

18 Outlook for ecostacking

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Introduction and background

The various chapters in this volume describe many components of ecostacking and the elements which can be relatively quickly taken into use to benefit growers in crop protection and agricultural production. In this Chapter, a synthesis of the concepts and potential of ecostacking is presented, along with outlining future strategies to employ such techniques.

By implementing ecostacking, we can effectively enhance the provision of ecosystem services in agricultural systems. This approach recognizes the interconnectedness of different components within the ecosystem and their collective contribution to the overall functioning of the agroecosystem. Through strategic management and conservation of functional biodiversity, we can optimize the delivery of multiple ecosystem services that are vital for sustainable and resilient agricultural production.

The term “ecostacking” was introduced in 2017 in an editorial by Heikki Hokkanen (Hokkanen 2017), and hence is a novel concept. The concept of ecostacking is a holistic and integrative approach to suppress pests (including diseases and weeds) in agricultural systems, and to ensure the optimum provision of other key ecosystem services (e.g., pollination, nutrient cycling). Pest control efficacy exploits the synergistic interactions among diverse ecosystem service providers, while considering the ecological dynamics and management practices within the cropping system. In this way we can reduce reliance on agrochemicals, promote biodiversity, and foster sustainable and resilient agricultural landscapes. Embracing ecostacking as a core principle in pest management holds great potential for addressing the challenges of pest control in the context of evolving environmental and societal demands.

The concept of ecostacking advocates the combination and optimization of multiple ecosystem services within a given agricultural context. This approach acknowledges the dynamic interactions among various service providers and emphasizes the need to consider the specific pedo-climatic conditions, crop characteristics, existing landscape elements, and socio-economic factors in a given area. By integrating these diverse elements, we can create synergistic effects that enhance the overall performance and resilience of agroecosystems.

Ecstacking is based on a systematic assessment of the functional biodiversity present in the agroecosystem. This involves identifying and quantifying key organisms, abundance, diversity, and functional traits that contribute to ecosystem services. Additionally, understanding the underlying ecological processes that govern the interactions among these organisms is essential. By unraveling service providers' ecological relationships, trophic interactions, and habitat requirements, we can effectively design strategies that promote their coexistence and mutually beneficial interactions.

¹ The sequence of authors is based on: first the series editors, followed by the editors of this book, and lead author of each chapter in alphabetical order. The affiliation and contact information for the authors are given at the respective chapters in this book.

Further research will enable optimizing its implementation to assess its long-term sustainability and scalability. Studies should explore the interactions between ecosystem service providers, identify critical factors influencing their effectiveness, and evaluate the economic and environmental benefits of ecostacking in different cropping systems and geographic regions.

The outlook for ecostacking is based on individual contributions concerning the vision and potential of the research presented in the book chapters for practical implementation and future directions.

INDIVIDUAL CONTRIBUTIONS BY CHAPTER AUTHORS, IN THE SEQUENCE AS IN THE BOOK

Priming of plant defenses

The potential of plant-induced defense in developing sustainable pest management strategies underscores the importance of further investigating these mechanisms. By doing so, we can gain a better understanding of how to harness the natural defense mechanisms of plants, ultimately leading to a more environmentally-friendly approach to agriculture. Plant-induced defense strategies could lead to the development of more effective and targeted pest management techniques. By utilizing the specificity of VOC (volatile organic compounds)-induced plant defense, we can selectively target harmful pests while minimizing the impact on non-target organisms and promoting the adoption of other ecostacking processes.

Plant-induced defense mechanisms have already been successfully used in a variety of pest management strategies. Practical examples of the application of these mechanisms include:

Push-pull strategy: In this strategy, certain plants are intercropped with the target crop to repel pests. The repellent plants emit volatiles that are unpleasant to pests, driving them away from the target crop. At the same time, attractive plants are placed around the perimeter of the target crop to lure pests away from the crop. This reduces the number of problems that damage the target crop and minimizes the use of chemical pesticides.

Use of natural enemies: Plant volatiles can also be used to attract natural enemies of pest insects, such as parasitic wasps or predatory mites. By planting specific plants that emit volatiles attractive to these natural enemies, farmers can encourage them to come to the area and prey on the pests, reducing the need for chemical pesticides.

Seed treatments: Seed treatments can also be used to trigger plant-induced defenses. For example, coating seeds with certain beneficial fungi or biorational products can induce systemic resistance in the plants, making them more resistant to a range of pests and pathogens.

These are just a few examples of the practical applications of plant-induced defense mechanisms in pest management. With continued research and development, we may discover even more effective and sustainable methods for managing pest populations while minimizing harm to the environment.

Plant-induced defenses have the potential to benefit both the environment and agricultural productivity. By incorporating plant-induced defense mechanisms into the ecostacking approach, we can create a more diverse and resilient ecosystem that is better equipped to withstand environmental stressors and support a wide range of organisms. This approach can help to promote a healthier, more sustainable food system, and lead to increased crop yields and reduced economic costs associated with pest damage.

Dual role of the entomopathogenic fungus *Beauveria bassiana* in insect pest management in the greenhouse

The development of agricultural production has led to an increasing demand for greenhouse crops. However, pests and diseases have become factors affecting greenhouse crop growth and yield. The extensive use of chemical pesticides has contributed to the increasing resistance in greenhouse pests, which has led to the need for alternative control measures.

Beauveria bassiana is a naturally occurring entomopathogenic fungus (EPF) that is found in soil and has already for a long time been used as a bio-insecticide for a variety of target pests. Recent research has also shown that *B. bassiana* can act as an endophytic fungus, colonizing the internal tissues of plants and helping to control plant diseases and herbivorous pest populations. This dual role of *B. bassiana* makes it a promising candidate for further research and development as a sustainable biocontrol agent.

Several studies have demonstrated the potential of *B. bassiana* as an effective biocontrol agent for a range of pests, including important greenhouse pests such as aphids, whiteflies, and thrips. These studies have shown that *B. bassiana* can infect and kill a high proportion of pest populations, without causing harm to beneficial insects or the environment. Furthermore, research has indicated that *B. bassiana* has the potential to act as a growth promoter, improving crop yields and enhancing plant growth.

In addition to its potential as a biocontrol agent, *B. bassiana* offers several other advantages as a sustainable pest management tool. Unlike chemical pesticides, *B. bassiana* is non-toxic and poses no risk of environmental contamination or harmful effects on human health. It is also relatively easy and inexpensive to produce, making it an attractive option for small-scale and low-income farmers.

The dual role of *B. bassiana* offers a promising avenue for incorporating plant-induced defense mechanisms into the ecostacking approach. This EPF can have a direct lethal effect on pests, as well as the ability to colonize plants as endophytes and, via this second function, inhibit the occurrence of pests and diseases. By colonizing plants internally, the fungus can be protected from environmental stresses and fluctuations, and have the potential to induce plant defense responses that can improve crop health and resilience. The interaction has been found to induce plant defense responses, such as the production of phytoalexins and changes in the expression of genes related to pathogenesis. For example, proteomic techniques have been used to demonstrate the induction of R gene-encoded proteins related to plant defense in *Phoenix dactylifera* leaves following *B. bassiana* colonization.

Moreover, the use of EPF as endophytes in pest management is advantageous compared to traditional methods. One study found that tomato plants endophytically colonized by *B. bassiana* had significantly higher levels of terpenes than control plants, while the weight of beet armyworms fed on colonized plants was considerably lower. Similarly, *Echinacea purpurea* plants colonized by *B. bassiana* exhibited increased levels of metabolites at high phosphorus levels.

By incorporating plant-induced defense mechanisms into the ecostacking approach by using suitable EPF such as *B. bassiana* as endophytes, we can create a more diverse and resilient ecosystem better equipped to withstand environmental stressors. Integrating EPF into pest management strategies can also reduce the use of chemical pesticides, which can harm the environment and non-target organisms. Omics technologies, such as transcriptomics, proteomics, and metabolomics, provide a powerful tool to investigate the molecular mechanisms underlying the interaction between EPF and plants. Ultimately, this knowledge can be applied to analyze the whole

process from genes to metabolites and then to biological phenotypes, enabling the creation of more effective and sustainable pest management strategies rooted in the principles of ecostacking.

Potential of plant-plant communication to improve sustainable pest control

Biogenic volatile organic compounds most often mediate plant-plant communication and can occur both above and below ground. Communication between insect or mite-damaged crop plants and healthy crop plants has been demonstrated in several studies, whereby herbivore-induced volatiles released by pest-attacked plants can activate chemical defense-related traits in the healthy neighboring plants. For example, common bean plants can signal the presence of spider mites directly to predatory mites via the emission of induced plant volatiles. At the same time, the induced volatiles of mite-attacked bean plants are responsible for mediating plant-plant communication that activates extrafloral nectar production in healthy neighboring bean plants. Extrafloral nectar is a suitable food and attractant to predatory mites, which occupy healthy plants and act as “bodyguards” on the healthy plants before spider mite attack.

Another type of plant-plant interaction is the passive adsorption of semi-volatile compounds of healthy emitter plants to the surfaces of crop plants. This is a passive temperature-dependent mechanism where the semi-volatile compounds released by a growing plant can affect a receiver plant by creating a protective mask on the plant surface to repel insect pests or prevent the germination of fungal pathogen spores. In this case, there is no activation of the receiver plant’s own chemical defenses.

Plant species diversification using secondary plants – such as cover crops or companion plants – has been found to improve crop plant performance in traditional agriculture. Positive effects on plant-insect interactions – such as more efficient pollination or improved insect pest resistance – have been observed in diversified agroecosystems. In general, plant-plant communication between the major crop plant and the companion plant has been found to be beneficial for crop plants in laboratory and greenhouse experiments. However, so far there is limited knowledge on how efficient plant-plant communication can be in specific plant species associations, which is especially relevant for plant selection in field agro-ecosystems. Volatile communication between plants and between plants and other organisms plays a vital role in the aims of ecostacking because it has multitrophic effects on the crop plant-based ecosystems.

Designing efficient cropping with companion plants needs to combine knowledge of pest biology, including their natural enemies and their responsiveness to host location cues and crop defenses. For example, traditionally the use of aromatic companion plants such as sacred basil and citronella grass have reduced insect damage on Chinese kale due to their repellent effects on *Brassica* pests (Huong et al. 2020) and rosemary plant with 1.5m distance reduces aphids on sweet pepper (Ben Issa et al. 2017). Neighboring peppermint plants can even activate chemical defenses in soybean and reduce moth damage (Sukekawa and Arimura 2018.) Marigold (*Tagetes* spp.) plants are efficient repellents against root nematodes of potato by releasing nematode repellents into the soil (Hooks et al. 2010). Therefore, for certain cultivar combinations of companion and crop plants, there are sufficient knowledge for ready uptake into practice, whereas for many systems a greater deal of research and field trials is required.

Better knowledge of suitable companion/cover crop plant species, volatile composition and information on the variability of key critical repellent compounds, is required to bring these techniques to agricultural practice. Cultivars rich in repellent compounds against the primary pest should be the most efficient plants used in diversified cropping systems.

Climate warming, along with problems caused by drought, rising sea levels, loss of biodiversity, and environmental pollution, are global issues that need to be considered by all governments. Alleviating these problems through well-considered environmentally friendly agricultural practices should be strongly backed financially. Governmental support to promote ecologically sustainable agriculture, including ecostacking, should be one of the core pathways for maintaining sustainable food chains globally.

Availability of suitable cultivars for scientifically developed ecostacking strategies and technologies will be essential for grower uptake. In addition, well-constructed informational material and clear correlation to reduced cost and/or increased yield, will be needed. Governmental subsidies may contribute to encouraging uptake of techniques that have environmental benefits, but that does not instantly yield economic gains.

Olfactory manipulation for pest control

Among the extensive assemblage of tephritid fruit flies, approximately 250 species are directly responsible for inflicting harm upon commercially cultivated or wild fruits and vegetables, consequently earning international recognition as phytosanitary pests. Within the Tephritidae family, four major genera stand out for their significance: *Ceratitis*, *Bactrocera*, *Anastrepha*, and *Rhagoletis* (Malacrida et al., 2007; Doorenweerd et al., 2018; Scolari et al., 2021). These genera encompass numerous species that pose significant threats to commercial agricultural production due to their economic impact on fruits and vegetables.

While identifying semiochemicals associated with male attraction in these flies has provided initial insights, translating this knowledge into practical attractant development has proven challenging. Many identified semiochemicals exhibit limitations, such as difficulties in isolation or synthesis and rapid degradation, hindering their practical use. Additionally, the unknown ratios of semiochemical components hamper the optimization of attractant formulations. Furthermore, the underlying molecular mechanisms governing semiochemical perception in tephritid flies remain largely unexplored. Addressing these bottlenecks is crucial for successfully developing efficient attractants and sustainable pest management strategies.

To overcome the challenges in attractant development, a comprehensive understanding of the molecular basis of chemoperception in tephritid flies is imperative. Recent advancements in research tools offer promising avenues to delve deeper into these mechanisms and pave the way for breakthrough solutions. CRISPR/Cas9-based genome editing provides a powerful tool for precise manipulation of the fly's genetic makeup, enabling the investigation of gene function related to chemoperception. By targeting specific genes associated with olfaction, researchers can uncover critical insights into the molecular basis of semiochemical perception and potentially identify key components involved.

Furthermore, the application of Alpha GO-guided protein structure prediction algorithms holds significant promise in elucidating the structure-function relationships of odorant receptors and other proteins involved in chemoperception. By accurately predicting the three-dimensional structures of these proteins, researchers can gain insights into their ligand-binding properties and develop a deeper understanding of their selective responses to specific semiochemicals. This knowledge will facilitate the design of novel attractants with enhanced specificity and efficacy.

In addition, the use of supercomputer-aided chemical engineering allows for efficient exploration of the chemical space, facilitating the design and synthesis of semiochemical analogs with improved stability and enhanced attraction properties. By harnessing computational modeling and simulations, researchers can accelerate the development of innovative attractants that are

environmentally friendly, economically viable, and compatible with sustainable pest management practices.

The integration of advanced research tools such as CRISPR/Cas9-based genome editing, Alpha GO-guided protein structure prediction, and supercomputer-aided chemical engineering offers promising prospects for unraveling the molecular basis of chemoperception in tephritid fruit flies. Overcoming the bottlenecks in attractant development is crucial for sustainable pest management strategies. By bridging the gap between molecular insights and practical applications, we can mitigate the economic losses caused by tephritid fruit flies and enhance the efficacy of attractant-based control measures in agricultural systems.

Adopting the ecostacking approach holds immense promise for developing sustainable pest management strategies. By utilizing the knowledge gained from studying the ecological interactions and molecular mechanisms of tephritid flies, we can design attractants that are highly effective, species-specific, and environmentally friendly. The integration of attractants based on ecological understanding not only increases the efficiency of trapping but also minimizes non-target captures and reduces the reliance on broad-spectrum insecticides.

Management of agricultural soils to support ecosystem services

Pest insects entering the soil at some stage in their life-cycle (often for pupation), or living in the soil as larvae (e.g., wireworms, root flies), often suffer high levels of mortality while in the soil due to entomopathogens or other factors (e.g., Razingger et al. 2014, Alyokhin et al. 2020, Erasmus et al. 2021). The extent of this mortality often remains unknown, and the soil component is frequently described as a “black box” in terms of population dynamics (e.g., Andrén and Balandreau 1999, Briones 2014, Custer et al. 2020). As this component of insect population regulation is practically absent in current agricultural fields (e.g., Zec-Vojinovic et al. 2006, Hokkanen and Menzler-Hokkanen 2017), there is an opportunity to utilize it for improved pest control by taking advantage of insect pest suppressive soils in the overall ecostacking approach. Improved biocontrol in the soil complements the mortality caused by predators and parasitoids, as well as other factors limiting the population size including competitors, plant secondary metabolites and induced resistance. As shown in Chapter 8 of this book, management of agricultural soils can easily be improved to ensure the build-up of entomopathogens by supporting their recycling, by avoiding the use of harmful agrochemicals, and by adopting minimum tillage.

To support entomopathogen survival and entomopathogen build-up in the soil, growers can establish suitable intercrops or cover crops (such as legumes). This is important for crops, which do not favour insects that have a soil-dwelling life-stage (e.g., cereals including maize). Further techniques already available include incorporation of suitable soil amendments (such as organic materials in the form of composts, biochar, or chitinous products) to stimulate the activity and persistence of beneficial antagonistic organisms in the soil, use of organic fertilizers when feasible, judicious and restricted use of pesticides, and by avoiding inversion tillage. Further research is needed to investigate the long-term impacts of these various techniques on the suppressiveness of soils to specific problem pests in different crops and cropping systems, their impact on other possible pest and beneficial organisms, and the optimal mix of tools for different ecological contexts.

The greenhouse environment: challenges for ecostacking

Greenhouse cultivation has evolved from traditional soil-based methods to more advanced soilless cultivation techniques. Soilless cultivation utilizes substrates such as sand, vermiculite, gravel, sawdust, and peat moss, combined with tailored nutrient solutions to meet the specific needs of plants. This innovative approach offers numerous advantages, including improved resource utilization, reduced environmental impact, and increased crop productivity. We need to explore the concept of ecostacking within greenhouse cultivation, and focus on its potential for enhancing pest control.

Soilless greenhouse cultivation presents a range of benefits that contribute to the development of ecostacking in agricultural production. By eliminating the need for soil, this method overcomes challenges associated with limited arable land and mitigates issues related to soil pollution, salinization, and acidification. Additionally, water usage is significantly reduced, with recycling systems enabling water conservation of over 90% (French & Roth, 2022). The absence of soil also reduces the incidence of soil-borne pests and diseases, providing a favorable environment for plant growth (Tzortzakidis et al., 2020).

Pests and diseases pose significant threats to crop production within greenhouses. Innovative techniques, such as the watershed algorithm, enable efficient and cost-effective pest identification (Xia et al., 2015). Recent progress in deep learning and object detection networks has further enhanced the precision of pest identification (Li et al., 2021). Moreover, researchers, such as Tay et al. (2021), have successfully implemented systems that employ Artificial Neural Networks and Adaptive Neuro-Fuzzy Inference Systems to estimate pest risk levels. By considering factors like internal temperature, humidity, human intervention, and real-time pest risk levels, these systems enable growers to implement appropriate pest management procedures. With continued advancements, it is plausible to anticipate the development of automatic pest management processes in the near future.

Ecstacking involves stacking ecosystem services to achieve improved pest control within greenhouse cultivation. By integrating multiple approaches and practices, it is possible to create synergistic effects that enhance pest management outcomes. Some of the critical components of ecstacking in greenhouse cultivation are discussed in Chapter 10 and include:

Biological Control: Beneficial organisms, such as predatory insects, parasitoids, and microbial agents, can be introduced to control pest populations. This approach reduces the reliance on chemical pesticides and promotes a more sustainable pest management strategy.

Habitat Manipulation: Designing greenhouse structures and landscapes to provide suitable habitats for natural enemies of pests can encourage their presence and enhance their effectiveness in pest control. Features like flowering plants, sheltering structures, and diverse plant communities can create a favorable environment for beneficial organisms.

Cultural Practices: Implementing proper crop rotation, sanitation, and plant selection techniques can disrupt pest life cycles and minimize pest infestations. These practices can help maintain a healthy and balanced ecosystem within the greenhouse.

Physical Barriers: Using physical barriers such as screens and nets can prevent pests from entering the greenhouse while allowing airflow and light penetration. This approach acts as a preventive measure and reduces the reliance on chemical interventions.

As greenhouse cultivation continues to evolve, the concept of ecstacking offers promising prospects for enhancing pest control strategies. Soilless cultivation provides a favorable foundation for implementing ecstacking practices, enabling efficient resource utilization and reduced environmental impact. By integrating various techniques such as biological control, habitat manipulation, cultural practices, and physical barriers, greenhouse growers can develop sustainable

pest management systems that minimize the reliance on chemical pesticides. Collaboration between researchers and end users is crucial for the successful transfer of technology and innovation, fostering a harmonious alignment of science, business, and society in the pursuit of effective and sustainable pest management practices in greenhouse cultivation.

Predatory mites for greenhouse pest management: possibilities for enhanced action via ecostacking

Greenhouses present unique challenges such as high temperatures and dry conditions, which create favorable environments for spider mite populations rather than for their phytoseiid mite predators, potentially disrupting or compromising biological control efforts. To address this issue, it is crucial to implement feasible biological control strategies that enhance the adaptability of predatory mites to conditions in the context of global warming and climate change.

The exploration of synergistic interactions between predatory mites and other control strategies presents a promising approach for achieving integrative management of tiny sap-sucking pests. The integration of host plant resistance alongside chemical control can enhance the effectiveness of management factors such as beneficial natural enemies (Khanamani et al. 2013, 2014, 2015; Alipour et al. 2016; Fathipour et al. 2019).

Research findings summarized in Chapter 11 of this volume emphasize the importance of developing adaptive strategies to enhance the efficacy of predatory mites in greenhouse environments. The identification and characterization of mechanisms driving pesticide resistance in predatory mites provide a foundation for the development of genetically enhanced strains. Through the use of advanced genomic tools and targeted genetic manipulations, we can optimize the resistance traits of these beneficial organisms, ensuring their efficacy in integrated pest management approaches. Such advancements pave the way for sustainable pest control solutions that can effectively mitigate pest populations, promoting environmentally friendly and economically viable agricultural practices.

By screening and selecting for dry-adapted and heat-tolerant strains of predatory mites such as *N. californicus* and *N. barkeri*, respectively, we can improve the ability of predatory mites to control spider mite populations under challenging climatic conditions. These advancements in understanding the molecular mechanisms behind thermal tolerance offer valuable insights for the practical application and utilization of stress-resistant strains in biological pest control, contributing to the development of sustainable and effective strategies for managing greenhouse pests.

Therefore, by leveraging the advantages of stress-resistant predatory mite strains, which have demonstrated enhanced adaptability to challenging environmental conditions, we can establish a synergistic approach that combines multiple control methods. This ecostacking strategy encompasses the integration of stress-resistant mite strains with other compatible pest management strategies, such as host plant resistance and biorational controls. The compatibility between stress-resistant mites and these strategies reinforces the effectiveness of biological control measures, allowing for a more sustainable and efficient approach to pest management in greenhouse systems.

By reducing the reliance on chemical pesticides, the ecostacking approach promotes environmentally friendly agricultural practices and helps mitigate the negative impacts associated with pesticide usage. Additionally, the integration of stress-resistant predatory mite strains offers improved predatory efficiency, enabling better control of sap-sucking pests that pose threats to greenhouse crops. This integrated approach has the potential to enhance the overall productivity and quality of vegetable, fruit, and ornamental production within greenhouse agricultural systems and

can achieve improved pest control outcomes while prioritizing sustainable and environmentally conscious practices.

RNAi promoted ecostacking concept: rebooting biological control for aphid pests

The integration of RNAi-based gene silencing as a pest control strategy with other control methods offers the potential for synergistic effects and enhanced efficacy. This approach explores utilizing RNAi as a "helper" in conjunction with other strategies (Niu et al. 2018). By explicitly targeting gene silencing through RNAi, this strategy aims to augment insect pest control by harnessing synergies with insecticides, biological control agents, plant defense inducers, and other complementary techniques.

The RNAi-based strategy can be tailored to target multiple genes involved in the intricate interactions between insect pests, their host plants, and pathogens. By adopting this diverse targeting approach, we adhere to ecological principles prioritizing diversity over singular target selection. Unlike traditional pest control methods that often focus on single factors, RNAi-based strategies address multiple facets of pest interactions. This broader scope reduces the likelihood of pests developing resistance and offers a long-term solution to managing pest populations effectively.

In the context of aphid control, the application of RNAi can be achieved through various methods such as spraying, transgenic plants, and planta delivery. These approaches facilitate the entry of exogenous double-stranded RNA (dsRNA) into aphids through two routes: penetration of dsRNA through the pest's integument and ingestion of dsRNA through pest feeding. To enhance penetration, formulations containing nanoparticles and other accessory ingredients can be utilized. These formulations play a crucial role in increasing the efficacy of dsRNA penetration.

In laboratory settings, dsRNA has primarily been delivered through injection or ingestion. The choice of delivery method is closely linked to how dsRNA is produced, which can follow two main strategies: transformative and non-transformative. In the transformative strategy, genetic engineering techniques introduce insecticidal dsRNA into plants. This approach combines dsRNA production and delivery, allowing pests to ingest the dsRNA during feeding directly. The first commercially available RNAi product for western corn rootworm control involved the transgenic expression of insecticidal dsRNA (Fishilevich et al. 2016).

The non-transformative strategy involves the exogenous synthesis of dsRNA and targets pests that can be controlled through spraying, similar to conventional insecticides. In this approach, the dsRNA spray is directly applied to plant leaves, making it particularly suitable for plant-chewing pests. A recent breakthrough in this field includes the announcement of a product designed to control the Colorado potato beetle using dsRNA spraying.

Microorganisms can also produce the dsRNA. Interestingly, some of these microorganisms are natural pathogens of insect pests and possess inherent delivery capabilities (Guan et al. 2021). This presents an intriguing avenue for dsRNA production and delivery, as the microorganisms can serve as carriers for the targeted delivery of dsRNA to pests.

The integration of RNAi with biological control approaches goes beyond the mere stacking of different pest control methods. RNAi acts as a "helper" to enhance the efficacy of various biological control tools, operating through both direct and indirect mechanisms. For example, the application of dsRNA via spraying can directly improve the killing speed of entomopathogenic fungi while ensuring the safety of entomophagous arthropods, thus promoting the stability of the ecosystem for pest management. Through proof-of-concept studies in RNAi-based aphid control, the combination

of dsRNA, entomopathogenic fungi, and lady beetles has demonstrated promising results in sustainably keeping aphid populations below the economic threshold. Further investigations are required to develop formulations of dsRNA and fungi with adjuvants, as they play a crucial role in reducing the necessary dosage from both dsRNA and fungi for effective pest control.

By exploiting the potential of RNAi, the field of pest control stands to benefit from a novel and promising approach. The wide array of candidate RNAi targets offers the opportunity to disrupt vital physiological processes in pests, leading to population suppression. Moreover, the versatility of delivery methods, including transformative strategies involving genetic engineering and non-transformative approaches utilizing dsRNA spraying, enhances the feasibility and applicability of RNAi-based pest control. This innovative approach, when combined with the concept of ecostacking, can revolutionize pest management by providing environmentally friendly solutions that target specific pests while minimizing harm to non-target organisms. Further research and development in this field promise to effectively reduce the economic and ecological impacts of insect pest infestations.

Integrating diversity to redesign agroecosystems

By simplifying agroecosystems to achieve economies of scales, conventional farming has become dependent on curative practices to control pests (i.e., pesticides, but also tillage against weeds) (Meehan et al. 2011; Gagic et al. 2021). The detrimental impacts of these control methods on the environment (Stavi et al. 2016; Pisa et al. 2021) calls for the development and implementation of radical alternatives that must be based on preventive tactics. There exists an array of agroecological practices known to prevent pest infestation and spread in crop fields (reviewed in Ch. 17). They all share the principle of maximizing functional biodiversity to strengthen biological regulations. While none of these practices can solely achieve an overall control of the multiple pests occurring (often simultaneously) in fields, the key lay on their integration through new agroecosystem design and management strategies.

Among other alternatives to conventional farming, organic and conservation agriculture incorporate several pest-preventive techniques. These alternative production systems represent very rich experiences (or prototypes, Bellon and Penvern 2014) as they realized a drastic reduction of curative techniques (i.e., synthetic inputs and soil plowing, respectively). The next frontier for agroecological engineering is to understand how to integrate practices of both systems to best adapt to local pedo-ecological conditions. Abandoning most (if not all) of the curative methods to control pests is undeniably challenging (see Ch. 15 for farmers' feedbacks). Future research on ecostacking will have to intensively invest in designing and testing novel integrative cropping systems on the one hand (e.g., Ditzler et al. 2021; Juventia et al. 2021), while analyzing farmers' successful redesign of their farms on the other hand (e.g., Boeraeve et al. 2020). Both on-station and on-farm research will have to feed one another, and be conducted in a diversity of environments to adapt to the local ecological (but also economic and sociological) contexts. By integrating diversity, the ecostacking perspective boldly commits to embracing complexity. It is definitively ambitious, but urgently necessary to address the challenges faced by today's agriculture.

Ecstacking as an alternative to the use of pesticides

The use of pesticides almost always will interfere more or less drastically with the beneficial processes contributing to ecostacking. Pesticide sprays even in other crops may severely compromise the biocontrol of pests, as in the case of pollen beetle *M. aeneus* parasitoids getting killed in the cereal fields following rapeseed in the crop rotation (see Chapter 14 in this Volume).

Reducing (if not eliminating completely) the application of pesticides by adopting appropriate ecostacking and supportive techniques in the agroecosystem will allow the ecosystem services to recover and reach their full potential with time. Replacing the negative ecological feedback loops resulting from pesticide use by positive ecological feedback processes, will bring the grower the benefits of the ecostacking approach. This includes improved biocontrol of pests, diseases and weeds, as well as enhanced pollination services.

How pesticide use interferes with ecosystem services in each crop and at a specific location needs to be assessed. Usually, as long as the cropping system is similar, the same principles are likely to apply over large regions. Thus, for the Finnish conditions, the interference of insecticide spraying against cereal aphids with the biocontrol of the pollen beetle in rapeseed will apply to the whole country. Similarly, the widespread pollination deficit affecting rapeseed yields (see Hokkanen et al. 2017), stemming primarily from insecticide applications, applies to most parts of the rapeseed growing area in the country. This information can readily be put to use and could be taken up by growers.

For growers, abandoning pesticide use is a difficult step to take. Further research is needed, particularly on alternative, innovative pest control techniques, which will protect crop yields while not interfering with ecosystem services. This will enable the establishment of the favorable ecological feedback mechanisms and achieve optimal ecostacking with time. Many of such innovative enabling technologies are presented in this book (e.g., RANi-based ecostacking, see Chapter 12; priming of plant defenses, see Chapter 3; olfactory manipulation, see Chapter 6; possible use of phoretic mites as vectors for antagonists, see Chapter 16; and many others).

The economic impact of integrating ecostacking into conventional IPM approach

Arthropods, weeds, and diseases threaten food security and natural resources, where many of the population derives their livelihoods. Yield losses experienced due to pests often result in food uncertainty and reduced revenues, particularly for rural communities in developing countries and farming enterprises in developed countries. In addition, pests also affect natural resources such as water, forest, and animals, thus reducing the income generated from the services provided by the natural resources, for instance, water supply for irrigation. The main goal of introducing innovations into agriculture, therefore, is to improve yields and thus livelihoods of agriculture-dependent households. While synthetic chemicals have been widely promoted as a remedy for managing invasive pests, most farming communities in developing countries are resource-constrained and have limited purchasing power to afford pesticides. Furthermore, chemical pesticides are often associated with high human and environmental risks. Mis(over)use of chemical pesticides is associated with increased pest resistance, pollution of natural resources, and negative impacts on ecosystem service providers such as pollinators, natural enemies and other beneficial insects.

Alternative innovations that are cost-effective and eco-friendly are recommended to control invasive pests in place of synthetic chemical pesticides. Integrated pest management (IPM) has been promoted globally as an alternative crop protection paradigm. While there are several definitions of IPM, it involves coordinated integration of multiple complementary methods, that targets different stages of a pest, and suppress them in a safe, cost-effective, and environmentally friendly manner. IPM definition is dynamic in design and implementation. As demonstrated in Chapter 18- Impact of Integrating Autodissemination with Male Annihilation Technique on Fruit fly Infestation- constant review and improvement of the IPM techniques are required to address the perpetual transposition of the target pest. Ecostacking, for instance, can be adopted in an IPM approach to enhance the performance of the innovation in reducing pest population, minimizing crop loss, and increasing

yield. The impact of integrating Auto Dissemination with the Male Annihilation Technique (MAT) on fruit fly infestation on mango yield is assessed in our context. While MAT involves attracting and trapping male fruit flies, the Auto Dissemination Technique (ADT) entails attracting the male fruit flies into a trap and allowing them to pick spores of an entomopathogenic fungus (*Metarhizium anisopliae* -based biopesticides) and spread them to their habitats thus infecting their conspecifics through their innate behavior. The results revealed that integrating MAT and ADT, reduced the *Bactrocera dorsalis* infestation rate by more than 48% than when using MAT alone, significantly reducing mango losses, improving yield, and hence increasing earnings of mango farmers. Therefore, integrating the ADT into the conventional IPM may improve sustainable management of the invasive fruit fly in SSA and improve economic gains via reduced yield losses.

Ecostacking strategies for avocado production in Florida

The application of ecostacking techniques as an integrated plant protection management strategy for Florida avocado groves is being developed. Supported by substantial evidence, some of these techniques offer viable options for immediate adoption, while many others need further research, development, and testing. Appropriate measures should be selected based on site-specific factors and practical considerations. By incorporating multiple components, the overall efficacy and impact of ecostacking techniques can be maximized, potentially yielding additive or synergistic effects. The primary objective is to harness ecosystem services provided by diverse organisms within the grove ecosystem while minimizing reliance on chemical pesticides to preserve beneficial organisms and their benefits. By capitalizing on the ecosystem services provided by various organisms within the grove ecosystem, ecostacking techniques promote natural pest control, pollination, and nutrient cycling.

Minimizing the reliance on chemical pesticides is crucial to maintaining a balanced and resilient avocado grove ecosystem. While chemical pesticides may provide short-term pest control, their usage can disrupt and compromise the activities of beneficial organisms. Consequently, adopting ecostacking techniques offers an alternative approach that reduces the need for chemical pesticides, thereby safeguarding the functionality of these beneficial organisms. We acknowledge that ecostacking techniques are not universally applicable and may yield varying results depending on the grove and pest dynamics. Therefore, flexibility and adaptability are crucial for long-term integrated plant protection management success.

One component of ecostacking techniques that could be readily implemented in many avocado groves is the use of cover crops. Field testing of these techniques is needed to verify the predictions derived from the scientific literature. Specifically, incorporating leguminous cover crops, such as white clover (*Trifolium repens*), sunn hemp (*Crotalaria juncea*), or velvet bean (*Mucuna pruriens*), between the rows has been shown to provide multiple benefits. Leguminous cover crops are known for their ability to fix atmospheric nitrogen through a symbiotic relationship with nitrogen-fixing bacteria. This nitrogen fixation process can contribute to the overall nutrient availability in the grove, reducing the need for synthetic nitrogen fertilizers. Moreover, leguminous cover crops can act as effective suppressors of weeds. The dense growth and shading provided by these cover crops can outcompete weeds, reducing their emergence and growth. This suppression of weeds not only helps to reduce competition for resources but also minimizes the reliance on herbicides for weed control, aligning with the principles of integrated plant protection.

In addition to nitrogen fixation and weed suppression, cover crops can serve as valuable habitats for beneficial organisms in the grove ecosystem. Introducing flowering cover crops, or allowing weeds such as *Bidens alba* to grow within the tree rows, can provide nectar and pollen resources, attracting and supporting pollinators. This, in turn, enhances pollination services and promotes fruit set and

yield in avocado trees. Flowering cover crops also serve as attractive habitats for natural enemies of pests, including predators and parasitoids. By providing alternative prey and shelter, these beneficial organisms contribute to biological control, helping to suppress pest populations naturally. In mango cultivation, Kleiman et al. (2022, Kleiman and Koptur 2023) showed that weeds promoted pollinators and beneficial insects.

Further integration of pollination services, mycorrhizal inoculation, and organic fertilizers in integrated plant protection management provides valuable ecostacking tools for enhancing the sustainability and productivity of Florida avocado groves. These practices are expected to contribute to improved pollination, enhanced biocontrol of pests and diseases, and optimized soil processes. However, continued research, field testing, and knowledge sharing is needed.

Developing and implementing ecostacking components specifically tailored to address the laurel wilt problem also require further research and development. Exploring the use of phoretic mites, fungal endophytes, mycoviruses, and leveraging avocado genetic diversity and semiochemicals offer exciting avenues for integrated plant protection against laurel wilt and associated pests. By conducting comprehensive studies in these areas, we can enhance our understanding of these components' ecological interactions and potential applications, ultimately advancing sustainable management strategies for the laurel wilt challenge in avocado groves.

Stacking the socio-cultural dimension

Agricultural systems are intrinsically socio-ecological systems where farmers are undoubtedly the central actors. Yet, their actions are influenced by many other players (i.e., their consumers, supply chain stakeholders upstream and downstream production, and regulatory and non-governmental institutions) notably when it deals with redesigning agroecosystems to reduce pesticide use (Thomine et al. 2022). Despite known agronomic and ecological benefits of crop and non-crop diversity (Hatt et al. 2018; Wan et al. 2020; Li et al. 2020), farmers' adoption of diversification practices remains low. Many studies have explored the willingness of farmers to diversify their farms, highlighting the factors affecting farmers' behavior, including the structural constraints they face (e.g., Lastra-Bravo et al. 2015; Meynard et al. 2018; Carlisle et al. 2022). Interestingly, Kleijn et al. (2019) suggested that public attitude and social motivations can significantly influence farmers' motivation to adopt biodiversity-based practices. This is what we could see through a case study on wildflowers strips (see Chapter 17), where the implementation of flower-rich semi-natural habitats aiming at supporting biodiversity in farms had positive psychological impacts on the farmers themselves, and generally improved their relationships with their neighbors. The importance of embellishing the landscape and improving the image society has on farming and farmers was especially highlighted by some of the producers interviewed. It suggests more systematically considering the socio-cultural benefits diversified farms and landscapes provide when conceiving, promoting, and implementing new agroecosystem designs. By embracing the inherent complexity of agricultural systems, ecostacking invites to integrate this socio-cultural dimension to agronomic and ecological innovations. In this way, triggering synergies is possible when practices enhancing the delivery of ecosystem services also improve the quality of life of farmers and society in general. For science, it notably implies conducting longer term research, as agronomic, ecological, and socio-cultural impacts may be observed through different time scales; develop multi-criteria indicators of performance to evaluate the diversity but also the intermingling of effects (e.g., Mottet et al. 2020); and more generally to adopt multi- and inter-disciplinary approaches, which realization—although largely advocated—is nonetheless not trivial (Ledford 2015).

ADDRESSING THE IMPLEMENTATION OF ECOSTACKING

Changes in cropping practices and pest management are difficult to achieve due to numerous, systemic “lock-ins”: growers are from many directions locked-in into the current production system. These include economic, logistic, social, and practical lock-ins. Researchers will have to break these lock-ins one by one. For example, compelling economic data need to be provided that the ecostacking approach will provide higher profits to the growers and enormous benefits to the local communities and society. Growers, decision-makers, and politicians all need to understand these arguments in order to gain their support for the proposed approach.

Policy-makers must understand the benefits of the ecostacking approach and start to favor these techniques. UN efforts to fight against the loss of biodiversity, such as the Convention on Biological Diversity (CBD), the Organic Agriculture Programme of FAO, and the UN Green Climate Fund (GCF), which provide financial support to developing countries to mitigate and adapt to climate change, are examples of international efforts to promote more sustainable food production. Similar UN programs in the future should promote ecostacking techniques as potential tools to fight against these global threats to food production. These global strategies should trickle down to localized policy-makers and encourage greater adoption of the ecostacking approach.

The implementation of ecostacking requires interdisciplinary collaboration and knowledge exchange among scientists, agronomists, policymakers, and farmers. Furthermore, at this stage, strong data sharing and collaborative work are needed between researchers to establish the data-based cumulative impacts of various strategies and synergy between different strategies. It is through these collaborative efforts that we can bridge the gap between scientific knowledge and on-the-ground application. Stakeholder engagement and participatory approaches play a vital role in the co-development and implementation of ecostacking strategies. By involving farmers and other stakeholders in the decision-making process, we can increase the likelihood of successful adoption and long-term sustainability of ecostacking practices.

Ecstacking is not a one-size-fits-all approach but rather a flexible framework that can be adapted to different agroecological contexts. The success of ecostacking strategies relies on continuous monitoring, evaluation, and refinement to ensure their effectiveness and adaptability over time. Stacking strategies should also be adopted based on available resources. By embracing a culture of adaptive management and learning, we can improve our understanding of the complex dynamics between functional biodiversity and ecosystem services and refine our strategies accordingly.

Ecstacking represents a transformative approach to integrated plant protection and sustainable crop production. By combining and optimizing the diverse components of functional biodiversity, we can harness the full potential of ecosystem services for enhanced pest control, pollination, and plant health. The success of ecostacking relies on a robust scientific foundation, interdisciplinary collaboration, and stakeholder engagement (see Fig. 1). Continued research, knowledge exchange, and practical implementation of ecostacking strategies are essential for achieving sustainable and resilient agricultural systems that reconcile ecological integrity, economic viability, and social well-being.



Fig. 1 Original logo for ecostacking, used by Hokkanen (2017). “Functional biodiversity in action on flower mixtures sown for biomass production in Bavaria, Germany”. Photo H. Hokkanen 2015.

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