

Microbiota and chemical ecology as biological levers for insecticide- and fungicide-free crop protection: A synthesis of WG3 breakthroughs

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Dr. İkbâl Agah İnce

Centre de Biologie Structurale, CNRS UMR 5048 - UM - INSERM U 1054, 29 rue de Navacelles 34090, Montpellier – France
Present address: Incubateur INITIUM de l'Université de Montpellier, 60 Rue des États Généraux, 34000, Montpellier, France

Abstract

European agriculture is under pressure to move beyond chemical pesticides, yet the alternatives are dispersed across separate scientific communities. This synthesis, prepared for Working Group 3 of the TOP-AGRI-Network, brings together three fronts of biological research that provide insecticide- and fungicide-free levers for crop protection: the plant and insect microbiota (the holobiont), the chemical ecology of insect–plant signalling (odorscapes and semiochemicals), and plant immunity within functionally diverse cropping systems. Across all three, the same shift recurs — from curative, broad-spectrum treatment to preventive, selective, biology-based protection. Microbe- and virus-based agents, semiochemical strategies and durable genetic resistance already reach the field, but their uptake is limited by field reproducibility, cost and an unsettled European regulatory landscape. Coordinated research and clearer regulation would let these levers be combined into the layered, preventive systems that a zero-pesticide agriculture requires.

1. Introduction and context

European crop protection remains largely curative and dependent on chemical pesticides, even as policy — the European Green Deal, the Farm to Fork strategy and successive pesticide-reduction targets — demands structural change rather than incremental adjustment. The TOP-AGRI-Network responds by pursuing a redesign of crop protection built on prophylaxis: preventing pest and pathogen pressure rather than treating it after the fact. Biological research supplies the mechanisms for that shift. This synthesis, contributed to Working Group 3, consolidates three converging fronts — microbiota and the holobiont, chemical ecology and odorscapes, and plant immunity within functionally diverse cropping systems — and treats them not as separate disciplines but as an integrated toolbox for insecticide- and fungicide-free protection. A thread running through the account is the interaction among insects, their pathogens, and their microbes, which recurs across the microbiota and chemical ecology fronts and links biological control to preventive design. The synthesis was prepared through cross-country exchange among Working Group 3 members and a structured reading of the literature, tracing each concept from its foundational work to the current state of the art.

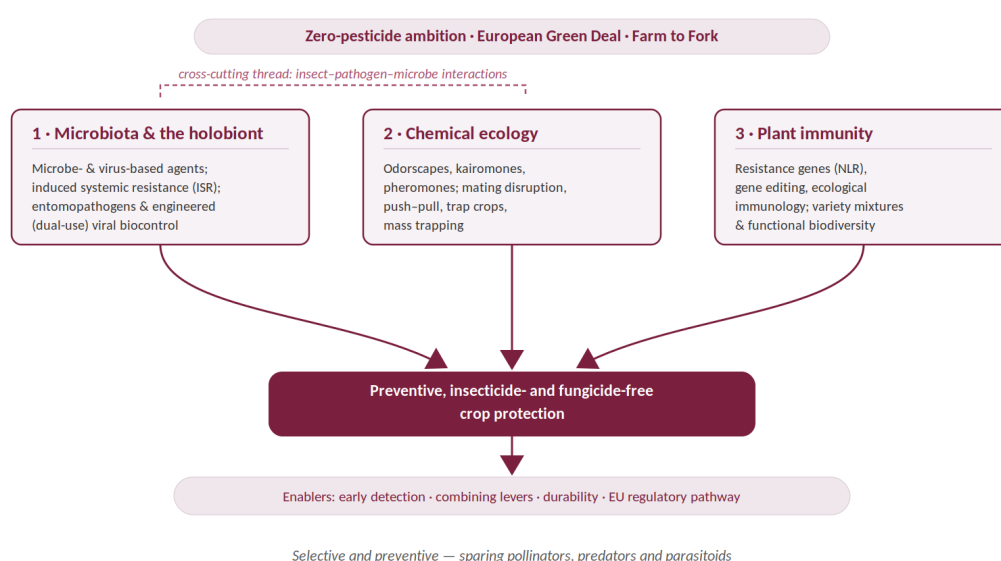


Figure 1. Three biological levers — microbiota and the holobiont, chemical ecology, and plant immunity within functionally diverse cropping systems — converging on preventive, insecticide- and fungicide-free crop protection. The cross-cutting insect–pathogen–microbe thread links the microbiota and chemical-ecology fronts.

2. Microbiota and the holobiont

Modern crop protection increasingly treats the plant not as an isolated organism but as a holobiont: the plant together with the microbial communities of its roots, shoots and internal tissues, whose combined activities shape nutrition, stress tolerance and health [13]. High-resolution definition of the root microbiome showed that these communities are structured and reproducible enough to be characterised and, in principle, managed [16]. Because microbes and the molecules they produce can substitute for agrochemical inputs, the composition of the plant microbiota becomes a manipulable determinant of crop resilience.

One of the clearest levers is microbe-induced immunity. Non-pathogenic rhizosphere bacteria trigger a plant-wide induced systemic resistance (ISR) effective against fungi, bacteria and viruses, acting through jasmonate and ethylene signalling rather than by directly attacking the pathogen [12]. Priming the crop's own defenses in this way is preventive by design, and it links the microbiota directly to the plant-immunity levers of Section 4.

The same priming principle extends to fungal microbiota. Arbuscular mycorrhizal fungi (AMF) confer mycorrhiza-induced resistance (MIR) against soil-borne pathogens such as *Phytophthora parasitica* in tomato, operating through crosstalk between salicylate and jasmonate signalling to prime localised and systemic defence responses [25][26]. *Trichoderma* spp., equally well established as fungal biocontrol agents, suppress soil-borne *Fusarium* through a combination of direct antagonism, JA-mediated ISR and reshaping of the rhizosphere microbiome [27]. Together, AMF and *Trichoderma* represent a fungicide-free layer of crop protection acting on the same preventive logic as bacterially mediated ISR.

Entomopathogenic fungi establish a further bridge between pest and disease control. When *Beauveria bassiana* and *Metarhizium brunneum* colonize plant tissues as endophytes, they alter the host volatile blend and reduce aphid performance, conferring resistance to both insect pests and phytopathogens [28]. Recent work shows that endophytic *M. brunneum* penetrates tomato leaves, directly infects leafminer larvae within their mines, and simultaneously primes systemic defence through ethylene- and jasmonate-related gene induction [29] — a dual insecticidal and disease-priming mode of action directly relevant to insecticide- and fungicide-free crop protection.

Turning these observations into reliable technology requires moving from correlation to causation. Synthetic microbial communities (SynComs) — defined, reconstructed consortia tested in controlled systems — let researchers establish which microbes cause which protective effects and engineer communities with predictable function [24]. Reproducibility under field conditions remains the central obstacle, but the approach converts the microbiome from a descriptive concept into a design space.

Alongside protecting the plant, microbes can directly control the pest. Microbial control of insects is long established: it runs from Metchnikoff's nineteenth-century use of the fungus *Metarhizium* and the early description of *Bacillus thuringiensis* to the foundations of insect pathology, and it is consolidated today in a large catalogue of entomopathogens — baculoviruses, *B. thuringiensis* and other bacteria, fungi and nematodes — deployed as selective biological insecticides [14]. Host specificity is the decisive contrast with broad-spectrum chemistry: an entomopathogen suppresses its target while sparing pollinators and natural enemies.

Insects themselves are holobionts. Their microbiota underpins nutrition, protection from pathogens, and immune priming, so microbiome-based thinking applied to crops also opens routes to managing pests through their microbial dependencies [15]. This connects to chemical ecology (Section 3), because insect-associated microbes help process the plant defensive chemistry that shapes host choice.

These strands converge in an emerging class of dual-use viral-engineering platforms. Functional-genomics mapping of insect-virus genomes, combined with rational or synonymous recoding, allows insect viruses such as densoviruses to be engineered in two directions: attenuated strains that protect farmed insects from viral collapse — a One Health biosecurity application for the fast-growing insect-farming sector — and, conversely, strains that selectively kill pest insects or reduce their feeding, acting as host-specific, insecticide-free biocontrol agents. This work is at an early stage — proof-of-concept, developing in European laboratories including at Montpellier — rather than a field-ready product, but it shows how evolutionary virology is extending the entomopathogen toolkit toward precisely targeted, engineerable protection.

3. Chemical ecology, odorscapes and semiochemicals

Chemical ecology reframes pest control as the management of information rather than of mortality. The field's foundations were laid when Karlson and Lüscher named the “pheromone” and Butenandt identified bombykol, the silkworm sex attractant, as the first chemically defined insect signal [1][2]. That work established a principle now

central to insecticide-free protection: insect behaviour is governed by defined molecules that can be identified, synthesised and deployed.

Plants participate actively in this chemical dialogue. When herbivores attack, plants release volatile organic compounds (VOCs) that recruit the herbivores' natural enemies — first shown when Dicke and Sabelis demonstrated that spider-mite-infested plants attract predatory mites, and generalised when Turlings, Tumlinson and Lewis found that caterpillar-damaged maize emits terpenoids that guide parasitic wasps to their prey [3][4]. These herbivore-induced plant volatiles (HIPVs) make the crop a signalling partner in its own defence.

Pests, in turn, exploit plant chemistry to find their hosts. Insects respond not to single compounds but to specific ratios within complex volatile blends, so the composition of a crop's odour — not merely its presence — determines whether a pest locates it [6]. These host-finding cues, the kairomones, are the levers that behavioural control manipulates. The insect's chemical world is further structured in space and time: odour plumes are turbulent and intermittent [5], and real-time measurement of the natural olfactory environment [8] led Conchou and colleagues to formalise the "odorscape" — the species-specific, habitat-dependent olfactory space through which an insect navigates [10]. Reshaping that odorscape, by masking attractive crop volatiles or adding more attractive decoys, reduces a pest's access to susceptible plants.

These principles have matured into deployable, largely non-toxic technologies. Trap cropping, in which a more attractive plant draws pests away from the crop [11], and mating disruption, in which synthetic sex pheromone saturates the air so that males cannot locate females, are long established; mating disruption is standard practice in European viticulture against grapevine moths. Cook, Khan and Pickett combined attractant and repellent stimuli into the push–pull strategy [7], which Khan and colleagues developed into a field-proven cereal system that also suppresses parasitic weeds [9]. Mass trapping and attract-and-kill formulations extend the same logic. The decisive advantage over broad-spectrum insecticides is selectivity: because each semiochemical targets a narrow set of species, beneficial and non-target insects — pollinators, predators and parasitoids — are largely spared, preserving the multi-trophic diversity on which agroecological regulation depends.

For the two value chains at the centre of this Action the relevance is direct: pheromone-based mating disruption is already deployed in viticulture, while VOC- and push–pull-based approaches are under development for aphid and lepidopteran pests of small grains. A frontier of particular promise is the chemical ecology of infection-related insect behaviour — how pathogens and entomopathogens alter the odour cues and host-seeking behaviour of insects — which could be exploited to engineer odorscapes that redirect pests or extend the reach of biological control agents.

Plant volatiles also prime neighboring plants against both pests and diseases. Exposure of citrus to the green leaf volatile (Z)-3-hexenyl propanoate activates immunity- and antioxidant-related genes, repels key citrus pests and attracts natural predators [30]; a subsequent transcriptomic study confirmed that the same compound activates SA/JA cross-signaling across citrus genotypes [31], indicating that semiochemical priming can reduce reliance on both insecticides and fungicides. Whether inducible plant defense traits are always compatible with natural-enemy communities providing biological control is not guaranteed: they can act as a double-edged sword, and breeders should target traits that reinforce rather than undermine existing biocontrol services [32].

4. Plant immunity, resistance genes and functional biodiversity

The genetics of resistance rests on Flor's gene-for-gene principle: a plant resistance (R) gene protects against a pathogen that carries the matching avirulence gene [17]. Jones and Dangl integrated this into a two-layer model of the plant immune system, in which surface receptors provide pattern-triggered immunity (PTI) and intracellular NLR (nucleotide-binding leucine-rich repeat) receptors provide effector-triggered immunity (ETI), the two locked in a co-evolutionary arms race with the pathogen [18]. This framework organizes how breeders understand and combine resistance genes.

Because pathogens evolve, single R genes tend to break down. McDonald and Linde showed that a pathogen's evolutionary potential — its population size, gene flow and reproductive system — predicts how quickly it will overcome resistance and argued for deployment strategies such as gene pyramiding and spatial or temporal diversity to keep resistance durable [19]. Durability, not mere presence, is therefore the goal for a preventive system.

Diversity is itself a control lever. In a landmark field experiment, mixing susceptible and resistant rice varieties reduced blast severity by 94% and enabled the abandonment of fungicide sprays, with yields matching or exceeding those of monocultures [20][21]. Such functional biodiversity — variety mixtures, niche complementarity and companion

planting — suppresses pests and pathogens at the field and landscape scale and underpins the redesigned cropping systems that a zero-pesticide agriculture requires.

Genome editing offers resistance without chemical inputs. Editing all three wheat MLO genes produced heritable, broad-spectrum powdery-mildew resistance in a staple cereal [22]. The European regulatory status of such crops is being reshaped: the Commission's 2023 proposal on new genomic techniques (NGTs) distinguishes edited plants that could have arisen naturally from those treated as conventional genetically modified organisms [P1], and the Council adopted the new rules in 2026, although final Parliament approval is pending and NGT plants remain banned in organic farming [P2].

Finally, ecological immunology reframes immunity as an ecological and evolutionary trait that carries costs and trade-offs [23]. This widens the available levers beyond single R genes to induced defences, tolerance and companion-plant effects, and connects plant immunity to the microbiota of Section 2 through holobiont-aware breeding. Taken together, genetic resistance and system-level diversity form the backbone of the preventive, insecticide- and fungicide-free protection this Action pursues.

5. From breakthroughs to biocontrol technologies

The three fronts are not competing alternatives but complementary layers of a single preventive system. A resistant or edited cultivar lowers baseline susceptibility; a managed microbiome primes the crop and suppresses soil-borne pathogens; semiochemicals and entomopathogens redirect or remove the pests that remain — each acting selectively and sparing beneficial organisms. Several of these levers are already commercial: microbial biocontrol agents and biostimulants, pheromone dispensers for mating disruption, and resistant cultivars and variety mixtures. Engineered entomopathogens, including the dual-use viral platforms discussed above, extend the range toward precisely targeted, host-specific agents. Combining layers multiplies their effect and slows the evolution of resistance, much as gene pyramiding does for single resistance genes. Realizing the full value of this toolbox depends on knowing when to act sensor- and molecular-based early detection of bioaggressors lets preventive measures be deployed before outbreaks establish, turning a set of biological tools into a coherent, anticipatory protection strategy.

6. Research gaps and Horizon Europe outlook

Common obstacles cut across the three fronts. Microbiome-based effects remain difficult to reproduce under field conditions; semiochemical strategies need cheaper delivery and validation beyond high-value crops; and genetic resistance must be made durable and, for edited crops, regulatorily viable. Shared enablers are equally decisive: reliable early detection, multi-actor deployment with farmers, and the economics of adoption. The most promising direction is integration — genotype-by-microbiota-by-immunity thinking (holobiont-aware breeding) and the deliberate combination of microbe- and semiochemical-based tools — which no single discipline can deliver alone. These gaps define a clear agenda for future Horizon Europe, ERC and Marie Skłodowska-Curie initiatives, and the community assembled by this Action is well placed to lead them, translating dispersed national advances into coordinated European research.

7. Conclusions

Taken together, the microbiota, the chemical ecology of insect–plant signalling and plant immunity within diverse cropping systems make preventive, insecticide- and fungicide-free crop protection scientifically credible rather than aspirational. Each front has moved from concept to field-relevant technology, and their interactions — particularly around insect–pathogen–microbe biology — point toward layered systems that reduce dependence on chemical pesticides. Consolidating these advances, and resolving the reproducibility, cost and regulatory gaps that limit them, is the contribution this synthesis offers to the TOP-AGRI-Network final report and to the wider transition towards a zero-pesticide agriculture.

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Supplementary Table S1 — the three biological levers

Front	Key mechanism	Biocontrol translation	Maturity	Key gap
Microbiota / holobiont	Holobiont: microbiota drive nutrition, stress tolerance and induced systemic resistance	Microbial biocontrol agents, biostimulants, SynComs; ISR-eliciting inocula	On market; field-variable	Field reproducibility; SynCom design; EU registration
Entomopathogens	Host-specific viruses/fungi/bacteria kill or suppress target pests	Baculovirus & Bt products; fungal agents; engineered / dual-use viral platforms	Registered (Bt, baculovirus)	Cost, speed, formulation; scaling; registration
Chemical ecology	Odorscape: insects navigate by volatile blends; semiochemical manipulation	Mating disruption, mass trapping, push-pull, trap crops	Field-deployed	Scaling beyond high-value crops; dispenser cost; EU rules
Immunity / functional biodiversity	Gene-for-gene → PTI/ETI; durability via diversity; genome editing	Resistant cultivars, variety mixtures, edited (MLO) lines	Mixtures proven; edited pre-deployment (EU)	R-gene durability; EU status of edited crops