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# An overview of NMR-based metabolomics to identify secondary plant compounds involved in host plant resistance

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**Abstract** Secondary metabolites provide a potential source for the generation of host plant resistance and development of biopesticides. This is especially important in view of the rapid and vast spread of agricultural and horticultural pests worldwide. Multiple pests control tactics in the framework of an integrated pest management (IPM) programme are necessary. One important strategy of IPM is the use of chemical host plant resistance. Up to now the study of chemical 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. Metabolomics allows the simultaneous detection of a wide range of compounds, providing an immediate image of the metabolome of a plant. One of the most universally used metabolomic approaches comprises nuclear magnetic resonance spectroscopy (NMR). It has been NMR which has been applied as a proof of principle to show that metabolomics can constitute a major advancement in the study of host plant resistance. Here we give an overview on the application of NMR to identify candidate compounds

for host plant resistance. We focus on host plant resistance to western flower thrips (*Frankliniella occidentalis*) which has been used as a model for different plant species.

**Keywords** Eco-metabolomic approach · Host plant resistance · NMR metabolomics · Secondary plant metabolites · Thrips (*Frankliniella occidentalis*)

## Introduction

Plants produce an immense number of secondary compounds to interact with beneficial or harmful organisms. These compounds mainly act as signal compounds and chemical defence (Wink 2003). Plant defence chemicals repel, restrain or kill plant enemies. An extensive overview of the different groups of secondary metabolites involved in plant defence has been given by Bennett and Wallsgrave (1994). The rich chemical diversity of plants is the result of ongoing evolutionary processes. Mutation and gene duplication lead to a continued formation of new chemical structures that will be selected for if they convey an adaptive advantage to the plant (Grotewold 2005; Benderoth et al. 2006). The more than 200,000 known secondary metabolites provide a potential source for the generation of host plant

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resistance and development of biopesticides (Hartmann 2007).

This is especially important in view of the rapid and vast spread of agricultural and horticultural pests worldwide. Years of selection for yield or palatability traits greatly reduced phenotypic and genetic diversity and may thus have led to loss of resistance (Kennedy and Barbour 1992). At the same time there has been a massive increase in international trade of plants and plant parts, leading to the accidental introduction of many pests. Journeys become faster and climatic differences between countries have little effect when movements are between green houses. In this way major pests on a range of crops, throughout the world, developed resulting in substantial economic losses.

Pest control mainly depends on the use of pesticides. The efficacy of chemical control is limited in part due to pests feeding inside plant organs and in part due to deposition of eggs into sub-epidermal tissues. Furthermore, most chemicals have a short-term effectiveness and frequent spraying is required. Such excessive use of pesticides has led to resistance of many pests to various insecticides as reviewed in Denholm and Rowland (1992). In addition, it has resulted in a residue problem on marketable crops. This gained special importance in the European Union due to the new regulations for maximum residue limits and safety intervals. At the same time adaptation to new European Union regulation regarding pesticide registration and application resulted in a decreased availability of insecticides (Coelho 2009). Besides the risks for human health, problems arise with toxicity towards non-target beneficial organisms and contamination of the environment.

Multiple tactics in the framework of an integrated pest management (IPM) programme are necessary. This approach represents an array of complementary methods with use of pesticides as the last resort (Dent and Elliott 1995). One important strategy of IPM is the use of chemical host plant resistance. Up to now the study of chemical 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. Looking at different compounds at the same time decreases the ability of pests to brake through host plant resistance. Metabolomics allows the simultaneous detection of a wide range of

compounds, providing an immediate image of the metabolome of a plant. One of the most universally used metabolomic approaches comprises nuclear magnetic resonance spectroscopy (NMR). It has been NMR which has been applied as a proof of principle to show that metabolomics can constitute a major advancement in the study of host plant resistance. Here we give an overview on the application of NMR to identify candidate compounds for host plant resistance focusing on host plant resistance to western flower thrips (*Frankliniella occidentalis*), which has been used as a model for different plant species.

### NMR-based metabolomics

NMR spectroscopy measures the resonances of magnetic nuclei such as  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  that interact with an external magnetic field (Hatada and Kitayama 2004). It offers non-invasive structural analysis of metabolites in crude extracts, cell suspensions, intact tissues or whole organisms allowing in vivo analysis (Fan and Lane 2008). NMR spectra are unique and specific for each single compound (Verpoorte et al. 2007, 2008) and can be used to identify metabolites of biological origin of which no a-priori knowledge is needed (Fan and Lane 2008). The latter makes the need for authentic standards, often a major barrier to structure determination, unnecessary. Definitive structural information with no restrictions relating to volatility, polarity or the presence of specific chromophores is given (Fan 1996; Verpoorte et al. 2008). The NMR method provides simultaneous access to both qualitative and quantitative information since the signal intensity is directly proportional to the molar concentration (Pauli et al. 2005). NMR requires minimal sample preparation, is highly reproducible (Fan 1996; Verpoorte et al. 2008) and allows a high sample throughput (Fan and Lane 2008). The quality of an NMR analysis is not dependent on the number of signals detected but on the number of metabolites identified. To facilitate the identification of metabolites databases of NMR spectra of common plant metabolites are needed. The development of such an extensive databases has contributed to the development of NMR, into a fast, convenient and effective metabolomic tool (Verpoorte et al. 2007, 2008), despite the low intrinsic sensitivity (Kopka et al. 2004; Krishnan et al. 2005). To enhance sensitivity,

selectivity and spectral resolution two-dimensional NMR is applied. Correlated spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) spectroscopy, heteronuclear single quantum coherence (HSQC) spectroscopy and heteronuclear multiple bond coherence (HMBC) spectroscopy improve metabolite identification by providing information on the relationship between the signals from two different nuclei (Ratcliffe and Shachar-Hill 2005; Ratcliffe et al. 2001).

### General application of NMR in plant metabolomics

NMR in plant metabolomics has a wide range of applications. Here we give a short overview of its different uses. For an extensive review we suggest Krishnan et al. (2005) and Ward et al. (2007). NMR has been used for quantitation of plant metabolites in mixtures and validation of reference materials as reviewed in Pauli et al. (2005). NMR analysis is a powerful tool for studying biosynthetic pathways using stable isotopes (e.g.  $^{13}\text{C}$ ) including stability and kinetic studies of intermediates as reviewed by Ratcliffe and Shachar-Hill (2005) and Fan and Lane (2008). NMR analysis in plants comprises investigations into the impact of environmental factors, such as growth conditions (Lommen et al. 1998; Avelange-Macherel et al. 2006; Pereira et al. 2006; Charlton et al. 2008) exposure to metals (Bailey et al. 2003; Le Lay et al. 2006; Jahangir et al. 2008a), herbicides (Ott et al. 2003) and cultural practice (Neelam et al. 2008). NMR has been applied to study plant development stages (Palama et al. 2009) and plant hybridization (Kirk et al. 2005), to characterize plant ecotypes (Ward et al. 2003; Arany et al. 2008), as well as comparing wild-type and transgenic genotypes (Noteborn et al. 2000; Le Gall et al. 2003a; Choi et al. 2004a; Piccioni et al. 2009). NMR spectroscopy has been used for chemotaxonomic analysis (Choi et al. 2004b, 2005; Kim et al. 2005), the analysis of food products (McGorrin 2009) and phytomedicines (van der Kooy et al. 2009).

### Application of NMR to study host plant resistance

Surprisingly, little use has been made of metabolomics to study interactions between plants and other

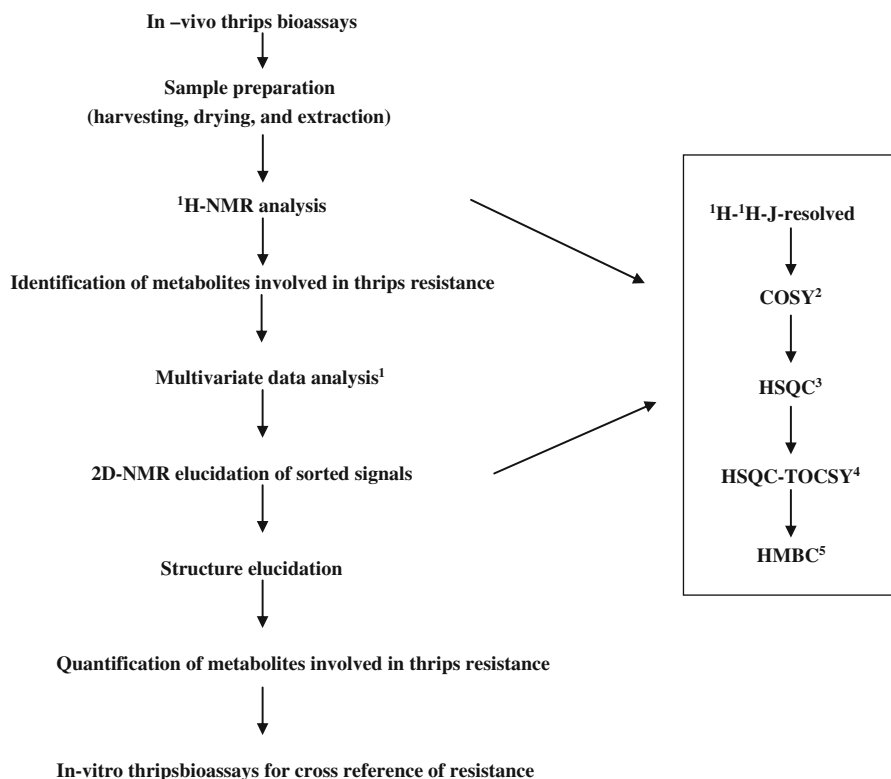
organisms (Allwood et al. 2008). However, NMR plays a dominant role in those studies which have used a metabolomic approach investigating plant-biotic relationships. It has been NMR which has been applied as a proof of principle to show that metabolomics can constitute a major advancement in the study of host plant resistance using western flower thrips as a model. We will therefore focus on the use of NMR to study host plant resistance to thrips.

### Host plant resistance to western flower thrips (*Frankliniella occidentalis*)

Leiss et al. (2009a, b) developed an eco-metabolomic approach (Fig. 1), based on NMR to identify candidate compounds for constitutive host plant resistance to western flower thrips (*Frankliniella occidentalis*). As a proof of principle this approach was applied to three different host systems, including *Senecio* as a wild plant (Leiss et al. 2009a), chrysanthemum as an ornamental (Leiss et al. 2009b) and tomato as a crop (Mirnezhad et al. 2009). Firstly, thrips-resistant and -susceptible plants were identified applying in vivo thrips bioassays. The most resistant and the most susceptible plants were then chosen for NMR metabolomics. One and two-dimensional NMR was performed and the resulting metabolomic profiles of the thrips-resistant and susceptible plants were analysed with multivariate statistics like principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) to identify the metabolites involved in thrips resistance. For cross reference of resistance the metabolites in question were then, if possible, confirmed by a thrips in vitro bioassay. In all three host systems used the metabolomic profiles of thrips-resistant and susceptible plants were significantly different (Fig. 2) leading to a range of different metabolites involved in thrips resistance (Table 1).

In the wild plant *Senecio* a second generation hybrids of *S. jacobaeae* and *S. aquaticus* was investigated for thrips resistance (Leiss et al. 2009b). Out of 33 hybrids the four most resistant and the four most susceptible ones were chosen for NMR analysis. Young and old leaves were investigated. As described in Leiss et al. (2009b) the thrips resistant hybrids contained significantly higher amounts of the pyrrolizidine alkaloids (PAs) Jacobine- and Jaconine

**Fig. 1** Eco-metabolomic approach to study host plant resistance in western flower thrips. <sup>1</sup>For multivariate data analysis principal component analysis (PCA) and partial least squares regression-discriminant analysis (PLS-DA) were applied. For two dimensional NMR <sup>2</sup>correlated spectroscopy, <sup>3</sup>heteronuclear single quantum coherence, <sup>4</sup>total correlated spectroscopy-heteronuclear single quantum coherence and <sup>5</sup>heteronuclear multiple bond correlation were used

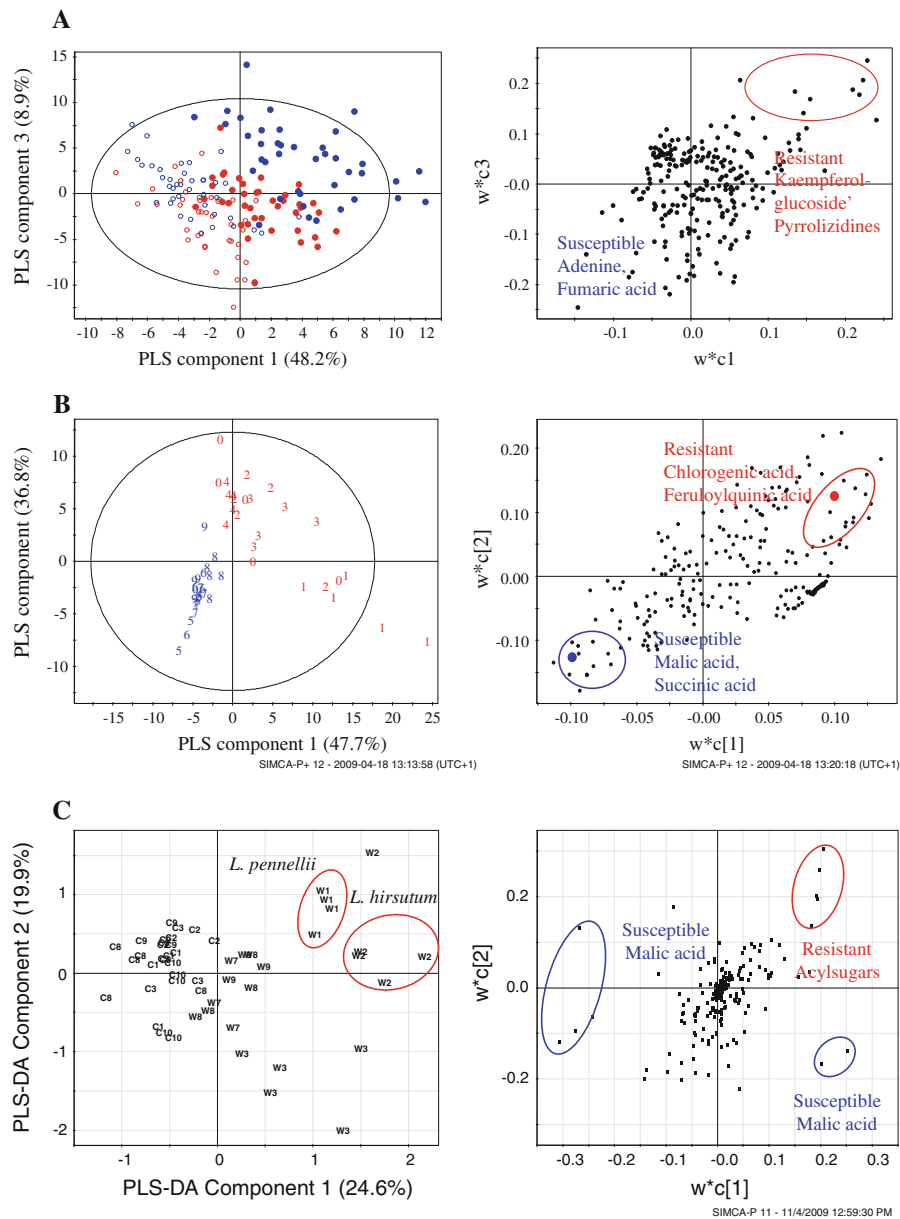


N-oxide and a flavanoid, kaempferol glucoside (Fig. 2a). PAs are regarded as constitutive defense compounds against generalist herbivores. They deter chewing insects such as caterpillars, locusts, beetles and aphids. In addition they show negative effects on fungi. Also flavanoids are known to be involved in plant resistance to herbivores. Kaempferol has a deterrent effect on generalist caterpillars and aphids. It is also effective against fungal pathogens. Young leaves of the thrips resistant plants showed significantly higher amounts of PAs and kaempferol glucoside compared to old leaves. In general young leaves contained more jacaranone. Analogues of jacaranone exhibited insecticidal activity on houseflies and generalist caterpillars. Young leaves being photosynthetically more active are more valuable plant parts compared to old leaves and thus need to be better defended. PAs are hepatotoxic to mammals, whereas kaempferol and jacaranone, showed antitumor activity on human cancer cell lines.

Ten cultivars, of which five were classified by breeders as thrips resistant and five as susceptible were tested for the ornamental chrysanthemum

(*Dendrathera grandiflora*) (Leiss et al. 2009a). As described in this paper the thrips resistant cultivars showed significantly higher amounts of the phenylpropanoids chlorogenic and feruloyl quinic acid (Fig. 2b). Phenols are known for their role in plant defense. Chlorogenic acid has been described as an antifeedant and digestibility reducer causing harmful effects on different caterpillar species, leaf beetles, leafhoppers and aphids. Chlorogenic acid also affects plant fungi and bacteria. Feruloyl quinic acid, which is closely related to chlorogenic acid, is an ester of quinic—and ferulic acid. The later is a precursor of lignin conferring rigidity to cell walls and as such it is linked to the resistance against stem borers and gall midges as well as against fungi. Both phenylpropanoids are not only implicated with the resistance to herbivores, they are also the most widespread natural plant dietary antioxidants and might be implicated in the prevention of cancer and cardiovascular diseases in humans.

For tomato 10 wild species and 10 cultivated lines were compared for thrips resistance (Mirnezhad et al. 2009). Cultivated tomatoes were much more



**Fig. 2** Score and loading plots of partial least square regression – discriminate analysis based on <sup>1</sup>H-NMR spectra of *Senecio* (A), chrysanthemum (B) and tomato (C) plants resistant (filled circle old leaves, open circle young leaves) and

susceptible (filled circle old leaves, open circle young leaves) to western flower thrips. The ellipse represents the Hotelling T2 with 95% confidence in score plots

susceptible to western flower thrips compared to the wild species. Three species/lines of each group, the wild and cultivated tomatoes, which were the most resistant or most susceptible, were subjected to NMR analysis. As described by Mirmezahad et al. (2009) the

thrips resistant tomatoes contained acylsugars (Fig. 2c) which are known for their negative effect on caterpillars, leafminers, whiteflies and aphids. In contrast to chrysanthemum tomato contained relatively small amounts of chlorogenic acid.

**Table 1** Metabolites involved in resistance to western flower thrips as identified by NMR

Metabolite	Negative effects on herbivores	Negative effects on pathogens	Effects on humans
Jacobine, Jaconine (Pyrrolizidine alkaloids)	<i>Choristoneura fumiferana</i> , Lepidoptera <sup>1</sup> ; <i>Spodoptera exigua</i> , Lepidoptera <sup>2</sup> ; <i>Mamestra brassicae</i> , Lepidoptera <sup>3</sup> ; <i>Locusta migratoria</i> , Orthoptera <sup>4</sup> ; <i>Aglossina discolor</i> , Hymenoptera <sup>5</sup> ; <i>Oreina calcaliae</i> , Coleoptera <sup>5</sup> ; <i>Oreina speciosissima</i> , Coleoptera <sup>5</sup> ; <i>Cylindrotoma distinctissima</i> , Diptera <sup>5</sup> ; <i>Miramella alpina</i> , Califera <sup>5</sup> ; <i>Brachycaudus cardii</i> , Homoptera <sup>6</sup> ; <i>Myzus persicae</i> , Homoptera <sup>4</sup>	Rhizosphere fungal communities <sup>7</sup> ; <i>Fusarium oxysporum</i> <sup>8</sup> ; <i>Fusarium sambucinum</i> <sup>8</sup> ; <i>Trichoderma sp.</i> <sup>8</sup>	Toxic <sup>9</sup>
Kaempferol-glucoside (Flavanoid)	<i>Mamestra configurata</i> , Lepidoptera <sup>10</sup> ; <i>Aphis fabae</i> , Homoptera <sup>11</sup> ; <i>Thrips tabaci</i> , Thysanoptera <sup>12</sup>	<i>Fusarium oxysporum</i> <sup>13,14</sup> ; <i>Fusarium culmorum</i> <sup>13</sup> ; <i>Verticillium albo-atrum</i> <sup>14</sup>	Cytotoxic activity on human cancer cell lines <sup>15–18</sup> ; Growth-inhibitory activity against oral pathogens <sup>19</sup> ; Antiviral and immunomodulatory effect <sup>20</sup>
Chlorogenic acid (Phenylpropanoid)	<i>Heliothis zea</i> , Lepidoptera <sup>21,22</sup> ; <i>Heliothis virescens</i> , Lepidoptera <sup>23</sup> ; <i>Heliothis subflexa</i> , Lepidoptera <sup>23</sup> ; <i>Pteris rapae</i> , Lepidoptera <sup>24</sup> ; <i>Spodoptera litura</i> , Lepidoptera <sup>25</sup> ; <i>Trichoplusia ni</i> <sup>26</sup> ; Lepidoptera; <i>Lymantria dispar</i> , Lepidoptera <sup>26</sup> ; <i>Agelastica alni</i> , Coleoptera <sup>27</sup> ; <i>Plagioderma versicolora</i> , Coleoptera <sup>28</sup> ; <i>Popillia japonica</i> , Coleoptera <sup>29</sup> ; <i>Dalbulus maidis</i> , Homoptera <sup>30</sup> ; <i>Aphis pomi</i> , Homoptera <sup>31</sup>	<i>Pseudomonas syringae</i> <sup>32</sup> ; <i>Phytophthora capsici</i> <sup>33</sup>	Prevention of cancer and cardiovascular diseases <sup>34–35</sup> ; Prevention of diabetes and obesitas <sup>37</sup>
Feruloyl quinic acid (Phenylpropanoid)	<i>Helicoverpa armigera</i> , Lepidoptera <sup>38</sup> ; <i>Ostrinia nubilalis</i> , Lepidoptera <sup>39</sup> ; <i>Sexamia nonagrioides</i> , Lepidoptera <sup>40</sup> ; <i>Metopolophium dirhodum</i> , Homoptera <sup>41</sup> ; <i>Rhopalosiphum padi</i> , Homoptera <sup>41</sup> ; <i>Sitobion avenae</i> , Homoptera <sup>41</sup> ; <i>Schizaphis graminum</i> , Homoptera <sup>42</sup> ; <i>Sitodiplosis mosellana</i> , Diptera <sup>43,44</sup> ; <i>Helicoverpa armigera</i> , Lepidoptera <sup>52</sup> ; <i>Tuta absoluta</i> , Lepidoptera <sup>53,54</sup> ; <i>Liriomyza trifolii</i> , Diptera <sup>55–57</sup> ; <i>Bemisia tabaci</i> , Homoptera <sup>58,59</sup> ; <i>Macrosiphum euphorbiae</i> , Homoptera <sup>60</sup> ; <i>Myzus persicae</i> , Homoptera <sup>59</sup> ; <i>Tetranychus urticae</i> , Prostigmata <sup>61</sup> ; <i>Tetranychus evansi</i> , Prostigmata <sup>62</sup>	<i>Fusarium gramineum</i> <sup>45</sup> ; <i>Fusarium oxysporum</i> <sup>46,47</sup> ; <i>Fusarium solani</i> <sup>47</sup> ; <i>Trichoderma harzianum</i> <sup>47</sup> ; <i>Puccinia coronata</i> <sup>48</sup> ; <i>Sclerotium rolfii</i> <sup>49</sup>	Inhibiting human cancer cell lines <sup>50,51</sup>
Acylsugars	–	–	–

<sup>1</sup> Bentley et al. (1984), <sup>2</sup> van Dam et al. (1995), <sup>3</sup> de Boer (1999), <sup>4</sup> Macel et al. (2005), <sup>5</sup> Hägele and Rowell-Rahier (2000), <sup>6</sup> Vrieling et al. (1991), <sup>7</sup> Kowalchuk et al. (2006), <sup>8</sup> Hol and van Veen (2002), <sup>9</sup> Cheeke (1988), <sup>10</sup> Onyilgha et al. (2004), <sup>11</sup> Lattanzio et al. (2000), <sup>12</sup> Wu et al. (2007), <sup>13</sup> Lorenc-Kukula et al. (2009), <sup>14</sup> Ouf et al. (1994), <sup>15</sup> Daniela et al. (2007), <sup>16</sup> Lee et al. (2007), <sup>17</sup> Li et al. (2007), <sup>18</sup> Campbell et al. (2006), <sup>19</sup> Cai and Wu (1996), <sup>20</sup> Arena et al. (2008), <sup>21</sup> Elliger et al. (1981), <sup>22</sup> Wiseman et al. (1992), <sup>23</sup> Bernays et al. (2000), <sup>24</sup> Huang and Renwick (1995), <sup>25</sup> Mallikarjuna et al. (2004), <sup>26</sup> Beninger et al. (2004), <sup>27</sup> Ikonen et al. (2002), <sup>28</sup> Jassbi (2003), <sup>29</sup> Fulcher et al. (1998), <sup>30</sup> Dowd and Vega (1996), <sup>31</sup> Miles and Oertli (1993), <sup>32</sup> Niggeweg et al. (2004), <sup>33</sup> Lizzi et al. (1995), <sup>34</sup> Laranjinha et al. (1994), <sup>35</sup> Sawa et al. (1999), <sup>36</sup> Noratto et al. (2009), <sup>37</sup> Narita and Inouye (2009), <sup>38</sup> Wang et al. (2006), <sup>39</sup> Mao et al. (2007), <sup>40</sup> Santiago et al. (2006), <sup>41</sup> Havlickova et al. (1996), <sup>42</sup> Cabrera et al. (1995), <sup>43</sup> Abdel-Aal et al. (2001), <sup>44</sup> Ding et al. (2000), <sup>45</sup> Bily et al. (2003), <sup>46</sup> El Modafar et al. (2001), <sup>47</sup> Gomez-Vasquez et al. (2004), <sup>48</sup> Delgado et al. (2002), <sup>49</sup> Sarma and Singh (2003), <sup>50</sup> Kampa et al. (2003), <sup>51</sup> Lee (2005), <sup>52</sup> Simmons et al. (2004), <sup>53</sup> Pereira et al. (2008), <sup>54</sup> Hawthorne et al. (1992), <sup>55</sup> Hawthorne et al. (1992), <sup>56</sup> Hartman and St. Clair (1999), <sup>57</sup> Kohler and St. Clair (2005), <sup>58</sup> Baldin et al. (2005), <sup>59</sup> Silva et al. (2008), <sup>60</sup> Simmons et al. (2003), <sup>61</sup> de Resende et al. (2008), <sup>62</sup> Saeidi et al. (2007)

## Host plant resistance to other insects and pathogens

Next to western flower thrips there is only one more study applying NMR on insects, investigating induced resistance. Widarto et al. (2006) showed that both the effect of the generalist *Spodoptera exigua* and the specialist *Plutella xylostella* on *Brassica rapa* were depended on the larval stage. While the second larval instars of the generalist increased levels of glucose, ferulic acid and gluconapin, the fourth instar caused an increase in levels of alanine and sinapoyl malate. Second larval instars of the specialist accumulated glucose, feruloyl and sinapoyl malate and gluconapin, whereas the fourth instars stimulated the plant to accumulate more sucrose.

Most NMR studies looking at the effect of pathogen infection on a host plant investigated induced resistance by analyzing the metabolome of the infected and non-infected hosts. Infection by phytoplasma *Catharanthus roseus* caused an increase of metabolites related to the biosynthetic pathways of phenylpropanoids and terpenoid indole alkaloids (Choi et al. 2004c). Fructose, in contrast to glucose, which accumulated in infected leaves, was involved in the phytopathogenicity in *Spiroplasma citri* (Andre et al. 2005). A range of compounds including chlorogenic acid,  $\alpha$ -linolenic acid analogues, sesquiterpenoids and diterpenoids were increased in tobacco infected with tobacco mosaic virus (Choi et al. 2006). Metabolic changes in *B. rapa* varied with the type of bacterial species. While threonine and GABA were detected to be the discriminating metabolites in Gram-positive bacteria treated plants, those treated with Gram-negative bacteria exhibited a significant increase in sinapoyl-malate, caffeoyl-malate and histidine (Jahangir et al. 2008b). Metabolomic changes in *B. rapa* to disarmed and tumor-inducing strains of *Agrobacterium tumefaciens* constituted significant differences in flavanoid, phenylpropanoid, sugar and free amino/organic acid contents (Simoh et al. 2009). The root-pathogenic oomycete *Pythium sylvaticum* caused an increase in indolics and a decrease in three phenylpropanoids upon infection of root cultures of *Arabidopsis thaliana* wild type (Bednarek et al. 2005). Fungal infection of *B. rapa* with *Leptosphaeria maculans*, *Aspergillus niger* or *Fusarium oxysporum* was associated with the presence of phenylpropanoids, flavanoids and glucosinulates, whereby specific

patterns were depended on fungal species and host plant cultivar (Abdel-Farid et al. 2009).

Relatively little attention has been given to use NMR to study constitutive host plant resistance to pathogens. There are two studies on the host plant resistance to fungi on grapes. In comparison to susceptible grapevine cultivars, those resistant to mildews accumulated inositol and caffeic acid, two compounds known to confer resistance to fungi (Figueiredo et al. 2008). Differentiation of grape cultivars based on their resistance to downy mildew was based on quercetin-3-*o*-glucoside and a *trans*-feruloyl derivative (Ali et al. 2009).

## Conclusion and future prospects

NMR-based metabolomics proved to be a promising tool to identify metabolites involved in host plant resistance. It can provide key information for the development and implementation of host plant resistance. As shown by the eco-metabolomic approach, studying thrips resistance, NMR led to the successful identification of different candidate compounds in three different plant species. Especially those compounds such as kaempferol and phenylpropanoids are very valuable candidates to develop host plant resistance in agricultural crops. Not only do they inhibit pests but they also show inhibiting effects on human cancer cell lines. Combining host plant resistance to pests with healthy food characters is a promising future prospect. Indeed, a tomato with increased amounts of kaempferol glucoside (Le Gall et al. 2003b) as well as a tomato with a doubled amount of chlorogenic acid (Niggeweg et al. 2004) have been engineered for dietary purposes.

The next challenge in studying herbivore resistance with metabolomics will be to undertake compartmentalized NMR-metabolomics to study composition of those tissues the insects feed upon. Thrips, for instance, are cell feeders ingesting whole plant cells, including the vacuoles in which many of the plant defense compounds, like pyrrolizidine alkaloids and phenylpropanoids are stored. Furthermore, thrips probing is characterized by shallow feeding in the epidermal tissue while, thrips penetration feeding effects mesophyll cells (van de Wetering et al. 1998).

NMR allows the simultaneous detection of a wide range of metabolites, providing an instantaneous

profile of the metabolome of the resistant plant. NMR applied to thrips resistance in different host systems, including wild plants, ornamentals and crops, showed that the eco-metabolomic approach is universal. It may be applied to any economically important agricultural ornamental or crop and, while thrips has been used as a model, it can be applied to any other key herbivore and pathogen. Once the metabolites involved in host plant resistance are known, the resistance of plants can be predicted on the basis of their metabolomic profile. Thus using NMR a single test may be sufficient in the future to predict resistance to different pests/pathogens simultaneously. At the time resistance to each pest/pathogen needs to be tested separately, leading to a whole array of time consuming and costly tests. Applying general standard protocols for NMR measurements, a greatly reproducible, high throughput NMR screening facility could be realized. It would establish the metabolomic profiles of ornamentals and crops resistant to the economically most important pests and pathogens and store these in a metabolomic profile library. Since NMR is the only metabolomic method where the physical characteristics of compounds will always be the same it is the method of choice for a long-lasting database (Verpoorte et al. 2008). Comparing the profiles in the data base with the profiles of screened plants can then predict resistance. Furthermore, with the opportunity to detect metabolites which are involved in the resistance to different organisms NMR may facilitate multi-resistance breeding.

NMR based metabolomics using the eco-metabolomic approach, comparing the metabolome of resistant and susceptible plants has a vast potential in the identification of secondary plant defense compounds involved in resistance to herbivores and pathogens, which will drive future resistance breeding and biopesticide development.

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