AFPP – CHEMICAL ECOLOGY: NEW CONTRIBUTIONS TO PLANT PROTECTION AGAINST PESTS
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APPLICATIONS OF CHEMICAL ECOLOGY IN AGRICULTURE: DEVELOPMENT OF INNOVATIVE INFOCHEMICAL-BASED MONITORING TOOLS AND STRATEGIES

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ABSTRACT

Harmful organisms must be monitored by appropriate and effective methods and tools. In agriculture, such tools should include trapping systems, field observations and warning, forecasting and diagnosis systems. The first example of my talk is on phytoplasmas, bacteria which are worldwide responsible for more than 700 different plant diseases and have an important economic impact. Apple proliferation (AP), pear decline (PD) and European stone fruit yellows (ESFY) cause severe crop losses in European fruit growing regions. Phloem feeding insects (psyllids, planthoppers) were identified as vectors, often one species transmitting a specific phytoplasma. All investigated psyllid species use chemical cues for the identification of their host plants during migration between different host plant species. The production of plant volatiles in some systems is influenced by phytoplasma infections which indirectly influenced the behavior of vector insects. For several vector insects, species-specific attractive and repellent compounds have been identified. Attractive compounds can be used in traps as lures for monitoring and mass trapping purposes. By combination of attractive compounds in traps and repellent compounds in dispensers these infochemicals may be used in push-and-pull strategies. In my second example I report on the development of a new method for egg monitoring of European grape berry moths by infochemicals for defining thresholds and timing for insecticide spraying. The pros and cons of the different strategies are discussed.

Keywords: Apple proliferation, Pear decline, ESFY, *Lobesia botrana*, *Eupoecilia ambiguella*, monitoring, mass trapping, push-and-pull strategy.

RESUME

Les organismes nuisibles doivent être suivis aux moyens de méthodes et d'outils adaptés. En agriculture de tels outils devraient comprendre des méthodes de piégeage, des observations de plein champ et des avertissements, des prévisions et des diagnostiques. Le premier exemple de ma communication porte sur les phytoplasmes, bactéries responsables au niveau mondial de plus de 700 maladies végétales et qui ont un impact économique important. La prolifération du pommier(AP), le dépérissement du poirier (PD) et la jaunisse du pêcher (European stone fruit yellow-ESFY) provoquent des pertes sévères dans les principales zones de production fruitières européennes. Les insectes se nourrissant sur le phloème des arbres (psylles, cicadelles) sont connus comme vecteurs avec souvent une seule espèce transmettant spécifiquement un seul phytoplasme. Toutes les espèces de psylles étudiées utilisent des indicateurs chimiques pour l'identification des plantes hôtes pendant leur migration sur différentes plantes hôtes. La production de ces composés volatiles végétaux dans certains systèmes est influencée par l'infection par le phytoplasme qui donc influence indirectement le comportement des insectes vecteurs. Pour plusieurs insectes vecteurs, des produits spécifiques soit attractifs soit répulsifs ont été identifiés. Les attractifs peuvent être employés dans des pièges comme appâts pour le suivi ou le piégeage de masse. En combinant les produits attractifs dans les pièges et les répulsifs dans des diffuseurs ces médiateurs chimiques peuvent être mis en œuvre dans le cadre de stratégies « push and pull ». Dans mon second exemple, je rapporte le développement de nouvelle méthode de suivi des œufs de tordeuses de la grappe au moyen de médiateurs chimiques en définissant des seuils et des périodes pour les applications d'insecticides. Le pour et le contre des différentes stratégies sont discutés

Mots-clés : proliferation des pommes, dépérissement du poirier, Jaunisse du pêcher ESFY, *Lobesia botrana*, *Eupoecilia ambiguella*, suivi, piégeage de masse, stratégie push-and-pull.

INTRODUCTION

Harmful organisms must be monitored by adequate methods and tools. In agriculture, such adequate tools should include trapping and observation systems in the field as well as scientifically sound warning, forecasting and early diagnosis systems. While the adult life stage will be the target for monitoring of pest insects by infochemicals regularly, in some cases other ontogenetic stages might need to be the target of monitoring depending of their biology and ecology (Gross and Gündermann 2016). I will get back to this in my second example (Key parameters influencing the number of trapped organisms from any specified distance of origin are a) the probabilities that the trap is found (findability), b) that the organism is captured after arriving at the trap (efficiency), and c) retained (retention) until the trap is emptied by the farmer (Miller et al. 2015).

The secret language of the earth's ecosystem involves a multitude of players that populate the playing field and occupy different rungs on the food chain (Wartenberg 2016). The actors include plants, plant feeding insects, insect feeding insects, plant parasites, insect parasites, pathogenic and beneficial microorganisms, vectoring organisms, pollinators, and more. Thus, fundamental research on the biology and ecology including chemically mediated multitrophic interactions of every target organism are needed (Fig. 1). The outcome of this research is the key for developing innovative and sustainable applications in phytomedicine. Besides pheromones also allelochemicals can be used as lures in traps, different classes of infochemicals can be combined to attract-and-kill strategies, push-and-pull or push-pull-kill strategies (Fig. 2). In my talk I will give two examples on my recent research on the development of innovative infochemical-based monitoring tools and strategies.

In my first example I will focus on the interaction and transmission of phytoplasmas, small bacteria which are worldwide responsible for more than 700 different plant diseases, and which have an important economic impact. Phytoplasma species belonging to the apple proliferation group are the economically most important fruit tree phytoplasmas and are widespread in the temperate regions of Europe. The three diseases apple proliferation, pear decline and European stone fruit yellows (ESFY) cause crop losses in European fruit growing regions of about 500 Million Euro each year. Phloem feeding insects (psyllids) were identified as vectors. We studied the role of allelochemicals for vector behavior and the influence of phytoplasma infections on volatile production of host plants. In this case emission rates of the attractive compounds, often plant produced kairomones, has to be multiple times higher than by using pheromones. Based on our results the development of chemically lured traps for monitoring has recently started.

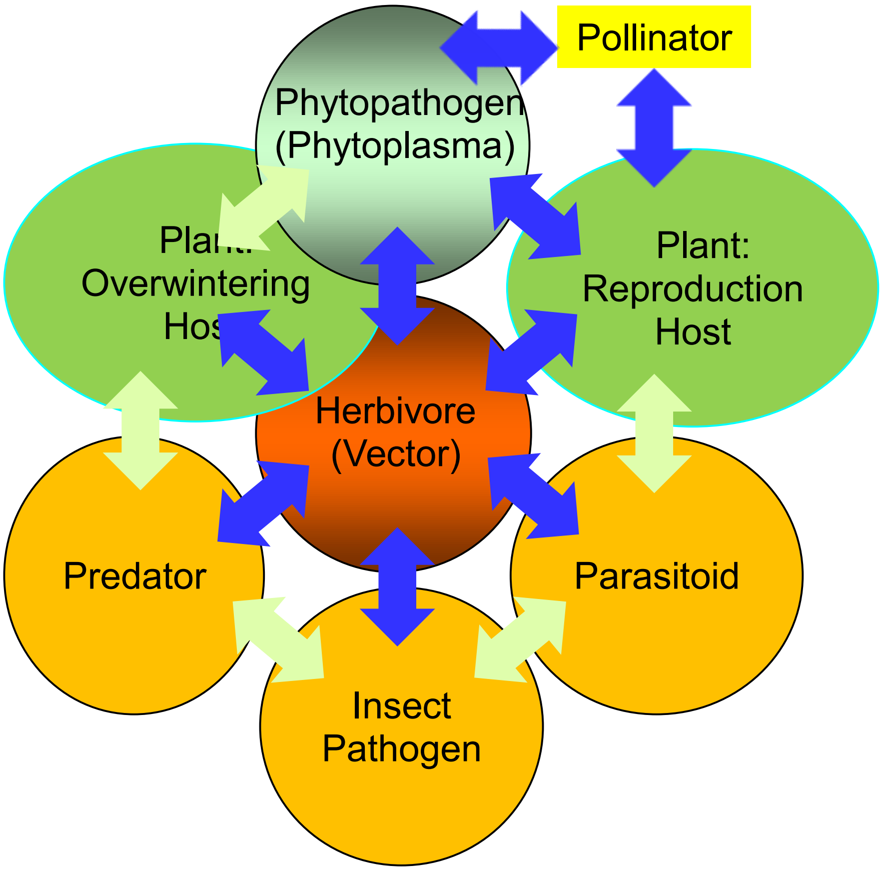
In my second example I will report a new method for egg monitoring of grape berry moths, which is currently under development in my group. The European grapevine moth *Lobesia botrana* and the European grape berry moth *Eupoecilia ambiguella* Hübner (Lepidoptera: Tortricidae) are the most damaging insect pests in European viticulture. In areas, where mating disruption as control strategy against the moths is not appropriate (e.g. small vineyards, highly structured landscapes), insecticides have to be applied. To achieve effective chemical control, insecticide treatments have to be conducted before hatching of the larvae.

For this purpose an egg monitoring is necessary, but counting of the small green eggs on green plant parts is not practicable. To prevent immoderate insecticide application, which would not be in compliance to integrated pest management (IPM) (Gross & Gündermann, 2016), a decision support system for growers is needed, which enables the timing and necessity of pest control.

Based on a quantifiable egg deposition, which is visible on a specific egg-monitoring card distributed in the vineyard, the winegrower should be able to draw conclusions for the infestation on the adjacent grapes.

Fig. 1: Fundamental research on multitrophic interactions between a herbivorous insect, its host plants (e.g. overwintering host plant and reproduction host plant), its antagonists (predators, parasitoids and insect pathogens), transmitted phytopathogens (e.g. phytoplasma) and interactions with pollinators. The chemically mediated interactions (in some cases complemented by visual, acoustical and haptic signals) are symbolized by arrows (Gross, original drawing).

Fig1: Recherche fondamentale sur les interactions multitrophiques entre un insecte phytophage, sa plante hôte(plant d'hivernation et plante de reproduction de l'insecte), ses antagonistes(prédateurs, parasitoïdes, et entomopathogènes), la transmission de phytopathogènes(ex. phytoplasmes) et les interactions avec les pollinisateurs. Les interactions par médiation chimique (parfois complétées par des signaux visuels, acoustiques, haptiques) sont représentées par des flèches (Gross, dessin original)



New strategies for phytoplasma vector control by semiochemicals

Phloem-inhabiting phytopathogens like *Candidatus* Phytoplasma are prokaryotes and obligatorily parasitize two different hosts: plants and vector insects. Phytoplasmas are polymorphic and among the smallest living organisms, measuring between 200-800 nm. Their lifestyle is obligatory parasitic, i.e. they live exclusively in phloem sieve tubes (Weintraub & Gross, 2013; Weintraub & Beanland, 2006). Plant vigor and fruit yield are seriously impaired by *Ca.* Phytoplasma infection. Depending on species, these phytopathogens cause symptoms such as leaf yellowing and rolling, stunting, tasteless and undersized fruits, tree decline and death. Moreover, phytoplasmas modulate key processes in plant development through inducing root and shoot proliferation (witches' broom) and an abnormal development of floral parts into leafy structures, called greening of flowers or phyllody (Weintraub & Beanland, 2006; Orlovskis *et al*., 2015).

Phytoplasmas are worldwide responsible for more than 700 different plant diseases and have an important economic impact. Phytoplasma species belonging to the apple proliferation group are the economically most important fruit tree phytoplasmas and are widespread in the temperate regions of Europe. The three diseases apple proliferation (AP), pear decline (PD) and European stone fruit yellows (ESFY) cause crop losses in European fruit growing regions of about 500 Million Euro each year (Strauss, 2009). Phloem feeding insects (Hemiptera: Psylloidea) were identified as vectors, often one species transmitting a specific phytoplasma. We studied in these different systems the role of semiochemicals for vector behavior and the influence of phytoplasma infections on volatile production of host plants.

Impact of chemical signals in multitrophic interactions between cultivated plants, insect vectors and phytoplasmas

Based on the knowledge of chemical orientation (Soroker *et al*., 2004; Gross & Mekonen, 2005; Gross, 2016), the influence of phytoplasma diseases on vector behavior was studied in detail. By analyzing the complex chemically mediated interactions between the apple proliferation phytoplasma (*Ca*. P. mali), its vector *Cacopsylla picta* Foerster and its alternate host plants (reproduction and overwintering host plants), it was shown that this phytoplasma lured its highly adapted vector to infected apple plants by changing the odor of the tree (Mayer *et al.*, 2008 a).

Psyllids that had no previous contact with *Ca*. P. mali, as well as already infected ones, were more attracted by volatiles emitted from phytoplasma-infected apple plants than from uninfected ones. Psyllids that had developed on infected plants without getting infected showed the opposite behavior (Mayer *et al*., 2008 a). Infected apple trees emitted higher amounts of the sesquiterpene β-caryophyllene when compared to uninfected ones. We confirmed the attractiveness of this sesquiterpene for *C. picta* in both olfactometer bioassays and field studies (Mayer *et al*., 2008 b). Synthetic β-caryophyllene was highly attractive to newly emerged adults of *C. picta* both when offered simultaneously with healthy apple odor and without. The psyllid's response was independent of its prior odor experience and infection status.

The interactions between the pathogen *Ca.* P. mali, the host plants, and the vector insect *C. picta* are complex and the proposed epidemiology of *Ca*. P. mali are summarized in the following: After overwintering on conifers (e.g. *Picea abies*) (Mayer *et al*., 2011), the adults of *C. picta* remigrate into apple orchards for reproduction. They mate and lay their eggs on their reproduction host plant *M. domestica*. After passing five nymphal stages, the adults of the new generation occur (Mayer *et al*., 2008 a). Soon after adult emergence (phenology stages BBCH 69-71 of the apple plant), the emigrants are able for uptaking *Ca*. P. mali from phloem of infected plant (acquisition feeding). Attracted by β-caryophyllene (Mayer *et al*., 2008 b), which is mainly produced by infected apples during this time, both males and females are lured to infected plants, increasing the number of psyllids, which acquire the phytoplasma (Mayer *et al*., 2008 a). Witches' brooms produced exclusively by infected plants increase their leaf surface and may support the emission of volatile β-caryophyllene. Shortly after feeding on apple, the adults emigrate to conifers where they stay until spring (Mayer *et al*., 2011). During this time, the phytoplasma can replicate within its vector and invade its salivary glands. After overwintering, the psyllids return to apple trees (remigrants) but now prefer to lay their eggs on uninfected plants, which increases the opportunity to transmit the phytoplasma (Mayer *et al.*, 2011). Which infochemicals regulate this egg-laying behavior still remains unknown and will be the focus of ongoing research. By developing on apple plants infected by *Ca*. P. mali, the nymphs of *C. picta* suffered higher mortality and remained smaller compared to the ontogenetic development on uninfected plants (Mayer *et al*., 2011). In contrast, infection by *Ca*. P. mali was tolerated by adults and seems to have no detrimental effect. Thus, females of *C. picta* evolved mechanisms to minimize harmful effects for their offspring emanated by the phytoplasma by avoiding oviposition on infected plants. This behavior ensures the development of a new, vital vector generation which is important for the spread of the phytoplasma. In conclusion, the complex multitrophic interactions between phytoplasma, plant and vector may result in both higher numbers of transmitting vector insects and a very effective transmission of the phytoplasma within the population. The effective transmission of *Ca*. P. mali was confirmed in long-term field surveys (Mayer *et al*., 2009; Jarausch *et al*., 2011).

Monitoring/mass trapping devices

Monitoring traps consisting of sticky foils or funnel traps filled with toxic fluids are often equipped with dispensers emitting synthetic sex pheromones. Besides the beating tray method (Müther & Vogt, 2003), they are widely used for monitoring the population dynamics of insect pests, which is the basis for decisions regarding chemical control and for calculating the optimal date for spraying insecticides. Thus, through monitoring the amount of chemical insecticides can be reduced.

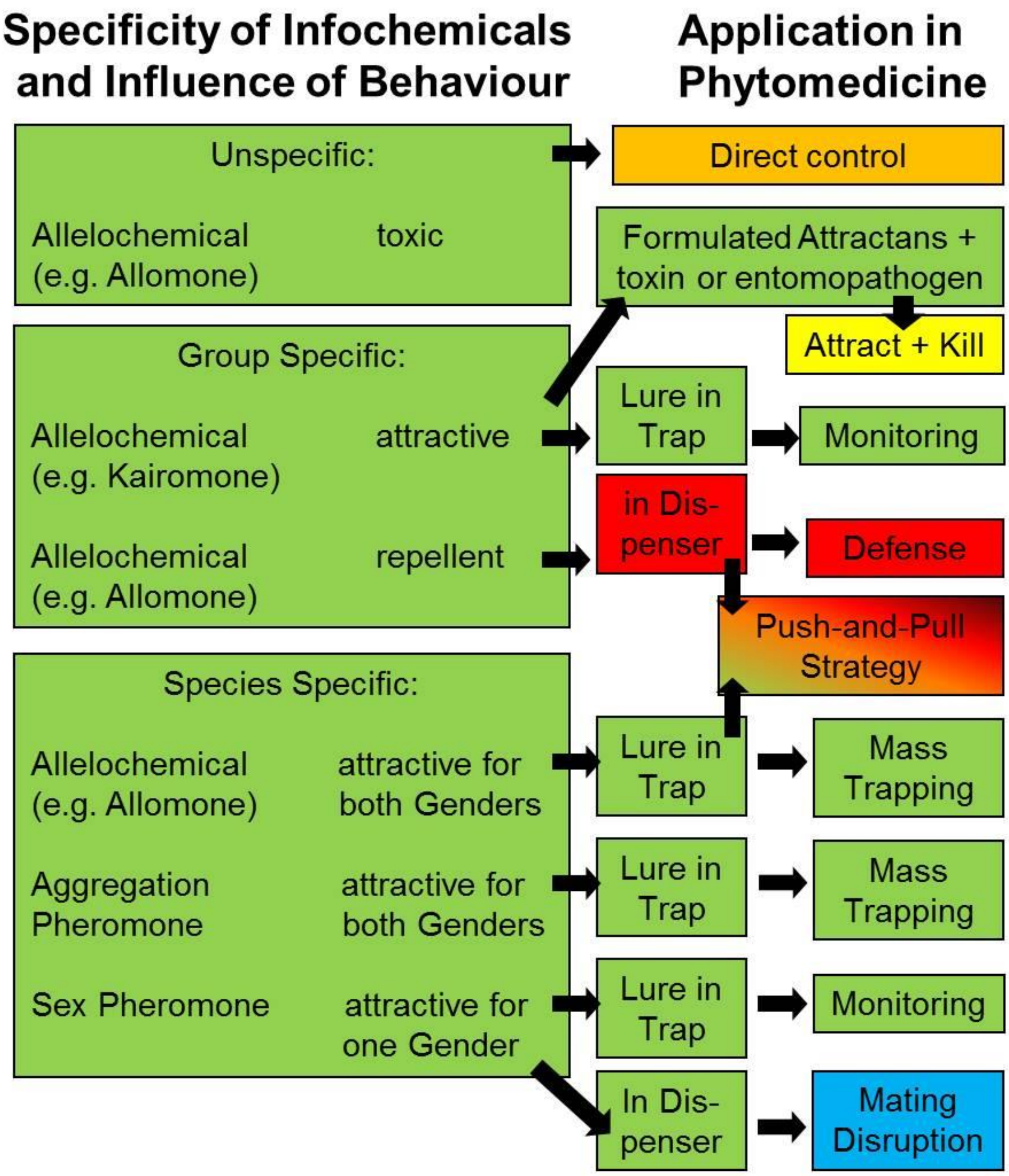
In recent years we started to exploit our findings by constructing traps with attractive allelochemicals like β-caryophyllene for the capture of psyllids (Eben & Gross, 2013). These volatile organic compounds may be produced constitutively (Soroker *et al*., 2004) or can be induced by plant pathogens (Mayer *et al*., 2008 a, b; Rid *et al*., 2016). Psyllids are small insects and the different species are morphologically very similar and can be only distinguished by specialists. Thus, the development of species specific traps could greatly reduce the efforts of growers necessary for identification. Deployed in apple orchards, sticky traps baited with β-caryophyllene dispensers caught significantly more adults (both males and females) of *C. picta* than the negative control traps containing an empty dispenser (Mayer *et al*., 2008 b). Compared to traps equipped with yellow foil, another attractive but not specific signal for many psyllids, the chemically lured traps caught fewer numbers of beneficial species like hoverflies and bumble bees.

For mass trapping, a specific pheromone or allelochemical is needed, which attracts both males and females. Sex pheromones are mainly produced by female insects and trap only males. Because the most crop losses due to herbivores are caused by larval stages of pest insects, which develop from eggs laid by females on the crop plants, the trapping of males may fulfill only monitoring purposes, but has no direct effect on pest insect populations (Gross & Gündermann, 2016). When aggregation pheromones were used, excellent results of the mass trapping technique could be obtained. Very high numbers of pest insects (West Indian sugarcane weevil, banana weevil, American palm weevil) were caught in Central and South America by using pheromone traps emitting male-produced aggregation pheromones of different weevil species (Giblin-Davis, 1996). For the apple psyllid *C. picta*, insect traps equipped with lures consisting of a highly attractive component, the species-specific β-caryophyllene, were developed for monitoring purposes (Mayer *et al*., 2008 a; Weintraub & Gross, 2013). Because this infochemical is attractive to both genders of phytoplasma vectoring psyllids (Mayer *et al*., 2008 b), it could also lay the basis for the development of mass trapping systems for sustainable control of these insects (Gross, 2013).

Recently we were able to publish a protocol on how to collect, analyze and determine behavior-modifying compounds in plant headspace and how to test promising candidates against phytoplasma vectors (Weintraub & Gross, 2013). This information can be used in the development of new kinds of traps for monitoring or mass trapping using behavior modifying infochemicals as lure. There is ongoing research on the development of new traps, lures and dispensers in my group, which are currently tested in field surveys.

Fig. 2: Different specificity of infochemicals (semiochemicals), their influence on target organisms' behaviour and their potential for innovative applications in phytomedicine (plant protection).

Fig. 2: Spécificités différentes des médiateurs chimiques , leurs influences sur le comportements des organismes cibles et leur potentiel pour des applications innovantes en protection des plantes


Specificity of Infochemicals and Influence of Behaviour
Application in Phytomedicine
Unspecific:
Allelochemical (e.g. Allomone)
toxic
Direct control
Formulated Attractans + toxin or entomopathogen
Attract + Kill
Group Specific:
Allelochemical (e.g. Kairomone)
attractive
Lure in Trap
Monitoring
Allelochemical (e.g. Allomone)
repellent
in Dis-penser
Defense
Push-and-Pull Strategy
Species Specific:
Allelochemical (e.g. Allomone)
attractive for both Genders
Lure in Trap
Mass Trapping
Aggregation Pheromone
attractive for both Genders
Lure in Trap
Mass Trapping
Sex Pheromone
attractive for one Gender
Lure in Trap
Monitoring
In Dis-penser
Mating Disruption

Push-and-pull strategies

Another possibility of innovative control of pest organisms is the so-called push-and-pull strategy, which involves manipulating insect behavior by the combined use of repellent (push) and attractive (pull) plants or infochemicals (Cook *et al*., 2007; Gross, 2013). For *Cacopsylla. Pruni (Scopoli)*, the only known vector of *Ca*. P. prunorum (European stone fruit yellows, ESFY), we have recently identified repellent chemicals (unpublished results). Together with attractive compounds in traps surrounding the orchard, dispensers filled with repellent compounds could be distributed within the orchard, in order to strengthen the effectivity of the traps.

Innovative attempts for the control of *Cacopsylla. pyri (Linnaeus).* could extend push-and-pull strategies to acoustic signals and include the use of both attractive chemical and acoustic signals in combination with repellent signals (also acoustic or chemical). The first behavior modifying chemical compounds for this species we currently identified (unpublished result) as well as the evidence of acoustic communication in this species has been published recently (Eben *et al.*, 2014).

Egg monitoring of grape berry moths

My second example presents the development of a new method for egg monitoring of grape berry moths. The European grapevine moth *Lobesia botrana(Hubner)* and the European grape berry moth *Eupoecilia ambiguella* (Lepidoptera: Tortricidae) are the most damaging insect pests in European viticulture. In 60-70% of the European vineyards these pest species are controlled by the mating disruption technique, an environmentally friendly technique using female sex pheromones for disturbing the mating behavior of this two pest species. In areas, where the mating disruption technique as control strategy against the moths is not appropriate (e.g. in small vineyards, highly structured landscapes), insecticides have to be applied for pest control. To achieve an effective chemical control, insecticide treatments have to be conducted before hatching of the larvae.

For this purpose an egg monitoring is necessary. Unfortunately, counting of the small green moth eggs on green plant parts is not practicable. To prevent immoderate insecticide application, which would not be in compliance to integrated pest management (IPM) (Gross & Gündermann, 2016), a decision support system for growers is needed, which enables the timing and necessity of pest control.

Based on a quantifiable egg deposition, which is visible on a specific egg-monitoring card distributed in the vineyard, the winegrower should be able to draw conclusions for the infestation on the adjacent grapes.

Such a tool, called "Moth Oviposition Card" (M-OVICARD) consisting of volatile and non-volatile compounds, supported by visual and tactile cues, is currently under development in a joint project of my group together with my colleague Christoph Hoffmann, the head of the laboratory for "Zoology and Integrated Plant Protection in Viticulture" (JKI, Siebeldingen, Germany). This tool should help to carry out insecticide applications very precisely, resulting in a reduction of plant protection products.

An app for mobile phones to facilitate the decision making for the grower is also in development (Sprute et al. 2016).

CONCLUSION

New attempts in the development of non-chemical methods for plant protection in IPM will help to enhance sustainable control of pest organisms and to reduce the amount of applied pesticides (Gross & Gündermann, 2016). Management strategies using chemical signals and/or other relevant signals for trapping insect vectors of phytoplasmas are environmentally friendly and could be combined with existing IPM strategies to reduce insecticide applications and to increase the control efficiency of important pest species in agriculture of this century.

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