



IMPACT



**Co-funded by
the European Union**

**Project Title: Integrated plant protection as an answer for climate change
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PR2/A1 Best Practices: New Diseases and Actions

Summary report

***Prepared by: MAICh
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Introduction

Anthropogenic climate change is exerting measurable and profound effects on agriculture that have become increasingly evident in the last two decades, and that are predicted to intensify in the future (Bebber *et al.*, 2003; Fischer *et al.*, 2013). Scientific consensus and bodies including the UN-FAO and the EC Directorate of Agriculture all acknowledge a clear trend of increasing average global temperatures and altered precipitation patterns (more intense rains leading to flooding interspersed with extended dry periods) and more frequent extreme weather events. These phenomena exert effects on crop plant growth and health, and also on pest and pathogen development. Global trade and the free movement of goods is resulting in the rapid distribution of existing and emerging pests worldwide, and climate change is allowing a greater proportion of newly-introduced pests to establish and thrive in their new environments. The net result of this effect is documented migration of insect, fungal and viral pests in temperate zones towards the poles of an average of 2.7km per year since 1960 (Bebber *et al.*, 2003). Also, in these regions the warmer conditions and milder winters also benefit existing insect populations by allowing a greater number of generations per year and more individuals to overwinter. The result is both larger amounts of insect damage, but also a greater capacity for the transmission of viral and bacterial diseases by vector species.

INPACT Deliverable PR1/A2 detailed the results of a survey conducted among farmers and agricultural stakeholders in the project member countries (Bulgaria, the Czech Republic, Greece, Hungary, Poland and Romania). There was a consensus among respondents that agriculture in this region is already being seriously affected by climate change, with the majority of participants from all countries reporting that drought, flooding, and other climatic phenomena are increasingly causing stress to their crops, resulting in less productive and less healthy plants that are more vulnerable to pathogen attack. They also reported fungal, oomycete and mildew infestations of increased frequency and severity, with the majority stating that prevailing climatic conditions have made existing treatments and regimes less effective. In the more northern countries milder winters have increased the numbers of overwintering insects and insect generations per year, leading to more crop losses both from insect damage or from the pathogens they transmit. In all partner countries either the introduction of novel pests and diseases or a decreased ability to control existing pests was reported.





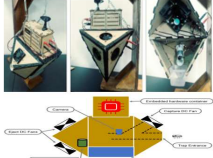

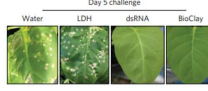
Results

The present document PR2/A1 represents a collection of Best Practices in Crop Protection (Table 1; Table 2) addressing plant protection issues of high or emerging importance in crop protection in the project member countries. The interventions chiefly address but are not limited to the crop groups of interest to the project (stone fruits, cabbage, tomato, pepper, root vegetables and cucumber). The practices included address viral, bacterial, fungal, and insect pests, and cover the entire spectrum of plant protection interventions – from preventative measures designed to increase the health, not just of the crop plants but of the whole agroecological system (including cultural control methods such as crop rotation, the use of trap crops, tillage, etc.), the use of beneficial microorganisms to boost plant health and to out-compete pathogenic organisms, and the use of resistant crop varieties or hybrids. The practices also include the use of novel mechanical control measures, including smart traps that use artificial intelligence to selectively kill only pathogenic insects, and the use of predators and natural enemies of pests such as entomopathogenic microorganisms. Plant protection protocols that combat pathogens using compounds less toxic to humans and to the environment, such as plant-derived pesticides and natural products, mineral oils, biorational insecticides, and insect growth regulators are favoured, but also exquisitely pathogen-specific technologies such as the use of RNA interference (RNAi) to specifically boost the immune plant system to combat pathogens. When no alternative exists to the use of synthetic insecticide compounds, their use via integrated pest management protocols that target applications, and minimise use and limit the exposure of non-target species is recommended as part of a holistic integrated pest management (IPM) approach.

Table 1. A list containing the INPACT Innovative and/or Environmentally-Friendly Crop Protection Practices.

no.	Innovative and /or Environmentally Friendly Practice in Crop Protection	Applicability (host/ pest/ pathogen)
1	Cross-Protection against <i>Pepino mosaic virus</i> Severe Strains in Tomato	tomato vs. <i>Pepino mosaic virus</i>
2	Mutagenesis of a host virus susceptibility gene using the CRISPR/Cas9 technology	tomato vs. <i>Tomato brown rugose fruit virus</i>
3	Insect Pest Monitoring - Camera equipped Traps	Various insect pests
4	<i>Beauveria bassiana</i> (white muscardine fungus) Entomopathogenic Fungus	<i>Cephus pygmeus</i> , <i>Helicoverpa armigera</i> , <i>Lobesia botrana</i> , <i>Popillia japonica</i> , <i>Spodoptera frugiperda</i> , thrips, aphids, whiteflies
5	Clay Nanosheets for Topical Delivery of RNAi against Plant Viruses - Nanophytovirology	Various phytopathogenic RNA viruses
6	Plant growth-promoting <i>Rhizobacteria</i> -mediated induction of systemic resistance	Phytopathogenic viruses/ Broad spectrum innate defence against pathogens
7	Stacking of ecosystem services: mechanisms and interactions for optimal crop protection,	Overall crop protection ecosystem management
8	Exogenous RNAi for sustainable Crop Protection	Against fungi, bacteria & viruses infesting crops
9	Gene Editing [CRISPR- Cas9 technology] in Crop Protection	Against fungi, bacteria & viruses infesting crops
10	Cover Crops are more effective than insecticides for managing pests	Insect pests
11	Effect of kaolinite clay on migrant alate green peach aphid in orchards	Peach aphid
12	Bio-insecticide for the efficient control of <i>Spodoptera</i> species and other Noctuid moths	Spodoptera species and other Noctuid moths
13	Effect of silicon on two major insect pests of tomato	Tomato insect pests (whiteflies, <i>T. absoluta</i>)
14	Biological control of western flower thrips using the entomopathogenic fungus <i>Beauveria bassiana</i>	Thrips (<i>Frankliniella occidentalis</i>) in various crops
15	Control of the tomato leaf miner with baculoviruses	<i>T. absoluta</i> in tomato
16	Mechanical control of peat fly larvae by sharp sand application	Peat fly larvae
17	Use of nudibranch ash extract to deter Spanish naked snails	Spanish naked snails
18	Control of aphids and thrips using a decoction of spindle berries & tansy	Aphids, thrips
19	Protection against mites using tobacco extracts	Mites
20	Crop rotation for crop protection in organic agriculture	Overall management of pests
21	Fungicidal plant protection product based on <i>Thymus vulgaris</i> essential oil	<i>Fusarium culmorum</i> , <i>Blumeria graminis</i> and <i>Pyrenophora teres</i> on cereals
22	EDN - Ethadinitrile	<i>Ips typographus</i> , <i>Ips duplicatus</i> x <i>Picea abies</i>
23	Bluefume - HCN	<i>Ditylenchus dipsaci</i> , <i>Aceria tulipae</i> , <i>Fusarium</i> sp.
24	'Rubelit' apple trees resistant to apple scab caused by <i>Venturia inaequalis</i>	Apple scab fungus (<i>Venturia inaequalis</i>)
25	Protection against <i>Peronospora destructor</i> (onion blight) using the essential oil of <i>Pelargonium graveolens</i>	<i>Peronospora destructor</i> in onions
26	Neem oil against insects	Small soft-bodied insects like aphids, mealybugs, mites, thrips & whiteflies
27	PREV-GOLD - orange oil	Powdery mildew, gray mold, spider mite species, moths and other insects with stinging and sucking mouthparts
28	Naturalis- L <i>Beauveria bassiana</i> insect parasite fungus	Numerous arthropod pests
29	<i>Bacillus thuringiensis</i> ssp <i>kurstaki</i> against lepidoptera	Lepidoptera
30	Caolin to manage whiteflies, <i>Ceratitis capitata</i> , oriental fruit fly	Whiteflies, <i>Ceratitis capitata</i> , oriental fruit fly
31	Use of Nemastar against <i>Steinemema carpocapsae</i>	<i>Steinemema carpocapsae</i>
32	Mating disruption techniques against moths	Moths
33	Effect of <i>Sorghum sudanese</i> (Sudan grass) as secondary crops	<i>Agrobis segetum</i> , <i>Elateridae</i> spp. larvae, <i>Delia brassicae</i> , <i>Phyllotreta atra</i> , thrips, <i>Tethranichus</i> spp.
34	Secondary effect of fenugreek / <i>Trigonella foenum-graec</i>	<i>Agrobis segetum</i> , <i>Elateridae</i> spp. larvae, <i>Delia brassicae</i> , <i>Phyllotreta atra</i> , thrips, <i>Tethranichus</i> spp.
35	<i>Heterothabditis bacteriophora</i> nematode against the vine weevil <i>Otiorynchus ligustici</i>	Vine weevil <i>Otiorynchus ligustici</i>
36	Use of Erwiphage bacteriophage products against fireblight (<i>Erwinia amylovora</i>)	<i>Erwinia amylovora</i> bacterium
37	Use of <i>Delphastus catalinae</i> against the tobacco whitefly (<i>Bemisia tabaci</i>) in greenhouse grown vegetables	Whitefly (<i>Bemisia tabaci</i>)
38	Combined use of <i>Beauveria bassiana</i> and <i>Arthrobotys oligospora</i> against cockchafer grubs	Cockchafer grubs
39	Orange oil as dormant oil against the overwintering forms of insect pests	Various insect pests
40	Diatomaceous earth to eliminate various bugs and pests	Various bugs & pests
41	Metarhizium: jack of all trades, master of many	Different arthropods
42	Natural Resistance Genes against Plant Viruses	Various plant infecting viruses

Table 2. A list of illustrative presentations of the 42 selected INPACT Innovative and/or Environmentally-Friendly Crop Protection Practices.

<p>INPACT Innovative & Environmentally-Friendly Methods in Crop Protection</p> <p>1. Cross-Protection (vaccination) against <i>Pepino Mosaic Virus Severe Strains</i> in Tomato</p> <p>Tomato (<i>Lycopersicon esculentum</i>) X <i>Pepino mosaic virus</i> (PepMV) PepMV is a mechanically-transmitted plant pathogen present throughout Europe that is chiefly controlled by the implementation of strict hygiene conditions. Infection of tomatoes with the most aggressive strain of the virus causes severe fruit marbling (Fig. 1.1) but pre-infection with mild isolates in areas where the disease is endemic can provide protection and prevent symptom development (cross protection).</p>  <p>Mechanism of Action & Use Cross protection was first described in 1929 by H. H. McKinney (Fig. 1.2), who demonstrated that inoculation with a mild strain of a particular virus can induce protection against a subsequent challenge with a severe strain of the same virus. During the 1990s, a natural phenomenon of immunity in plants was discovered: a sequence-specific RNA-based mechanism that protects plants from invading pathogens called RNA interference or silencing. Cross protection is the result of RNA silencing and in the case of PepMV, the symptoms in "vaccinated" tomato plants following infection with severe strains in the field can be greatly reduced (Aguero et al., 2018). Furthermore, V10, a natural crop protection product developed by Valto and distributed by Koppert Biological Systems, is used to prevent the emergence of PepMV.</p> <p>ERASMUS+ project INPACT – www.inpactproject.eu Ref: Aguero et al., 2018. <i>Frontiers in Plant Science</i> 9, Article 1810; https://www.frontiersin.org/newsdetail.aspx?id=52448</p>	<p>INPACT Innovative & Environmentally-Friendly Methods in Crop Protection</p> <p>2. Mutagenesis of a host virus susceptibility gene using the CRISPR/ Cas9 technology</p> <p>Tomato (<i>Lycopersicon esculentum</i>) X <i>Tomato brown rugose fruit virus</i> (ToBRFV). ToBRFV is an rapidly-spreading virus that affects tomato plantations, where losses can reach 100% (Zhang et al., 2022; Fig. 2.1). ToBRFV is transmitted mainly <i>via</i> contaminated seeds, or mechanically through standard horticultural practices. First reported in the Middle East in 2015, multiple ToBRFV outbreaks have been reported throughout Europe in recent years (Fig. 2.2). ToBRFV can break down genetic resistance to tobamoviruses conferred by the R genes Tm-1, Tm-2, and Tm-22 in tomato and L1 and L2 alleles in pepper. Currently, no commercial ToBRFV-resistant tomato cultivars are available.</p>   <p>Mechanism of Action & Use Ishikawa and co-workers (2022; Fig. 2.3) used the CRISPR/Cas9 technology to mutate four tomato homologues of TOBAMOVIRUS MULTIPLICATION1 (TOM1), an <i>Arabidopsis</i> gene, which is essential for tobamovirus multiplication, conferring resistance to ToBRFV in tomato plants.</p> <p>ERASMUS+ project INPACT – www.inpactproject.eu Ref: Zhang et al., <i>Mol.Plant Path.</i> 23,1262-1277; Ishikawa et al., 2022. <i>Plant Phys.</i> 189, 679-686</p>																																																																				
<p>INPACT Innovative & Environmentally-Friendly Methods in Crop Protection</p> <p>2. Mutagenesis of a Host Virus Susceptibility Gene using the CRISPR/ Cas9 Technology</p> <p>TABLE 3 Summary of studies that have employed CRISPR/Cas9 strategies for the targeting of host susceptibility genes</p> <table border="1"> <thead> <tr> <th>Plant species</th> <th>Name of the susceptibility (S) gene targeted</th> <th>Virus name</th> <th>Reference</th> </tr> </thead> <tbody> <tr> <td><i>Arabidopsis thaliana</i></td> <td>Atorf44E</td> <td>Turnip mosaic virus (TMV)</td> <td>Pyret et al. (2016)</td> </tr> <tr> <td></td> <td>atf4E1</td> <td>Clover yellow virus (CYV)</td> <td>Bastis et al. (2019)</td> </tr> <tr> <td><i>Hordeum vulgare</i> (barley)</td> <td>orf4E1</td> <td>Barley mild mosaic virus (BaMMV)</td> <td>Hoffie et al. (2021)</td> </tr> <tr> <td><i>Manihot esculenta</i> (cassava)</td> <td>ncBP-1/2</td> <td>Cassava brown streak virus (CBSV)</td> <td>Gomez et al. 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Electronic traps can eliminate pests without the use of pesticides, and smart traps with IoT (Internet of Things) capabilities and computer vision can selectively target specific pests.</p>  <p>Mechanism of Action & Use The trap consists of three components: a) an embedded system with a camera, GPS sensor, and motor actuators, b) a database service provider, and c) a web application that displays data through a configurable heat map. When a beetle enters the trap, successive images are processed, and compared with standard CBB body characteristics. If identification is positive, the capture fan directs the CBB into a cage where it is imprisoned and destroyed (Fig. 3.2; Figuredo et al., 2020).</p>  <p>ERASMUS+ project INPACT – www.inpactproject.eu Ref: Figuredo et al., 2020. <i>JMP Pest Science</i>, 94, 203-217</p>
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<p>INPACT Innovative & Environmentally-Friendly Methods in Crop Protection</p> <p>4. <i>Beauveria bassiana</i> (white muscardine fungus) Entomopathogenic Fungus</p> <p>White muscardine fungus [<i>Beauveria bassiana</i>] x various insect pests [<i>Cephus pygmeus</i>, <i>Helicoverpa armigera</i>, <i>Lobesia botrana</i>, <i>Papilio japonica</i>, <i>Spodoptera frugiperda</i>, <i>thrips</i>, <i>aphids</i>, <i>whiteflies</i>]</p> <p><i>Beauveria bassiana</i> is a fungus that grows naturally in soils throughout the world that parasitizes various arthropod species causing white muscardine disease (Fig. 4.1). It is used as a biological insecticide to control a number of pests, including termites, trips, whiteflies, aphids and various beetles. In culture, <i>B. bassiana</i> grows as a white mould and produces many dry, powdery conidia. The spores are sprayed on affected crops as an emulsified suspension or wettable powder (Wikipedia).</p>  <p>Mechanism of Action & Use Entomopathogenic fungi are a group of fungi living in soil that infect insects by penetrating their cuticle, eventually killing and feeding on them (Dara, 2017). After invading insect hosts, <i>B. bassiana</i> produces a variety of toxins (secondary metabolites) including beauvericin, bassianin, bassianolide, beauvericoides, tenellin, oosporein, and oxalic acid, that help <i>B. bassiana</i> to parasitize and kill the hosts (Wang et al., 2021).</p> <p>ERASMUS+ project INPACT – www.inpactproject.eu Ref: Dara et al., <i>Am. J. Plant Sc.</i> 8, 1224-1233; Wang et al., 2021. <i>Front. In Microbiology</i> 12, 705343.</p>	<p>INPACT Innovative & Environmentally-Friendly Methods in Crop Protection</p> <p>5. Clay Nanosheets for Topical Delivery of RNAi against Plant Viruses - Nanophytovirology</p> <p>Tobacco, tomato X <i>Cucumber mosaic virus</i> (CMV) Phytoviruses are highly destructive plant pathogens, causing significant agricultural losses due to their genomic diversity, rapid and dynamic evolution, and the general inadequacy of management options such as chemical means.</p>  <p>Mechanism of Action & Use dsRNA is the triggering molecule of RNA silencing. Non-toxic, biodegradable, layered double hydroxide (LDH) clay nanosheets can be loaded with dsRNA. After spraying onto plants the LDH breaks down, and plant cells take up the dsRNA causing topical silencing of homologous RNA viruses (Mitter et al., 2017). A single spray with dsRNA-loaded LDH (BioClay) was shown to provide viral resistance for at least 20 days. The method seems promising means to protect against plant viruses (Fig. 5.2; Fig. 5.3) and bacterial diseases (Fig. 5.3; Ren et al., 2022).</p>  <p>ERASMUS+ project INPACT – www.inpactproject.eu Ref: Mitter et al., <i>Nature Plants</i>, 2017; Ren et al., 2011, 11, 891.</p>																																																																				

INPACT Innovative & Environmentally-Friendly Methods in Crop Protection

6. Plant growth-promoting *Rhizobacteria*-mediated induction of systemic resistance

Tobacco / various plant viruses [CMV, TYLCV, TSWV]
 Plant growth-promoting rhizobacteria (PGPR) are diverse groups of plant-associated microorganisms, which can reduce the severity or incidence of disease by antagonism with bacteria and soil-borne pathogens, as well as by eliciting a systemic resistance defensive response in host plants (Meena et al., 2020).

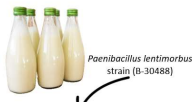


Fig. 6.1

Mechanism of Action & Use
 Soil inoculation with *Paenibacillus lentimorbus* (B-30488) isolated from cow's milk increased plant vigour, while significantly decreasing (91%) *Cucumber mosaic virus* (CMV) RNA accumulation in systemically-infected tobacco leaves (Kumar et al., 2016; [Fig. 5.5.3.1](#)). In this study, defence-related enzyme production induced by CMV-infection was ameliorated in B-30488-treated plants, suggesting that systemic induced resistance mediated the protection against CMV.




Fig. 6.2

ERASMUS+ project INPACT – www.inpactproject.eu
 Ref: Meen et al., 2020. J. Basic Microb. 60, 828-860; Kumar et al., PLOS One 2016, <https://doi.org/10.1371/journal.pone.0163350>.

INPACT Innovative & Environmentally-Friendly Methods in Crop Protection

7A. Stacking of ecosystem services: mechanisms and interactions for optimal crop protection, pollination enhancement and productivity – EcoStack [EU-funded project]

Pest(s) X Host Range: mainly insect pests

Project aims & Method mechanism: EcoStack project will develop and support ecologically, economically- and socially-sustainable crop production via stacking & protection of functional biodiversity ([Fig. 7A.2.4](#)).

More specifically:
 a. It will assess sustainable crop production needs based on functional biodiversity, using an interactive forum of stakeholders,
 b. It will evaluate and optimise the role of main off-crop habitats supplying ecosystem services for crop production,
 c. It will design and test in-crop interventions, which support the generation of ecosystem services (Hokkanen et al., 2017) within the crop, and which may carry over to the next crop in the rotation,
 d. It will develop, design & implement integrated systems for optimised provision of ecosystem services and use of plant protection tools, with focus on ecological, economic and social sustainability of integrated systems.




Fig. 7A.1




Fig. 7A.2

Photos by Rothamsted Research Limited (UK)

ERASMUS+ project INPACT – www.inpactproject.eu
 Ref: <https://www.ecostack-h2020.eu/>; Hokkanen et al., 2017. Arthropod-Plant Interactions 11, 741-742.

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7B. Agroecosystem services: A review of concepts, indicators, assessment methods & research perspectives

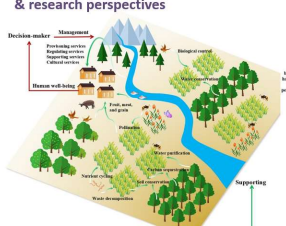


Fig. 7B.1. Ecosystem services within the agroecosystem. Biodiversity is the basis of agroecosystem, and provide many ecosystem services which are usually affected by social management (Liu et al., 2022).

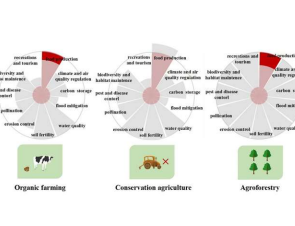


Fig. 7B.2. Comparison of conventional intensification (shown in red) and alternative farming approaches (shown in grey) for AES trade-offs (Liu et al., 2022).

ERASMUS+ project INPACT – www.inpactproject.eu
 Ref: Liu et al., 2022. Ecological Indicators 109218.

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8. Exogenous RNAi for sustainable Crop Protection:

Pest(s) X Host Range: potentially ALL

Introduction: Pioneering early work on the overexpression of the chalcone synthase gene (van der Krol et al., 1990) in petunia and Tobacco etch virus (Lindbo & Dougherty, 1993), led to the identification of the phenomenon present in many eukaryotic organisms, RNA-activated sequence-specific RNA degradation. The Nobel Prize in Physiology/ Medicine 2006 was shared by A. Fire and C. Mello for the discovery that double-stranded (ds) RNA triggers suppression of gene activity in a homology-dependent manner, a process named RNA interference (RNAi) (Fire et al., 1998).

Mode of action: the accumulation of dsRNA in plant cells triggers RNAi through its recognition and cleavage into 21-24 nt small interfering (siRNAs) by an RNaseIII-like enzyme called DICER. siRNAs guide a nuclease complex referred to as the RNA-induced silencing complex (RISC) to homologous single-stranded (ss) mRNAs that are degraded. Researchers have learned how to trigger RNAi for specific genes, which can result in better disease and pest resistance ([Fig. 8.1 & 8.2](#)).



Fig. 8.1



Fig. 8.2

ERASMUS+ project INPACT – www.inpactproject.eu
 Ref: van der Krol et al., 1990. PMB 14, 457-466; Lindbo & Dougherty 1993, Virology 189, 725-733; Fire et al., 1998. Nature 391, 806-811

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Foliar application of clay-delivered RNA interference for whitefly control



Fig. 8.3

Can dsRNA be applied curatively and preventatively?

Treatment scenarios:
 24 hours post-infection
 6 days post-infection (first symptoms)
 8 days post-infection (first pustules)
 16-18 days post-infection (established infection)



Fig. 8.4

Can we control fungal/oomycete diseases with exogenous RNAi?

Testing different RNA application methods



Fig. 8.5



Fig. 8.6

ERASMUS+ project INPACT – www.inpactproject.eu
 Ref: <https://www.youtube.com/watch?v=trk6GXvHcU>; <https://www.youtube.com/watch?v=UcN8IZnqAR0> [Risk Awareness]

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9. Gene Editing [CRISPR-Cas9 technology] in Crop Protection

Pest(s) X Host Range: potentially ALL

Mode of action: CRISPR is a family of DNA sequences found in the genomes of prokaryotic organisms derived from DNA fragments of bacteriophages that had previously infected the prokaryote. Cas9 (or "CRISPR-associated protein 9") is an enzyme that uses CRISPR sequences as a guide to recognize and cleave specific strands of DNA that are complementary to the CRISPR sequence. CRISPR-Cas9 is a technology that can be used to edit genes within organisms (Jinek et al., 2012). This editing process has a wide variety of applications including basic biological research, development of biotechnological products, and treatment of diseases ([Fig. 9.2.4](#); Karavolias et al., 2012).

The development of the CRISPR-Cas9 genome editing technique was recognized by the Nobel Prize in Chemistry in 2020, which was awarded to E. Charpentier and J. Doudna.

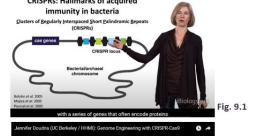


Fig. 9.1




Fig. 9.2

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 Ref: Jinek et al., 2012. Science 337, 814-821; Karavolias et al., 2021. Front Syst. Food Systems 07. <https://www.youtube.com/watch?v=Su4xVYB7K0>; <https://www.youtube.com/watch?v=TdBAHexVzyc>

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SCIENTIFIC REPORTS

ARTICLES

Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion

Ricardo Olivares^{1,2,3}, Chenghui Ji⁴, Genesio Almeida-Granda^{1,2,3}, José C. Higuera-Sagasti^{1,2,3}, Álvaro Pérez Quintana^{1,2,3}, Ting Li⁵, Joon-Seok Eom⁶, Cheolho Yi⁶, Harun Nguyen⁷, Ba-Mi⁸, Florence Auguy⁹, Galina Solov'yeva¹⁰, Yan T. Lian¹¹, Gerhard S. Dörner¹², Schirineh Ghassebi¹³, Sarah M. Schmidt¹⁴, Ines H. Stamm-Landst¹⁵, Catalina Vera Cruz¹⁶, Boris Szovák¹⁷, Wolf B. Frommer^{18,19}, Frank R. Wilhelm²⁰ and Bing Yang²¹

Fig. 9.3

Establishing RNA virus resistance in plants by harnessing CRISPR immune system

Tang Zhang¹, Qiang Zhang², Xu Yi³, Hong An⁴, Yang Zhao⁵, Siqun Ma⁶ and Guohua Zhou⁷

Fig. 9.5

Establishing a CRISPR-Cas-like immune system conferring DNA virus resistance in plants

Xiang Jiao¹, Huihui Zhang², Yi Zhang³, Yanping Wang⁴ and Cailin Guo⁵

Fig. 9.6

ERASMUS+ project INPACT – www.inpactproject.eu

Ref: <https://www.youtube.com/watch?v=7hwuKLVtE>

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10. Cover Crops are more effective than insecticides for managing pests

Insect pests X corn (*Zea mays*) – potentially ALL insects

Cover crops, planted to cover the soil rather than for harvest can regulate soil erosion, soil fertility, soil quality, water, weeds, pests, diseases, biodiversity and wildlife in an agroecosystem. Cover crops may be an off-season crop planted after harvesting of the cash crop, be planted between crop plants, or grow over winter.

Mode of action: Increasing scientific evidence indicates that no-till & cover crops support populations of resident arthropod predators and protect annual crops from insect pests. On the other hand, the use of neonicotinoid seed coatings, represents a common practice against early-season insect pests. The interaction between preventive pest management (PPM), integrated pest management (IPM) and the conservation practice of cover cropping was investigated by Rowen and co-workers (2022) in a 3-year

corn-sow (*Zea mays-Glycine max* L.) rotation, the response of invertebrate pests and predators to PPM and IPM, with and without a cover crop.

Results: PPM in year 1 decreased predation compared to a no-pest management control. Contrary to expectations, the IPM strategy requiring a single insecticide application, was more disruptive to the predator community than PPM, likely because the applied pyrethroid was more acutely toxic to a wider range of arthropods than neonicotinoids. Enhanced early-season cover was more effective at reducing pest density and damage than either intervention-based strategy. As part of a conservation-based approach to farming, cover crops can promote natural-enemy populations that can support effective biological control of insect pest populations.

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Ref: Rowen et al., 2022. *Ecological Applications* 32, e2598

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11. Effect of kaolin clay on migrant alate green peach aphid in orchards

Peach [*Prunus persica* L.] X *Myzus persicae*

The green peach aphid, *Myzus persicae* (Hemiptera: Aphididae), is a serious pest in peach and nectarine orchards (Fig. 11.1). Direct feeding damage caused by heavy infestations early in the spring leads to leaf curling and severe disturbances in shoot growth. *Myzus persicae* is an efficient vector of the *Plum pox virus* that causes the notorious "sharka" disease.

Mechanism of Action & Use:

Surround® WP Kaolin Clay forms fine film coatings of microscopic mineral particles are sprayed onto plant surfaces (Fig. 11.2), acting as a protective barrier that either controls or suppresses pests, while at the same time many beneficial pests that do not feed on the plant surfaces generally remain unharmed. It protects fruit against direct sunburn and heat stress damage, promotes plant health, which leads to more efficient photosynthesis and higher yields under extreme light and heat growing conditions. Autumn applications might be an alternative to the insecticides commonly used in spring to control aphids in orchards. The use of kaolin clay to impede aphid egg laying in autumn reduces winter egg-laying by about 50%. It is not sufficient to control aphid colonies in the spring, but it could be used as part of a supervised control strategy, combined with the application of mineral oils in late winter.

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Ref: Garcin, A., Millan, M. 2015. *Infos-Citri*, (311), 36-43. <https://grownaturally.com/products/surround-kaolin-clay-insecticide>

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12. Bio-Insecticide for the efficient control of Spodoptera species and other Noctuid moths

Wide range of crops X *Spodoptera* spp., *Helicoverpa armigera*

Noctuid moths (Lepidoptera: Noctuidae) (Fig. 12.1) are polyphagous pests with a cosmopolitan distribution, damaging many economically important crops. They are widespread in distribution throughout Asia, Africa, Australia and the Mediterranean Europe. Noctuid moths have a high reproductive rate and cause heavy losses to crops. Larvae (Fig. 12.2) feed gregariously on plant leaves and later eat almost every plant part.

Mechanism of Action & Use:

Nomu-Protec is based on the insect-pathogenic fungus *Metarhizium rileyi* (previously known as *Nomuraea rileyi*) that infects and controls lepidopteran insect pests, especially those of the family Noctuidae. *Metarhizium rileyi* spores can either penetrate through the cuticle or enter the larvae through ingestion during feeding. Once inside the larvae, the fungus grows and multiplies, killing the larvae by internal tissue destruction. Between 2-4 days after the initial infection the larva stops feeding and dies 5-7 days later. Once the larva has died the fungus sporulates (Fig. 12.3), therefore having the ability to remain in the environment and re-infect the next generation of pests. Nomu-Protec also shows effective reduction in feeding damage shortly after infection. Recommended are 4 weekly applications of 300 g/ha and 600 g/ha, starting at first appearance of insect pest with good spray coverage and at higher humidity.

ERASMUS+ project INPACT – www.inpactproject.eu

Ref: Grijalbo, E. P. et al. 2018. *Fungal biology*, 122 (11), 1069-1076. <https://www.undermet.com/product/nomu-protec/>

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13. Effect of silicon on two major insect pests of tomato

Tomato X *Tuta absoluta*, *Bemisia tabaci*

Tomato is attacked by several insect pest species, among which, the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Fig. 13.1) and the tomato leaf miner *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) (Fig. 13.2) are of greatest importance. Vast application of pesticides is harmful to the environment, human health and may increase the risk of pest resistance on insect populations. One of the promising strategies which are compatible with organic farming is application of silicon for enhancing plant vigor and resistance to pest damage on various agricultural crops.

Mechanism of Action & Use:

Silicon is known to enhance the crop resistance to biotic and abiotic stresses through physical and allelochemical mechanisms. AB Yellow® silicic acid formulation could be applied in two ways soil drench treatment or foliar spraying, with 2% Si concentration. The Silicon applications significantly decreased the population of immature of both whiteflies and tomato leaf miner on tomato crop in the greenhouse. Si-foliar spraying is more effective in reducing the population density of these key pests compared to Si-soil drench application.

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Ref: Alyouss, A. et al. *Silicon* 14, 3019–3025 (2022). <https://doi.org/10.1007/s12633-021-01091-7>; <https://reel-agro.com/abyellow.html>.

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14. Biological control of western flower thrips using the entomopathogenic fungus *Beauveria bassiana*

Vegetables, fruits, ornamentals X *Frankliniella occidentalis*

Western flower thrips, *Frankliniella occidentalis* (Fig. 14.1), is one of the most destructive pests of vegetables, fruits and ornamental crops worldwide, causing extensive damage by direct feeding of the crop and transmitting economically important viruses (Fig. 14.2).

Mechanism of Action & Use:

BotaniGard ES is a highly effective biological insecticide containing *Beauveria bassiana*, an entomopathogenic fungus that attacks a long list of troublesome crop pests (not plants) like aphids, thrips, whitefly, spider mites, mealybugs, root aphids and more.

This naturally occurring myco-insecticide works on contact and thorough coverage is required to achieve control. The applied spores attach to the insect, germinate and penetrate through the insect cuticle (skin). The fungus then grows rapidly within the insect, causing mortality in 7-10 days (Fig. 14.3). The product could be used in greenhouse, nursery, vegetables, etc. Effectiveness is NOT dependent on high relative humidity. The fungus controls ALL stages of the most troublesome crop pests.

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Ref: Dawari et al., 2021. *Bulletin of Entomological Research*, 111 (6), 688-693; <https://www.planetnatural.com/product/botani-gard-es-insecticide/>


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15. Control of the tomato leaf miner with baculoviruses

Tomato (*Lycopersicon esculentum*) X *Tuta absoluta*
 The tomato leafminer *Tuta absoluta* (Meyrick 1917) (Lepidoptera: Gelechiidae) is a devastating pest, causing losses of up to 100% (Fig. 15.1). Originating in South America, it has been spreading throughout the Mediterranean region but also continental Europe, the Middle East and Africa. The larvae of the pest (Fig. 15.2) mine into leaves and fruits, which can fast lead to complete crop loss. Many tomato leafminer populations are resistant to a wide array of pesticides, both chemical and biological. An innovative tool for its control is the use of the insect specific viruses of family *Baculoviridae*.

Mechanism of Action & Use:
 The viral bio-insecticide Tutavir contains a *Phthorimaea operculella* granulovirus (PhopGV) for highly effective and selective control of the tomato leafminer. Once this natural pathogen is ingested by a suitable insect host, it reproduces within gut cells of the insect causing it to become ill and die. Due to its high compatibility with pollinators, beneficials and other inputs, Tutavir is the best candidate for integrated pest management programs. Because of its new and unique mode of action, Tutavir is an important tool for resistance management in conventional and biological production systems. Tutavir is applied at 100 ml/ha, 5 weekly applications. Assessment of the pest severity is made on 50 leaves per plot.



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Ref: Gómez Valderrama, J. A., et al. 2018. *Journal of Applied Entomology*, 142(1-2), 67-77; <https://www.anderematt.com/product/tutavir/>

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16. Mechanical control of peat fly larvae by sharp sand application

Peat fly (*Bradysia* spp.) on potted plants in greenhouses
 The peat fly (Fig. 16.1) is a common pest whose larvae eat the hair roots of potted plants and seedlings grown in greenhouses (Fig. 16.2). Peat-based soil is a major pest of the larvae. Quartz sharp sand (Fig. 16.3) placed on the surface of the growing medium results kills the majority of the larvae.

Mechanism of Action & Use:
 Quartz sharp sand causes mechanical damage to the larvae as they move. The peat fly cannot reproduce in the presence of the quartz crystal material and cannot harm the seedlings. This method can be used for pansy, kohlrabi, pepper and aubergine seedlings. (Fig. 16.4-5).

Mix 1 cubic metre of potting soil mixture with 10% quartz soil. The mixture can be used specifically for seedlings. This is not a good method to use in the open field.



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Ref: Nougaret & Lapham (1928). *Technical Bulletin USDA* 20, p. 1-38.


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17. Use of nudibranch ash extract to deter Spanish naked snails

The Spanish nudibranch (*Arión vulgaris*) x multiple fruit and vegetable field cultivations
 Also known as the killer snail this nudibranch (Fig. 17.1) has recently appeared in Vegetable and fruit farms in Romania. In addition to vegetation, it also eats other snails. The pest particularly affects vegetable and fruit farms (Fig. 17.2), but also eats other snails. An aqueous suspension of the ash made by burning the snails has been observed shown to be used to reduce *A. vulgaris* infestations.

Mechanism of Action & Use:
 Some farmers have been using the locust control method of the early last century with success against slugs. According to Theresa von Bellerdsdorf, during locust epidemics they would collect the locust larvae, burn them alive and spread the ash on the crop by mixing it with water. The operation needs to be performed 1-2/year, on a regular basis. It does not completely eliminate the snails, but there is no invasion. Burn the snails on a beech wood fire, put about 200 g of ash (two handfuls) in 200 l of water and mix it (tradition says you have to mix it for an hour, until the solution becomes uniformly opalescent). This should be dispersed in the evening. Not harmful to plants. It is worth the whole area at first, then later only the edges of the area where the snails may come from. (Fig. 17.3-5).



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Ref: Chailier-Scott, 2013. *HortTechnology* 23, p814-819.

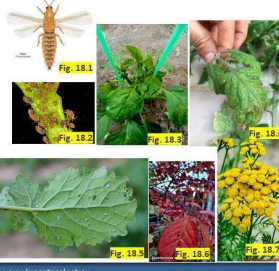
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18. Control of aphids and thrips using a decoction of spindle berries & tansy

Thrips (order *Thysanoptera*), and aphids (*Aphidoidea* spp.)
 (Fig. 18.1-2) are among the most common and destructive pests found in greenhouses, open fields and gardens. They weaken plants by sucking sap, and causing deformation (Fig. 18.3-5) and act as vectors for plant viruses. Aphids also leave deposits of honeydew which favour sooty mould growth. An aqueous decoction of spindle (*Euanymus europaeus*) berries (Fig. 18.6) and tansy (*Tanacetum vulgare*, Fig. 18.7) contains alkaloids and other active substances. When used to spray plants it can effectively reduce infestation levels.

Mechanism of Action & Use:
 Preparation: add 50-60 gr of spindle berries and 100 gr of tansy in 5 liters (lt) of water, boil for 20 minutes, let stand for 12 hours, strain and dilute to 10 lt. You can put in 50 gr sugar as a glue and bait. It can be used for cabbage, peppers, tomatoes, potatoes where lice appear. Spray early in the morning or during the evening. This is a stomach poison for aphid pests. Does not burn and can be used on very young plants. It is a universal aphid repellent, but very effective against thrips in peppers.



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Ref: Jacobs et al., 2019. *New Phytologist*.

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19. Protection against mites using tobacco extracts

Mites, *Acariforme* and *Parasitiforme* arachnids x multiple plants
 Mites, small eight-legged arthropods of the orders *Acariforme* and *Parasitiforme*, (Fig. 19.1), identifiable with the use of a magnifying glass, are destructive plant pests that feed on the cellular fluids of plants, weaken them, reduce vigour and can ultimately cause plant death (Fig. 19.2). Aqueous tobacco extracts can effectively kill arachnid mites.

Mechanism of Action & Use:
 Tobacco decoction, or tobacco steeped in water (Fig. 19.3), can be used against mites. Home grown L.H. uses home-grown tobacco. The business tobacco also burns the plants.

Put 50 gr dried tobacco to 10 l of water (Fig. 19.4). After fermentation (approx. 5 days), spread the juice over the plants. Prefer to spray at dawn or in the evening, avoiding the sun. Use on cucumbers, peppers, eggplant, celery. In summer, you can ferment tobacco like nettles.



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Ref: Lal Jot et al., (2013). *BioInfoLet* 10, p422-424.

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20. Crop rotation for crop protection in organic agriculture

The majority of open field crops, some greenhouse crops x multiple pests.
 Crop rotation is the practice of planting/sowing different crops in succession on the same plot of land to improve soil health, optimize soil nutrients and control pests and weeds. A simple crop rotation may contain two or three plants, while a complex rotation may contain a dozen or more (Fig. 20.1). According to EU Regulation No 2092/91: "Pests, diseases and weeds shall be controlled by a combination of the following measures: - the application of an appropriate crop rotation system" Annual rotation limits the spread of pathogens and pests (root and stem diseases, nematodes, etc.), as well as the proliferation of weeds that are dominant in certain crops. It has long been observed that even two to three years of non-rotational cultivation increase disease susceptibility and insect damage. For this reason, monoculture production is practically unfeasible without a significant level of chemical plant protection (Fig. 20.2).

	Plant 1	Plant 2	Plant 3	Plant 4
First year	Beet	Spring barley or red clover 2 years old	Red clover 2 years old	Winter wheat
Second year	Spring barley Red clover or red clover 2 years old	Red clover 2 years old	Winter wheat	Beet
Third year	Red clover 2 years old	Winter wheat	Beet	Spring barley or red clover 2 years old
Fourth year	Winter wheat	Beet	Spring barley or red clover 2 years old	Red clover 2 years old
Fifth year = first year	Beet	Spring barley or red clover 2 years old	Red clover 2 years old	Winter wheat

Fig. 20.1. An example of a multi-year open-field crop rotation scheme.



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Ref: Regulation (EEC) No. 2092/91, <http://archive.biokontrol.hu/cms/hu/szakok/kozvetlen/nyovenytermeszes/361-a-vetesforgo-szobalyainak-ertelmezese>

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21. Fungicidal plant protection product based on *Thymus vulgaris* essential oil

Pathogen(s) X Host Range: *Fusarium culmorum*, *Blumeria graminis* & *Pyrenophora teres* on cereals



Mechanism of Action & Use:
 The innovative aspect of this product is that the fungistatic agent *Thymus vulgaris* essential oil is incorporated into biopolymer microdroplets to increase its persistence in the crop. The product has been shown to significantly reduce fungal pathogen infestation and mycotoxin content in grain when applied at a rate of 200-400 l/ha using conventional sprayers. The product has been patented but is yet not on the market.

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Ref: <https://tdw.upu.cz/webapp/tesdb.ota.htm>

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22. EDN - Ethadinitrile

Pest(s) X Host Range: *Ips typographus*, *Ips duplicatus* x *Picea abies*



Mechanism of Action & Use:
 Ethadinitrile (EDN) is an insecticide highly effective against all developmental stages of bark beetles and other wood-boring insects. The product is prepared as a gas and supplied in 50 kg cylinders. The treatment of harvested timber takes place in forest landfills, where logs are sealed in polyethylene sheeting and fumigated for 10 hours. This product leaves no harmful residues. The product can be used under the exception of the so-called "Emergency conditions in plant protection".

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Ref: Hnátek et al. (2019). *Agromanuál*; Douša et al. (2021). *Agronomy*, 11 (2), 208.

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28. Naturalis-L *Beauveria bassiana* insect parasite fungus

Mechanism of Action & Use (cont.):

Naturalis-L is a bioinsecticide based on the entomopathogenic fungus *Beauveria bassiana* (strain ATCC 74040). Compared to many other strains of *Beauveria*, Naturalis-L infects a very wide range of economically damaging pests such as whitefly, mites, thrips and some groups of flies. In addition, the vegetable oil dispersion (OD) formulation means that Naturalis-L has a long shelf-life, is easy to use and gives excellent efficacy in real-world conditions.

The mode of action of Naturalis-L makes it a perfect tool for the control of pests and mites on vegetables, fruit crops and ornamentals. Naturalis-L can be successfully used in both organic production and integrated pest management programmes, especially if a reduction in residue levels and a number of traditional chemical sprays is desirable.

Naturalis-L does not leave any chemical residues and there is no harvest interval, so it can be applied through the entire life of the crop. As both an insecticide and miticide, Naturalis-L fits perfectly into pest management programmes aimed at minimizing the risk of resistance to conventional insecticides. Furthermore, Naturalis-L is compatible with beneficial insects and it is non-toxic to bees and pollinators.

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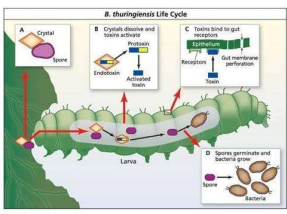

Ref: <https://jaragro.co.uk/news/product-news/naturalis-l-the-versatile-bioinsecticide-for-all-protected-edible-and-non-edible-crops>

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29. *Bacillus thuringiensis* ssp *kurstaki* against lepidoptera

Pest(s) X Host Range: lepidoptera caterpillar control in various crops

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Ref: <https://www.researchgate.net/publication/312111111>

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29. *Bacillus thuringiensis* ssp *kurstaki* against lepidoptera (page 2 of 2)

Mechanism of Action & Use (cont.):

Bacillus thuringiensis kurstaki (Btk) is a gram-positive, rod-shaped bacterium native to the soil in a wide range of regions globally. A subspecies of *Bacillus thuringiensis*, Btk controls Lepidoptera. This order includes gypsy moths, cabbage loopers, tomato hornworms and grape leaf skeletonizers.

One of the many advantages to using Btk is that it does not pose a threat to other animals or insects outside of the order *Lepidoptera* in the environment once it has been sprayed or ingested by the target pest. Similar to *Bacillus thuringiensis israelensis*, birds and other predators can feed on the infected pests without ingesting toxic chemicals. As with most biological control measures, Btk applications will be most effective when made early in the pest's life cycle, particularly during the larvae's 1st and 2nd instars. Once ingested, the alkaline environment of the caterpillar's digestive system triggers the Btk bacterium to release a crystalline protein, a type of endotoxin, which paralyzes the caterpillar's digestive tract. The caterpillars will stop feeding and die shortly after this occurs.



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30. Kaolinite to manage whiteflies, *Ceratitis capitata*, oriental fruit fly

Pest(s) X Host Range:

Kaolinite is clay mineral is based on the aluminium silicate compound $Al_2Si_2O_5(OH)_4$. The active ingredient of Surround® WP is calcined kaolin, a biological insect repellent registered by the EPA, in a powder formulation. In order to be effective against insects, Surround® WP should be applied as a prevention and sprayed before the appearance of insects. Surround® WP reduces pest pressure and can delay or eliminate the need for conventional insecticide spraying. Adult individuals of the pest become heavily coated with kaolin particles within 24 hours of spraying. The insects then get busy trying to remove these particles from their bodies, unable to feed or lay eggs and thus die.

It forms a grayish coating on the surface of the leaf, so attention should be paid to the last application before harvesting.

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Ref: <https://nwdistrict.ifas.ufl.edu/phog/2019/03/01/using-kaolin-to-manage-whiteflies-a-novel-approach/>





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31. Use of Nemastar against *Steinernema carpocapsae*

Pest(s) X Host Range:

Infective juveniles of a threadworm that parasitizes a large variety of insect prey, especially beetles, fleas, cutworms (*Agrotis* spp.), and other moths. 100% safe to humans and pets. Primarily used for the control of beetles, fleas, cutworms and other moths in soil. The infective juveniles of nemastar® are ambush predators, and are most effective against mobile prey. Once caught, they crawl inside the prey through breathing spiracles or other orifices, release a beneficial bacteria to break down the pest's internal organs, and feeds on the bacterial slurry. The nematodes then breed in the cadaver, which eventually breaks apart releasing more nematodes into the soil.

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Ref: <https://www.bioforce.co.nz/products/nemastar.html>






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32. Mating disruption techniques against moths

Pest(s) X Host Range: *Grapholita funebrana*, *Tortricidae* spp.

For the effective operation of air circulation, the size of the field cannot be smaller than 4 hectares and the field must be located in the same direction as the wind direction. Pheromones are species-specific, and individuals of different sexes do not find each other for mating. Moth monitoring requires weather data and pheromone traps. The dispensers are placed before the end of the biofix date determined on the basis of the heat amount. The pest control is successful, but the appearance of new *Tortricidae* species is expected.

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Ref: <https://www.bioforce.co.nz/products/nemastar.html>

41. *Metarhizium*: jack of all trades, master of many

Potentially ALL insects & plants

What it is: *Metarhizium* is a genus of highly abundant fungi that grow naturally in the soil with several identities. They are best known for their ability to infect and kill many different arthropods, but most are also saprophytes, rhizosphere colonizers and beneficial root endophytes, with the ability to switch between these different lifestyles.

Mode of action: These fungi are able to degrade, penetrate and assimilate the insect cuticle using a combination of cuticle-degrading enzymes and mechanical pressure (Fig. 41.1a,b). Onward transmission of *Metarhizium* requires the death of the host as the insect cuticle is breached to release the conidial spores.

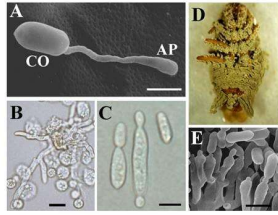


Fig. 41.1.

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Ref.: https://silkipathdb.swu.edu.cn/fungi_meoni/arsef23; Gao et al., 2011. *PLoS genetics* 7(1): e1001264.

42. Natural Resistance Genes against Plant Viruses

Potentially ALL plants & viruses

What it is: Virus infections of crops are persistent and cannot yet be combated in the same way as many animal viruses, through provocation of an active immune response. The best strategy is one of avoidance by physical separation of the pathogen and host, or through the deployment of genetic resistance that prevents or limits the extent of the infection.

Mode of action: To date, the majority of characterized pathogen dominant resistance (R) genes from plants provide monogenic dominant resistance. Those characterized at the molecular level mostly confer resistance to fungal or bacterial pathogens, but there are currently 12 examples of such genes conferring resistance to viruses (Fig. 42.1).

Gene	Host	avr	Pathogen	Reference
W	Tobacco mosaic virus (TMV) (Carnivorous)	—	Tobacco etiolosa	Whitaker et al. (1964), Bollen et al. (1986), Ecker et al. (1998), Lamborn et al. (2002), Wang and Pinner (1998)
NSP2	Tobacco mosaic virus	Movement proteins	Tobacco	Lamborn et al. (2002)
AV1	Tobacco etiolosa virus	Coat protein	Tobacco	Wang and Pinner (1998)
AV2	Tobacco etiolosa virus	Coat protein	Tobacco	Lamborn et al. (2002)
AV3	Tobacco etiolosa virus	Coat protein	Tobacco	Lamborn et al. (2002)
AV4	Tobacco etiolosa virus	Coat protein	Tobacco	Lamborn et al. (2002)
AV5	Tobacco etiolosa virus	Coat protein	Tobacco	Lamborn et al. (2002)
AV6	Tobacco etiolosa virus	Coat protein	Tobacco	Lamborn et al. (2002)
AV7	Tobacco etiolosa virus	Coat protein	Tobacco	Lamborn et al. (2002)
AV8	Tobacco etiolosa virus	Coat protein	Tobacco	Lamborn et al. (2002)
AV9	Tobacco etiolosa virus	Coat protein	Tobacco	Lamborn et al. (2002)
AV10	Tobacco etiolosa virus	Coat protein	Tobacco	Lamborn et al. (2002)
AV11	Tobacco etiolosa virus	Coat protein	Tobacco	Lamborn et al. (2002)
AV12	Tobacco etiolosa virus	Coat protein	Tobacco	Lamborn et al. (2002)

Fig. 42.1.

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Ref.: Maule et al., 2007. *Mol. Plant Path.* 8 (2), 223-231.

Concluding Remarks

The INPACT Best Practices presented in this document represent a series of innovative and/or environmentally-friendly agricultural practices to increase crop health and manage pest infestations in some of the most important crop groups of the member countries, with particular reference to emerging pests. Broadly, the practices may be divided into groups according to action/target, although a significant number of the Best Practices confer multiple benefits across different pathogen groups, or in addition to pesticidal control actions also provide plant growth stimulation, etc.

The majority of the Best Practices address insect pests. This is unsurprising as insects can either cause damage in their own right by directly feeding on crop plants, but also many species are the vectors of significant plant disease-causing organisms and the presence of novel insect pests have been detected in all partner countries. The interventions include the use of natural products such as plant decoctions and aromatic plant oils derived from neem, orange, thyme etc., and the use of compounds which act as physical barriers, such as kaolin emulsions and sharp sand, which have the advantages of low cost and minimal environmental impact. Other methods involve the biological control of insects using their natural predators, including beetles, nematodes or parasitic wasps. Several practices employ entomopathogenic fungi, which can be used to combat major pest species including thrips, and which have recently been demonstrated to confer additional benefits to host plants by acting as antagonists of other pathogens and as promoters of plant growth. The use of *Bacillus thuringiensis* (Bt) against moth pests and in particular *Tuta absoluta*, the devastating pest of greenhouse and field tomato cultivation is also described. The use of pheromones to disrupt insect mating, and the use of fumigation techniques that kill pests without leaving residues are also described, as is the use of highly discriminatory smart traps.

Another important group of practices concern plant protection through cultural control measures. These measures which act to increase the functional biodiversity of the agricultural system include the use of crop rotation, cover crops and intercropping with various species, and the use of plant growth promoting microorganisms such as *Rhizobacteria* and *Trichoderma*. One practice describes how by combining ('stacking') various agroecological ecosystem services, an agricultural system may be engineered to provide optimal crop protection, pollination enhancement, and productivity without the requirement for synthetic inputs of fertilisers and pesticides. The use of resistant crop-plant varieties developed by traditional breeding techniques is included, and also the use of CRISPR/Cas9 technology to accelerate the development of new crop lines resistant to pathogens

by using genome editing techniques. The use of advanced crop surveillance techniques in order to detect pests and pathogens in a timely manner, which in turn allows for their containment and the use of milder and less environmentally-harmful containment/eradication measures is also presented.

The effects of climate change in the project region have resulted in greater problems being reported due to fungi, and in particular mildew. To combat fungal infections, Best Practices describe the use of *Ampelomyces quisqualis*, a hyperparasitic fungus that selectively targets powdery mildew, but also the use of fumigants and aromatic essential oils – particularly prepared by procedures such as microencapsulation, thus limiting environmental exposure while increasing the persistence of the treatment.

Against plant viral pathogens two important practices – the use of cross-protection by pre-infection with a mild viral strain is described to protect against *Pepino mosaic virus* infection of tomato cultivation, but also an improved implementation of RNAi technology that induces specific antiviral resistance in crop plants, which through incorporation of the RNA effector into nano-clay sheets (nanotechnology) greatly improves the persistence and effectiveness of the treatment. For the important emerging viral pathogen *Tomato brown rugose fruit virus* for which there are no effective treatments, the use of resistant tomato cultivars (created by CRISPR/Cas9 deletion of a host susceptibility factor) is described. The use of a bacteriophage preparation to combat the bacterial pathogen of root vegetables *Erwinia amylovora* is also described.

Together, the INPACT Best Practices represent an accessible resource informing farmers and agricultural stakeholders of state-of-the-art methodologies in crop plant protection.

Cited Literature

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