EIP-AGRI Focus Group
Sustainable ways to reduce pesticides in pome and stone fruit production

Mini Paper 6

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Title: Innovative approaches for a sustainable future plant protection

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Introduction

Pests and pathogens are a major cause for economic losses in agriculture. Especially the production of pome and stone fruits of high quality require numerous applications of biological and chemical pesticides across the season. Moreover, climate change and the increasing number of invasive species lead to new challenges to control pests and pathogens which will require an increasing number of pesticide applications. On the other hand, in the European Green Deal, the European Commission proposed a 50% reduction of pesticide applications by 2030. To achieve this ambitious goal and to reduce negative side effects to non-target organisms and the environment, alternatives to the common chemical and biological pesticide applications are needed. Currently, many researchers across the world are investigating how crops can be protected against pests and pathogens in a more sustainable way by increasing the plant resistance and adaptability, reducing the fitness and damage potential of pests and pathogens, developing physiological alternatives to chemical pest control, and developing new direct application tools. Here we highlight a selection of innovative approaches for a future sustainable control of pests and pathogens (Figure 1).

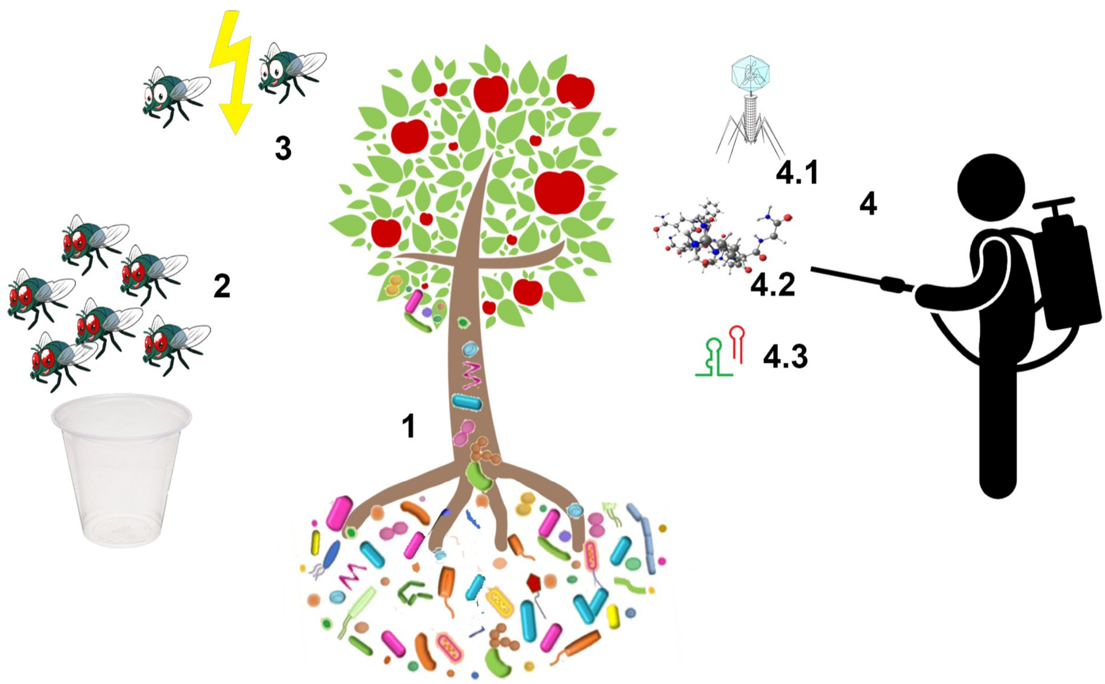

Figure 1: Schematic overview of different techniques: 1) Enhancing the fitness of the plant by influencing their phytobiome, 2) reducing the fitness of populations via sterile and incompatible insect technique, 3) influencing the mating behaviour via biotremology and 4) direct application of bacteriophages (4.1), peptides (4.2) and RNAi (4.3)

1. Enhancing plant fitness

Plants live in close association with a dynamic **phytobiota** (consisting of a macrobiota and microbiota associated with the plant), in particular the microbes that inhabit the phyllosphere (the areal region of the plant colonized by microbes), the endosphere (the microbes inside the plant) and the rhizosphere (the soil in which plants roots grow). Recent evidence highlights the crucial role of microbiota in plant performances, fitness and health (Chaney and Baucom, 2020), as well as nutrient acquisition and the attraction of pollinators and of predators and parasites of herbivores (Etemadi et al., 2018). Plants are able to actively recruit and modulate their microbiota to selectively enhance beneficial function over the negative. This complex network of ecological interactions is finely regulated by chemical signaling such as volatile compounds (VOCs), phytohormones and root exudates. Homeostatic balance between both microbe-microbe and host-microbe interactions is critical for plant health. The perturbation of this balance is often referred to microbial dysbiosis and it may represent an important mechanism of disease (Kemen, 2014).

Current methods to manipulate the plant microbiome concentrated on the addition of single bacterial or fungal strain acting as biological control agent (BCAs) and/or plant growth promoting agent (PGPs). More recently, multi-strain products or combined application of different strains to different plant organs have been commercially developed. However, BCA and PGPs often showed erratic results highly depending on the environmental and cultural conditions. Indeed, BCA isolated from other crops and not adapted to the specific plant microbiota face a hostile environment that may prevent their establishment and functioning. Moreover, also in case they can colonize the plant, their effect on the taxonomical and functional structure of native phytobiota could lead to unexpected negative effects.

A possible solution to this problem is the **application of synthetic communities** (SynCom). SynCom integrates the 2nd-generation of microbiome manipulation allowing reshaping with high precision the microbial composition in the phytobiota with desired functional traits (Venneman et al., 2020). SynCom can be built by mixing and applying to plants native selected strains with complementary modes of action (e.g. plant promotion, induction of resistance, direct inhibition of the pathogens) and minimal inter-specific competition among the different component of the community. SynCom approach is still at its dawn and needs more systematic and standardized studies to harness its full potential.

The main limiting factor of SynCom is related to their strict relation with the crop plant they are developed for, limiting their commercial use to other crop species. Moreover, their application still needs to be optimized for practical use. Finally, no information is available on the long-term effects of phytobiota manipulation. Indeed, even our knowledge on the impact of xenobiotic pesticides on plant phytobiota is still very limited and no information is currently available on the effect of the pesticides-induced shift in phytobiota on crop physiological processes and resistance (Sangiorgio et al., 2022). In the light of the 'One Health' paradigm, the pesticide-induced changes in the structure and functions of phytobiota may pose risks to human health by influencing human microbiota upon consumption of fresh produces, increasing the spread of cross-resistance against different antimicrobials and leading to the accumulation in fruit and vegetables of residues of intermediate products of microbially metabolized pesticides. Despite the use of BCA is generally regarded as a sustainable disease control measure, we lack information on their effects on phytobiota ecology and their use may pose unexpected threats to plant health.

2. Reducing population fitness

There are a number of ways how insects can be modified to negatively influence their fitness, reducing their population density and damage potential.
The **sterile insect technique** (SIT) is a biological control tool where males are exposed to ionizing radiation or sterilizing chemicals causing mutations in the sperm (Dyck et al. 2021). The males are then released in the field. After crossing with wild females, viable or fertile offspring are rarely produced. Releasing a high number of sterilized males will create therefore an increasing number of unsuccessful crossings and a subsequently a lower number of viable offspring which will reduce the reduction and eventually elimination of natural populations. Although initially this approach was designed to eradicate insect populations it is nowadays considered as a method to suppress local populations. SIT has successfully established for several insect vectors such as *Culex pipiens, Aedes albopictus* and Tsetse flies, but also important pest species including several Tephritid, Coleoptera and Lepidoptera species (Klassen et al. 2021). This approach showed promising capacity in suppressing or even locally eradicating insect populations, it has several technical and financial constrains requiring an efficient mass-rearing system and an efficient storing and releasing protocol.

A similar approach is the **incompatible insect technique** (IIT) release of males which are incompatible with wild females (Nikolouli et al. 2020). The endosymbiont *Wolbachia* is probably the most common symbiont and is known to cause cytoplasmic incompatibility in natural field populations, where infected males are incompatible with uninfected females. *Wolbachia* causes embryo mortality when infected males mate with uninfected females. Artificial transinfection studies and laboratory, semi-field and field applications showed that this endosymbiont is able to suppress the natural population if *Wolbachia*-infected released males mate with uninfected females (or females infected with an incompatible *Wolbachia* strain). Currently, this technique is being adapted to combat

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Drosophila suzukii and other pest species. A limiting factor of the incompatible insect technique, however, is that the unintentional release of *Wolbachia*-infected females would result in a fixation of the *Wolbachia* strain in the natural field population and would therefore make the method ineffective. An adequate sexing system is therefore essential.

A promising approach is therefore the **combination of SIT and IIT**. Generally, females are more sensitive to radiation than males (see Nikolouli et al. 2020). The unintentional release of *Wolbachia*-infected females would therefore result that sterile females would not transmit *Wolbachia* to their offspring and therefore hinder the establishment and the subsequent replacement of naturally uninfected females which would make the method inefficient. The potential of this approach was shown in a case study in mosquitoes where the release of millions artificially *Wolbachia*-infected and irradiated males of *Aedes albopictus* were released in two isolated islands in China resulting in an almost complete elimination of natural field populations (Zheng et al. 2019).

These methods are highly target specific and therefore it virtually has no negative effect on non-target organisms. Given the current trends of more sustainable insect control methods, SIT, IIT and its combination are therefore considered novel environmentally friendly tools to control insect pests. However, risk assessment studies have to be performed case-by-case to assess potential risk to non-target organisms. While the intentional release of insects with lower fitness and/or insects which are infected with a natural widespread bacterium like *Wolbachia* might represent limited risks to non-target species, the potential elimination of a species in natural populations might represent a risk to the local ecosystem which needs to be evaluated.

3. Semio-physical methods to manipulate insect behaviour

Semiochemicals, especially insect sex pheromones are widely used to control populations of insect pests (e.g. pheromones applied in orchards to disrupt the mating process of the codling moth). In addition to the well-studied pheromones and kairomones, also semiophysicals such as light, sounds and vibrations can be used to manipulate insect behavior and thus control the population (Hill et. al., 2019). An interesting novel technique is **Biotremology,** which studies the production, transmission, and reception of mechanical vibrations by organisms and their effects on behaviour for the mating disruption of insect pest species.

Since several insect pests are not, or not fully dependent on odors to find a mate, pheromones are often not sufficiently effective for their control (Avosani, S., Verrastro. 2022). As an alternative pest control strategy, physical stimuli such as vibrations, can be used singularly or combined with semiochemicals in order to manipulate insect-insect and insect-plant interactions. They can be used to orient target organisms (attraction/repellence), to prevent or elicit specific behaviors (inhibition/promotion) or to disrupt intraspecific communication which is often used to mediate reproduction (Nieri, R. 2021).

Researchers from Italy successfully applied the theoretical concept of vibrational mating disruption in an organic vineyard, to control the population of American grapevine leafhopper *Scaphoideus titanus*, which relies almost exclusively on vibrational signals during the process of pair formation (Mazzoni et. al. 2019). By reproducing the rivalry vibrational signal emitted by *S. titanus* males in plants and thereby disrupting the communication necessary for mating, the number of mating events was reduced by up to 70% under laboratory and semi-field conditions.

However, the potential of vibrational signals, emitted by tremors (colloquially called shakers), goes beyond leafhopper control, and promising results are also available for other crops and insect pests (Nieri 2022). Laboratory experiments as well as semi-field and field tests have shown that the brown marmorated stink bugs (*Halyomorpha halys*) and the Asian citrus psyllid (*Diaphorina citri*) can be trapped more efficiently using multimodal approaches combining vibrational signals with attractants. Moreover, laboratory experiments have shown that various Heteroptera species (e.g. shield bugs (*Parastrachia japonensis*), borrower bugs (*Adomerus rotundus*) and stinkbugs (*H. halys*)) use vibrations to synchronize hatching highlighting the potential to adapt this technique to various systems.

Biotremology seems to have great potential for innovative pest control solutions in the future. Although, to date, only few species have been studied in detail and the available literature is still scarce compared to semiochemicals, the field of study is rapidly developing (Nieri 2021). Being produced by electronic devices, tremors could be quickly integrated into high-tech smart agriculture systems. However, in order to successfully use vibrational signals to control until now little-studied target species extensive knowledge of the insects' biology, activity, mating behavior, communication modality (dependence on physical stimuli such as vibration signals, sounds, light, or on chemicals or multimodal) and their signal production and transmission systems must be obtained.

In addition to the basic and applied technical expertise in the field of biotremology also the regulatory framework for this new technology should be investigated. The regulatory framework for the use of vibrational signals in pest control, and thus the guidelines for risk assessment, are currently unclear. In order to avoid negative side effects, studies on the effects on non-target species (e.g., parasitoids) should be conducted before the technology can be used on a large scale.

4. Direct application targeting pest and pathogens

4.1 Bacteriophages

Bacteriophages are virus that infect bacteria and archaea that may present two types of life cycle. The first, known as lytic cycle, leads to the death of the bacterial host. On the other hand, the lysogenic cycle results in phage genome integration inside host DNA. Phages are among the most promising agents of biological control, as they have no toxic effects, are self-replicating, and highly host-specific (Czajkowski, 2019). Bacteriophages have been successfully applied to control fire blight (*Erwinia amylovora*) in apple with an efficacy of control up to 84% (Schwarczinger et al., 2017). However, currently no phage-based products are registered as a plant protection product or biopesticide in Europe (EFSA Database of active substances for plant pesticides; URL: **https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/**).

To be effective, phage application should be tailored on a clear understanding of bacterial pathogen biology and disease epidemiology (Holtappels et al., 2021). Thus, one of the main constrain to phage efficacy is related to inappropriate application time. Phage application should also take in consideration the pathogen intraspecific variability which may limit phage effectiveness. For this reason, the commercial products usually rely on phage cocktails. Despite the successful examples of phages in reducing bacterial diseases were achieved in field, challenges persist regarding the fact that lytic bacteriophages increase bacterial mutation rates, and bacteria may also evolve bacteriophage resistance.

Finally, phages are sensitive to environmental conditions (e.g. UV light, temperature etc.) that limits their persistency. Based on these considerations, integrating phage use with fixed spray system (FSS) regulated by precise risk model and DSS may overcome these limitations. In fact, FSS may allow a frequent and timely application of low volume of phages based on the disease epidemiological cycle and the real-time monitoring of risk conditions.

4.2 Peptides

Peptides are single linear chains of amino acids that can be used for the development of pesticides. These compounds affect specific pathways within the targeted pest or pathogen. Specifically, research has shown that peptides sourced from spider venom can interfere with voltage-gated calcium and sodium ion channels. This leads to an insecticide activity similar to the insecticide class pyrethroids. Ongoing research worldwide has identified a number of peptides that have different activity on pests and pathogens, and these are available for reference at DINeR, an EU funded database for insect peptide research (**http://www.neurostresspep.eu/diner**).

Peptides have ongoing challenges for scaling to agriculture. Being produced through yeast fermentation, peptide yields have not consistently been efficient enough to allow competitive pricing when compared to conventional synthetic insecticides. Additionally, peptides are easily broken down within the gut of many insects, requiring a mechanism to pass through the gut lining. This may be partially managed by increasing concentration, but future work on gut disruptors to improve peptide uptake will be critical for technology advancement.

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Although there are several companies working to commercialize peptides, there is only one company to-date that has products available for sale within the United States and under review in the EU. Vestaron, a Unites States of America based company, has introduced two peptide active ingredients that have been applied on pome and stone fruits, mainly against lepidopteran pests such as Codling moth, Oblique banded leafroller and Oriental fruit moth. Their use of a mixture of a gut disruptor and peptide have provided experimental results that show activity as good as or better than conventional insecticides and growth regulator chemistries within tree fruits. Pricing is comparable to modern, reduced risk chemistries such as chlorantriloprile. Of additional value is that these pesticides work effectively with current farm equipment on the farm, allowing for tank mixing of nutrients, other pesticides, and adjuvants, and have limited activity on off-target organisms (Clark et al., 2019).

Antimicrobial peptides (AMPs) are very effective against several bacterial pathogens including *Erwinia amylovora* (Mendes et al., 2021) and *Xanthomonas arboricola* pv. *pruni* (Camò et al., 2019) the causative agent of fire blight in pomefruit and bacterial spot of stone fruits, respectively. In Europe, the company AMPbiotech (Girona, Spain) produce several AMPs for the use in agriculture.

4.3 RNAi against diseases and pests

RNA interference (RNAi) is a natural process in plants to inhibit gene expression to resist the virulence of pathogens represents a practical method of manipulating cellular processes and plant defenses. Moreover, various RNAi-related pathways were detected in insects allowing to target the expression of species-specific genes which are essential for the development (Christiaens et al., 2020).

Double-stranded RNA (dsRNA) RNAs from pathogens or pests can contribute to defense suppression in the host plant, whereas host RNA may switch off pathogen virulence (Wang et al., 2017). In addition, to cross-kingdom RNA trafficking, some fungal pathogens can also uptake sRNA from the environment opening the possibility of exogenously apply RNA to interfere with pathogen virulence. This new-generation of RNAi fungicides is powerful, environmentally friendly, and can be easily adapted to control multiple diseases simultaneously (Wang et al., 2017). This approach has been successfully tested for scab (*Venturia inaquealis*) (Borah and Konakalla, 2020). Another possible approach relies on suppressing host susceptibility genes to confer resistance against a specific pathogen. For example, host gene silencing via RNAi has been demonstrated to confer resistance against *Podosphaera leucotricha* (powdery mildew) (Pessina et al., 2016).

Moreover, the ingestion of double-stranded RNA (dsRNA) can trigger the silencing of specific target genes necessary for the gene function for insect development and physiology and can therefore be used as a species-specific tool to control pest species (Baum et al. 2007).

dsRNA can be either directly injected to target pests, ingested by feeding to transgenic crop plants, or on plants where dsRNA was introduced by soaking or trunk injection (Liu et al. 2020). While gene silencing can be induced directly by transgenic plants which can produce a dsRNA that silences a critical gene in insects. However, these plants are considered genetically modified organisms (GMO) which complicates a broader application in Europe. Alternatively, *in vitro* synthesized dsRNA can be sprayed to crops which will then be ingested by phytophagous insects after feeding on the plant. This approach has been tested successfully in various chewing and sucking pests such as the Colorado potato beetle, whereas phloem feeding insects are less likely affected by foliar applications of dsRNA (reviewed in Liu et al., 2020).

The sequence-dependent mode of action of RNAi's allows to target the expression of specific genes in a target insect, while leaving the non-target insect species unharmed. An RNAi based approach would be therefore more efficient and selective than conventional pesticides (Christiaens et al., 2020a). However, the RNAi sensitivity of insects varies substantially across insect species and therefore alternative formulation strategies and improvement of cellular uptake as well as stability of RNAi in the environment need to improve (Christaens et al., 2020b). Apart from the creation of new and more effective and stable RNAi-based products additional risk and benefit studies as well as studies focusing on societal acceptance are needed to integrate RNAi-based approaches into a more sustainable pest management strategy (Taning et al., 2021).

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5. Conclusion

Sustainable agricultural production needs efficient alternative tools to control pests and pathogens. Here we described various novel tools which have the potential to be integrated in a sustainable organic and integrated pest management strategy. Our minipaper is not intended to be an exhaustive list of alternative tools, we rather aimed on focusing on some case studies of different techniques. While some techniques like SIT are currently being implemented in some agricultural systems across the world, other tools like biotremology are still in the early stage of development. Much more research is needed from building a bridge between basic research and applied research to studies on the potential chances and risks of new methods. To make this area of research interesting for developer, researcher and start-ups, clear regulatory framework and risk assessment guidelines for these methods are needed that are not subject to the Regulation (EC) No 1107/2009 yet. Moreover, faster registration of substances that can be considered as natural and/or low risk substances are needed. In addition to the risk assessment, for organic farming a separate investigation of the technologies may be required to ensure the conformity with the principles of organic farming. In conclusion, innovative tools come from innovative ideas and therefore all actors, from the farmer to researcher and developer need to be innovative for a sustainable future plant protection.

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