticide that prohibits subsequent mating [8]. The purpose of this research project is to take the attract-and-kill strategy beyond

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what has been achieved for other insect pests by identifying grapevine volatiles responsible for attraction of the grape moths to their host plants. These volatile plant secondary products will be incorporated together with the sex pheromones to increase efficacy of attract-and-kill lures for the grape moths. The rationale of the approach is based on the fact that encounters between the grape moth sexes occur within vineyards, and females need to sense the grapevine for oviposition. To determine whether the grape moths can perceive grapevine volatiles, the odours of flowers, leaves and fruits of the vine have been collected on a porous polymer and thermally desorbed onto a high-resolution gas chromatographic column with the antenna of a grape moth as an electroantennographic detector. Subsequently, gas chromatography-mass spectrometry analysis of the biologically active fractions has permitted the identification of a range of significant chemostimuli for the grape moth antennal receptor cells in the different host plant substrates. In fact, both male and female grape moth antennal receptor cells are equally sensitive to plant volatiles, explaining how males find females in the context of the host plant and how female grape moths find suitable sites for egg laying.

Adding plant volatile attractants to the existing pheromone lures for the moth pests of grapevines could provide a number of advantages to the attract-and-kill strategy of control. Stronger attractants for the grape moths would permit the deployment of a reduced number of lures per unit surface of vineyard. Incorporating relatively cheap plant volatiles in the lures would permit a reduction in the amount of the relatively expensive pheromone required, thus contributing to cost savings. Combining pheromones and plant volatiles would establish a broader base for attraction of the moths to the insecticide-treated lures, thus insuring a more efficient knockdown effect by the insecticide and so contribute to its lifespan. Finally, the use of host plant volatiles raises the prospect of attracting females, thereby broadening the action spectrum of the lures to affect both sexes of the target species.

Project 10, R. Pezet: Resistance of Grape to Grey Mould (Botrytis cinerea) *and Downy Mildew* (Plasmopara viticola)*: Biochemistry and Disease Development*

The goal of this research project is to investigate resistance mechanisms of grape to two important fungal diseases (grey mould and downy mildew). This study will establish some histological, biochemical, and molecular criteria useful in grape breeding for an early selection of plant resistance to these diseases.

The development of *Botrytis cinerea* (grey mould) is linked to its necrotrophic character. It cannot develop in young and healthy tissues. But a quiescent form of this parasite was determined inside young grape berries, where it constitutes an important pathogenic potential. Resistance to grey mould can be defined as the capacity to reduce the importance of the quiescent form of the parasite and to limit its development in mature berries. Factors leading to resistance may be constitutive or induced. Constitutive resistance factors are mainly phenolics. The special role by tannins during berry development was demonstrated [9] and polygalacturonase-inhibiting-proteins (PGIP) were also identified. The factors act as growth inhibitors, conidial germination inhibitors, fungal enzyme inhibitors, or participate in mechanical barrier formation (lignification).

This project is directly linked to Project 1a, which is devoted to the same two pathogens with the common goal to increase knowledge of resistance mechanism of grapevine to fungal diseases and to promote and develop management methods connected with agronomic practices. Whereas Project 1a will study the genes involved in resistance processes and a potential systemic acquired resistance, this project investigates the final products (metabolites) involved in these phenomena. The identification of antifungal metabolites, induced or constitutive, and the studies of the enzymatic equipment of the parasites will help to understand the plant–pathogen interactions and constitute a bridge between the genetic research and the analysis of active end products.

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[1] J.-M Renaud, G. Tsoupras, R. Tabacchi, *Helv. Chim. Acta* **1989**, *72*, 929.

[2] G.-M. Dubin, A. Fkyerat, R. Tabacchi, *Phytochemistry* **2000**, *53*, 571–574.

[3] E. Abou-Mansour, E. Couché, R. Tabacchi, *Phytopathologia Mediterranea* **2003**, in press.

[4] T.C.J. Turlings, J.H. Loughrin, P.J. Mc Call, U.S.R. Röse, W.J. Lewis, J.H. Tumlinson, *Proceedings of the National Academy of Sciences of the United States of America* **1995**, *92*, 4169–4174.

[5] H.T. Alborn, T.C.J. Turlings, T.H. Jones, G. Stenhagen, J.H. Loughrin, J.H. Tumlinson, *Science* **1997**, *276*, 945–949.

[6] T.C.J. Turlings, H.T. Alborn, J.H. Loughrin, J.H. Tumlinson, *Journal of Chemical Ecology* **2000**, *26*, 189–202.

[7] H. Arn, S. Rauscher, P. Guerin, H.-R. Buser, *Agriculture, Ecosystems and Environment* **1988**, *21*, 111.

[8] P.-J. Charmillot, D. Hofer, D. Pasquier, *Entomologia Experimentalis et Applicata* **2000**, *94*, 211.

[9] C. Perret, R. Pezet, R. Tabacchi, *Chimia* **2003**, *57*, 608.