Lecture Supplements

Plant Bacteriology

Bacterial Disease Management-Part 1

Compiled by N. Hassanzadeh

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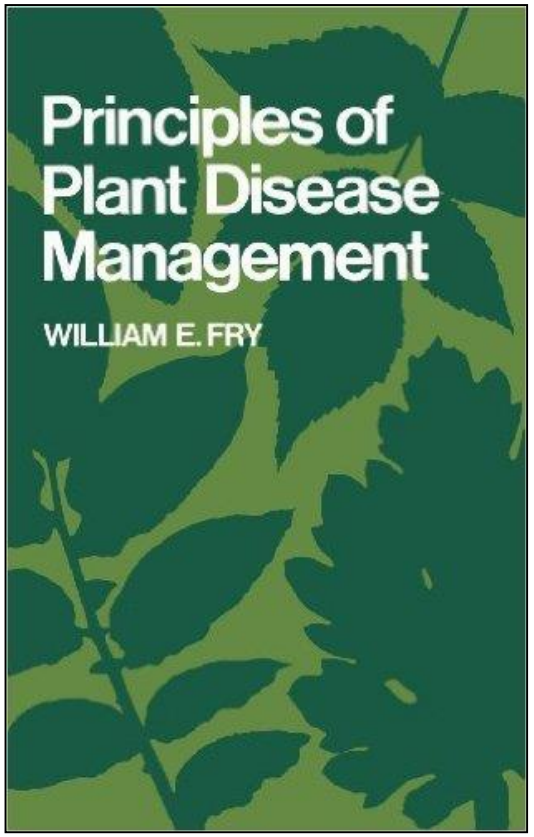

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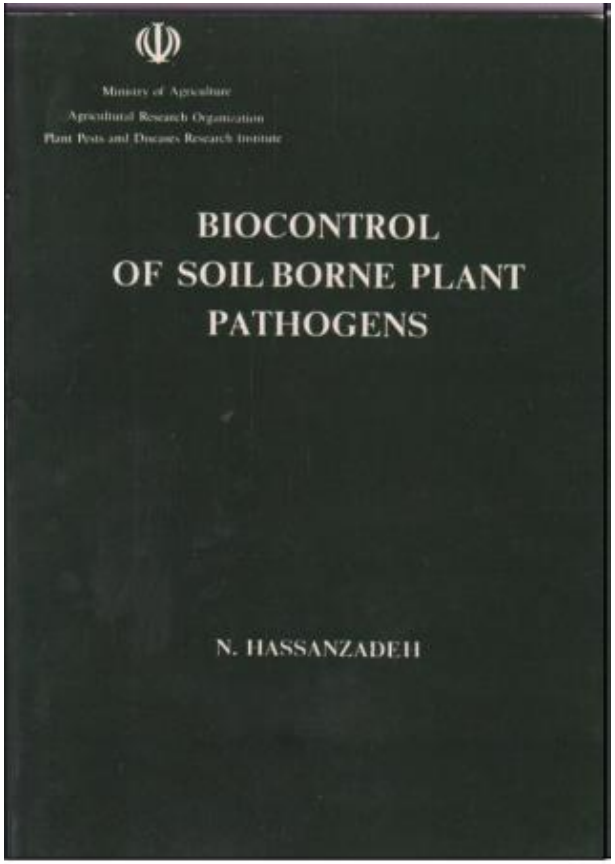

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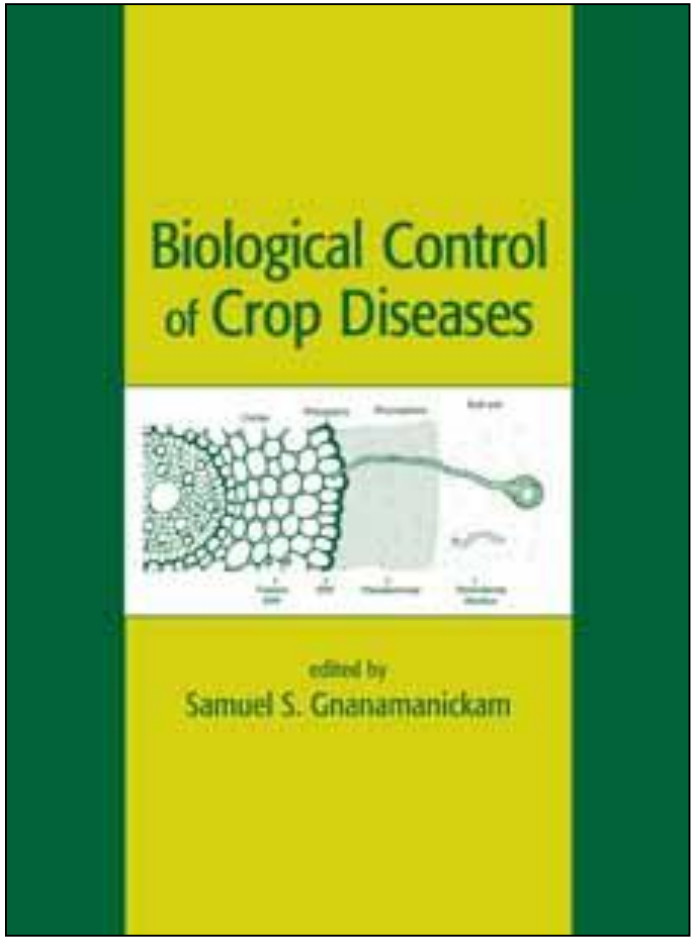

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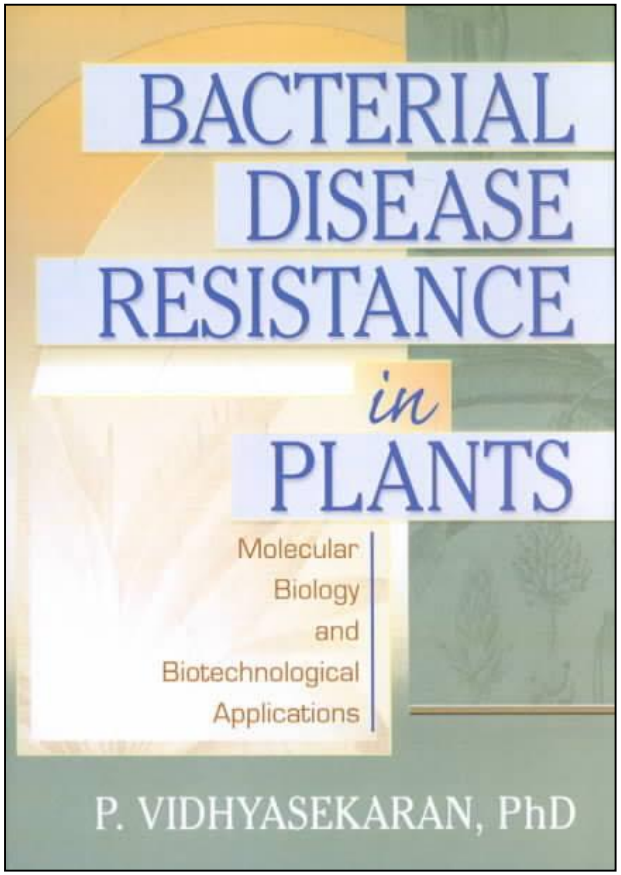

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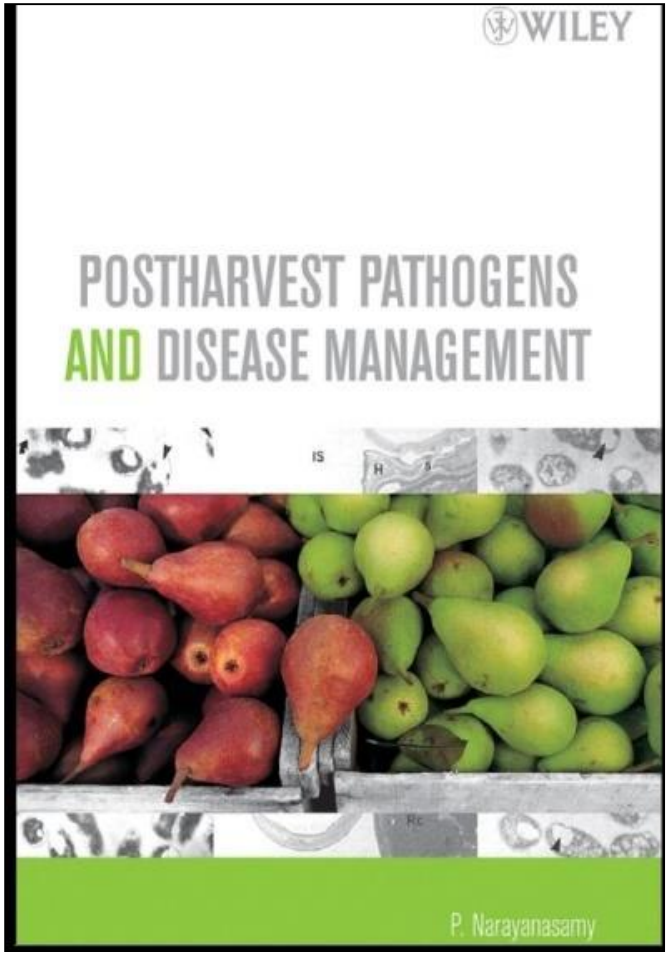

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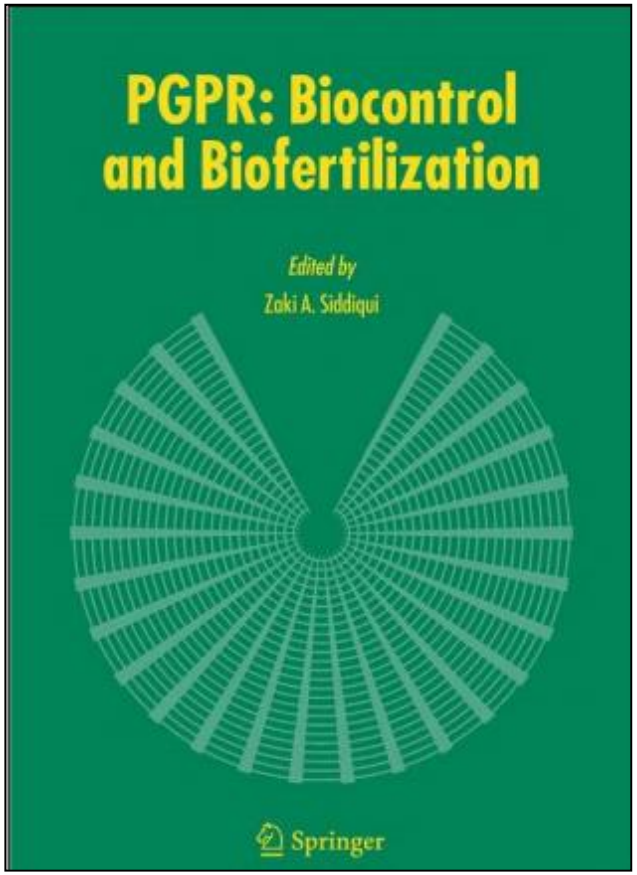

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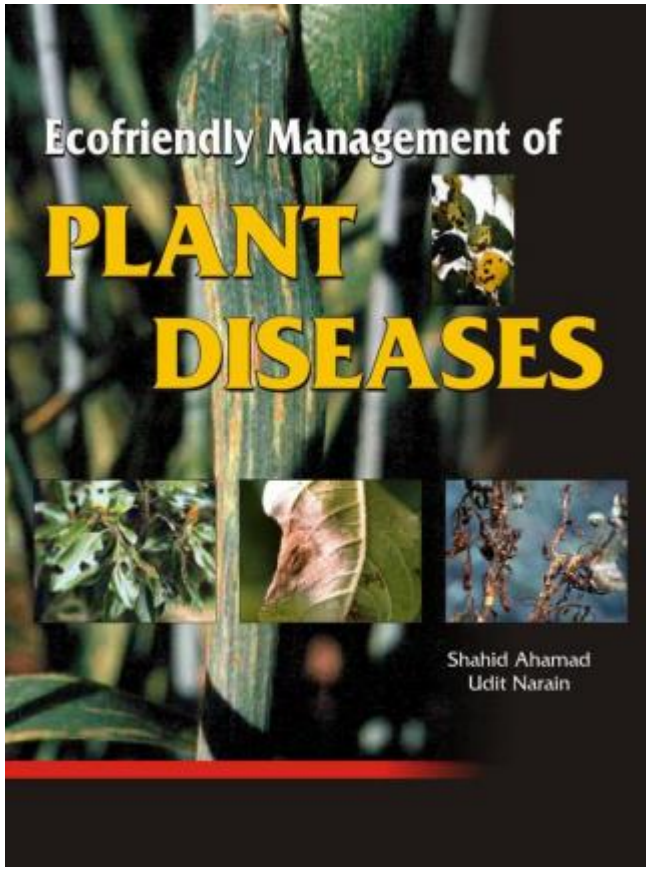

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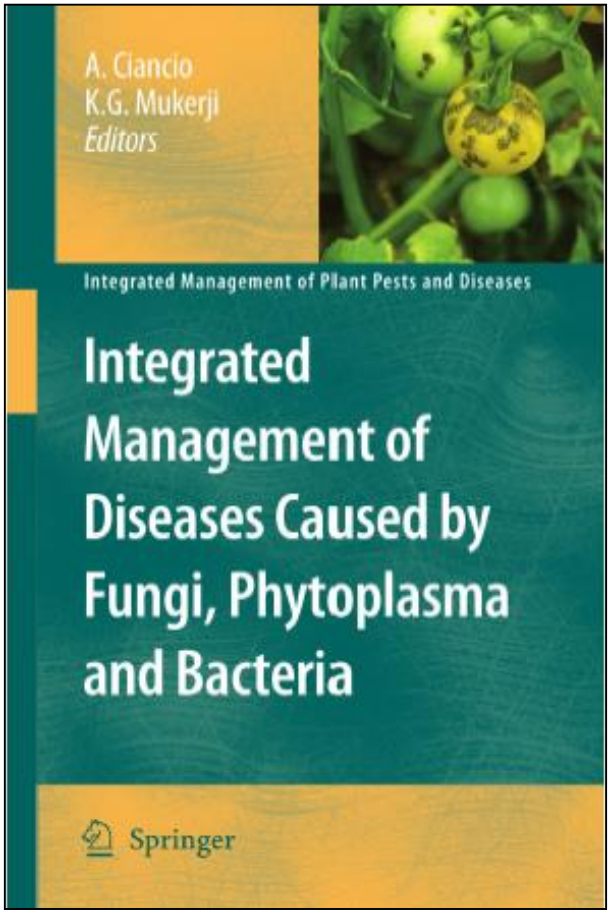

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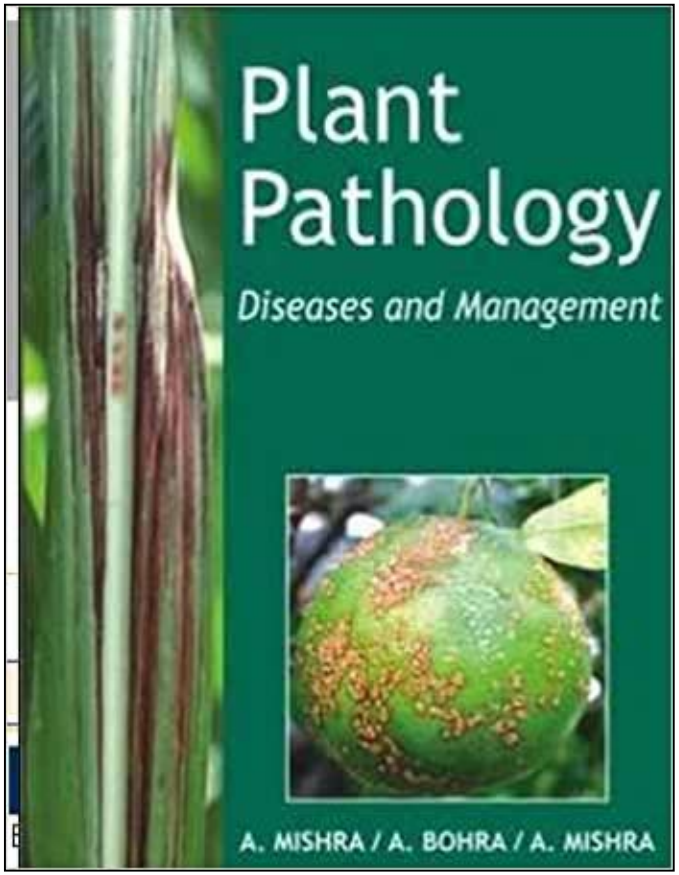

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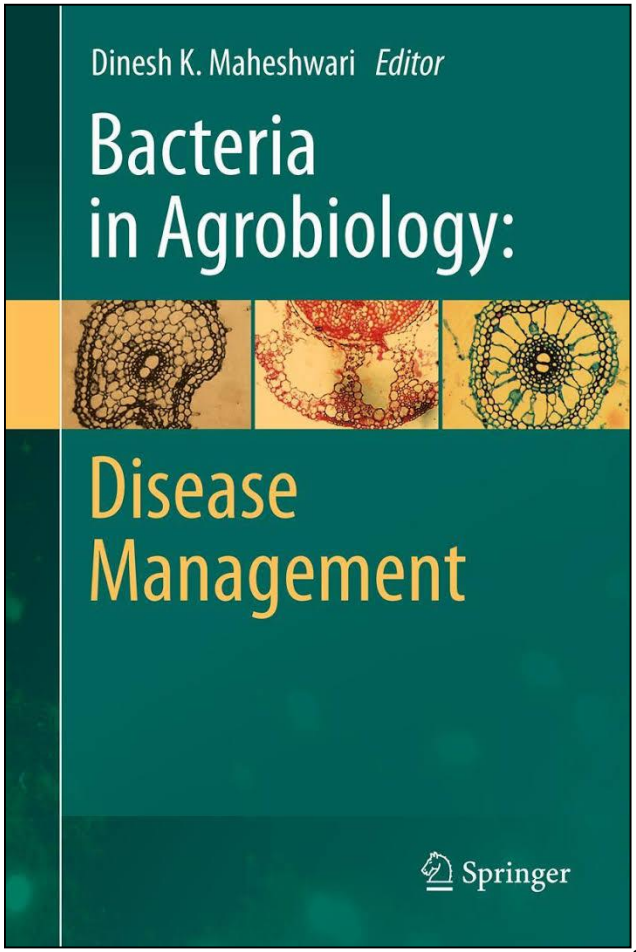

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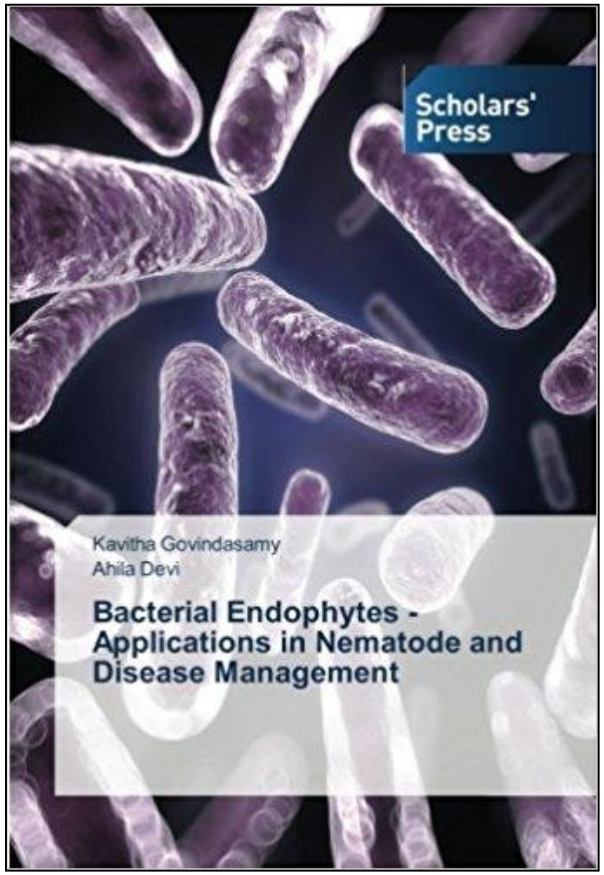

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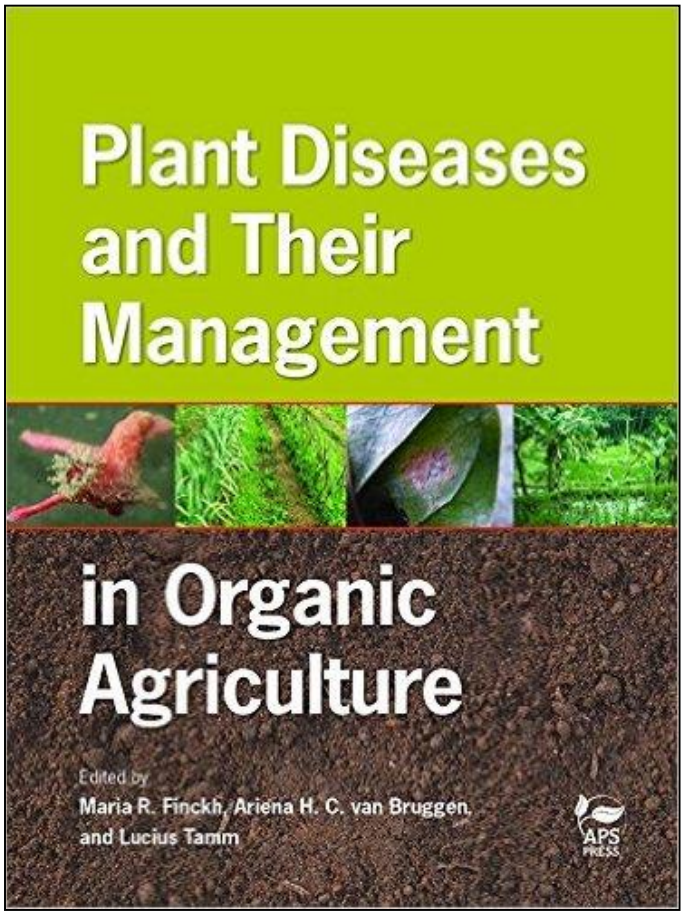

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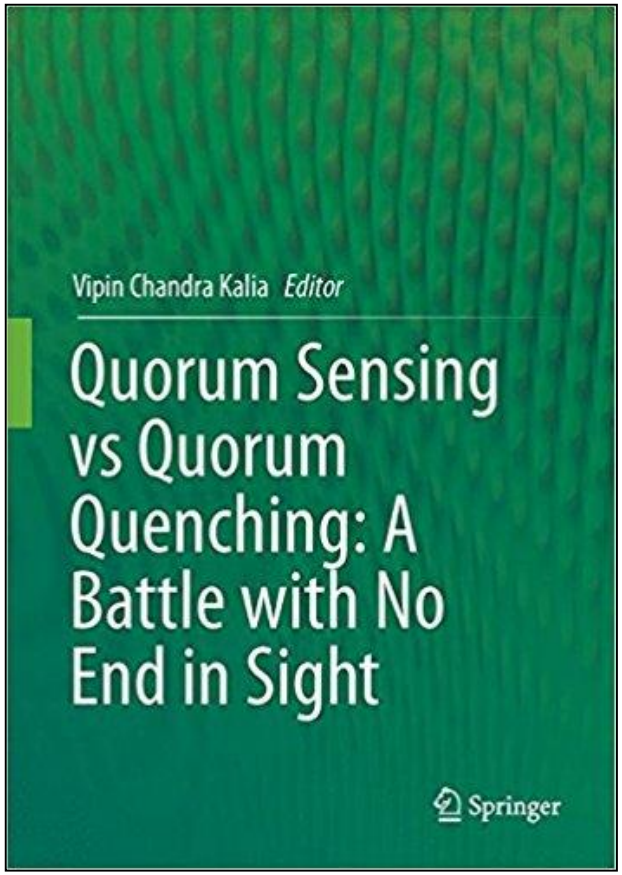

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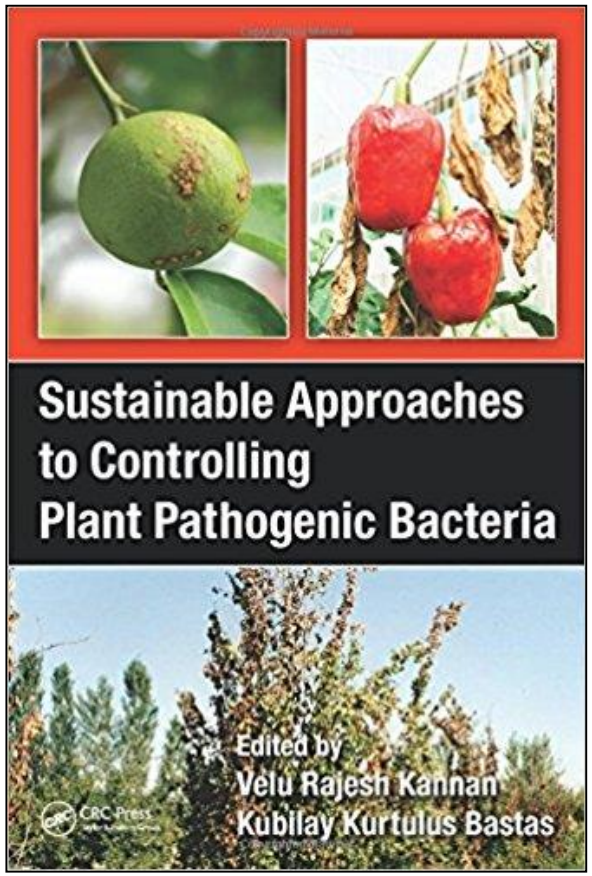

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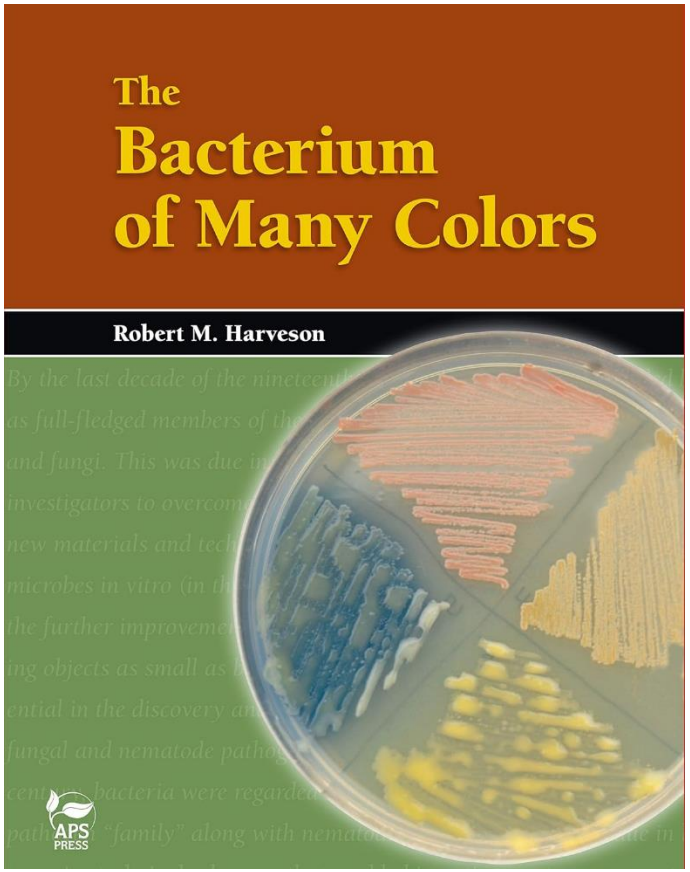

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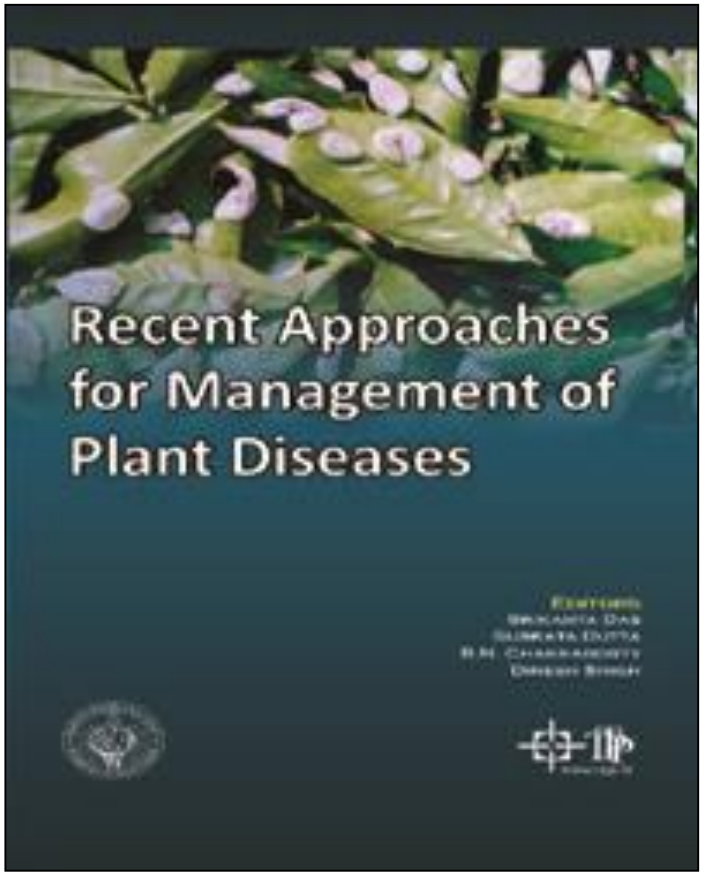

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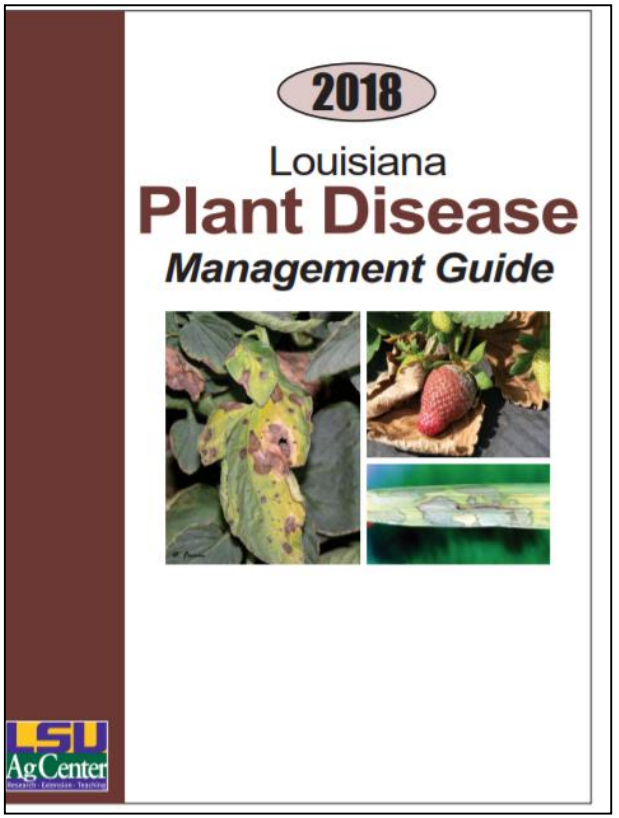

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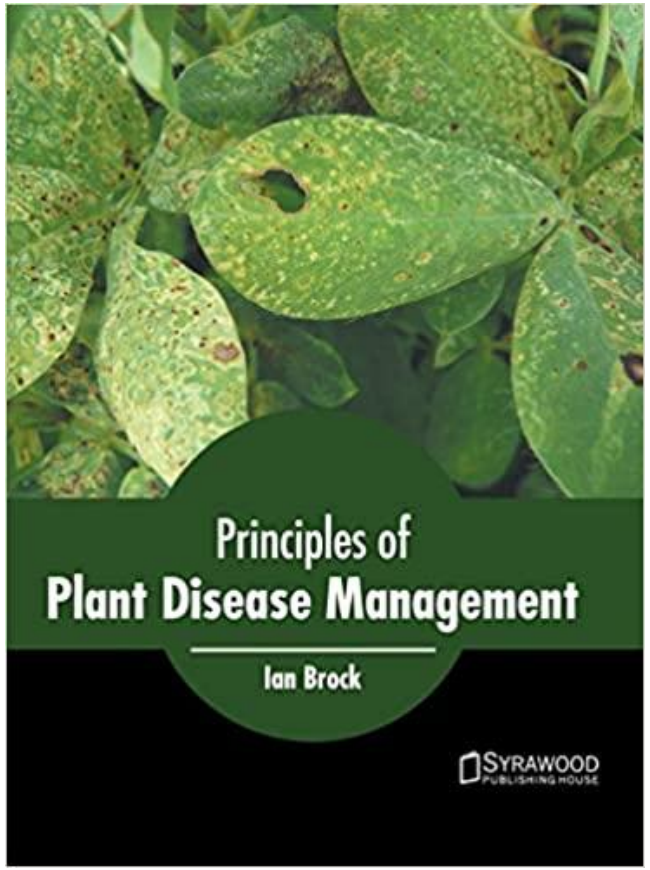

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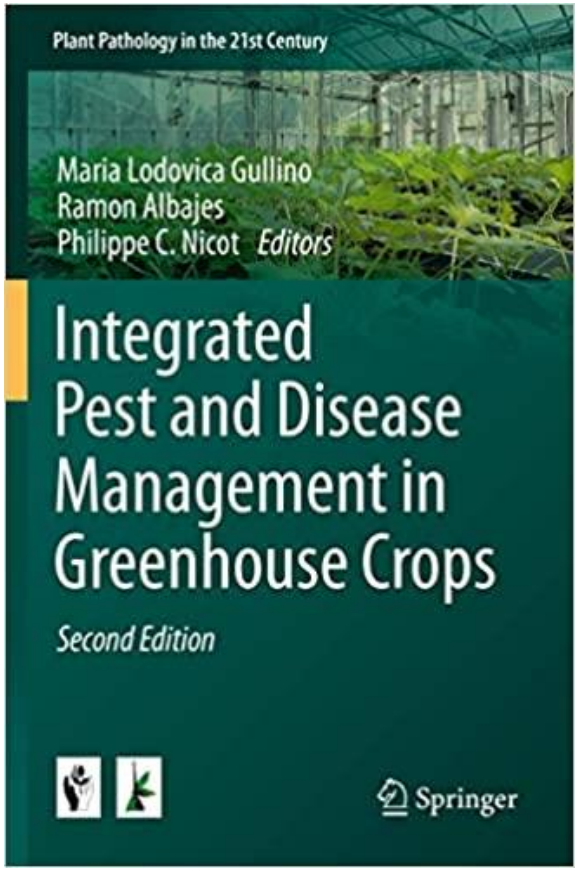

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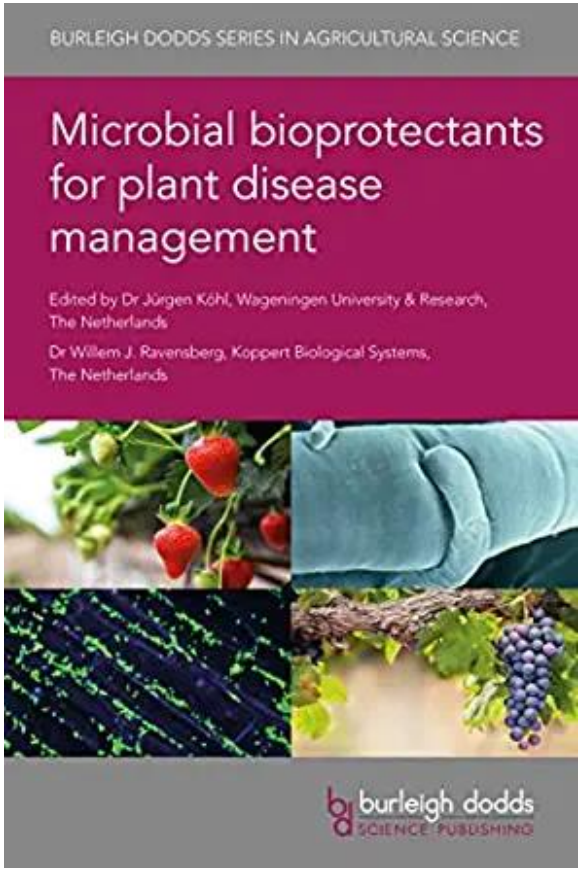

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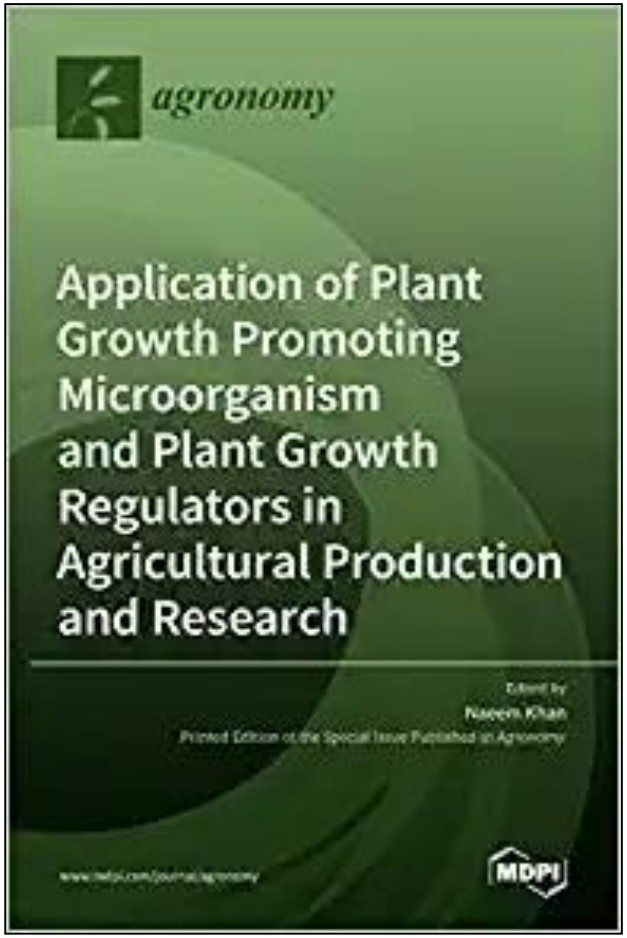

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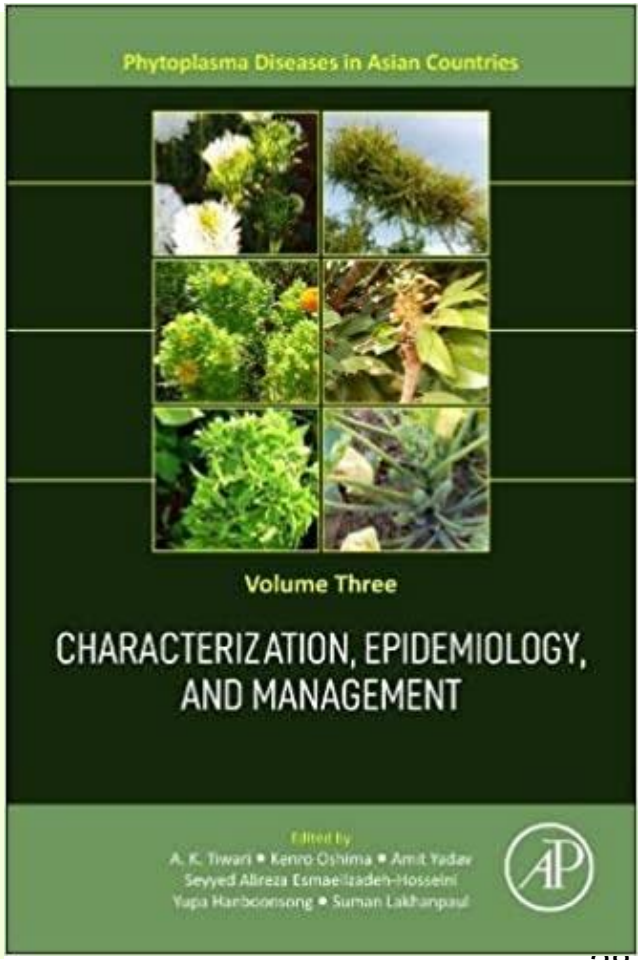

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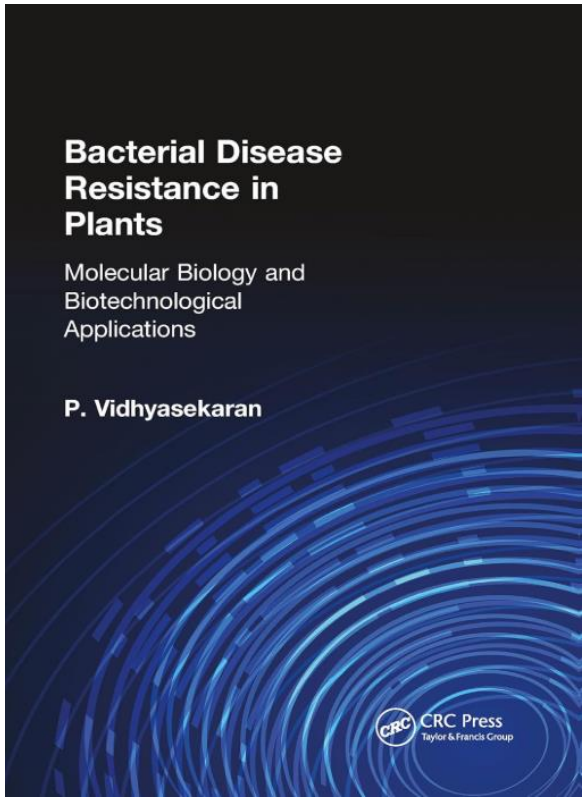

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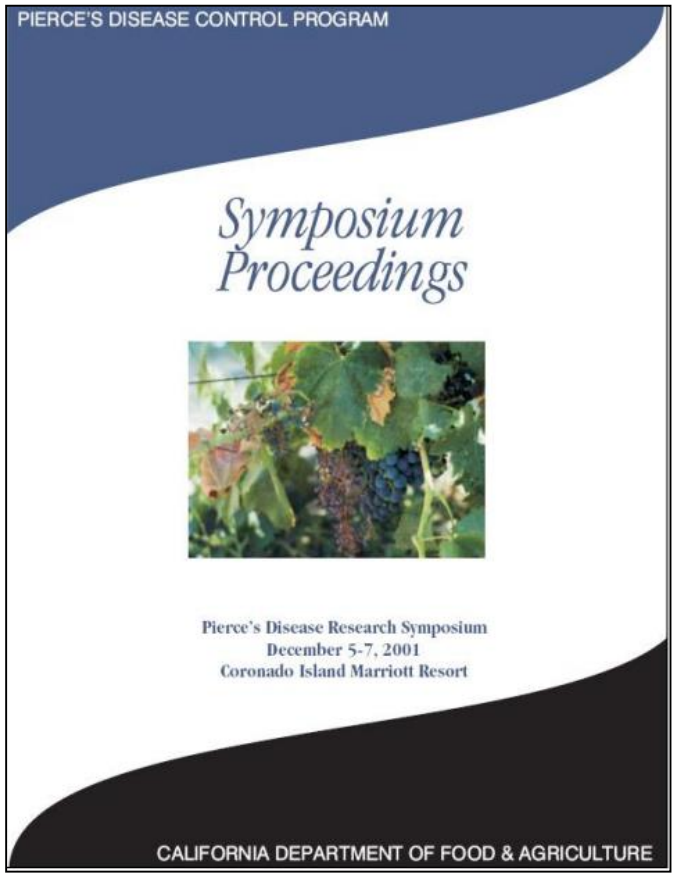

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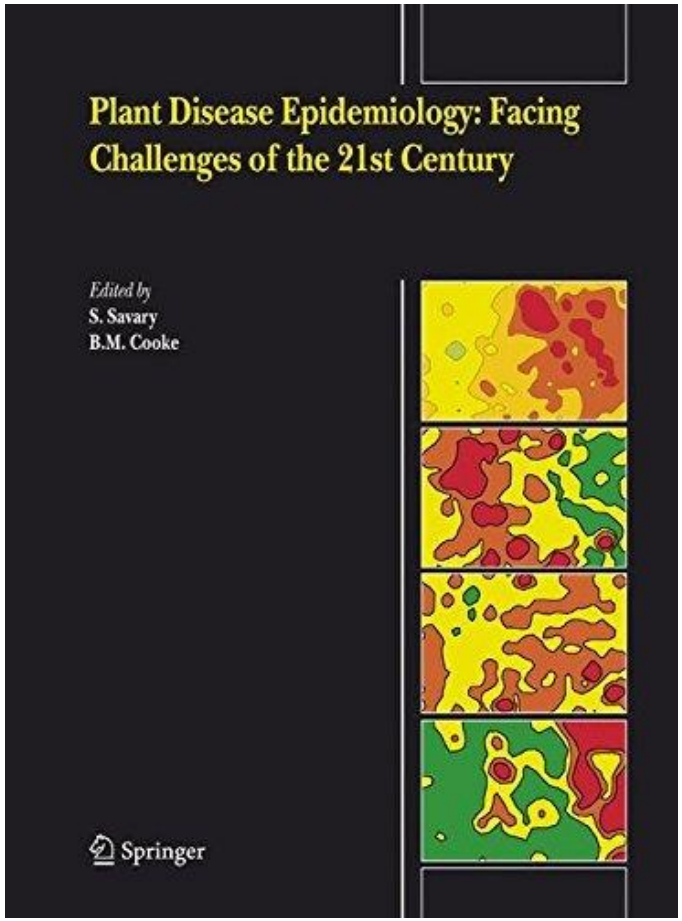

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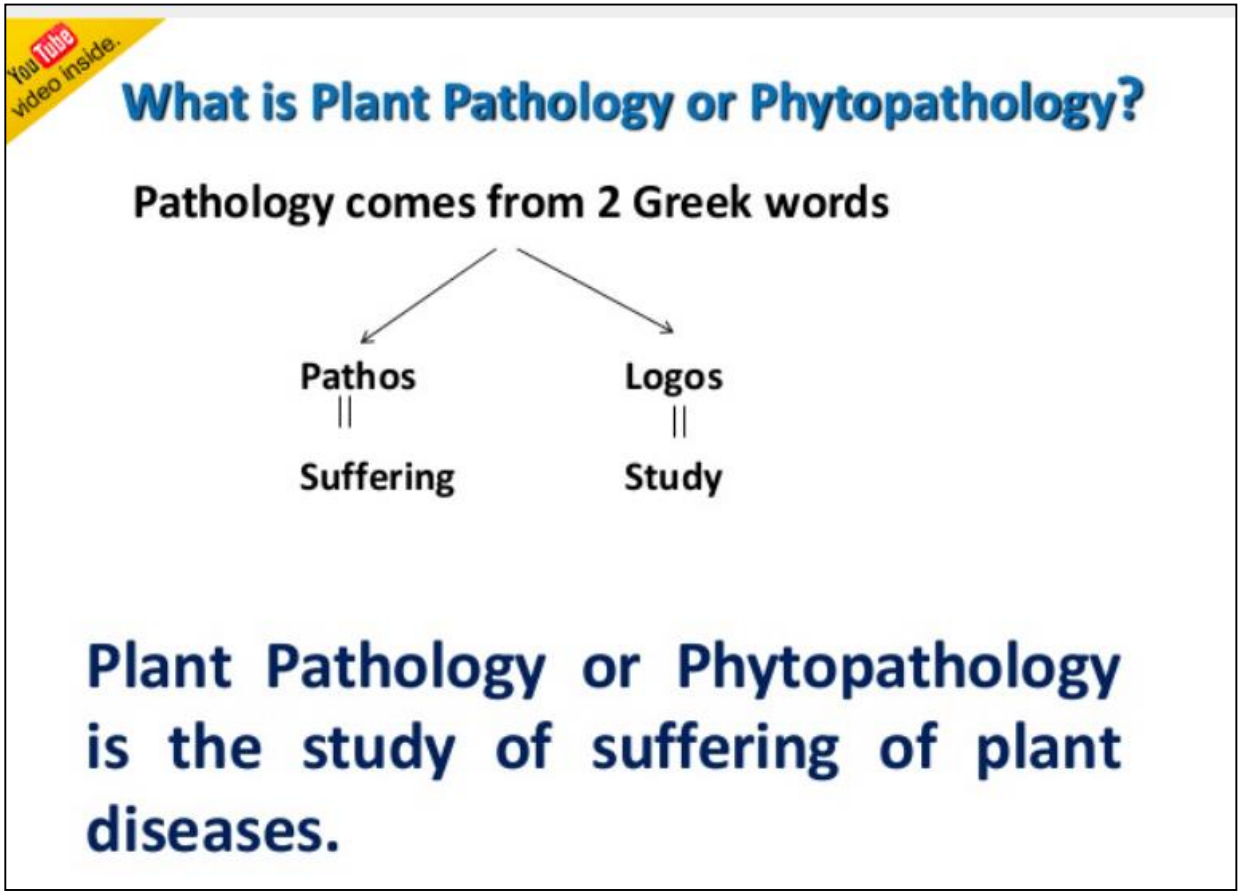
What is a plant pathology?

A plant is diseased when it's not at ease



What is a plant disease?

A plant is diseased when it's not at ease

- A plant is diseased when its chemistry or structure has been altered in a continuous way.
- The disease continuously alters normal functions of the plant.
- This definition tells us that a leaf pulled off a tree is not a disease but instead an injury because the alteration is not continuous.
- The lack of normal functions over a period of time results in a plant with undesirable symptoms.

Causes of plant disease

Infectious and noninfectious plant diseases

- Plant diseases can be:

1. Infectious (The primary agents of plant disease are fungi, bacteria, viruses and viroids, nematodes, parasitic seed plants, transmitted from plant to plant), or
2. Noninfectious. Noninfectious diseases are usually referred to as disorders.

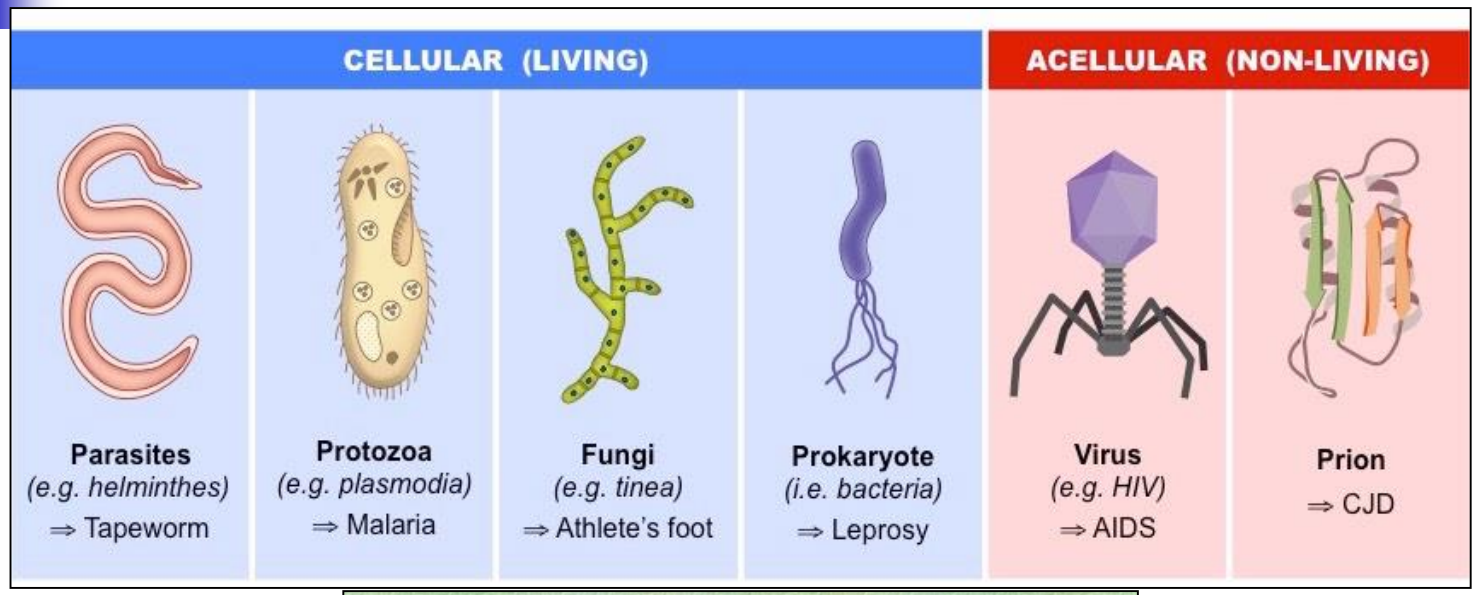
- Common plant disorders are caused by:

1. deficiencies in plant nutrients,
2. waterlogged or polluted soil, and
3. by polluted air.

The Gale Encyclopedia of Science,2008;..

Causes of plant disease

Infectious plant diseases

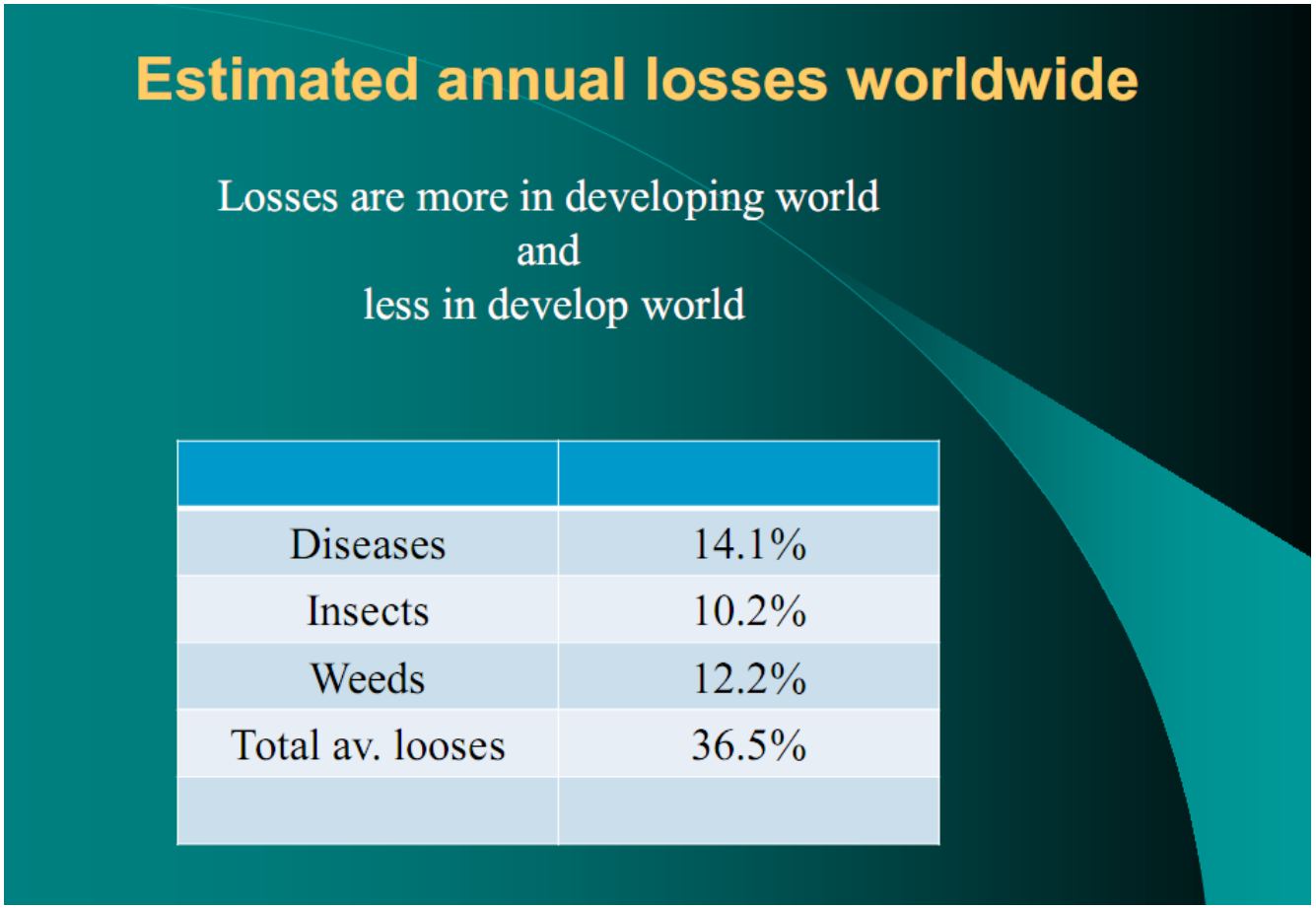


- **Produced by Living Agents (Biotic)**
  - **Fungi**
  - **Bacteria**
  - **Viruses, Viroids, Mycoplasmas Spiroplasms**
  - **Nematodes**
  - **Parasitic Vascular Plants**

Economics of plant diseases

Bacterial diseases and crop losses

Crop losses due to disease and pests
Annual losses worldwide



Diseases	14.1%
Insects	10.2%
Weeds	12.2%
Total av. looses	36.5%

Crop losses due to disease and pests
Worldwide and USA

- All crop pests (pathogens, arthropods, and weeds) combined cause:

1. Preharvest losses of 42%
2. An additional 10% loss after harvest.

- Of these:

- 13% are due to plant pathogens,
- 15% to arthropods, and
- 13% to weeds.

Estimated annual crop losses worldwide Agrois,2005

Attainable crop protection (2002 prices)	$1.5 trillion
Actual crop production (~36.5%)	$950 billion
Production without crop protection	$455 billion
Losses prevented by crop protection	$415 billion
Actual annual losses to world crop production	$550 billion
Losses caused by disease only (14.1%)	$220 billion

Crop losses due to disease and pests
Worldwide and USA

- According to some estimates over 30% of the world's crops are lost in the field, another 15% are destroyed during transit and storage.
- Crop losses in the USA:
  - 9.1 billion lost to disease
  - 7.7 billion to insects
  - 6.2 billion to weeds.

Crop losses due to disease and pests Worldwide

- Plants make up 80 percent of our food but are under constant and increasing threat from pests and diseases.
- Every year, up to 40 percent of global food crops are lost to plant pests and diseases.
- This leads to

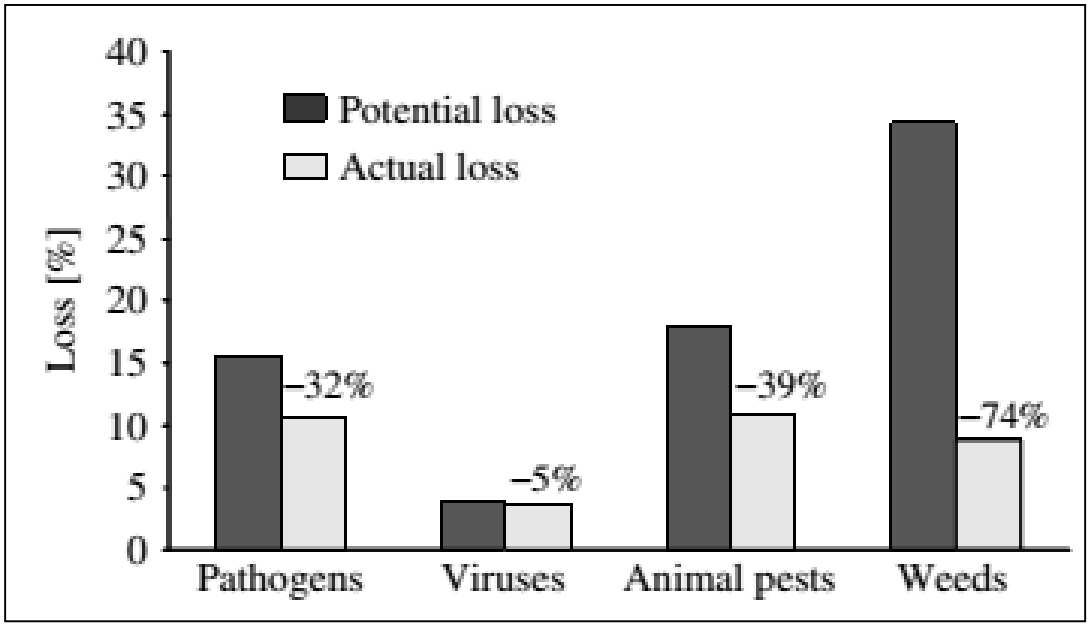
1. annual agricultural trade losses of over $220 billion,
2. leaves millions of people facing hunger, and
3. severely damages agriculture – the primary income source for poor rural communities.

FAO,2020

Crop losses due to disease and pests

Efficacy of pest control worldwide in reducing loss caused by pathogens, viruses, animal pests and weeds

- The efficacy of control of pathogens and animal pests only reaches 32 and 39%, respectively, compared to almost 74% for weed control.



Folnovic,2015

Crop losses in the United States
Introduced pathogens into the USA

- In the United States alone, plants are subject to attack by over 50,000 different pathogens, primarily fungi, viruses, bacteria, and nematodes.
- Economically less important than diseases caused by fungi and viruses.
- About 65% of U.S. crop losses are due to nonindigenous (introduced) pathogens, amounting to an estimated cost of $137 billion annually.

Madden and Wheelis,2003; Fletcher *et al.*,2006

Crop losses due to bacterial diseases
Bacterial diseases impacts

- Plant pathogenic bacteria impact innumerable and valuable agricultural crops, causing hundreds of millions of dollars in damage each year (Jackson,2009).
- However, phytopathogenic bacteria cause devastating effects on plant productivity and yield.

The major types of plant-pathogenic bacteria

Genus/species	General disease symptoms
Gram-negative bacteria	
Acetobacter spp.	Pink disease of pineapple fruit
Acidovorax spp.	Leaf blight, leaf spots/streak
Agrobacterium spp.	Crown gall, hairy root formation
Burkholderia spp.	Vascular wilts, rots
Enterobacter spp.	Cankers, leaf spots and rots
Erwinia spp.	Vascular wilts, dry necrosis, leaf spots and soft rots
Gluconobacter oxydans	Pink disease of pineapple fruit
Pantoea spp.	Vascular wilts, rots
Pseudomonas spp.	Leaf spots, vascular wilts, soft rots
Ralstonia spp.	Vascular wilts
Rhizobacter daucus	Bacterial gall of carrot
Serratia marcescens	Crown and root rot of lucerne
Xanthomonas spp.	Leaf spots, vascular wilts, stem cankers
Xylella fastidiosa	Pierce's disease of grape
Xylophilus ampelinus	Bacterial blight of grape
Gram-positive bacteria	
Arthrobacter ilicis	Holly bacterial blight
Clavibacter spp.	Vascular wilts, cankers
Curtobacterium spp.	Silvering disease, vascular wilts
Nocardia vaccinii	Blueberry gall
Rathayibacter spp.	Gumming disease
Rhodococcus fascians	Leafy gall
Streptomyces spp. (*S. scabies*)	Potato scab

Plant Pathologist's Pocketbook,2002

Top 10 plant pathogenic bacteria in molecular plant pathology

The list includes, in rank order

Rank	Bacterial pathogen
1	*Pseudomonas syringae* pathovars
2	*Ralstonia solanacearum*
3	*Agrobacterium tumefaciens*
4	*Xanthomonas oryzae* pv. *oryzae*
5	*Xanthomonas campestris* pathovars
6	*Xanthomonas axonopodis* pathovars
7	*Erwinia amylovora*
8	*Xylella fastidiosa*
9	*Dickeya* (*dadantii* and *solani*)
10	*Pectobacterium carotovorum* (and *Pectobacterium atrosepticum*)

Bacteria garnering honorable mentions for just missing out on the Top 10 include *Clavibacter michiganensis* (*michiganensis* and *sepedonicus*), *Pseudomonas savastanoi* and *Candidatus Liberibacter asiaticus*.

Mansfield *et al.*,2012

Examples of severe losses caused by plant bacterial diseases

Disease	Location	Comments
A. Bacterial Diseases		
Citrus canker	Asia, Africa, Brazil, U.S.	Caused eradication of millions of trees in Florida in 1910s and again in the 1980s and 1990s.
Fire blight of pome fruits	North America, Europe, Asia	Kills numerous trees annually.
Soft rot of vegetables	Worldwide	Huge losses of fleshy vegetables.
B. Phytoplasmal Diseases		
Peach yellows	Eastern U.S., Russia	Historical, 10 million peach trees killed.
Pear decline	Pacific coast states and Canada	Millions of pear trees killed.

Disease management considerations

- Integrated pest management best multiple prong(composed of) approach:

1. Importance of the disease - economics - health issues;
2. Availability of resistance;
3. Reliable and simple screening techniques;
4. Availability and effectiveness of other control mechanisms.

Disease Control vs Management

Control

- Goal: Zero disease
- Qualitative assessment
- Disease present?
  - Yes or no
- Elimination, prevent, or exclude disease.
- Reality: Impractical or impossible.

Management

- Goal: Reduced disease
- Quantitative assessment
  - Amount present
  - Disease progress over time
- Maintain disease below acceptable thresholds
- More practical.

Priorities for disease control

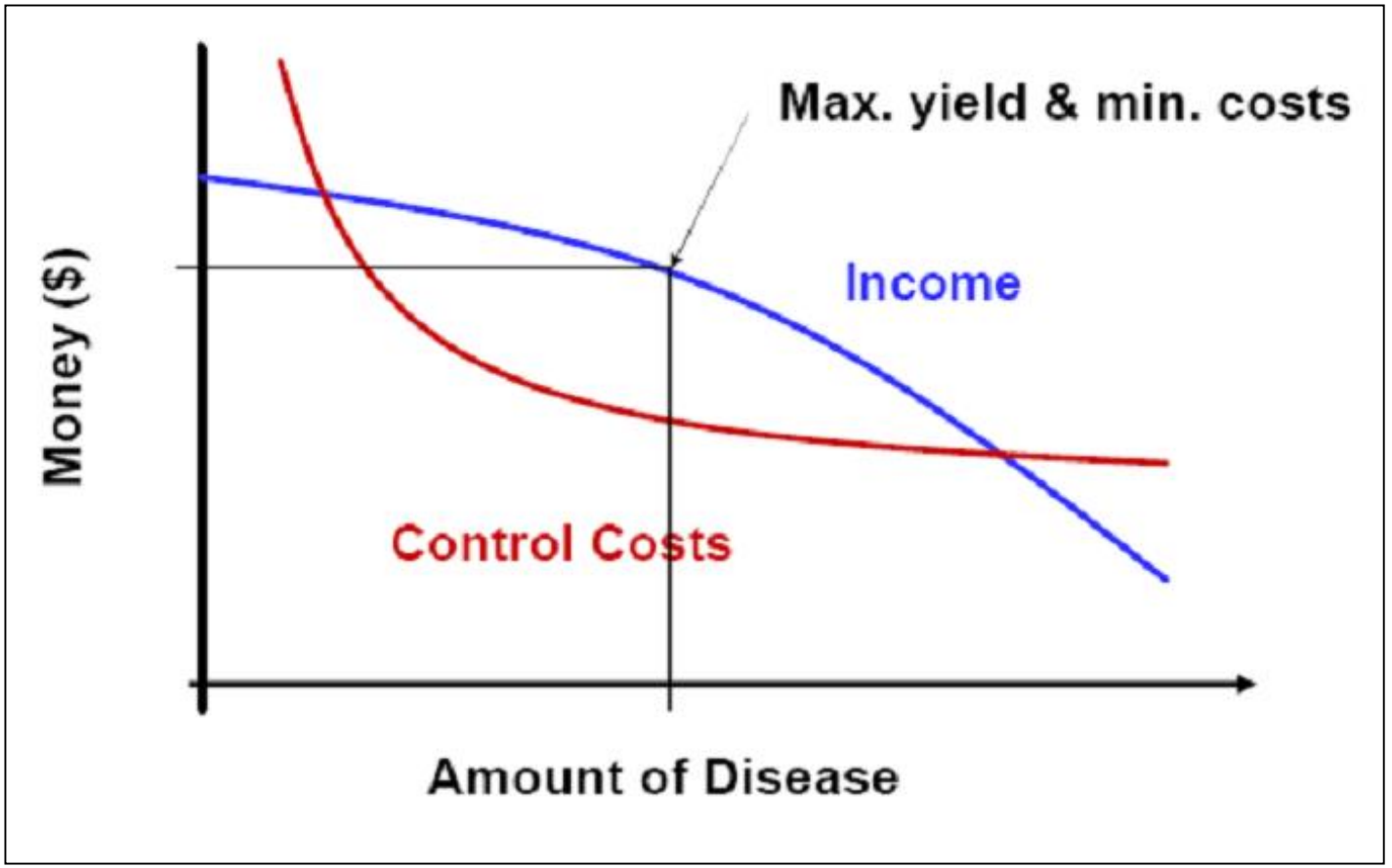
- Yield is affected by:

1. Inoculum levels of pathogens, and
2. The severity of the diseases they cause.

- Disease severity is the measure of damage done by a disease.
- The measurement of all three factors is therefore necessary in order to set:

1. Priorities for control,
2. Predict yield losses, and
3. Evaluate control measures.

Crop incomes vs. Control costs



Plant pathology
Challenging for controlling plant diseases

- Plant pathology is both a basic and an applied science.
- The role of plant pathology in our rapidly changing world is of increasing importance.
- Our discipline is connected with very relevant

1. social and economic issues.
2. environmental protection and conservation,
3. food safety and security, and
4. climate change, to name a few.

As the ever-increasing world population demands more to consume, we must respond with improved methods of disease control that are less destructive to the environment.

Gullino,2008

Plant pathology

Challenging for controlling plant diseases

Environmentally persistent pathogens

- There is growing concern worldwide about environmentally persistent pathogens.
- This new dimension of research on pathogens is making considerable progress for human pathogens but it has received little attention for plant pathogens such as *P. syringae*.
- For such studies, it is essential to have reliable techniques for the isolation and/or identification of natural populations that can be present at low concentrations in substrates other than infected tissues such as rivers.

Guilbaud *et al.*,2015

Plant pathology
Challenging for controlling plant diseases

- Improvements in agricultural technology require attention to basic science with applications that can be quickly focused to solve specific crop production problems.
- We are unique and indispensable because we represent an integrated science, a discipline that brings together components of many sciences such as botany, plant physiology, and microbiology.
- We must constantly adapt and effectively implement our research findings.

Gullino,2008

Plant pathology

Challenging for controlling plant bacterial diseases

- Plant diseases caused by bacteria are a major economic liability to agricultural production.
- Disease control has been a major challenge for many bacterial diseases.
- This challenge is a direct result of:

1. Pathogen variability;
2. High probability for mutation or gene transfer in the pathogen when confronted with resistance genes or bactericides;
3. High pathogen multiplication rate during optimal conditions for disease development, and
4. Lack of adequate chemical-based approaches for control.

Jones *et al.*,2007

Plant pathology

Challenging for controlling plant bacterial diseases

- Disease control is best achieved using an integrated management approach by combining:

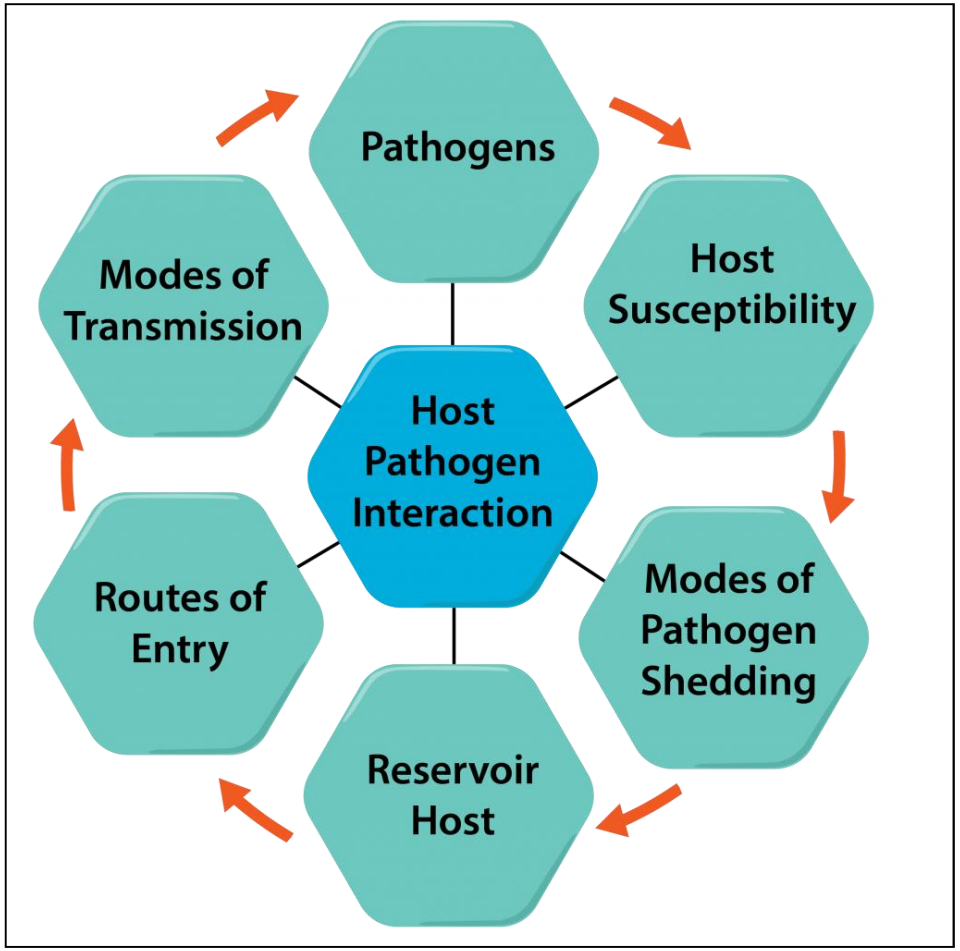
1. Proper cultural practices,
2. Chemicals such as bactericides, or
3. Plant activators where applicable,
4. Introgression of plant resistance genes, and
5. Biological control strategies.

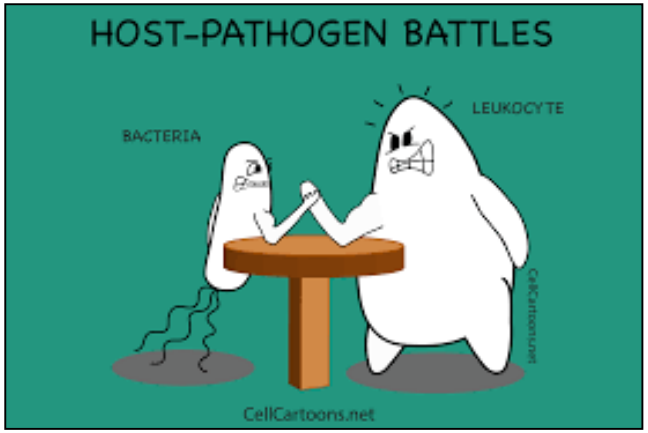
Jones *et al.*,2007

Plant disease management

Host-Plant Interaction

Challenging for controlling plant bacterial diseases





Plant disease management

Underexplored niches in research on plant pathogenic bacteria

Research areas

- In brief:

1. Effective monitoring and surveillance system for rapid and accurate diagnostics of emerging and re-emerging plant diseases. Bacterial diseases likely to cause severe losses in the future.
2. Identifying hidden/multiple pathogens/polymicrobial diseases partnerships
3. Phytobiomes (plant microbiomes/plant probiotics)

- Endophytic phytobiomes,
- Rhizosphere/soil phytobiomes.

Bacteria are estimated to occupy between 7% and 15% of the total root surface area.

Plant disease management

Underexplored niches in research on plant pathogenic bacteria

More details on management research areas

- Major disease of major staple crop (APS compendium lists approximately 100 pathogens of soybean, of which only 35 are economically important).
- Disease of understudied staple crop (e.g. plantains, oil palms and cassava)
- Major disease of high-value specialty crop or developing nation crop
- Effective disease management would expand cropping zone
- Commodity group or international non-government organization support
- Current control methods environmentally undesirable
- Pathogen persistence in environment
- Pathogen colonization of plant surface or vasculature
- Pathogen latent or commensal stage
- Pathogen seed transmissibility
- Pathogen insect transmissibility
- System has unique biology (e.g. *Agrobacterium tumefaciens*)
- Plant-associated human pathogen
- Pathosystem has potential impact on medical biology.

Allen *et al.*,2009;..

Plant disease management

More details on management research areas

- Epidemiology
- Evolution of diseases
- **Bio/nanotechnology**
- **Microbial/pathogen diversity**
- **Identification of hidden partnerships(synergists)**
- International quarantine mechanisms
- Ecology of biocontrol
- Soil-borne disease control
- Foliar and above-ground disease control
- Postharvest disease control
- Commercialization
- Regulations and risk assessment
- Integration

Plant disease management

More details on management research areas

- **Biotechnological approaches:**
  - Recombinant DNA technology
  - Risk assessment
  - Consumer forces
- Biopesticides:
  - Bioassay techniques for development of biopesticides and transgenic plants;
  - Biopesticides for control of key pests in export crops (apples, kiwifruit, stone fruit, avocados).

Chris Hale,2009

Plant disease management

More details on management research areas

- **Common bacterial pathogens of plants and animals:**
- There are pathogens that are quite adept (highly skilled) at attacking both plants and animals. e.g.

1. *Erwinia* spp.: A well-known cause of a variety of wilt diseases in plants, including bacterial fire blight of apples and pears.
2. *Burkholderia cepacia*: The causal agent of soft rot in onion, can cause life-threatening infections in CF(cystic fibrosis) disease as human wounds and abscesses)patients.
3. *P. aeruginosa*: the best studied cross-kingdom pathogen.

Plant disease management

More details on management research areas

- **Common bacterial pathogens of plants and animals:**

1. It is just possible that during the process of evolution, *Salmonella enterica* (causes of food poisoning and infected fruits and vegetables), and
2. Pathogenic *E. coli*, presently characterized as plant-associated bacteria, may become plant pathogens.

- It is a matter of great concern for plant bacteriologists.

Thind,2013; García and Hirt,2014

Plant disease management

More details on management research areas

- **Common bacterial pathogens of plants and animals:**

1. Plants play a critical role in the life cycle of human enteric bacterial pathogens.
2. Also, animal/human bacterial pathogens and plant pathogens have some common mechanisms such as:

- Type III secretion systems and their effectors, and
- Transcriptional regulators, which function in both the hosts.

Thind,2013; García and Hirt,2014

Plant disease management

More details on management research areas

- **Microbial/pathogen diversity:**
- Study on genetic and pathogenic characteristics of *Dickeya* (*Erwinia*) *chrysanthemi* was indicated:

1. A shift in Ech type population on seed potatoes as the weakly macerating and HR^- isolates to strongly macerating and HR^+ isolate.
2. The weakly macerating and HR^- isolates with optimum temperature of 25-28°C have been repressed during the past five years by strongly macerating and HR^+ isolates with higher optimum temperature.

Van Vaerenbergh *et al.*,2009

Plant bacterial Diseases
Host Range

- The host ranges of individual bacterial pathogens vary greatly.

1. Some are very wide: e.g. *Agrobacterium tumefaciens, Pseudomonas syringae* pv. *syringae* and *R. solanacearum,* which all affect many genera and various plant families.
2. Some are more restricted: such as *E. amylovora*, which affects a number of genera, nearly all in the family *Rosaceae.*
3. Others have very narrow host ranges: often a single species, or a few species in a single genus, e.g. most pathovars in the genus *Xanthomonas* or the species *P. syringae*.

- In most instances, the species showing wide host ranges are heterogeneous, showing divisions into strains of differing biovars or pathovars, races, etc.

Plant Pathologist's Pocketbook,2002

Plant disease management

Research areas

1. Emerging & re-emerging plant disease/pathogens

- Emerging infectious diseases (EIDs) pose threats to conservation and public health.
- **Emergence of agents:**
- Previously known agents whose role in specific diseases has previously gone unrecognized.
- **Re-emergence of agents:**
- Whose incidence of disease had significantly declined in the past, but whose incidence of disease has reappeared. This class of diseases is known as re-emerging infectious diseases.

Plant disease management

Research areas

Emerging & re-emerging plant disease/pathogens

Disease	Comments
Bacterial leaf blight of rice *Xanthomonas oryzae* pv. *oryzae*	Destructive in Japan and India; spreading.
Bacterial wilt of banana(Moko disease) *Ralstonia solanacearum* (race 2, biovar 1)	Destructive in the Americas; spreading elsewhere.
Pierce's disease of grape *Xylella fastidiosa*	Deadly in southeast U.S.; spreading into California.
Citrus variegation chlorosis *Xylella fastidiosa*	Destructive in Brazil; spreading.
Citrus greening or dragon disease *Candidatus* Liberibacter asiaticus, africanus and americanus	Severe in Asia; spreading.
Dickeya species: an emerging problem for potato production in Europe.	Since 2004-5 a new pathogen, *D. solani*, spreading across Europe via trade in seed tubers and is causing increasing economic losses.

Bacterial diseases likely to cause severe losses in the future

Emerging & re-emerging disease/pathogens

Disease	Comments
Zebra chip of potato '*Candidatus* Liberibacter solanacearum' (CLso)	Destructive in Japan and India; spreading. It was first identified in the mid-1990s in Mexico, and now is present in Central and North America and in New Zealand. Severe epidemics occurred in the southwestern United States in the mid-2000s.

PowerPoints/pdf files/Monographs
Emerging and re-emerging plant diseases

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PowerPoints/pdf files/Monographs
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Plant disease management

Research areas

Emerging & re-emerging plant disease/pathogens

- Various emerging plant diseases responsible for a large amount of crop destruction every year all over the world and the challenges that the agricultural sector face to overcome this problem.

Infectious diseases likely to cause severe losses in the future

Emerging & re-emerging disease/pathogens

- Due to the significant threat of new and re-emerging plant diseases and pathogens, the United Nations declared 2020 the International Year of Plant Health.
- New and re-emerging plant diseases threaten global ecosystems, health, food security, and economy, which are particularly vulnerable due to geographic expansion, climate change, modified land use, and the increased use of agrochemical including insecticides, herbicides, fungicides, and nematicides in agricultural practices.

Janssen and Nehela,2022

Infectious diseases likely to cause severe losses in the future

Emerging & re-emerging disease/pathogens

- Novel and emerging plant disease can be caused by a broad range of organisms that include fungi, bacteria, bacteria, viruses, and phytoplasmas, and it produces occasionally important crop losses of global economic importance.
- Recent research and developments such as the use of molecular biology have led to improved technologies for faster and better detection of pathogens.
- Conventional epidemiology has changed and now includes molecular factors, ecology, and evolution as new challenges for plant pathology research.

Janssen and Nehela,2022

Infectious diseases likely to cause severe losses in the future

Emerging & re-emerging disease/pathogens

- Consumer demands for healthier food and sustainability of food production have made many farmers switch to integrated disease management strategies.
- On the other hand, global climate changes and increased traffic of people and goods are leading to the emergence of new diseases, or the re-emergence of diseases from the past, putting modern agriculture in a constantly alert situation.

Janssen and Nehela,2022

Plant disease management

Research areas

Emerging & re-emerging plant disease/pathogens

- Research topics of interest include, but are not limited to:

1. the ecology, epidemiology and ecological genomics of emerging plant diseases,
2. the emergence and evolution of invasive traits (e.g. modeling virulence/antimicrobial resistance) in plant pathogens,
3. the role of climatic and/or phytobiome changes in disease emergence.
4. Much is known about crop plant emerging infectious diseases (EIDs), but there is little information about wild-plant EIDs, suggesting that their impact on conservation is underestimated.

Anderson *et al.*, 2004; Ohio State University

Plant disease management

Research areas

Emerging & re-emerging plant disease/pathogens

- In recent decades, the issue of emerging and reemerging infectious diseases, especially those related to viruses, has become an increasingly important area of concern in plant health.
- Such diseases in a plant context are generally insect- or seed-transmitted, and changes associated with global warming, and accidental introduction of vectors or infected materials in new areas facilitated by global trade, may affect their incidence, severity and diffusion.

www. frontiersin.org/research-topics/13001/emerging-and-reemerging-plant-viruses

Plant disease management

Research areas

Emerging & re-emerging plant disease/pathogens

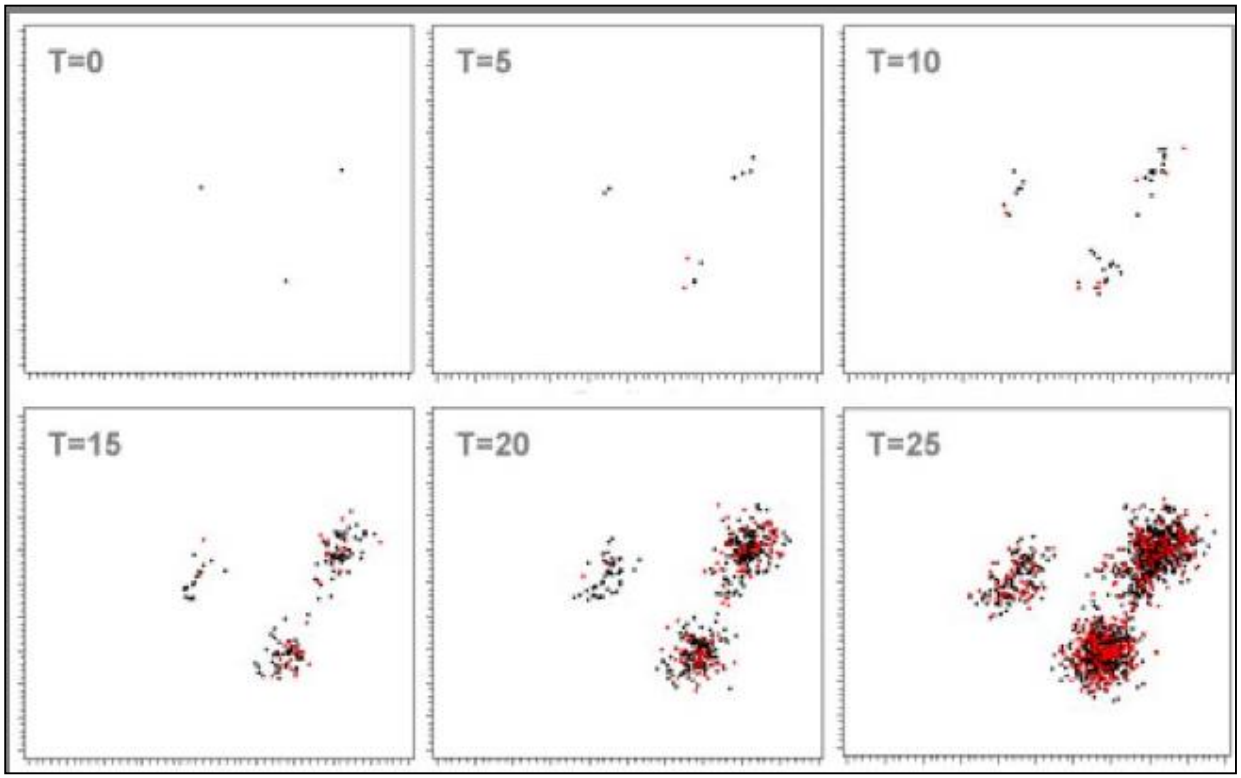
1. The extensive global trade of agricultural products is fueling opportunities for short-, medium-, and long-distance movement of plant pathogens as well as insects that transmit pathogens.
2. Changing regional and global climatic conditions are driving changes in the geographic distribution of plant diseases.
3. In addition, new plant pathogens are emerging when organisms adapt to new plant hosts or cultivars.
4. Existing pathogens are also re-emerging following the development of chemical resistance or changes in agricultural management practices and plant varieties.

APS,2019

Plant disease management

Research areas

Emerging & re-emerging plant disease/pathogens



Infectious diseases spread not randomly but around initial infections.

APS,2019;…

Infectious diseases likely to cause severe losses in the future

Emerging & re-emerging disease/pathogens

- The multidisciplinary links between plant pathology and other disciplines; disease management, including:
- Precision agriculture, plant growth and development, and decision analysis and disease risk; the development and use of new and novel plant protection chemicals; new ways of exploiting host genetic diversity including host resistance deployment; a new perspective on biological control and microbial interactions; advances in surveillance and detection technologies; invasion of exotic and re-emerging plant pathogens; and the consequences of climate change affecting all aspects of agriculture, the environment, and their interactions(Jeger *et al.*,2021).

Exotic pathogens cause severe damage in natural populations in the absence of coevolutionary dynamics with their hosts. **Exotic and invasive species** are two types of non-native species. Non-native species can be found in a second ecosystem apart from the ecosystem they evolved from. They are called exotic species. When an exotic species becomes harmful to the ecosystem, it is called an invasive species. For Europe, up to now, the threat arises from an endemic vector acquiring and spreading *X. fastidiosa* as an exotic and introduced pathogen.

Management of emerging plant diseases/epidemics

Emerging & re-emerging disease/pathogens

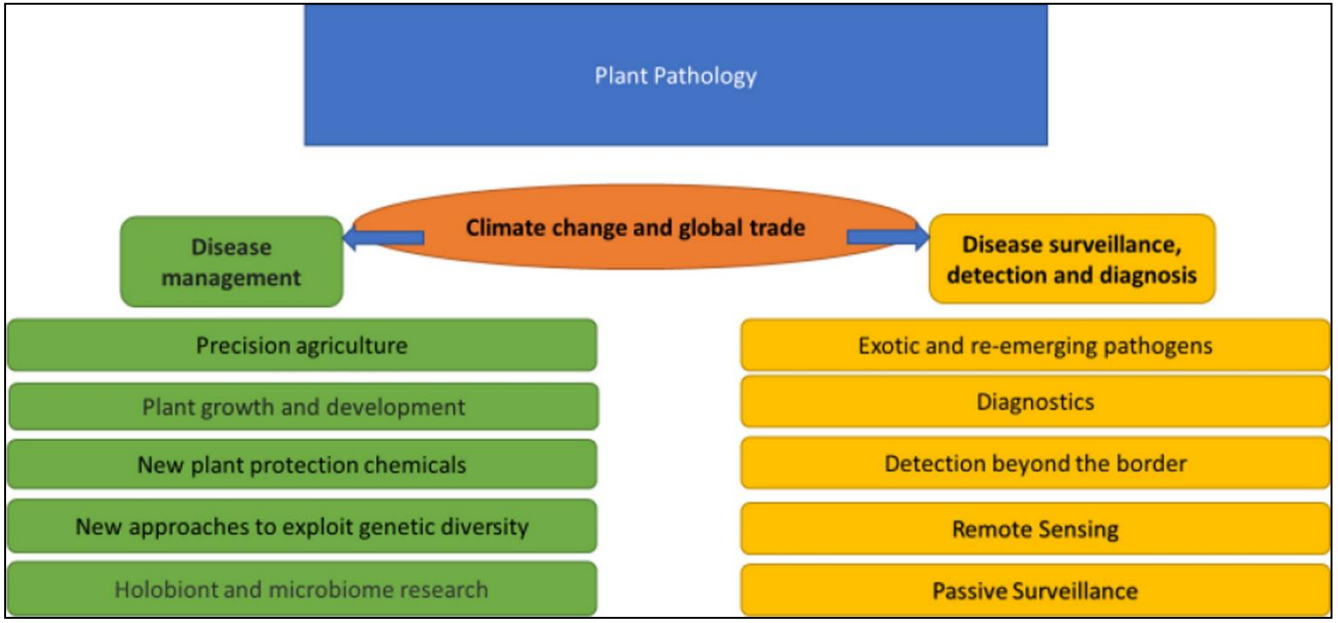
- A multifaceted approach is needed to prevent pathogen introduction, minimize pathogen movement across national and state borders, and meet the ongoing challenges posed by new and re-emerging pathogens.
- This approach requires:

1. An effective monitoring and surveillance system;
2. Rapid and accurate diagnostics;
3. Predictive knowledge of the risk of pathogen introductions; and,
4. The development of effective prevention and mitigation(the action of reducing the severity) measures.

APS,2019;…

Management of emerging plant diseases/epidemics

Emerging & re-emerging disease/pathogens



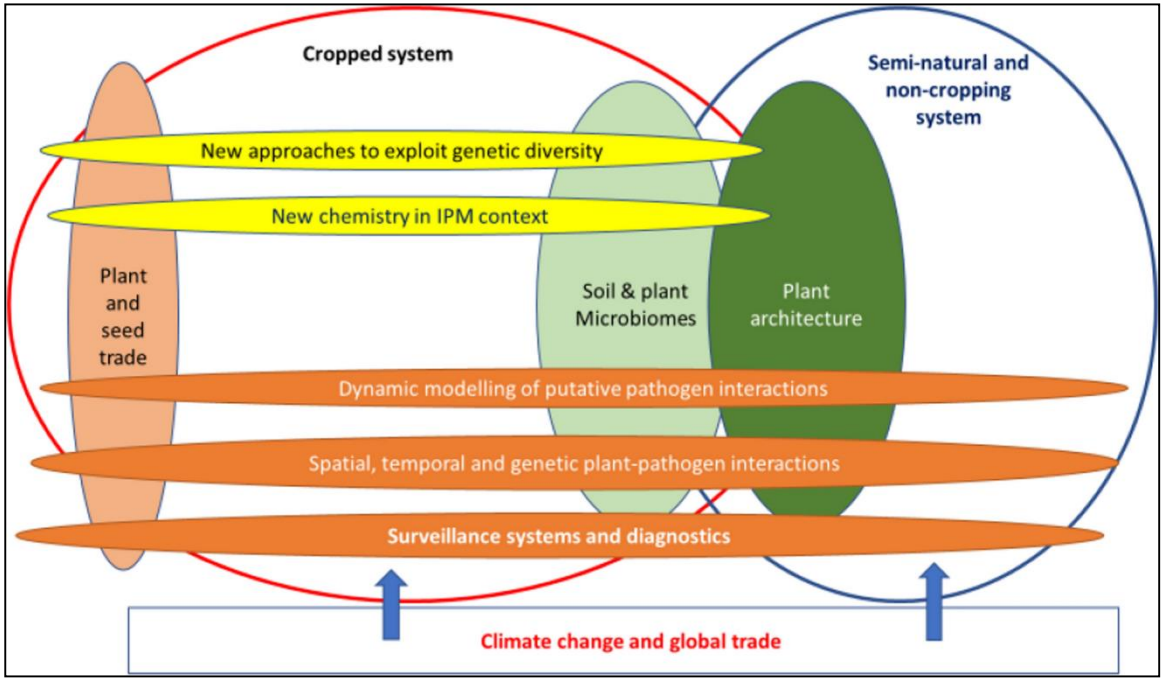
Schematic showing topics within plant pathology where multidisciplinary approaches in research have been developed but need further implementation as described in this review. The two arms of the schematics are shown for ease of presentation. Cross links between the two are present and for some there has been wider involvement of farmers, landholders, regulators, and other participants, but in all areas there will be a need for improvement to meet future challenges as discussed in this review.

Holobiont' consists of a host and its associated microbiota, the 'microbiome'.

Jeger *et al.*,2021

Infectious diseases likely to cause severe losses in the future

Emerging & re-emerging disease/pathogens



Schematic showing how the interlocking of different strands of multidisciplinary research in plant pathology should develop to meet the cropping, food security and environmental challenges of the coming decades. The diagram shows the continuum between cropped and non-cropped systems. Genetic and plant chemistry research will contribute from seed to mature plant performance. An understanding and management of host–pathogen interactions and epidemiology will benefit from research across the continuum. **Climate change and the global trade in commodities will drive the introduction** and spread of exotic pathogens into both cropped and non-cropped systems with the concomitant need for improved and linked surveillance and diagnostic systems (Jeger *et al.*,2021).

Plant disease management

Research areas

2. Identifying hidden partnerships

- Plants face multiple pathogens and there are hints that some pathogens function best in pairs:
- Synergists
- Hidden partnerships
- Mixed infections, but this area has been little explored.

Allen *et al.*,2009;..

Plant disease management

Research areas

Identifying hidden partnerships

- The similarity in symptoms means it is often difficult to distinguish these diseases visually, especially when mixed infections occur.
- Indeed, often no attempt is made during field assessment to discriminate within a disease complex.
- Overall, our study highlights that the occurrence of mixed infection is common and widespread, with important implications for wheat disease management and breeding strategies.

Justesen *et al.*,2021

Plant disease management

Polymicrobial Diseases

Complex Plant Diseases(hidden partnerships)

- **Synergistic Pathogen-Pathogen Interactions:**

1. **Bacteria-Bacteria Interactions**
2. **Virus-Virus Interactions**
3. **Mixed Interactions**

Lamichhane and Venturi,2015

Plant disease management

Polymicrobial Diseases

New approaches are needed for studies of complex plant diseases

1. The authors performed the isolation of pathogen on culture growth media.
2. In addition, other more specific (e.g., immunofluorescence or PCR) or generic (e.g., morphological identification) assays were used.
3. However, currently we have new knowledge and techniques which may facilitate the understanding of the total microbial species involved in plant diseases as well as the underlying mechanisms.

Lamichhane and Venturi,2015

Plant disease management

Polymicrobial Diseases

New approaches are needed for studies of complex plant diseases

- In the modern era of biodiversity surveillance, techniques such as next-generation sequencing (NGS) have enabled high-throughput analyses of complex microbial populations.
- In the last 10 years, metagenomic projects have been combined with NGS technologies boosting studies in microbial ecology at a very fast pace.

Metagenomics or community genomics is the study of genetic material recovered directly from environmental samples, consisting of the genomes of many individual organisms.

Lamichhane and Venturi,2015

Plant disease management

Bacteria-Bacteria Interactions

Host	Disease	Causal agents
Tomato	Pith necrosis	*Pseudomonas corrugate*, *P. mediterranea*, *P. Marginalis* and *P. cichorii*
Mulberry	Wilt	*Enterobacter asburiae* and *Enterobacter* sp.
Broccoli	Head rot	*P. marginalis*, *Pectobacterium carotovorum*, *P. fluorescens*, and *P. viridiflava*
Potato	Zebra complex	*Candidatus liberibacter solanacearum* and *Candidatus liberibacter psyllaurous*

Potato Zebra chip

Lamichhane and Venturi,2015

Plant disease management
Bacteria-Bacteria Interactions

Interaction between *Pseudomonas savastanoi* pv. *savastanoi* and *Erwinia toletana* in olive knots:

- *Pseudomonas savastanoi* pv. *savastanoi* (PSV) which is a pathogen of olive trees that can cause tumors once it gets to the inside of the plant.
- PSV in the presence of the endophytic bacteria *Erwinia toletana* could induce a significantly bigger tumor.

Venturi, Current aspects of European endophyte research,2012

Plant disease management

Identifying hidden partnerships

Bacteria-Bacteria Interactions

Interaction between *Pseudomonas savastanoi* pv. *savastanoi* and *Pantoea agglomerans* in olive knots:

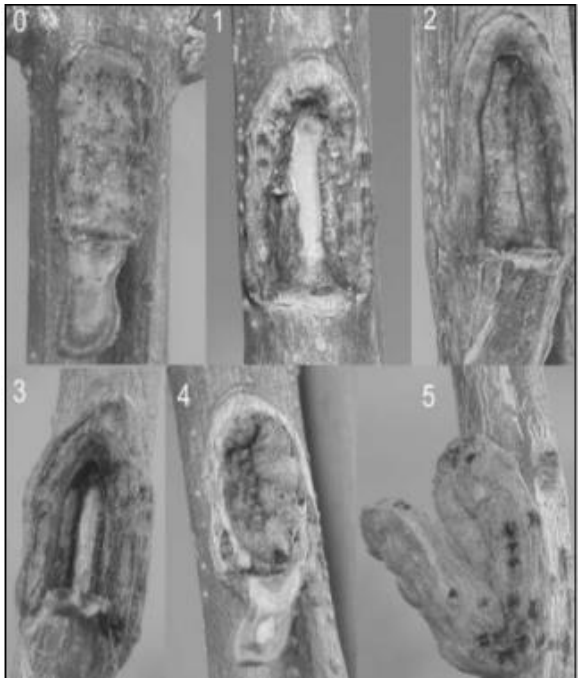
- *Pantoea agglomerans* was found associated with the pathogen *Pseudomonas savastanoi* pv. *savastanoi* in 70% of the olive knots examined.
- In some cases the association of *P. agglomerans*, which in culture was found to produce indole-3-acetic acid but not cytokinins, with *Ps. savastanoi* resulted in an increase in the size of knots.

Marchi *et al.*,2006

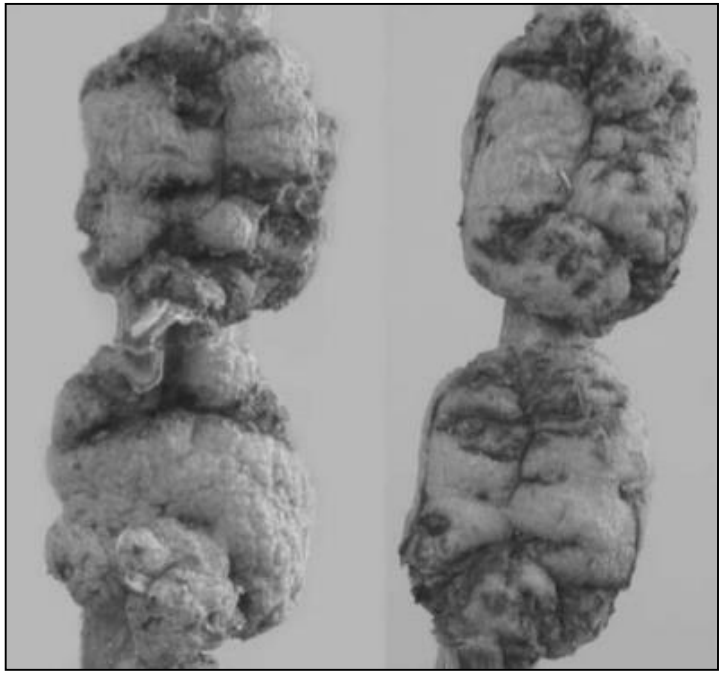
Olive knot

Bacteria-Bacteria Interactions

Interaction between *Pseudomonas savastanoi* pv. *savastanoi* and *Pantoea agglomerans* in olive knots



Symptoms on 1-year-old olive stems 60 days after inoculation with 1) *Pantoea agglomerans*, 2) with *Pseudomonas savastanoi* or 3) with a suspension of those bacteria mixed in a ratio of 1:1.



Knot morphology 120 days after inoculation with a suspension of *Pseudomonas savastanoi* and *Pantoea agglomerans* mixed in a ratio of 1:1 (left) or with a suspension of *P. savastanoi* (right).

Marchi *et al.*,2006

Plant disease management

Bacteria-Bacteria Interactions

Identifying hidden partnerships

Biocontrol agent interrupt interaction between *Pseudomonas savastanoi* pv. *savastanoi* and *Pantoea agglomerans* in olive knots:

- A dominant population of *P. agglomerans* at the inoculation site tended to depress the growth of *Ps. savastanoi*, probably because of:

1. competition for space and nutrients between these bacteria, and
2. by means of antibiotic production by *P. agglomerans*.

Marchi *et al.*,2006

Plant disease management

Identifying hidden partnerships

Pectobacterium - *Clostridium* partnership

Interaction between *Clostridium* and *Pectobacterium* species in potato soft rot disease:

- *Clostridium* and *Pectobacterium* species are routinely found together in decaying vegetables and both can cause disease on their own.
- Although potatoes are mostly starch, *Pectobacterium* curiously cannot degrade starch, while *Clostridium* efficiently breaks down this polymer.
- Close relatives of *Pectobacterium*, such as *Klebsiella*, can metabolize starch.

Allen *et al.*,2009

Blossom Blight of Kiwifruit

Hidden partnerships (synergists)

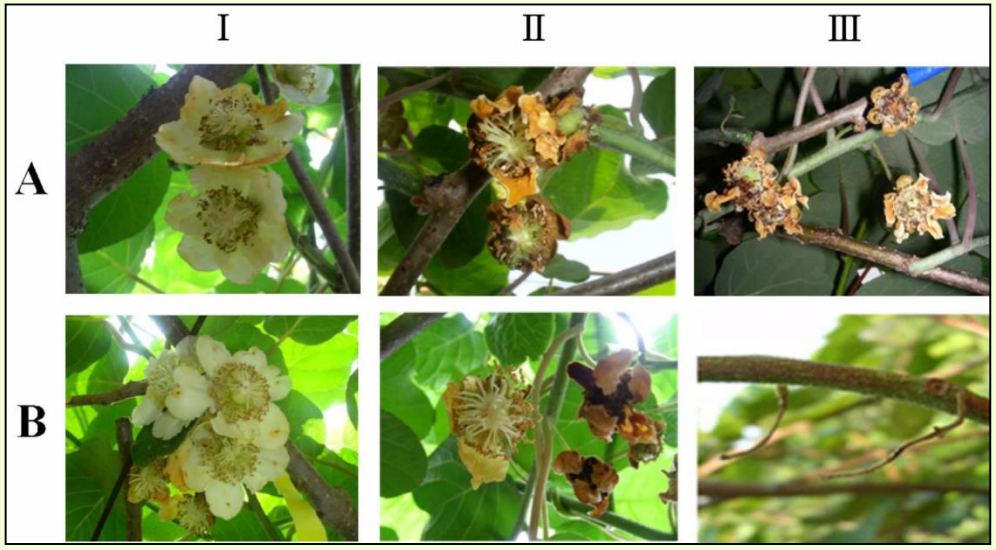
Pseudomonas syringae pv. *syringae* and *P. fluorescens*

- Symptoms on kiwifruit flowers caused by:

A. *P. s.* pv. *syringae* TDS2, and

B. *P. fluorescens* KDK8.

- *Pss* primarily affected the stamen, while *P. fluorescens* caused rotting of all internal tissues of buds or flowers.



Lee *et al.*,2009

Blossom Blight of Kiwifruit

Bacteria-Bacteria Interactions

Association of *P. syringae* pv. *syringae* with *Bacillus pumilus* in causing leaf and twig dieback of Asian pear

- A gram positive bacterium(*Bacillus pumilus*) was frequently isolated alone or in combination with *Pseudomonas syringae* pv. *syringae* from naturally infected twigs of field-grown pears.

Li *et al.*,2009

Oak decline

Hidden partnerships (synergists)

Brenneria quercina and *Serratia* spp.

- *Serratia* sp. is also an opportunistic human pathogen that can be found in plants.
- Pathogenicity tests suggested that the Spanish *Brenneria quercina* and *Serratia* isolates are able to survive and grow on oak trees, and to produce bark symptoms.
- Also, the fact that the studied isolates satisfied Koch's postulates supports the hypothesis that both bacteria are causal agents of oak disease.
- The pathogenicity of *Serratia* has not been previously reported as a plant pathogen.

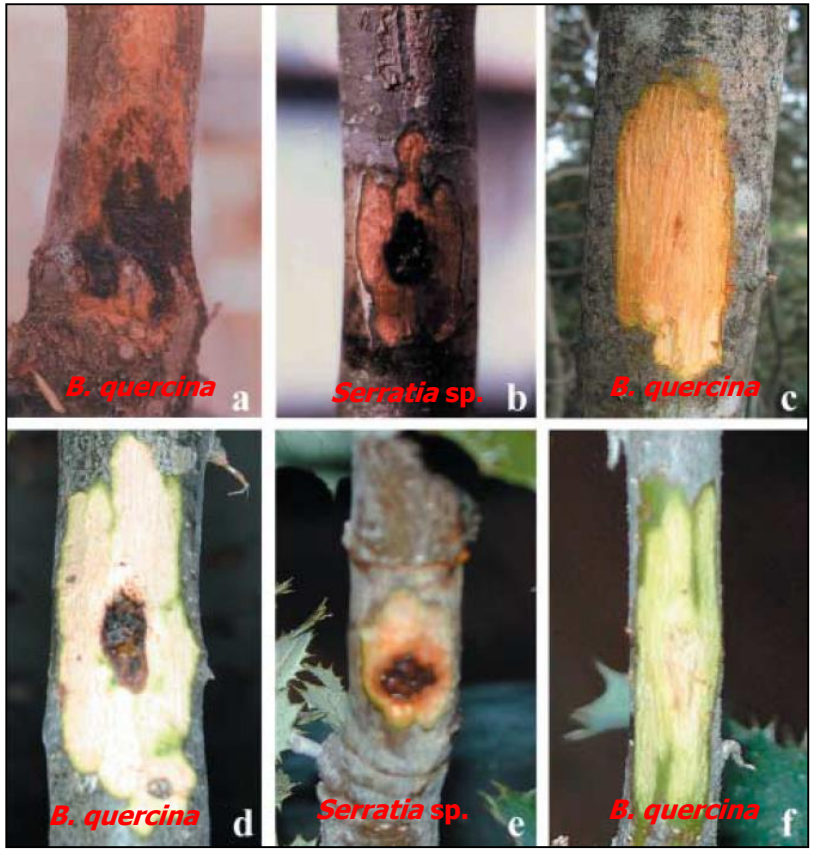
Poza-Carrión *et al.*,2008

Oak decline

Hidden partnerships (synergists)

Brenneria quercina and *Serratia* spp.

- Symptoms 3 months after artificial inoculation of:
  1. *Quercus pyrenaica* (a, b, c) and
  2. *Quercus ilex* (d, e, f)
- Both cultivars were inoculated with:
  1. *Brenneria quercina* isolate 1467-a (a and d), and
  2. *Serratia* isolate N-78-a (b, e).
- Negative control with 10 mM $MgCl_2$ (c and f).



Poza-Carrión *et al.*,2008

Oak decline

Hidden partnerships (synergists)

Brenneria quercina and *Serratia* spp.

- The data point to the possibility of the *Serratia* isolates being pathogens of trees, but confirmation between field symptoms and *Serratia* isolates it is still required.
- Alternatively *Serratia* spp. may be secondarily associated with infected oaks as a saprophyte and displaces *B. quercina* at later stages of the disease.

Poza-Carrión *et al.*,2008

Tomato pith necrosis

Bacteria-Bacteria Interactions

Partnerships (synergists)

- Tomato pith necrosis is thus far a leading example of co-infection due to synergistic interactions among several bacterial pathogens.
- Overall, eight bacterial species namely *Pseudomonas cichorii*, *P. corrugata*, *P. viridiflava*, *P. mediterranea*, *P. fluorescens*, *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* and *Dickeya chrysanthemi* can cause tomato pith necrosis alone or in association with the other bacterial species.
- The severity of the disease is greatly enhanced when co-infection of one or more bacterial species occurs.

Lamichhane and Venturi,2015

Association of *Pantoea agglomerans* with the citrus bacterial canker disease in Iran

Pantoea* has gained the ability to induce canker on citrus. This event can occur by transmission of parts of PAI from *Xanthomonas* to *Pantoea

- Symptoms on leaf surface of grapefruit developed 5-14 days after inoculation by *Pantoea* (left) and *Xcc* (right) isolates.

- Canker like symptoms (b) on adaxial (a) and abaxial (c) leaf of grapefruit. Symptoms developed 60 days after inoculation by *Pantoea*.

Note: *Erwinia herbicola* as the causal agent of citrus fruits blister was already reported in west Mazandaran, Iran (Nazeriyan *et al.*,2000).

Sherafati *et al.*,2014;..

Rice seeds and seedlings rots

Bacteria-Bacteria Interactions

Partnerships (synergists)

- *Burkholderia gladioli* was isolated at significantly higher proportions than *B. glumae* in the rice fields sampled.
- Bacterial recovery from seedlings inoculated with the combination of both pathogens showed high levels of *B. gladioli* but almost negligible levels of *B. glumae*.
- Both pathogens significantly reduced root development.
- Only *B. glumae* significantly affected the growth of the coleoptile(a sheath protecting a young shoot tip in a grass or cereal).
- Additionally, *B. gladioli* inhibited the growth of *B. glumae* *in vitro*, with average inhibition halos of 29.6 mm.

Riera-Ruiz *et al.*,2018

Fern distortion syndrome (FDS)

Multiple species of endophytic fluorescent pseudomonads

- Recreation of FDS symptoms of frond deformities by inoculation with fluorescent pseudomonads from diseased plants.
- Examples of distortions evident at 12 months after inoculation.
- A = water control
- C-F = bacteria from inside rhizomes of ferns with FDS symptoms
- G-H = rhizosphere bacteria from ferns with FDS symptoms.

Kloepper *et al.*,2013

Fern distortion syndrome (FDS)

Multiple species of endophytic fluorescent pseudomonads

	
Recreation of FDS symptoms of vena roja. Inoculation with fluorescent pseudomonads from rhizomes of diseased plants.	Recreation of FDS symptoms of internal discoloration of rhizomes. Inoculation with fluorescent pseudomonads from rhizomes and the rhizosphere of diseased plants.

Kloepper *et al.*,2013

Plant disease management

Identifying hidden partnerships

Xylella fastidiosa and *Methylobacterium* spp. partnership

Interaction between *Xylella fastidiosa* and *Methylobacterium* spp., in citrus variegated chlorosis disease(CVC):

- The endophytic bacteria *Methylobacterium* spp., occupy the same ecological niche as *Xylella fastidiosa* subsp. *pauca* (*Xfp*) in citrus plants.
- Recently, an interaction between *Methylobacterium* species and *Xfp* was strongly indicated.

Lacava *et al.*,2008

Plant disease management

Identifying hidden partnerships

Xylella fastidiosa and *Methylobacterium* spp. partnership

Interaction between *Xylella fastidiosa* and *Methylobacterium* spp., in citrus variegated chlorosis disease(CVC):

- Lacava *et al.*,2004 suggested that CVC symptoms in citrus plants could be a result of the population balance between:

1. Endophytic bacteria *Methylobacterium* spp.,
2. *Curtobacterium flaccumfaciens*, and
3. *Xylella fastidiosa* subsp. *pauca* (CVC strains).

Lacava *et al.*,2008

Plant disease management

Identifying hidden partnerships

Xylella fastidiosa and *Methylobacterium* spp. partnership

Interaction between *Xylella fastidiosa* and *Methylobacterium* spp., in citrus variegated chlorosis disease(CVC):

- This interaction may occur by *Methylobacterium* spp. synthesis of pathological factors, such as siderophores, which may be used by *Xfp* (Simionato *et al.*,2006).
- The ability of *X. fastidiosa* to use siderophores produced by endophytic bacteria as source of iron was confirmed.

Lacava *et al.*,2008

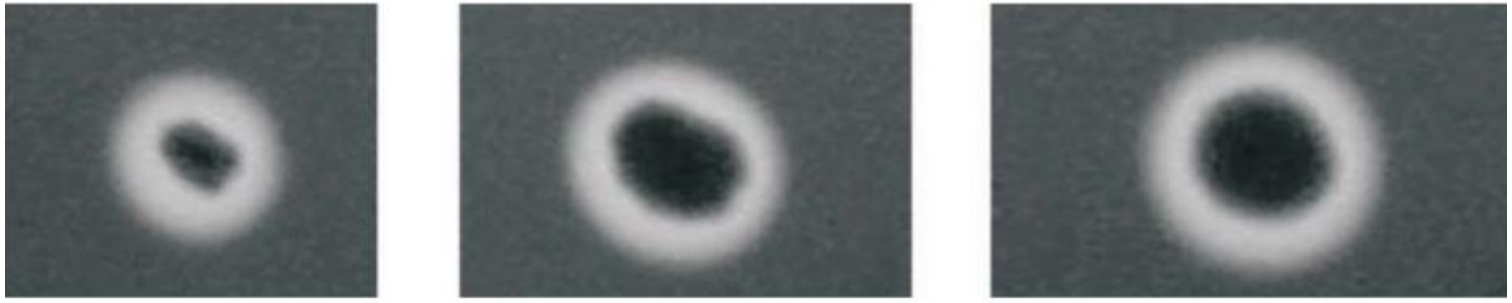
Plant disease management

Identifying hidden partnerships

Xylella fastidiosa and *Methylobacterium* spp. partnership

1. Production of siderophore by *Methylobacterium* spp.:

- A positive siderophore reaction by the CAS method (Chromeazurol S agar) shows a yellow halo surrounding the bacterial colonies of *Methylobacterium* grown under iron-limiting conditions.
- Three strains of *Methylobacterium* (AR5.1/5, AR5.1/6, and AR1.6/2) produced siderophore in a plate culture.



Three strains of *Methylobacterium* show yellow haloes in different size surrounding each bacterial colonies.

Lacava *et al.*,2008

Plant disease management

Identifying hidden partnerships

Xylella fastidiosa and *Methylobacterium* spp. partnership

2. Preparation of supernatant containing siderophores from growth culture of *Methylobacterium*:

- The siderophore producing strains of *M. mesophilicum* were individually grown in Fe-free MM9 broth to stimulate the production of siderophores.
- The supernatant was collected by centrifugation at 3,000 g for 5 min.
- Filtered through a 0.22 µm membrane filter.
- The supernatant containing siderophores was added to a final concentration of 0.2, 2, 20, 100% (v/v) to PW broth medium without a source of iron.

Lacava *et al.*,2008

Plant disease management

Identifying hidden partnerships

Xylella fastidiosa and *Methylobacterium* spp. partnership

3. Inoculation of culture broth media+supernatant containing siderophores with *Xfp*:

- PW broth with supernatant (positive control) and without supernatant (negative control) were inoculated with *Xfp* containing 10^4 viable *Xfp* cells.
- After inoculation, the tubes were incubated at 28°C for 20 days, and the growth of *Xfp* was evaluated at λ = 600 nm using an Ultrospec 3000 spectrophotometer.

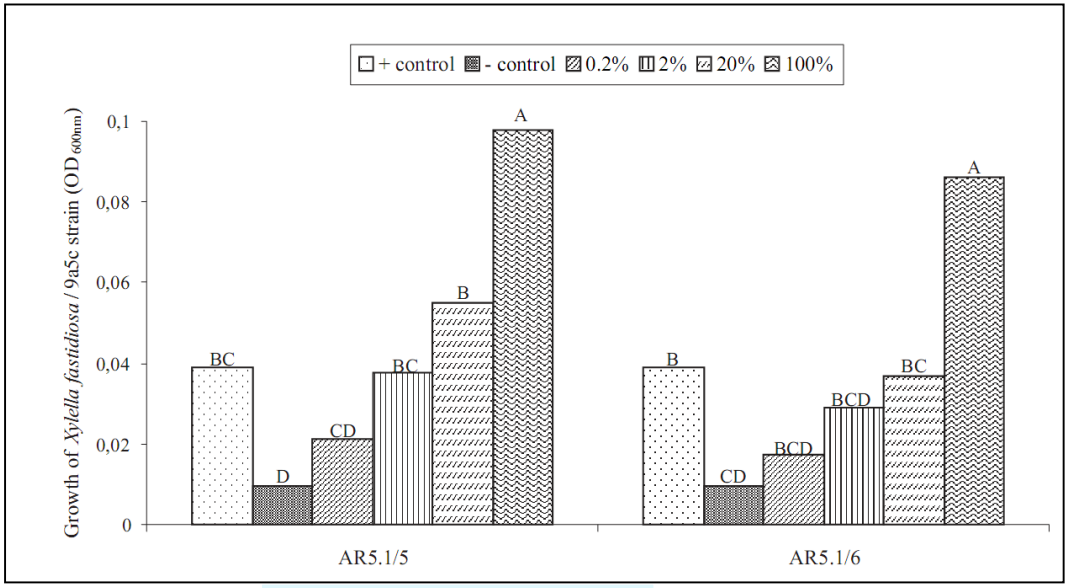
Lacava *et al.*,2008

Plant disease management

Identifying hidden partnerships

Xylella fastidiosa and *Methylobacterium* spp. partnership

- It was shown the growth of *Xylella fastidiosa* subsp. *pauca* is stimulated by the presence of a supernatant siderophore of endophytic *Methylobacterium mesophilicum* (AR5.1/5 and AR5.1/6).
- More conc. of siderophores were resultant more *Xfp* growth.



Different conc. Of supernatant siderophore of *Methylobacterium* prompts XF growth.

Lacava *et al.*,2008

Plant disease management

Bacteria-nematode Interactions

Hidden partnerships (synergists)

Interactions are also known to occur between the disease-causing bacteria *Clavibacter* spp., *Pseudomonas* spp. and *Agrobacterium* spp., and species of the nematode genera *Meloidogyne*, *Pratylenchus*, *Anguina* and *Ditylenchus*:

- Two well-known examples of nematode-bacteria interactions are that of:

1. *Meloidogyne* spp. and *Ralstonia solanacearum* causing bacterial wilt of many crops (tobacco, potato, tomato, aubergine), and
2. The ear cockle nematode, *Anguina tritici*, and *Clavibacter tritici* causing a disease in wheat referred to as 'tundu' in India.

Plant Pathologist's Pocketbook,2002

Plant disease management

Bacteria-Fungi Interactions

Fungi and bacterial partnership

Various pathogenic fungi and bacterium associated with brown apical necrosis of walnut fruit.

- There are a few reports in the literature of plant disease complexes involving association of more than one pathogenic microbial phyla.
- An example is brown apical necrosis of walnut fruit where numerous plant pathogenic fungi (*Fusarium, Alternaria*, *Cladosporium*, *Colletotrichum*, and *Phomopsis*) and a bacterium (*Xanthomonas arboricola*) are involved (Belisario *et al.*,2002).

Scotton *et al.*,2015

Plant disease management

Bacteria-Fungi Interactions

Fungi and bacterial partnership

Various pathogenic fungi and bacteria associated with cotton seed discoloration.

- Studies of various bacterial species isolated from discolored seed did not conclusively show a cause for the disorder.
- But association of various fungal and bacterial pathogens, all of which require wounds for initial infection end to boll rots.

Bell *et al.*,2004

Plant disease management

Identifying hidden partnerships

Fungi and bacterial partnership

- Symptoms from seed borne pathogens at boll opening.
- The number of pathogenic bacterial isolates obtained from 20 cotton seeds, each from different bolls, were:

1. *Pantoea agglomerans* (10 in No.);
2. A bacterium putatively identified as *Pantoea stewartii* (4), and
- *Agrobacterium tumefaciens* (2).

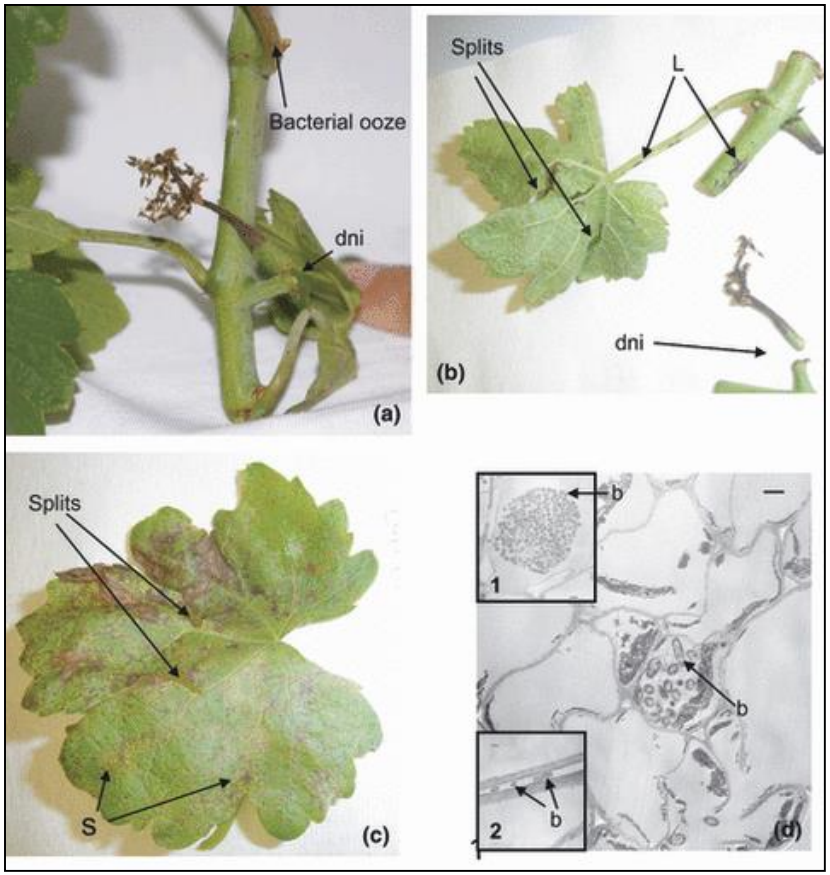
Pathogen	Symptoms
Fusarium semitectum	Completely rotted, tan-brown color .Both tight and matted locks
Alternaria alternata	Tight locks, tan-gray color
Phoma exigua	Tight locks, tan-gray color
Curvularia lunata	Completely rotted and matted, dark gray color
Verticillium nigrescens	Tight locks, tan color; and dark spots on partially loose white locks
All Bacteria	Tan to dark brown spots and streaks on mostly loose white locks

Bell *et al.*,2004

Plant disease management

Pseudomonas syringae pv. *syringae* precursor to fungal infections

- In cool, wet conditions that favour *Botrytis cinerea* the fungus rots damaged grapevine parts, including leaves, inflorescences and fruit.
- The induction of growth and sporulation of *B. cinerea*, a necrotrophic fungus, from asymptomatic latency following infection by *Pseudomonas syringae* pv. *syringae* (bacterial inflorescence rot), a biotrophic bacterium, is an important new finding.
- The results suggest that Pss-induced cell damage can be a precursor to overt infection invasion by the necrotroph and further cellular decay (mixed infections).



Whitelaw-Weckert *et al.*,2011

Wet rot of roots

Mixed Infections

Bacteria and yeast associated with sugar beet root rot at harvest

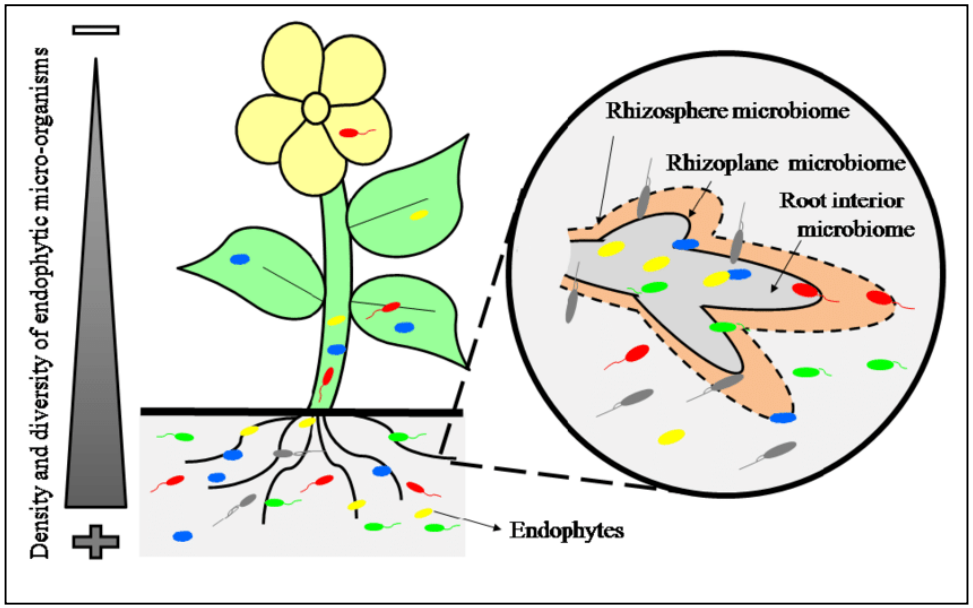
- Bacteria:
- *Lactobacillus*, *Leuconostoc*, *Acetobacter*, *Gluconobacter*, *Enterobacter*, *Escherichia*, *Pectobacterium*, *Serratia*, *Pseudomonas*
- Yeast
1. *Pichia*
2. *Candida*
- Isolated from harvested sugar beet.

Strausbaugh and Gillen,2008; Lamichhane and Venturi,2015

Plant microbiomes

Endophytic and rhizosphere microbiomes

- Endophytes live within intercellular spaces, tissue cavities, or vascular bundles without harming the host and often benefit the host.
- The rhizosphere and endophytic microbiomes ensure plant health.



Plant microbiomes

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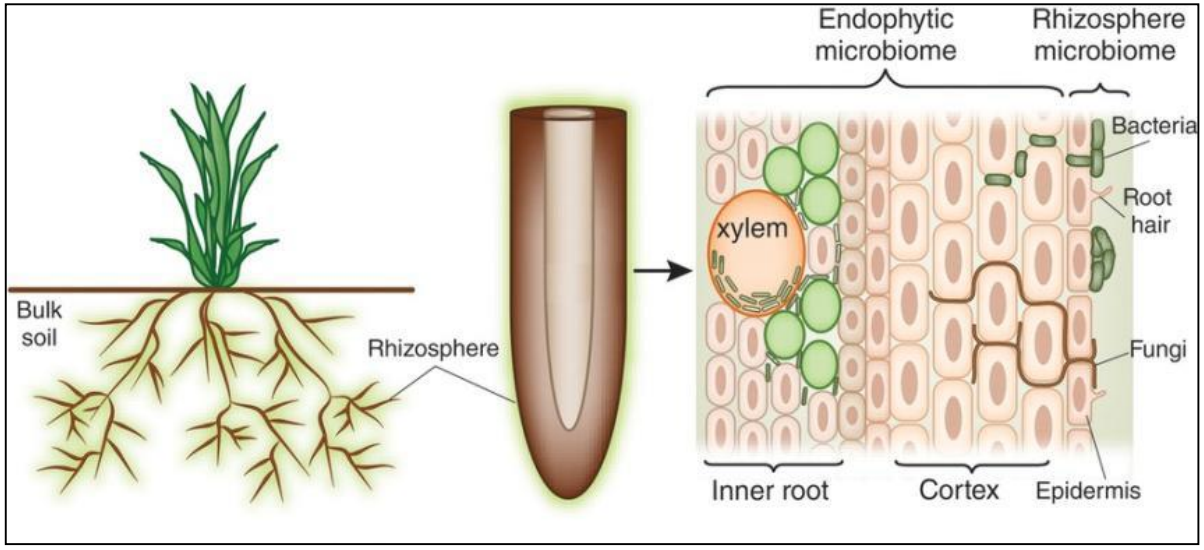
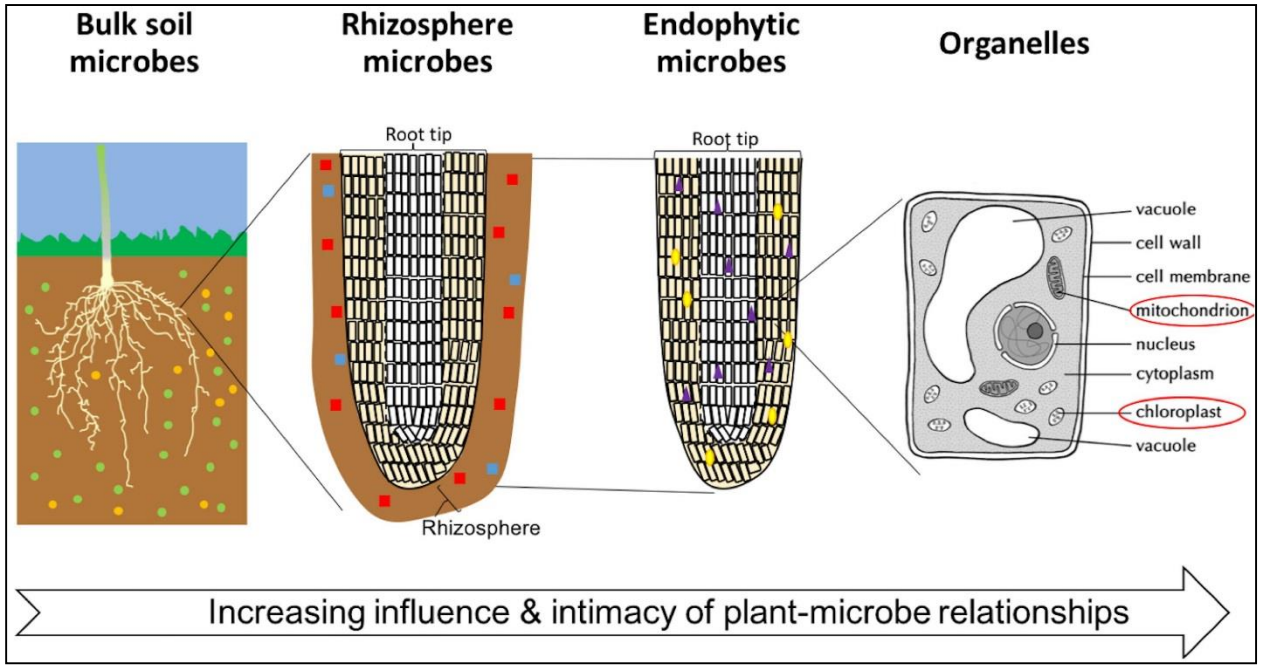


Hirsch & Mauchline,2012;..

Plant microbiomes

Endophytic and rhizosphere microbiomes



The degree of intimacy and influence of the plant-microbe interactions. Microbes are represented by small colored (red, green, yellow, purple, and blue) shapes. Diversity and number of microbes is variable between soils, distance from plant roots, crop species, and plant tissue.

Backer *et al.*,2018

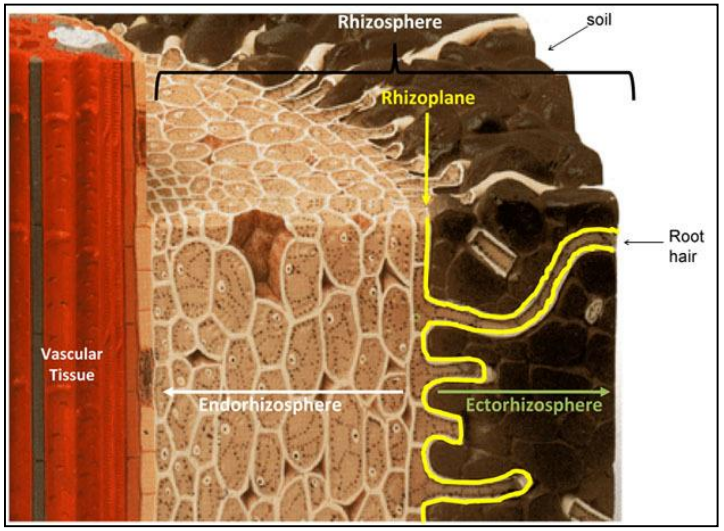
Plant microbiomes

New definition of rhizosphere

- In 1904, Lorenz Hiltner first coined the term "rhizosphere" to describe the plant-root interface.
- In the years since, the rhizosphere definition has been refined to include three zones which are defined based on their relative proximity to, and thus influence from, the root.

1. The endorhizosphere includes portions of the cortex and endodermis in which microbes and cations can occupy the "free space" between cells (apoplastic space).
2. The rhizoplane is the medial zone directly adjacent to the root including the root epidermis and mucilage.
3. The outermost zone is the ectorhizosphere which extends from the rhizoplane out into the bulk soil.

- As might be expected because of the inherent complexity and diversity of plant root systems, the rhizosphere is not a region of definable size or shape, but instead, consists of a gradient in chemical, biological and physical properties which change both radially and longitudinally along the root.



McNear Jr.,2013

Phytobiomes

Microbiomes

Plant and human microbiomes

Projects:

1. The human microbiome Project;
2. The plant microbiome Project.

- The human microbiome: at the interface of health and disease.
- The skin microbiome: potential for novel diagnostic and therapeutic approaches to some diseases.

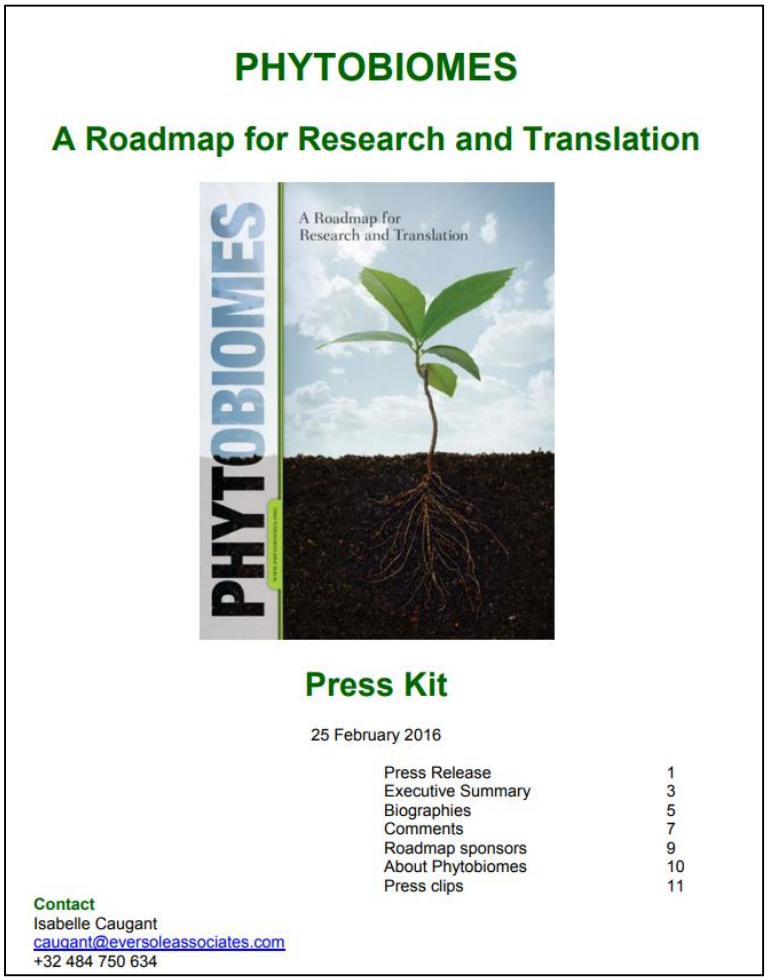
www.Phytobiomes.org

Phytobiomes website

A Roadmap for phytobiomes research and translation

Phytobiomes website: www.phytobiomes.org

About Phytobiomes:
PHYTO = related to plants
BIOME = a community of plants, microbes and animals living together in a particular climate and physical environment.



PHYTOBIOMES

A Roadmap for Research and Translation

Press Kit

25 February 2016

Contact
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Phytobiomes website

A Roadmap for phytobiomes research and translation

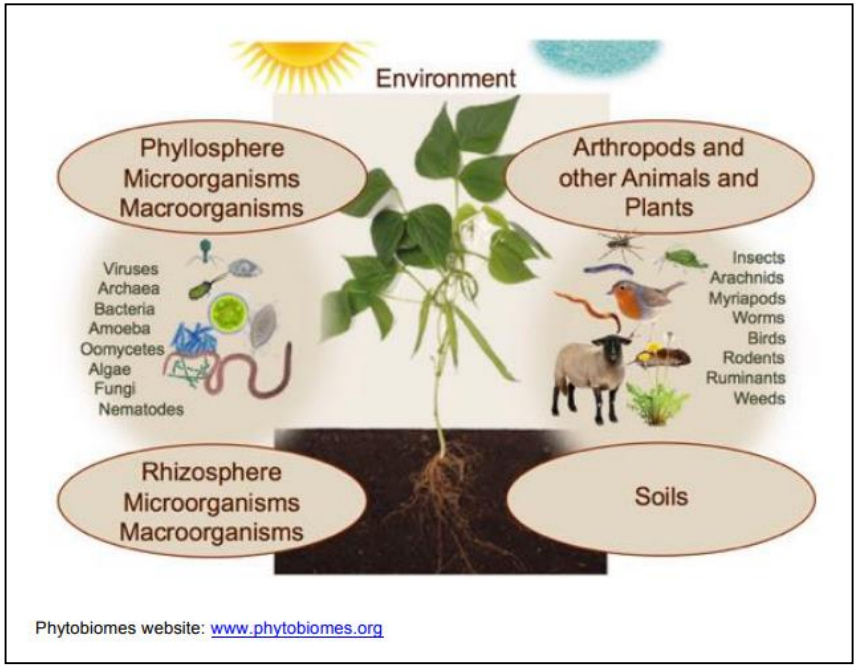
- The Phytobiomes Roadmap offers a new vision for agriculture in which sustainable crop productivity is achieved through a systems-level understanding of diverse interacting components.

Specifically, a vision for phytobiomes is that by 2050, all farmers will have "the ability to use predictive and prescriptive analytics based on geophysical and biological conditions for determining the best combination of crops, management practices, and inputs for a specific field in a given year."

Phytobiomes website

A Roadmap for phytobiomes research and translation

- PHYTOBIOMES consist of plants, their environment, and their associated micro- and macroorganisms. These organisms, which may be inside, on the surface, or adjacent to plants, include a wide diversity of microbes (viruses, bacteria, fungi, oomycetes, and algae), animals (arthropods, worms, nematodes, and rodents), and other plants.



The environment includes the physical and chemical environment influencing plants and their associated organisms, and therefore, the soil, air, water, and climate. The sphere of relevance of phytobiomes is quite broad, spanning from crops (commodity crops, fruits, vegetables, forest, and specialty and bioenergy crops), rangelands, grasslands, and natural ecosystems to consumer products, including the quality, nutritional value, and safety of our foods.

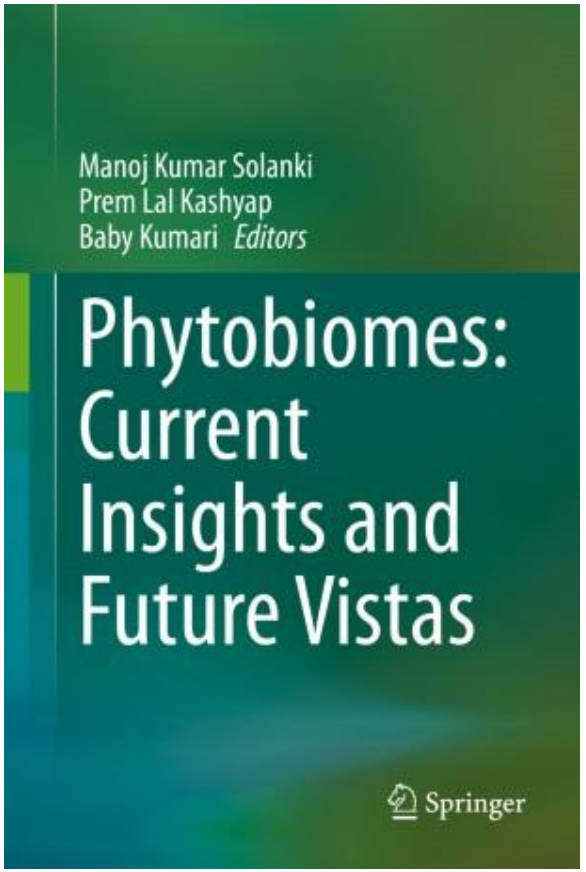
Phytobiomes Journal

The American Phytopathological Society (APS)

- Phytobiomes is a new open-access journal published by APS.
- This high-quality journal focuses on transdisciplinary research that impacts the entire plant ecosystem

Phytobiomes
Phytobiome book

- Phytobiomes: Current Insights and Future Vistas
- Editors: Manoj Kumar Solanki, Prem Lal Kashyap and Baby Kumari.
- Springer
- 2020
- 698 pages.



Plant disease management

Research areas

What is a Phytobiome?

- Phytobiome = A plant (phyto) in a specific ecological area (biome).
- It includes the plant itself, the environment and all organisms living in, on or around the plant.
- **Phytobiomes** are well-defined as a network of interactions by diverse microbiota with bacteria, archaea, fungi, viruses, and protists.
- The phytobiome is analogous to probiotic (beneficial microbes) studies in humans (e.g. gut microbiome).
- The microbiome-based approaches is a need for sustainable agriculture.

Dickman,2015; Ray *et al*., 2020

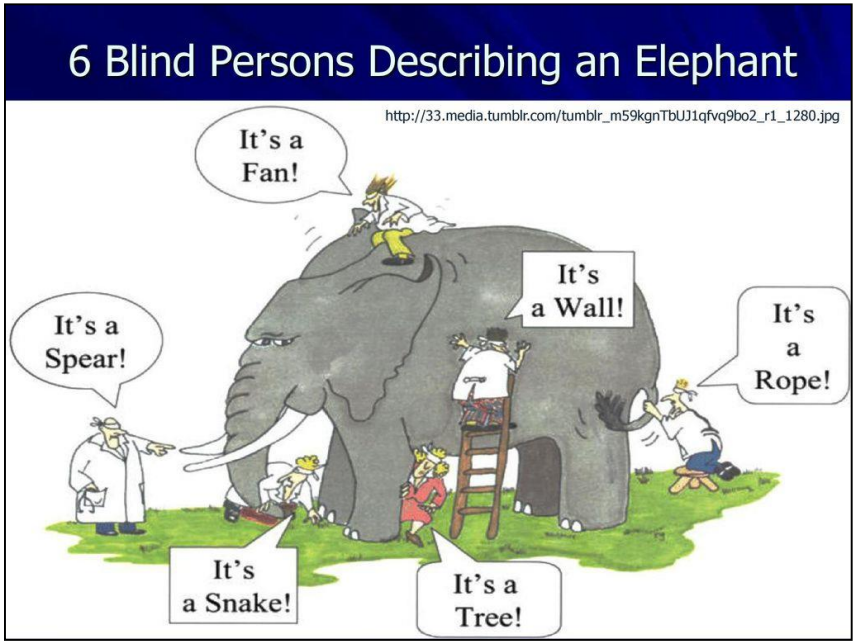
Phytobiomes

What is the phytobiome?

Plant microbiomes are components of phytobiome

- Understanding and application of microbiomes to advance agriculture requires:

1. Interdisciplinary, systems level approaches;
2. Consideration of interactions in context (the phytobiome).

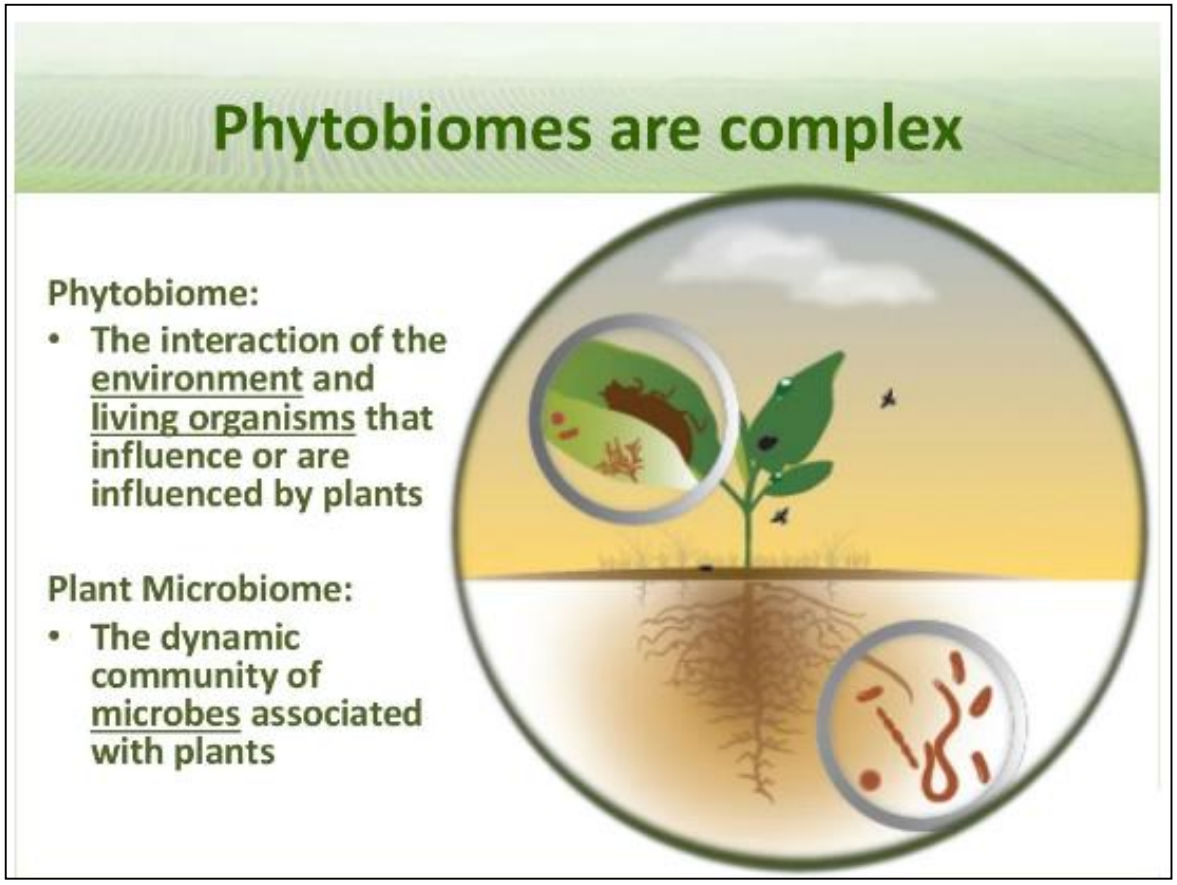


We have to remember that what we observe is not nature in itself, but nature exposed to our method of questioning. - Werner Heisenberg (Theoretical physicist).

Phytobiomes

Microbiomes

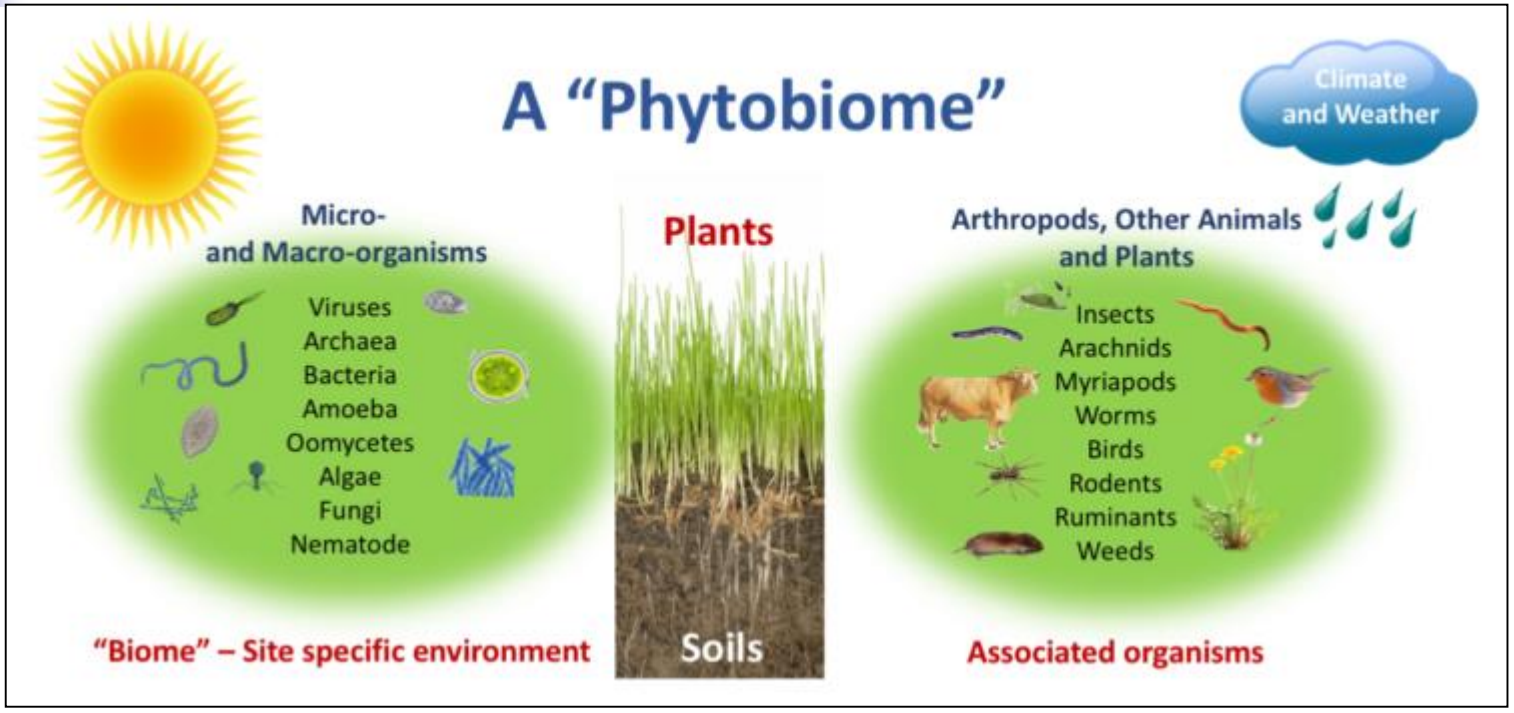
Plant microbiomes are components of phytobiome



Leach,2017

Phytobiomes

Phytobiomes have an important role in the sustained health and productivity of plants and plant ecosystems



Plants grow in association and interaction with complex communities of organisms, environmental conditions, and management practices. A biome is a large collection of flora and fauna occupying a major habitat. The term "Phytobiomes" encompasses all of this complexity.

International Phytobiomes Conference,2018

Plant disease management

Phytobiomes

Why the phytobiomes approach?

- Managing with attention to the whole phytobiome as opposed to one component (such as soils or nutrients alone) can:

1. Increase resilience to water and nutrient limitations and heat stress.
2. Increase resilience (toughness) to the ongoing emergence of new pests and pathogens.
3. Reduce crop losses due to pathogens and pests without relying solely on pesticides.
4. Enhance safety, quality and nutrition of our food supply.
5. Reduce reliance on external inputs to sustain crop productivity.
6. Regenerate the land.
7. Increase profitability.

Phytobiomes

Microbiomes

Why the phytobiomes approach?

- The health of soil plays an essential role in the ability of plants to produce food, fuel, and fiber for a growing world population.
- To keep pace, the total area of cultivated land worldwide has increased over 500% in the last five decades with a 700% increase in the fertilizer use and a several-fold increase in pesticide use.

Banerjee *et al.*,2019

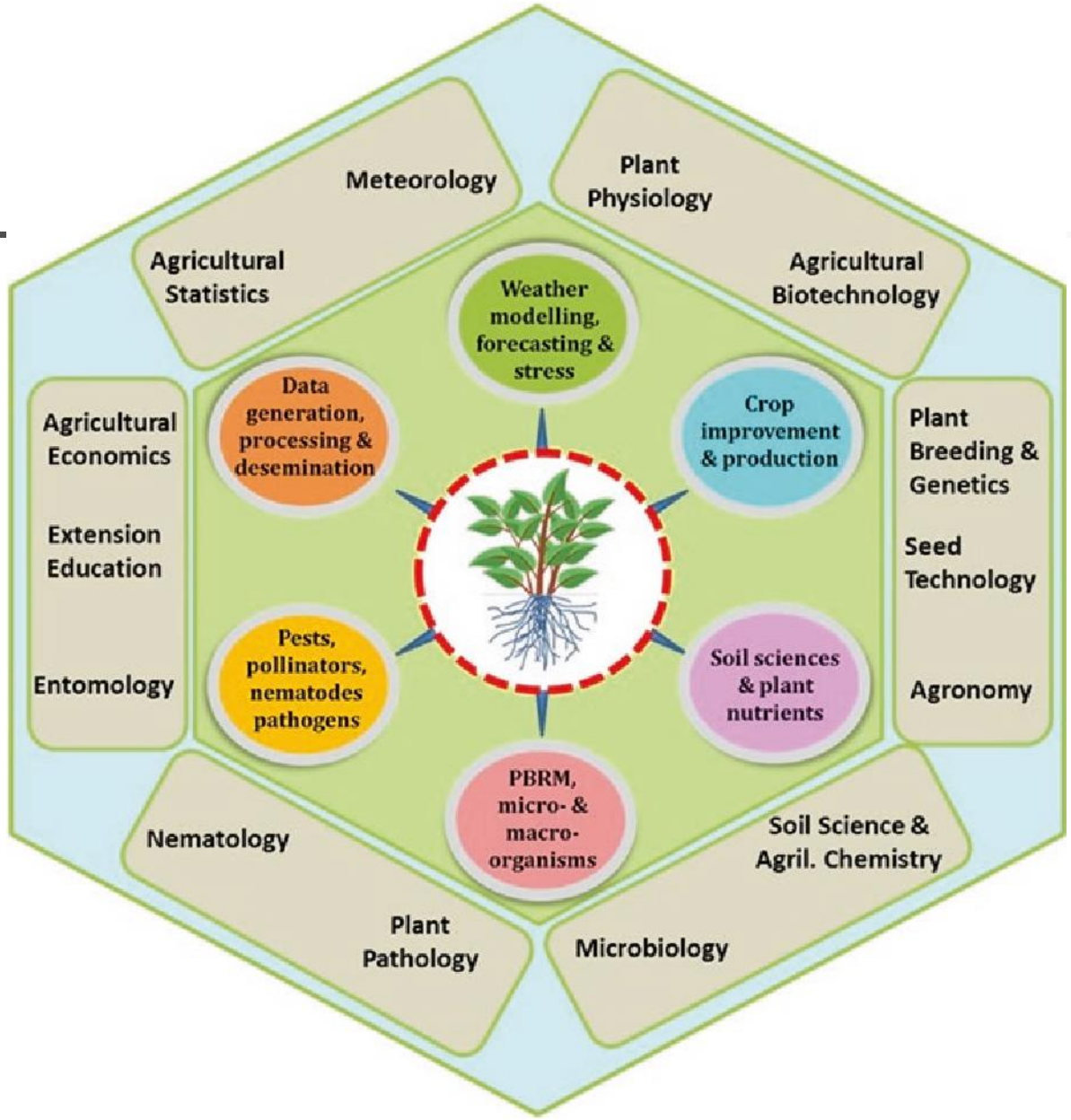
Phytobiomes

Interactions within phytobiomes are dynamic and complex

- Because interactions within phytobiomes are dynamic and complex, there is a need to build a foundation of systems-level knowledge of various phytobiomes.
- This includes:

1. an understanding of how the different components interact, and
2. influence each other to empower the development of predictive and prescriptive analytics for use in next generation precision agricultural systems.
3. Knowledge of the phytobiomes network of can be translated into new tools for agroecosystem management/health.

This chapter mainly emphasizes on phytobiome related to soil fertility, nutrient cycling, plant growth, and soil health.



Kumar and Kumari,2020

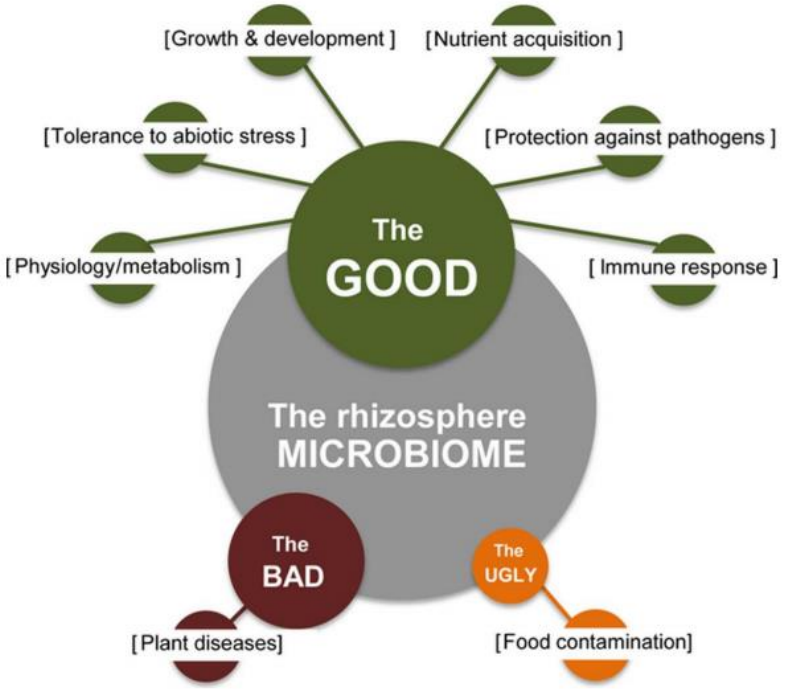
Phytobiomes

Translating phytobiome discoveries into products

Phytobiome

Microbiomes influence plant traits

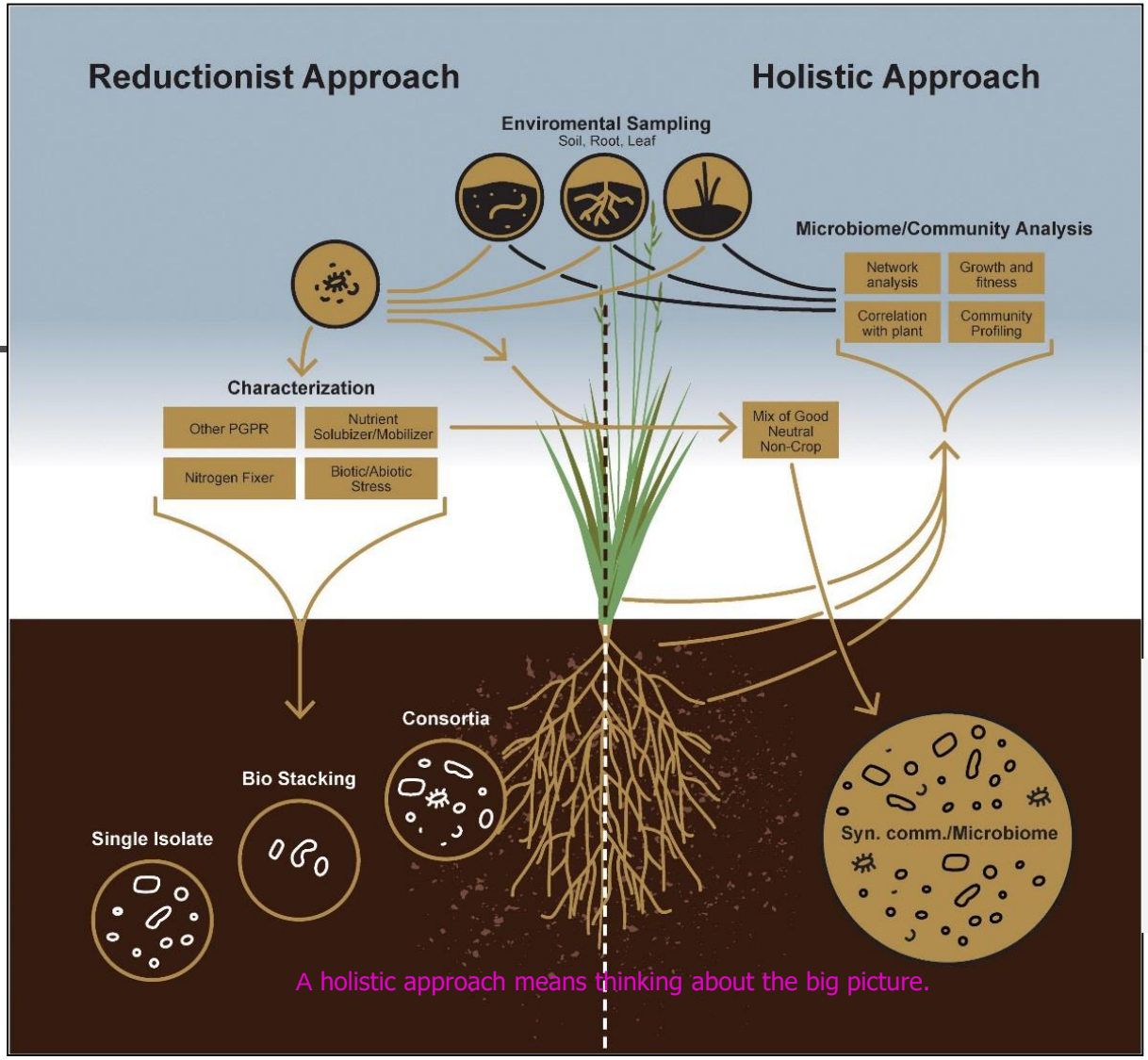
Also, microbiomes influence plant traits!



Mendes et al., 2013. FEMS Microbiology Reviews

- **Disease resistance** (Mendes et al., 2011; etc.)
- **Abiotic stress tolerance** (Lau & Lennon, 2012; Bainard et al., 2013; etc.)
- **etc**

Leach,2015



Reductionist approach:

- Single isolate,
- Bio Stacking
- Consortia.

Holistic approach:

- synthetic microbial communities (SynComs)

Phytobiome(Plant microbiome)

A schematic comparison between individual microorganism-based reductionist approach and microbial community-based holistic approach

Ray *et al.*,2020

Phytobiomes

Plant microbiomes

Insights on mechanisms disease/resistance

- Phytobiomes studies may:

1. provide more precise insights into the mechanisms and consequences of disease (and resistance);
2. identify indicators of disease (and resistance) progress.

Leach,2015

Phytobiomes

Plant microbiomes

Role of phytobiomes in plant disease control

- Plant disease may be influenced by phytobiome members beyond the host and the pathogen.
- Host defenses may be modulated by microbes and insects.
- We will focus on recent discoveries of the:

1. influence of plant-associated insects and microbes on plant disease outcomes, and on
2. how this knowledge may be translated into applications for disease management.

Phytobiomes

Plant microbiomes

Microbiomes can protect plants against pathogens/pests

- Plants are subject to infection by diverse microbial pathogens as well as herbivory by insect and nematode pests.

Mendes *et al.*,2011

Phytobiomes

3. Endophytic microbiomes/Endophytic microorganisms (EMOs)

- Plants are associated with:
- Micro-and nano-organisms such as beneficial endophytic bacteria and fungi, which:

1. Live inter and intracellularly in plants;
2. Without inducing pathogenic symptoms, while
3. Interacting with the host biochemically and genetically.

- Other possible sources of endophytes include:

1. the phyllosphere,
2. the anthosphere (flowers) and seeds.

Plant microbiomes

Bacterial microbiome associated with endophytic bacteria

Endophytic microbiomes

- Endophytes are typically non-pathogenic microbes that at some period in their life cycles colonize the interior spaces of plant tissues.
- In other words, endophytes lives within a plant for at least part of its life cycle without causing apparent disease.

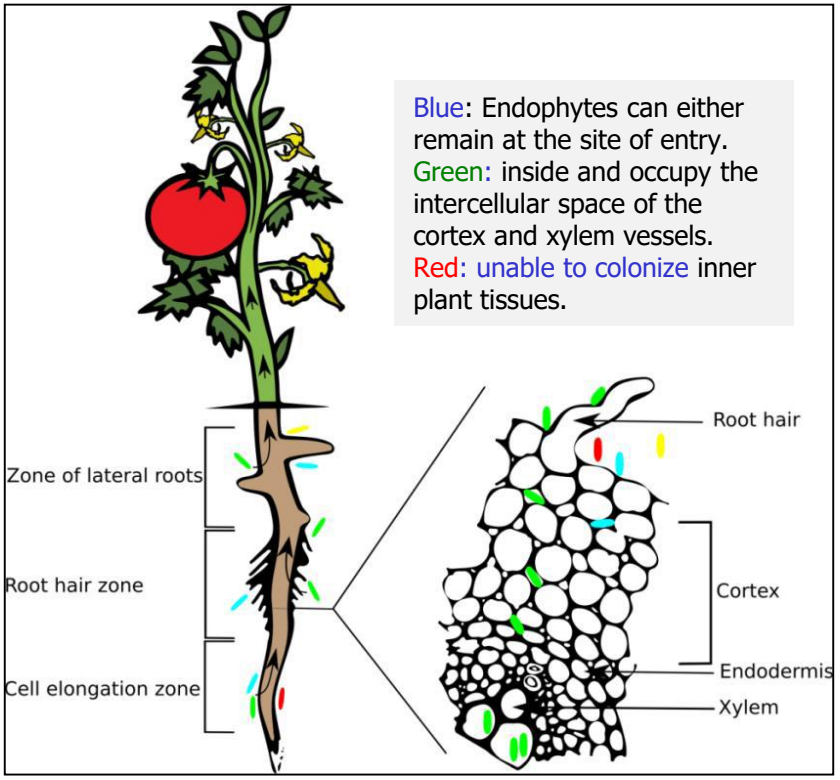
Zhang *et al.*,2019; Wikipedia,2020

Endophytic microbiomes

Endophytic microorganisms (EMOs)

The hidden world within plants

- Endophytes can either remain at the site of entry (indicated in blue), or
- move deeper inside and occupy the intercellular space of the cortex and xylem vessels (indicated in green).
- Red and yellow represent rhizospheric bacteria/rhizosphere microbiomes which are unable to colonize inner plant tissues.



Bacteria can enter a plant at several root zones as indicated above.

Malfanova,2013;..

Endophytic microorganisms

The hidden world within plants

Plant species

1. Nearly 300,000-500,000 plant species that exist on the earth, each individual plant is host to one or more endophytes.
2. But most likely, there is not a single plant species devoid of endophytes.

Ciancio & Mukerji (eds.),2008;..

Endophytic microbiomes

More than thousand bacterial endophytes were collected and characterized

- During more than ten years of endophyte research at the AIT(Austrian Institute of Technology) more than thousand bacterial endophytes were collected and characterized.
- The precise role of endophytes in plants is not yet known.
- However, their capability to thrive within the host tissues away from microbial competition and environmental degradation has made endophytes potential candidates for use in agriculture.

Prasad and Dagar,2014;..

Plant disease management

Function of endophytic microorganisms

Endophytic microbiomes

- The role of endophytic microorganisms in plants can be divided into two categories based on types of activity:

1. Growth promotion;
2. Disease control;
3. Adaptation of host plants to environmental stresses.

Prasad and Dagar,2014; Tadych and White, 2019

Endophytic microbiomes

Endophytic microorganisms

Which bacteria can be found as endophytes?

- More than 200 bacterial genera from 16 phyla have been reported as endophytes.
- These include both:

1. Culturable, and
2. unculturable bacteria.

- The most predominant and studied endophytes belong to three major phyla:

1. Actinobacteria,
2. Proteobacteria, and
3. Firmicutes.

Malfanova,2013;…

Examples of reported bacterial endophytes and plants harboring them

A. Growth promotion

- So far, considerable number of plant growth promoting endophytes (PGPEs) have been successfully isolated from a large diversity of plants and found to be beneficial for plant growth, yield and crop quality, including strains in the bacterial genera of *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Klebsiella*, *Pseudomonas*, *Rhizobium* and *Serratia*.

Li *et al.*,2016

Examples of reported bacterial endophytes and plants harboring them.

Endophytic bacteria	Host Plant species	Reference
α Proteobacteria		
Azorhizobium caulinodans	Rice	Engelhard *et al.*, 2000
Azospirillum brasilense	Banana	Weber *et al.*, 1999
Bradyrhizobium japonicum	Rice	Engelhard *et al.*, 2000
Methylobacterium mesophilicum**a**	Citrus plants	Araujo *et al.*, 2002
Methylobacterium extorquens	plants Scots pine, citrus	Araujo *et al.*, 2002; Pirttilä *et al.*, 2004
Rhizobium (Agrobacterium) radiobacter	Carrot, rice	Surette *et al.*, 2003
β Proteobacteria		
Burkholderia cepacia**b**	Yellow lupine, citrus plants	Araujo *et al.*, 2001; Barac *et al.*, 2004
γ Proteobacteria		
***Citrobacter* sp.**	Banana	Martínez *et al.*, 2003
Enterobacter sakazakii**a**	Soybean	Kuklinsky *et al.*, 2004
Enterobacter asburiae	Sweet potato	Asis and Adachi, 2003
***Erwinia* sp.**	Soybean	Kuklinsky *et al.*, 2004

Eltabee Youghy *et al.*,2014

Function of endophytic microorganisms
Plant growth promoting endophytes (PGPEs)
Growth promotion

- The PGPEs promote plant growth by various mechanisms include production of phytohormones, siderophores, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, nitrogen fixation, and phosphates solution.
- Due to their beneficial effects on growth and health for host plants, PGPEs have the potential for use in the friendly, sustainable and organic agriculture.

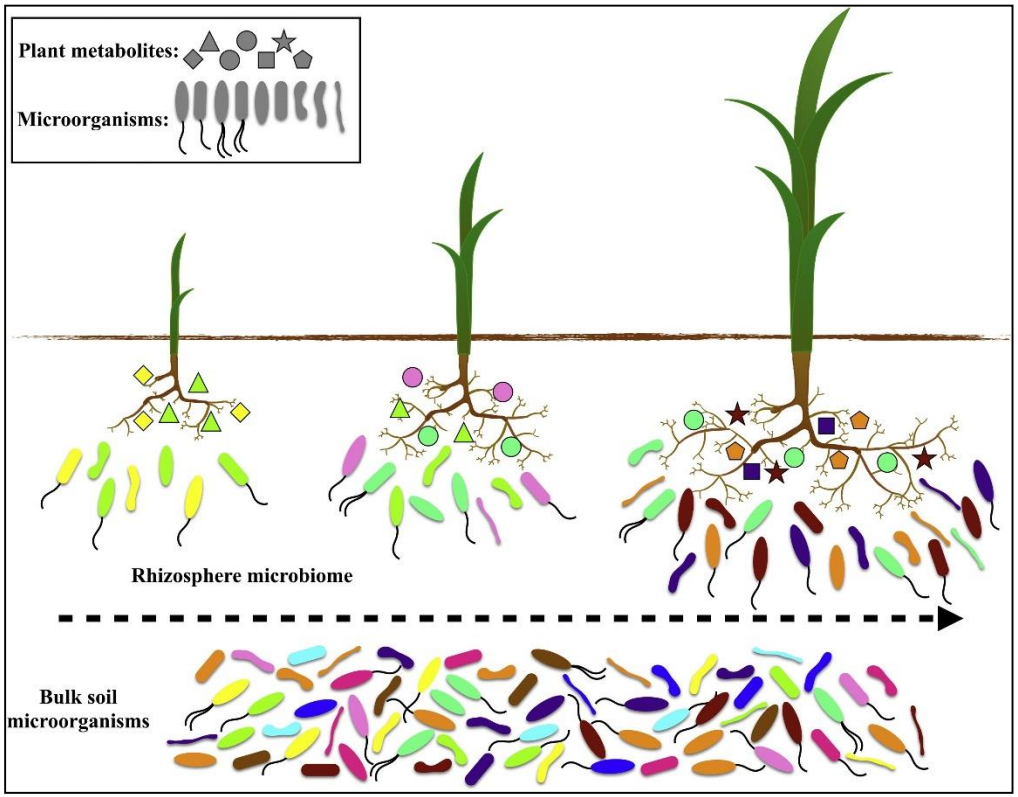
Li *et al.*,2016

Selected factors involved in endophytic bacterial colonization & interaction with plants reported by Hardoim *et al.*,2008.

Class	Function	Microorganisms
Chemotaxis	Motility	*Pseudomonas fluorescence*
Colonization	Type IV pillus	*Pseudomonas fluorescence*
	Twitching motility	
	Isoflavonoid efflux pump	*Azoarcus sp.*
		Azorhizobium sp. Agrobacterium tumefaciens
Interactions with plant metabolism	Ethylene modulation	*P. ptida*
	Plant growth promotion	*Bacillus subtils and B. amyloliquefaciens*
	Induced systemic resistance	*B. subtils and B. amyloliquefaciens*
	Indole-3 acetic acid	*Several plant associated bacteria*
		Acetobacter diazotrophicus
	Biological nitrogen fixation	*Azoarcus sp.*

Eltabee Youghy *et al.*,2014

Function of endophytic microorganisms
Plant growth promoting endophytes (PGPEs)
Growth promotion



Root exudates in an annual grass synchronize with microbial substrate use promoting microbial community assembly.

Zhang *et al.*,2021

Endophytic microbiomes

Function of endophytic microorganisms

B. Disease control

- The use of these endophytic microorganisms to control plant-pathogenic bacteria and fungi is receiving increased attention as a sustainable alternative to synthetic pesticides and antibiotics.
- In order to reduce inputs of pesticides and fertilizers and add value to eco-friendly agriculture in Europe, it will be important to develop inocula of biofertilizers, stress protection and biocontrol agents.
- But there are currently bottlenecks limiting the development of endophytes for use in biotechnology and agriculture.

Endophytic microbiomes

Function of endophytic microorganisms

B. Disease control

- The use of these endophytic microorganisms to control plant-pathogenic bacteria and fungi is receiving increased attention as a sustainable alternative to synthetic pesticides and antibiotics.
- In order to reduce inputs of pesticides and fertilizers and add value to eco-friendly agriculture in Europe, it will be important to develop inocula of:

1. biofertilizers,
2. stress protection, and
3. biocontrol agents.

Endophytic microbiomes
Function of endophytic bacteria
C. Growth promotion, and disease control

- Endophytic bacteria are believed to elicit plant growth promotion in one of two ways:

1. Directly by producing phytohormones such as auxin or cytokinin or by producing the enzyme 1-aminocyclopropane- 1-carboxylate (ACC) deaminase, which lowers plant ethylene levels, and
2. Indirectly by preventing pathogen infections via antifungal or antibacterial agents, by:

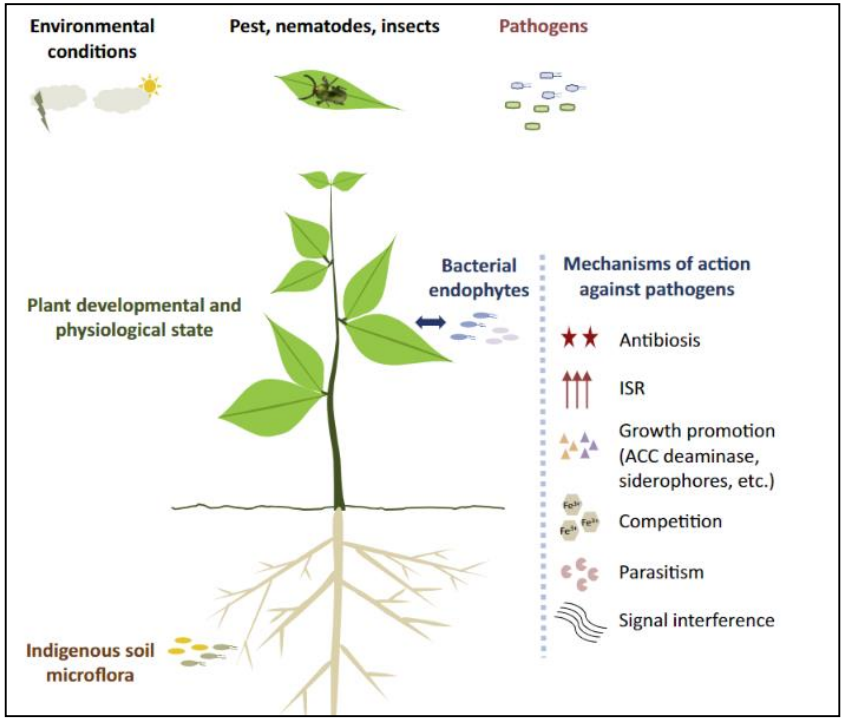
- ✓ outcompeting pathogens for nutrients by siderophore production, or by
- ✓ establishing the plants systemic resistance(ISR).

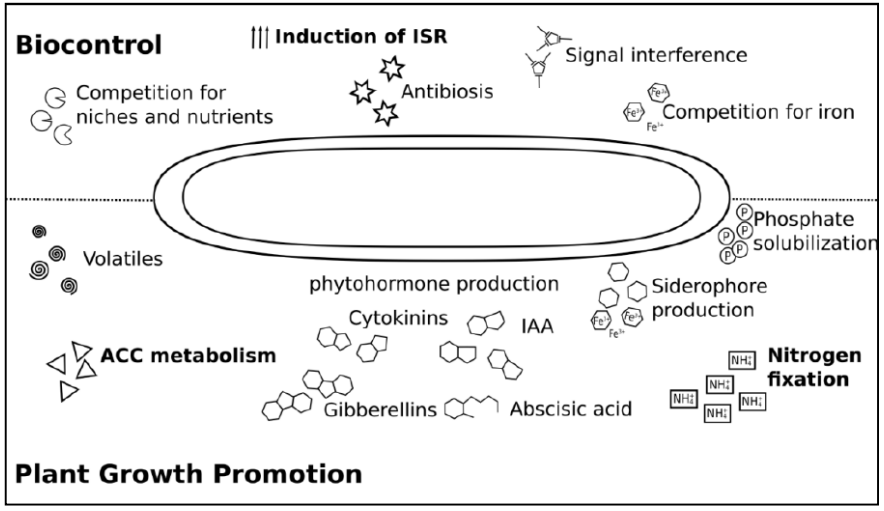
Prasad and Dagar,2014

Endophytic microbiomes

Function of endophytic microorganisms

Growth promotion, and disease control





It has been proposed that PGPR may enhance plant growth by lowering the plant ethylene levels. ACC (1- aminocyclopropane-1-carboxylate), is a precursor of ethylene.

Malfanova,2013; Eljounaid *et al.*,2016;..

Endophytic microbiomes

Functions of endophytic microorganisms

D. Other beneficial effects

- Helping plants acquire nutrients via:

1. nitrogen fixation,
2. phosphate solubilization, or
3. iron chelation,

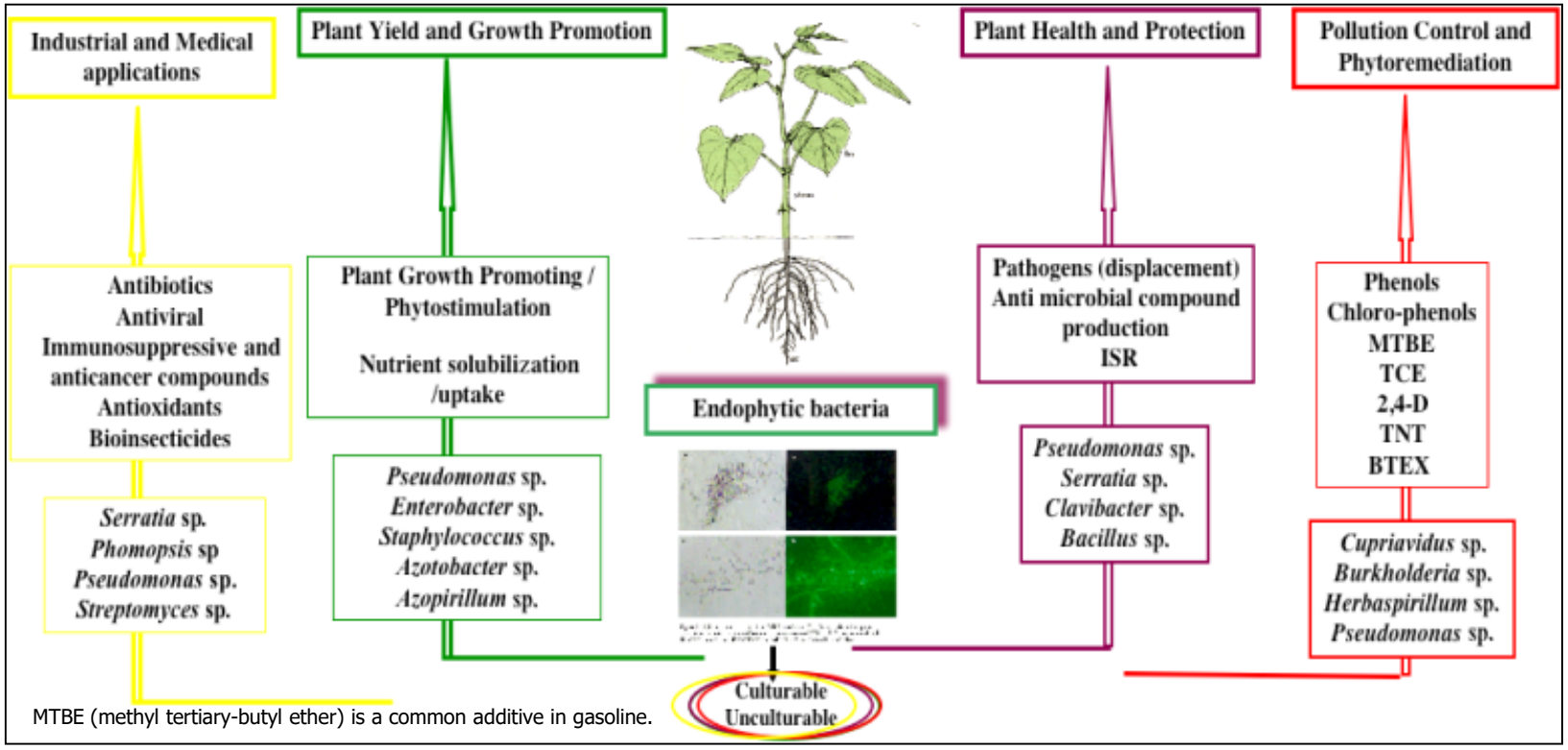
- increased:

1. drought resistance,
2. thermal protection, and
3. survival under osmotic stress such as salinity-osmotic stresses(salt stress).

Endophytic microbiomes

Function of endophytic microorganisms

Other beneficial effects including disease control



Schematic diagram of the different plant–bacterial endophyte interactions that have been studied and their applications.

Ryan *et al.*,2007

Endophytic microbiomes

Function of endophytic microorganisms

E. As bio-fertilizers

- In recent years, bacterial endophytes used as bio-fertilizers for improving crop production.
- Thus, the exploitation of plant growth promoting endophytes (PGPEs) as one of the best options to increase biomass yield of the energy crops on marginal lands has become a hot research subject with more attention both from academia and industry.

1. For instance, *Bacillus* sp. SLS18 promoted the biomass production of sweet sorghum.
2. The growth of poplar tree was improved up to 60% after inoculation with different endophytic strains.

Li *et al.*,2016

Endophytic microbiomes

May endophytes be or become pathogens?

- It is worrisome that there may be human or opportunistic pathogens among plant endophytes.
- Most fungal grass endophytes are considered mutualistic with their hosts.
- Some endophytes seem to be latent pathogens, and infections may proceed under certain conditions.

Rivas *et al.*,2004, isolated several endophytic slow-growing bacterial strains from roots of *Beta vulgaris* affected by tumour-like deformations. They proposed the name *Bradyrhizobium betae* sp. nov.(Moliszewska *et al.*,2016).

Rosenblueth and Martínez-Romero,2006

Plant microbiomes

Bacterial microbiome associated with the rhizosphere

4. Rhizosphere microbiomes

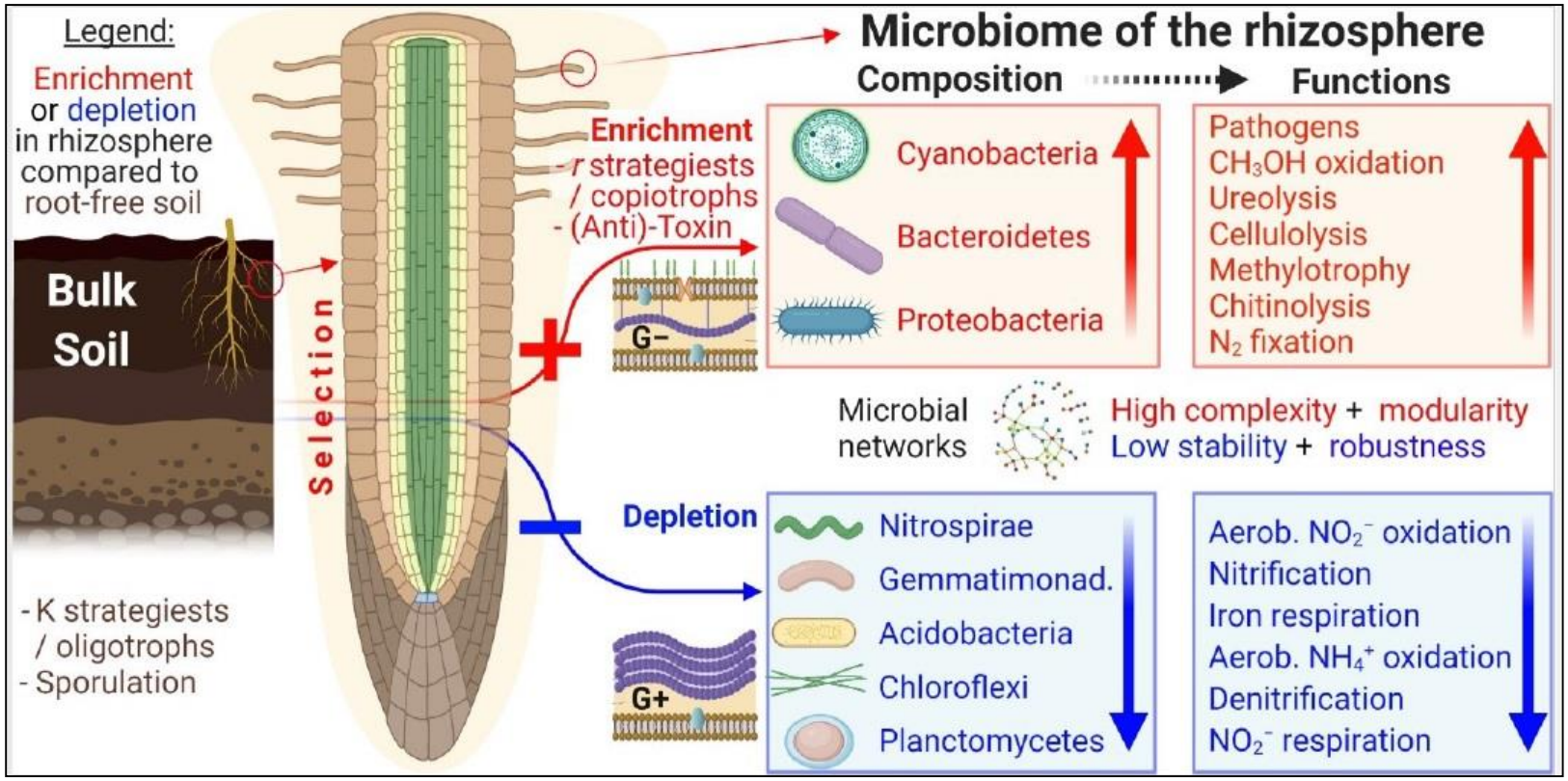
- The impact of rhizosphere microorganisms on health and disease.
- Many members of the rhizosphere microbiome are beneficial to plant growth, also plant pathogenic microorganisms colonize the rhizosphere striving to break through:

1. the protective microbial shield, and
2. to overcome the innate plant defense mechanisms in order to cause disease.

Mendes *et al.*,2013

Plant microbiomes

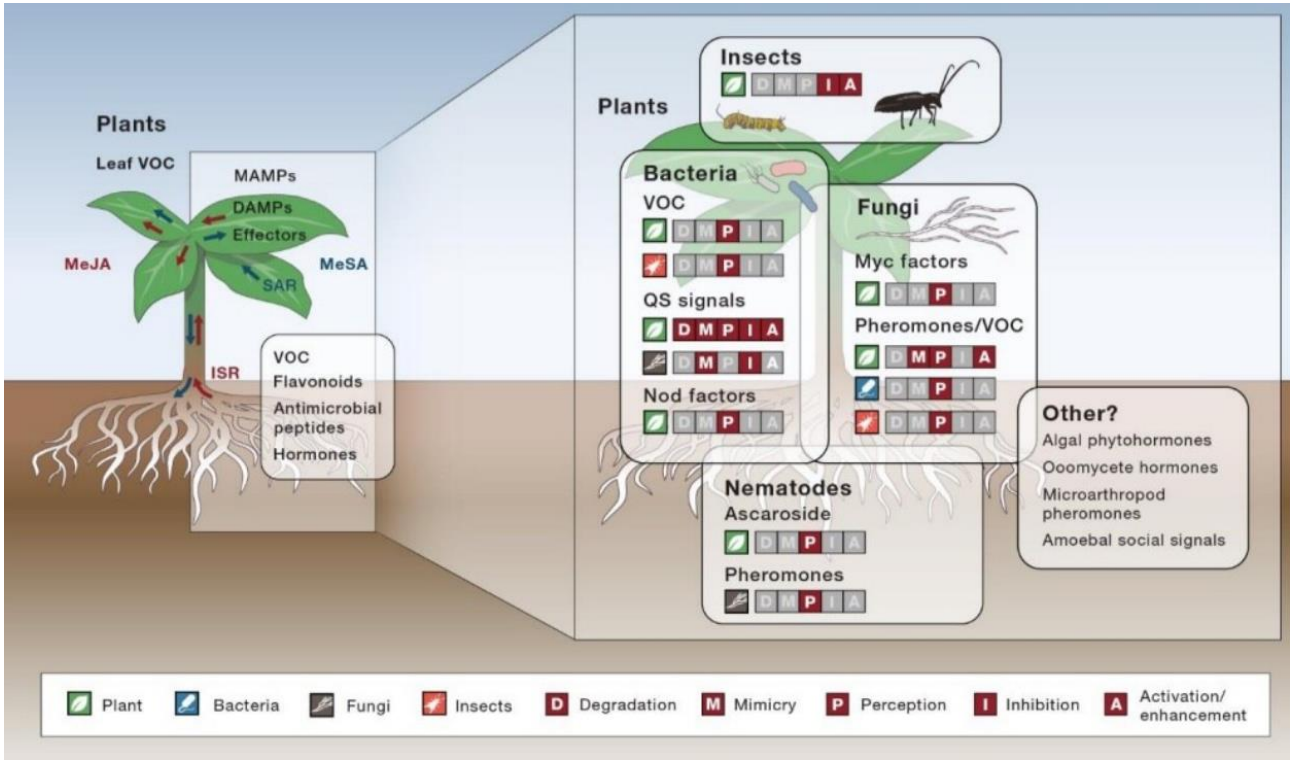
Rhizosphere microbiomes



Ling *et al.*,2021

Plant microbiomes

Communication among phytobiome members



The two plant volatiles **MeJA** Methyl salicylate (**MeSA**) and methyl jasmonate (**MeJA**) vapors increased plant resistance.

Leach *et al.*,2017

Plant microbiomes

Bacterial microbiome associated with the rhizosphere

Manipulation of rhizospheric microbiomes

- Rhizosphere is the factories of microorganisms because most diversity of microbes found in rhizosphere ecosystem than other ecosystem.
- Plant health depended on the rhizosphere of its root zone.
- Manipulation of rhizospheric microorganisms will affect the overall impact on plant growth and crop production.

Manoj *et al.*,2018

Plant microbiomes

Bacterial microbiome associated with the rhizosphere

Manipulation of rhizospheric microbiomes

- Manipulation in the sense of change the composition of microorganisms means:

1. The increase the no. of beneficial microbes like siderophore producing, phosphorus solubilising, zinc solubilising, nitrogen fixing bacteria, etc.
2. In turn, affect the growth of harmful organisms, which overall increase the plant growth and crop production.

Manoj *et al.*,2018

Plant microbiomes

Manipulation of rhizospheric microbiomes

- Manipulation of the plant microbiome has the potential to:

1. reduce the incidence of plant disease,
2. increase agricultural production,
3. reduce chemical inputs, resulting in more sustainable agricultural practices.

- This goal is seen as vital for sustaining the world's growing population.

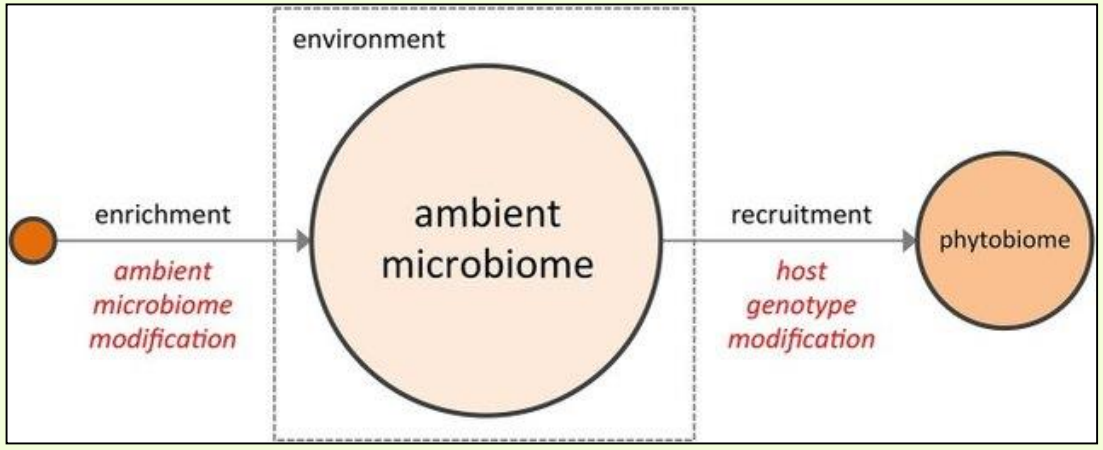
Manoj *et al.*,2018

Phytobiomes

The two complementary strategies for enhancing a crop phytobiome

- The two complementary strategies for enhancing a crop phytobiome are:

1. direct modification of the ambient microbiome, e.g., the bulk soil microbiome(BSM), via inoculants or soil transfers, and
2. the development of host genotypes better able to recruit a superior microbial assembly from the ambient microbiome.



Hale *et al.*,2014

Endophytic microbiomes

Molecular mechanisms of other bacteria-plant interactions

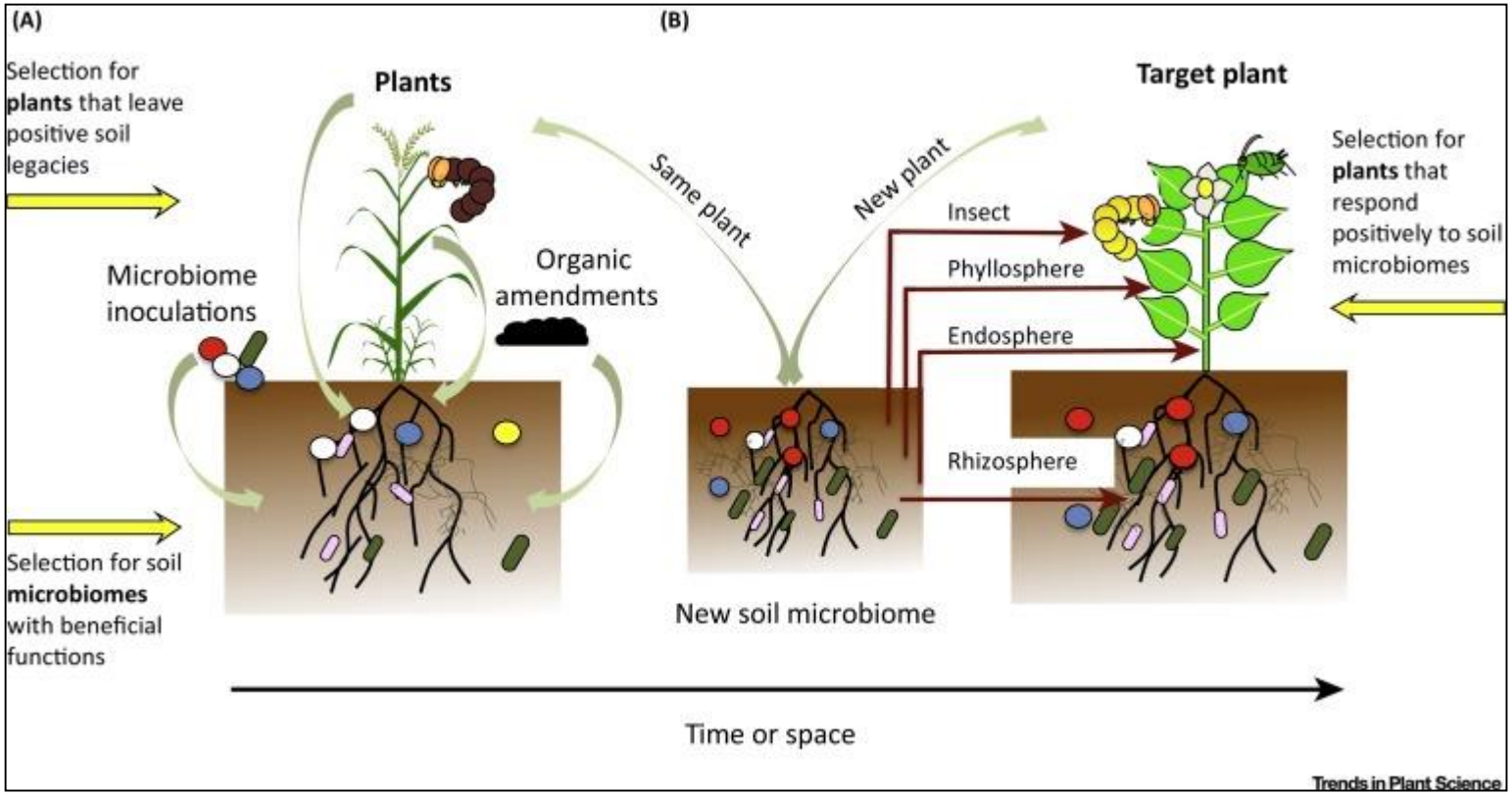
- The presence of different endophytic species in soybean depended on:

1. the plant genotype,
2. the plant age,
3. the tissue sampled, and also on
4. the season of isolation (Kuklinsky-Sobral *et al.*,2004).

Rosenblueth and Martínez-Romero,2006

Soil microbiomes

Manipulation of microbial population in rhizosphere directly affect the plant health and productivity of plant. This strategies will be used for control the plant disease



Pineda *et al.*,2017;Manoj *et al.*,2018

Plant microbiomes

Bacterial microbiome associated with the rhizosphere

Rhizosphere microbiomes

- Infected plants perceive pathogen invasion in roots or shoot and subsequently increase the secretion of microbe-stimulatory compounds in non-infected roots.
- These stimulants can recruit and activate plant-beneficial microorganisms.
- Beneficial microorganisms can:

1. Induce resistance (IR) directly, or
2. Produce pathogen-inhibitory compounds.

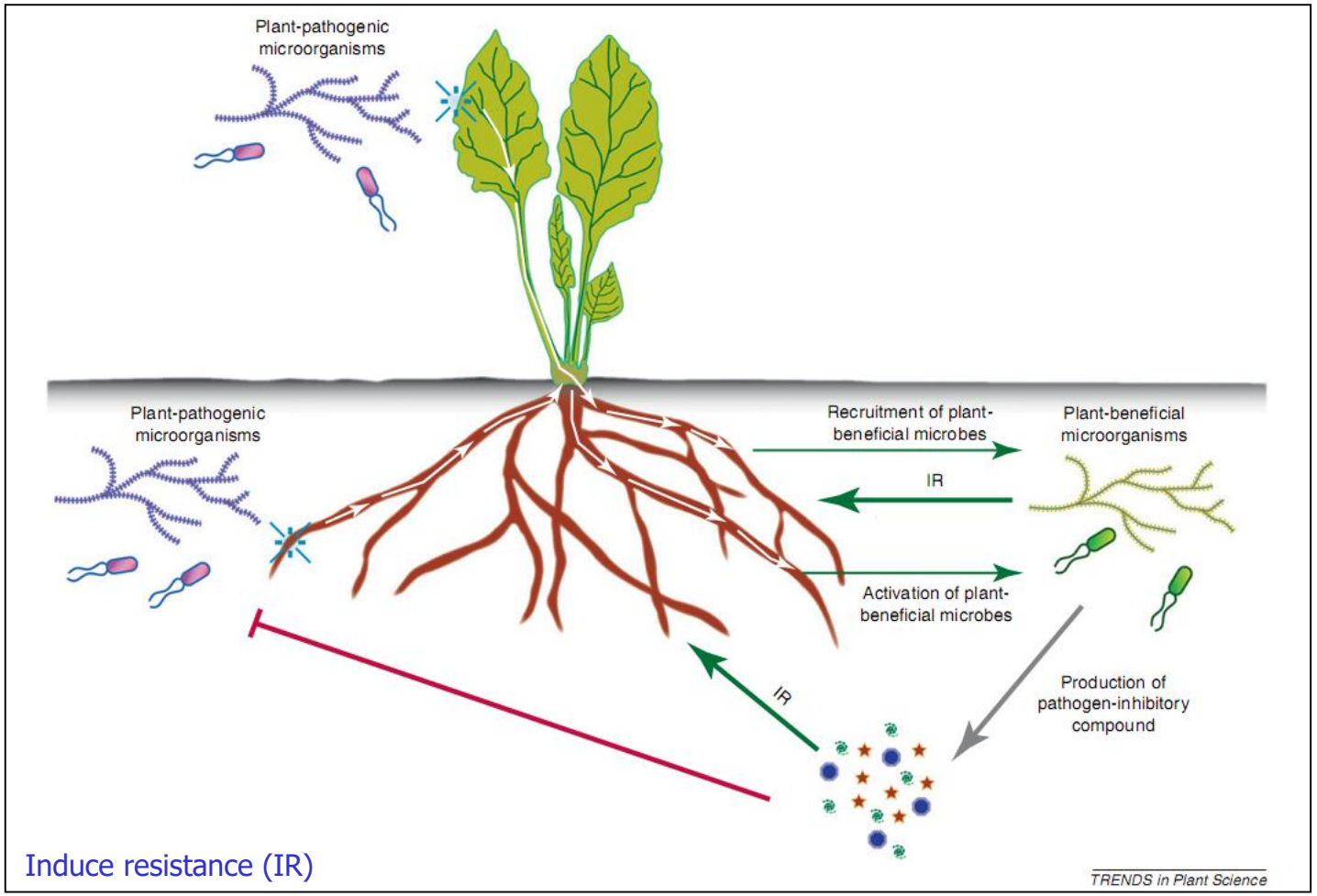
- Some pathogen-inhibitory compounds are known to induce resistance themselves.

Berendsen *et al.*,2012

The rhizosphere microbiome

Interactions in the rhizosphere

Microbiome to the rescue



Berendsen *et al.*,2012

Plant phytobiomes

Soil microbiomes

Steering soil microbiomes to suppress aboveground insect pests

- Soil microbes are a major source of the plant microbiome and recent advances show that they are key components of plant resistance against aboveground attackers.
- Soil-borne microbes affect aboveground herbivorous insects through a **cascade of molecular and chemical changes in the plant**.
- Knowledge of these microbe-plant-insect interactions is mostly limited to one or a few microbial strains.

Pineda *et al.*,2017

The rhizosphere microbiome
Interactions in the rhizosphere

- These beneficial micro-organisms are now called plant probiotics (Picard and Bosco,2007), and include:

1. mycorrhizal fungi+ helper bacteria
2. antagonistic fungi, and
3. the large group of Plant Growth Promoting Rhizobacteria (PGPR).

Most soil and plant scientists feel that the well known term PGPB and PGPR is simple and informative enough.
Earlier, it was plain biofertilizers, then bioinoculant arrived and now slowly plant probiotic.

Bosco and Picard,2008;…

Plant microbiomes
Ectomycorrhizal symbiosis
Mycorrhiza Helper Bacteria

- Some soil bacteria have been shown to have **beneficial effects upon the establishment** of ectomycorrhizal symbioses.
- Some of these bacteria, known as Mycorrhiza Helper Bacteria (MHBs), have been shown to stimulate ectomycorrhiza formation, root and shoot biomass. E.g.
- *Arthrobacter*
- *Azospirillum brasilense*
- *Azotobacter*
- *Bacillus*
- *Burkholderia*
- *Paenibacillus*
- *Pseudomonas*
- *Streptomyces*
- *Klebsiella*

Frey-Klett *et al.*,2007

The rhizosphere microbiome and plant health
Interactions in the rhizosphere
Plant probiotics/phytobiomes/microbiome

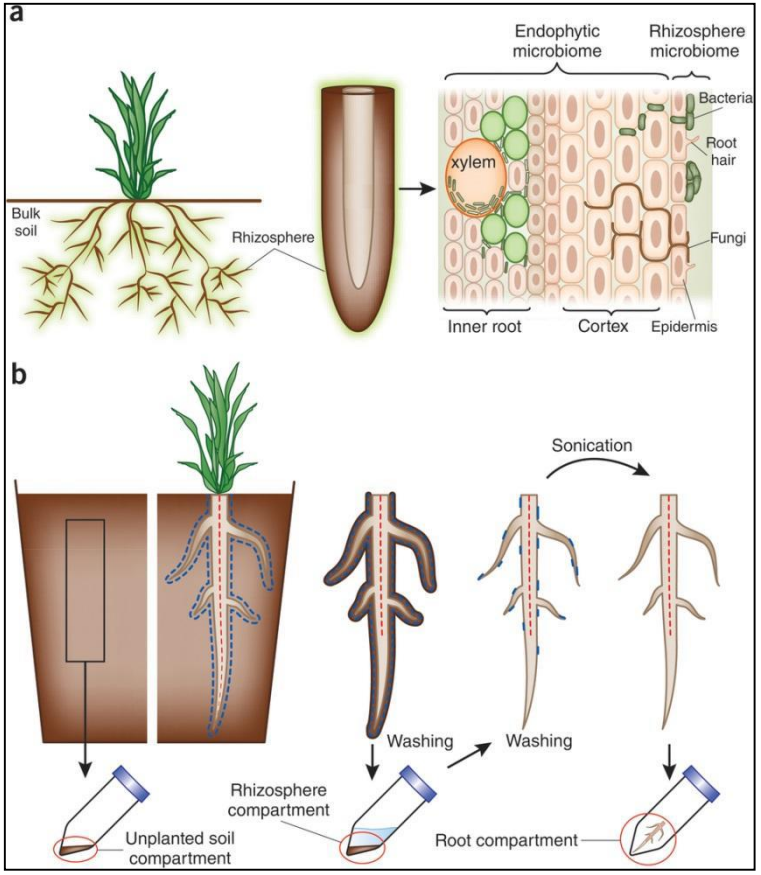
- Several model organisms for plant growth promotion and plant disease inhibition are well-studied including:
- The bacterial genera:
- *Azospirillum*
- *Rhizobium*
- *Serratia*
- *Bacillus*
- *Pseudomonas*
- *Stenotrophomonas*
- *Streptomyces*
- The fungal genera:
- *Ampelomyces*, *Coniothyrium*, and *Trichoderma*.

Song *et al.*,2012

Plant microbiomes

Do plants control their microbiome composition?

- Two recent root metagenomic or community genomics studies:
- DNA extracted from microbes in:
  1. the seed,
  2. rhizosphere, and
  3. endophytic compartments, and
  4. soils.
- amplicon sequencing.

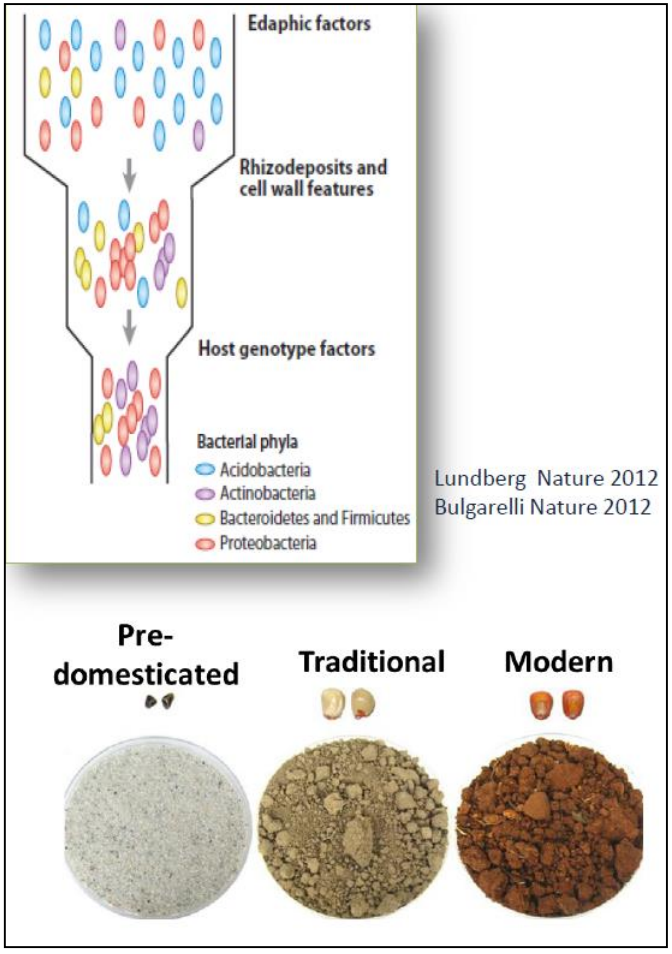


Jan E. Leach; Johnston-Montje *et al.*,2014;..

Plant microbiomes

Plants can select microbiome

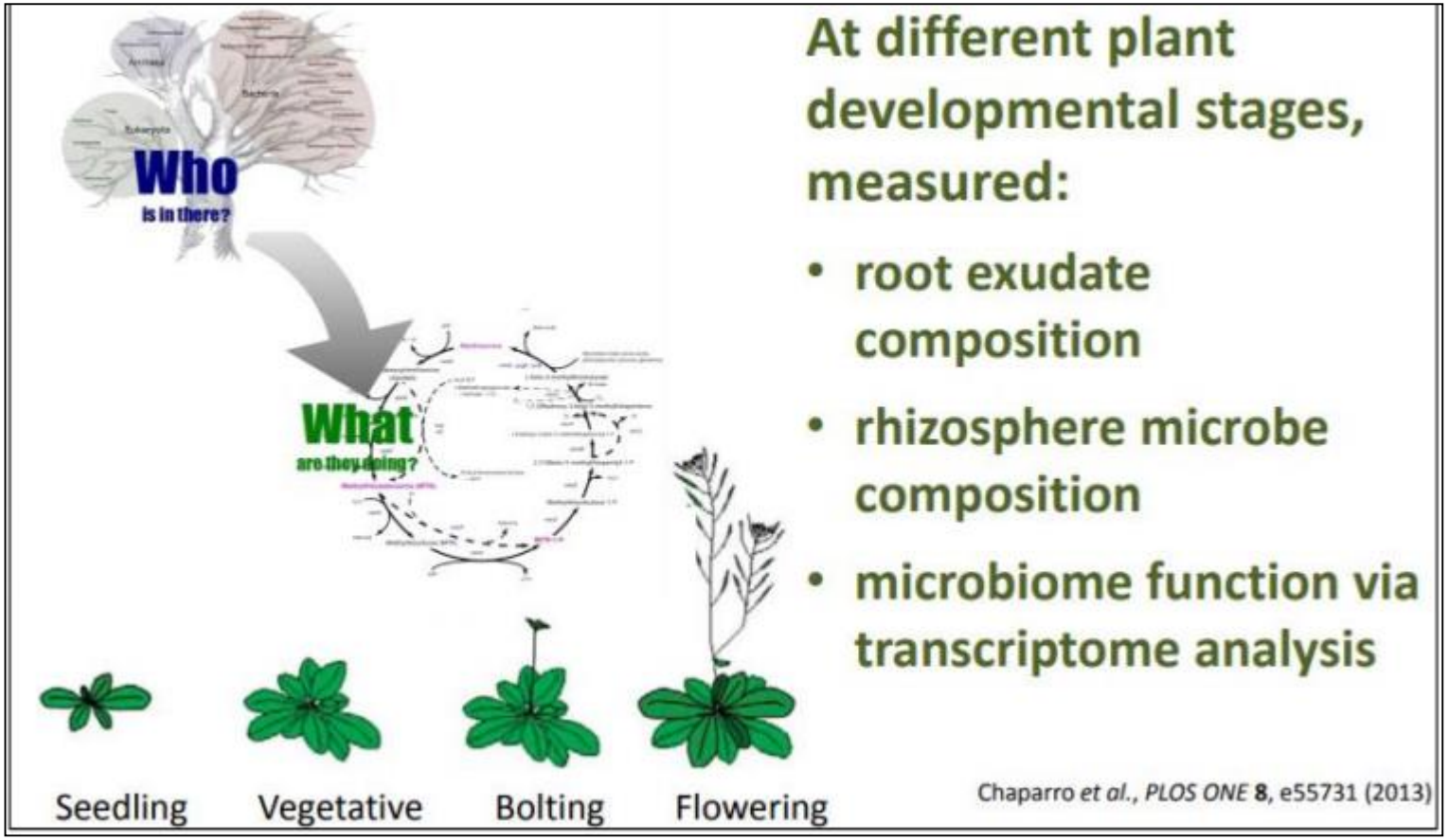
- Plant genotype – dependent selection fine-tunes the internalized microbial community profiles.
- Plants can transmit bacterial endophytes from generation to generation through seed.



Johnston-Montje *et al.*,2014

Plant microbiomes

How do plant roots influence the rhizosphere microbiome composition?



Leach,2015

Plant microbiomes

Plants can select microbiome

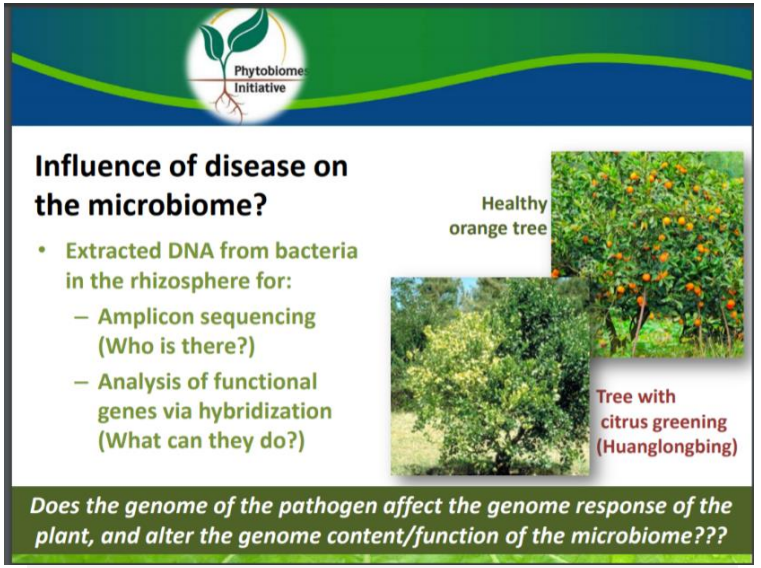
1. **Can we breed plants that select for a beneficial microbiome?**
2. Have we inadvertently(accidentally) selected against plant traits that help support beneficial microbes by breeding for high yield under conditions of high inputs and soil tillage?
3. What is the potential for identifying new, more successful biocontrol agents?

Plant microbiomes

Influence of disease on microbiomes

Rhizosphere communities on infected trees were different from those on uninfected trees

- Any changes in the core-microbiome composition or function leads to:
  1. Debilitative, or
  2. destructive diseases in humans as well as plants.
- Rhizosphere microbiome on trees with citrus greening are different from those on uninfected trees.
- Disease is associated with shifts in the microbiome composition
- Microbiome shifts diagnostic for disease.



Citrus greening disease caused by ca. Liberibacter spp. is associated with shifts in the microbiome composition.

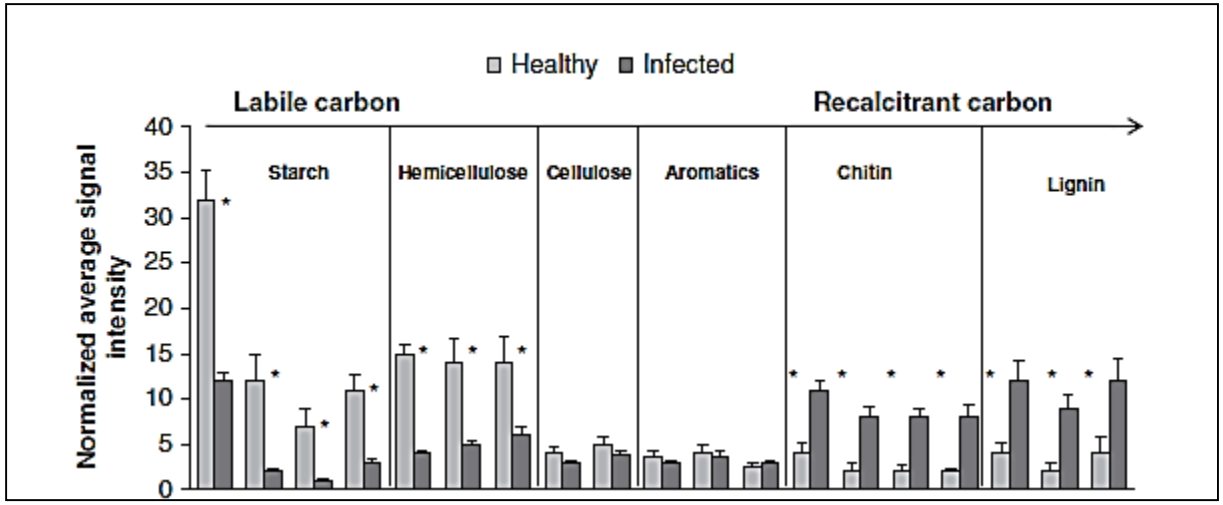
Trivedi *et al.*,2012

Plant microbiomes

Disease is associated with shifts in the microbiome composition

Microbiome shifts diagnostic for disease

- Functional shift: away from use of easily degraded/labile carbon sources(soluble) to more recalcitrant forms (insoluble).
  - consistent with carbohydrate repartitioning during citrus greening disease (photosynthate to roots…)



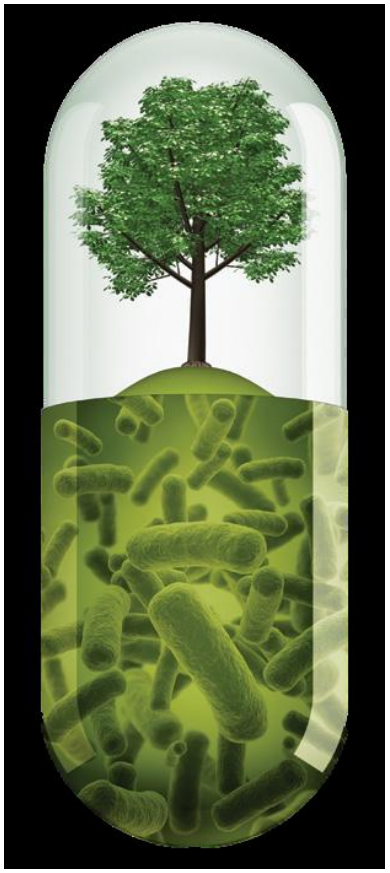
Trivedi *et al.*,2012

Plant microbiomes

Phytobiomes

The Future

1. Management strategies that create disease-suppressive microbial communities.
2. Plants that select for and maintain beneficial microbiomes.



Brett Ryder

Plant microbiomes

Phytobiomes

The Future

- Smart microbes that detect and treat disease/destroy pests.

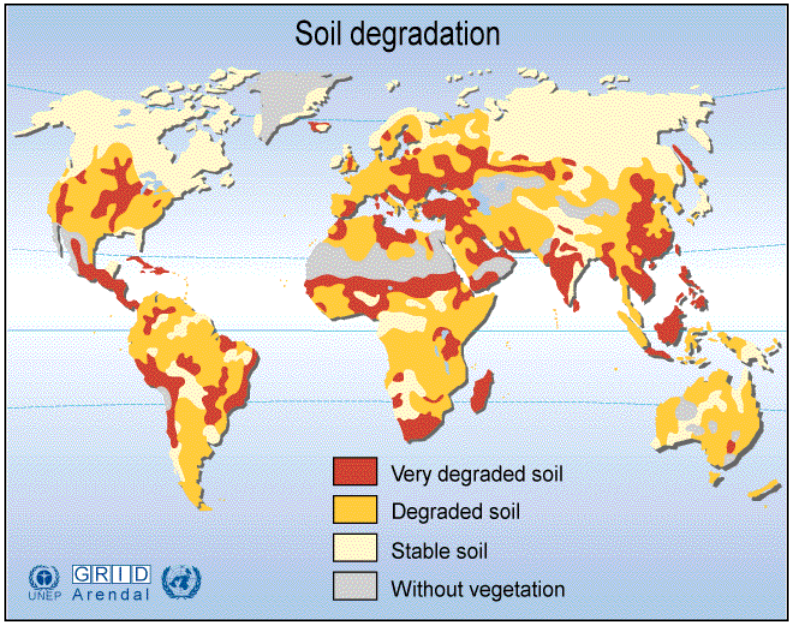
Brett Ryder

Plant microbiomes

Managed/engineered microbiomes

The Future

- Managed/engineered microbiomes that promote
  1. sustained crop productivity;
  2. rebuild depleted/degraded soils;
  3. produce with less water;
  4. produce in changing climate.



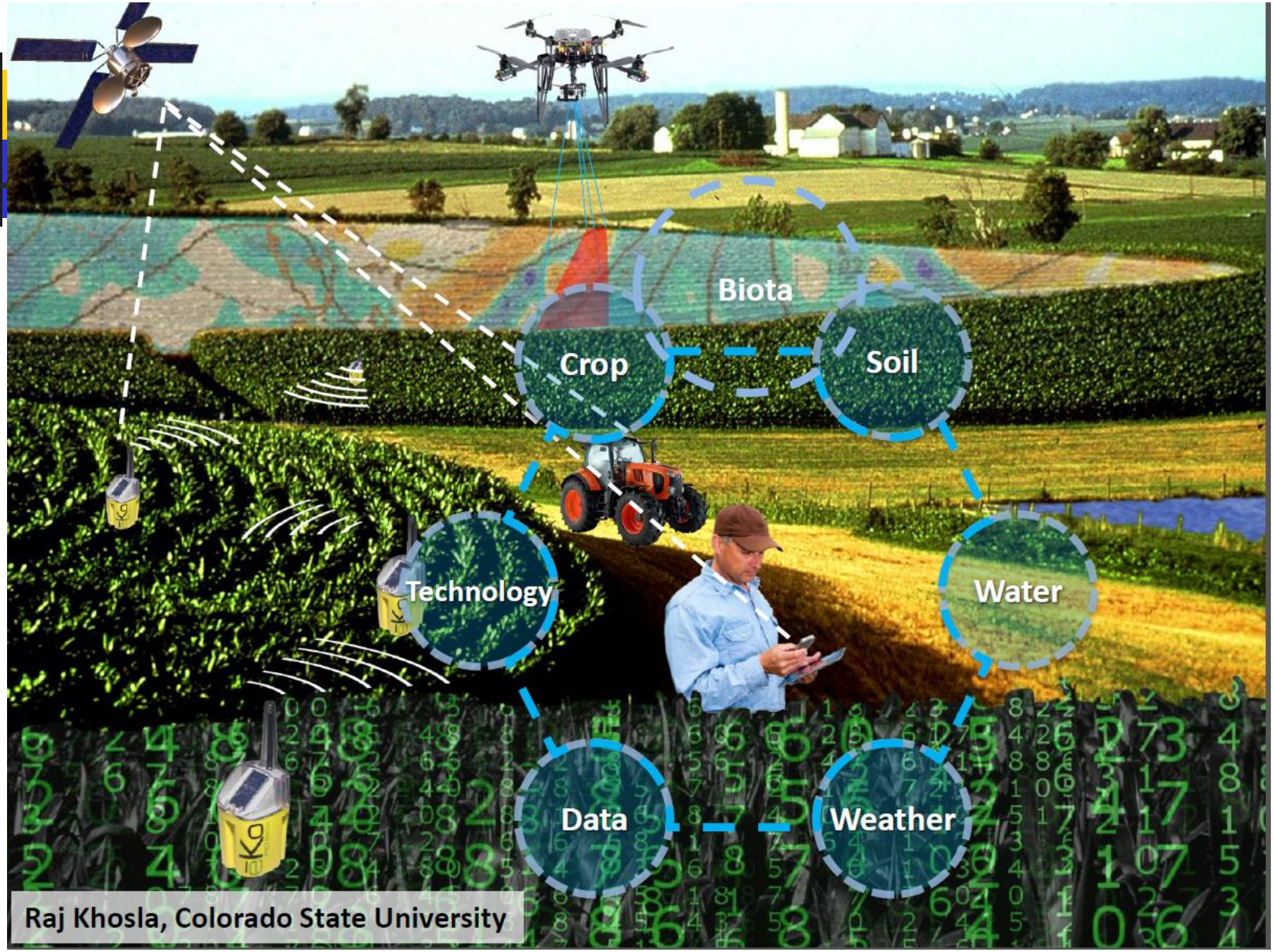
Source: UNEP

*1. 5 billion people depend on degraded lands for survival!

Leach,2015

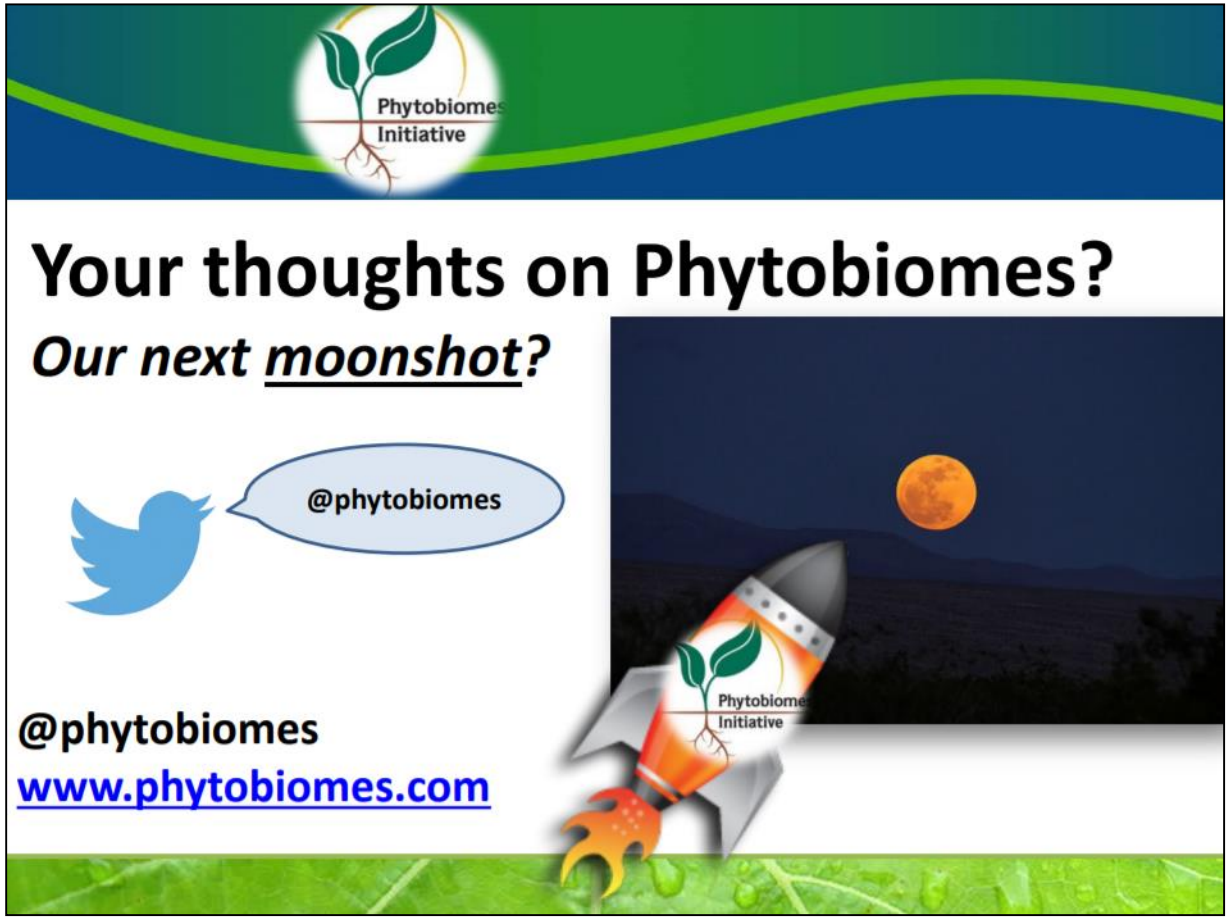
Building Partnerships

Leach,2015



Using drone and Satellite

Your thoughts on Phytobiomes?



Leach,2015

Plant microbiomes

How do we assess microbiomes?

Metagenomics and Metaproteomics

1. Metagenomics or community genomics is the study of genetic material recovered directly from environmental samples, consisting of the genomes of many individual organisms.
2. Metaproteomics: study of all protein samples recovered directly from environmental sources.

Plant microbiomes

How do we assess microbiomes?

Metagenomics and Metaproteomics

- It was identified that the 1-2% of microbes were culturable while 98-99% microbes were non-culturable.
- Interaction in rhizosphere with plant participated both types of microbes culturable as well as non-culturable.
- So now attention require to study the non-culturable microbes and its effect on the plant.
- The development of sequencing technologies it is now possible to study non-culturable microbes.

Manoj *et al.*,2018

Plant microbiomes
Metagenomics and Metaproteomics

- Metagenomics means to study of culturable as unculturable microbe.
- Metagenomics is based on studies of ecological diversity of uncultured microorganisms using molecular biology.
- For the metagenomic analysis of microbial populations, the total content of nucleic acids from a broad range of environmental samples is used, including:
- bacterial,
- Viral, and
- human gut metagenome.

Lara-Victoriano *et al.*,2011: Manoj *et al.*,2018

Plant microbiomes

How do we assess microbiomes?

Metagenomics and next-generation sequencing (NGS)

- Advances in next-generation sequencing (NGS) have allowed significant breakthroughs in microbial ecology studies.
- This has led to the rapid expansion of research in the field and the establishment of "metagenomics", often defined as the analysis of DNA from microbial communities in environmental samples without prior need for culturing.

Oulas *et al.*,2015

Plant microbiomes

Metagenomics and Metaproteomics

- It refers to the total extraction of DNA or RNA and, sometimes, microbial protein samples.
- Once DNA samples are extracted, amplification is carried out by PCR and followed by sequencing.
- This is how a genomic library is constructed, which is made up of millions of random DNA fragments.
- The next step is to determine which genes are present and their role, through cloning techniques.

Lara-Victoriano *et al.*,2011

Plant microbiomes

Next generation sequencing (NGS)

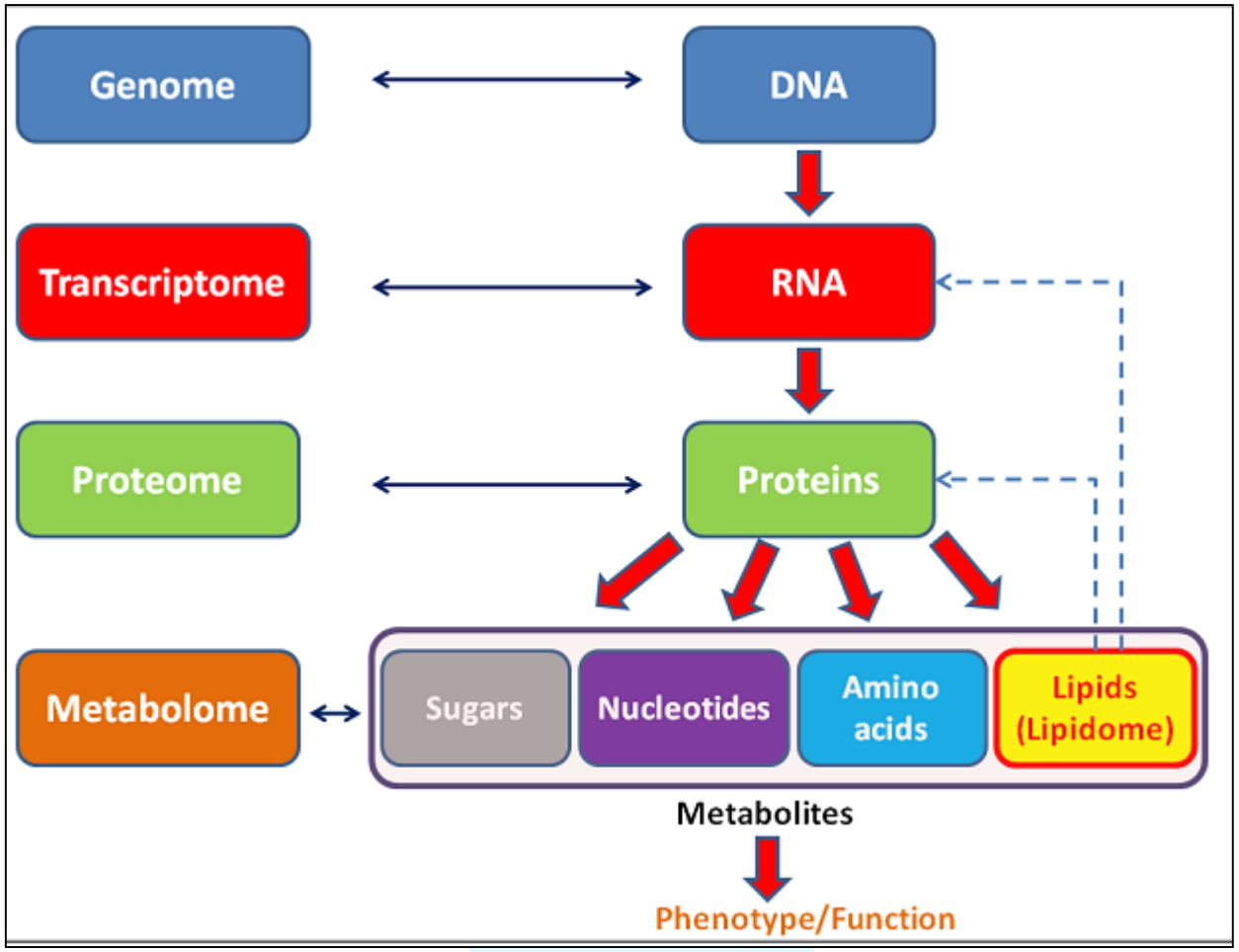
- Genomic analyses of individual strains or metagenomics studies of whole microbial communities may provide insight in to the

1. composition or diversity, and
2. physiological potential of endophytes associated with plants.

Akinsanya *et al.*,2015

Plant microbiomes

How do we assess microbiomes?

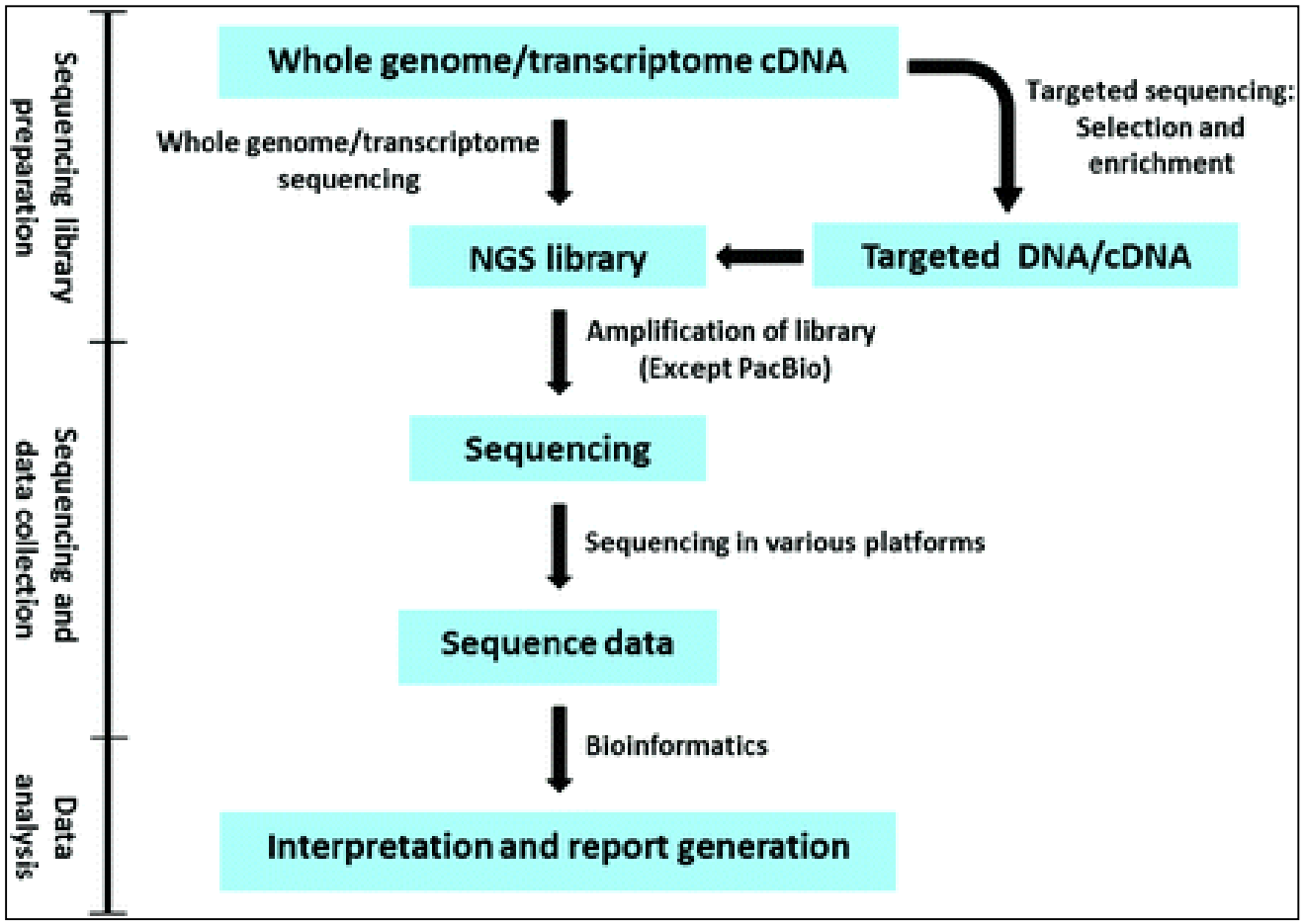


Wikipedia,2017

Plant microbiomes

How do we assess microbiomes?

Metagenomics and next-generation sequencing (NGS)

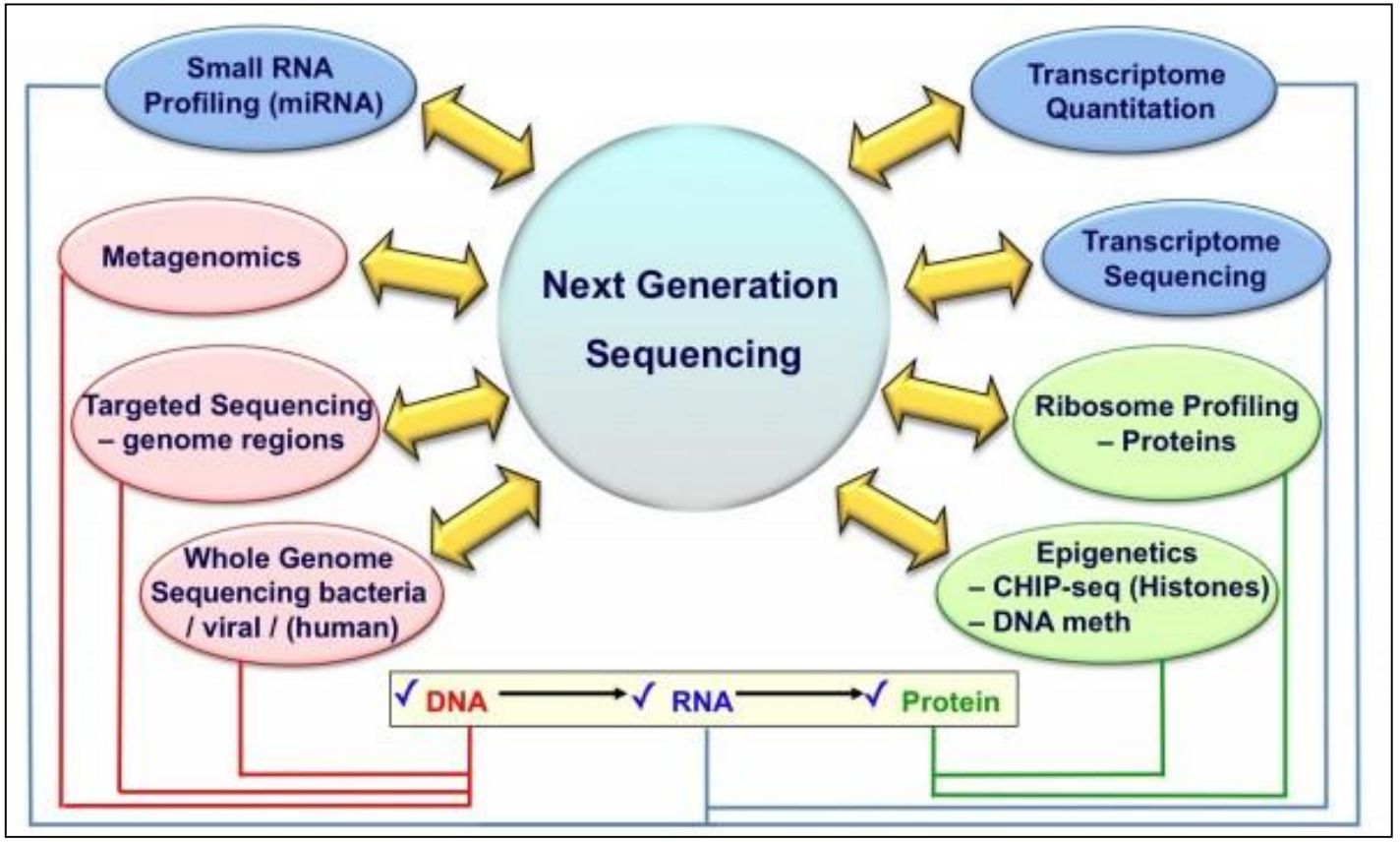


Chandran,2017

Plant microbiomes

How do we assess microbiomes?

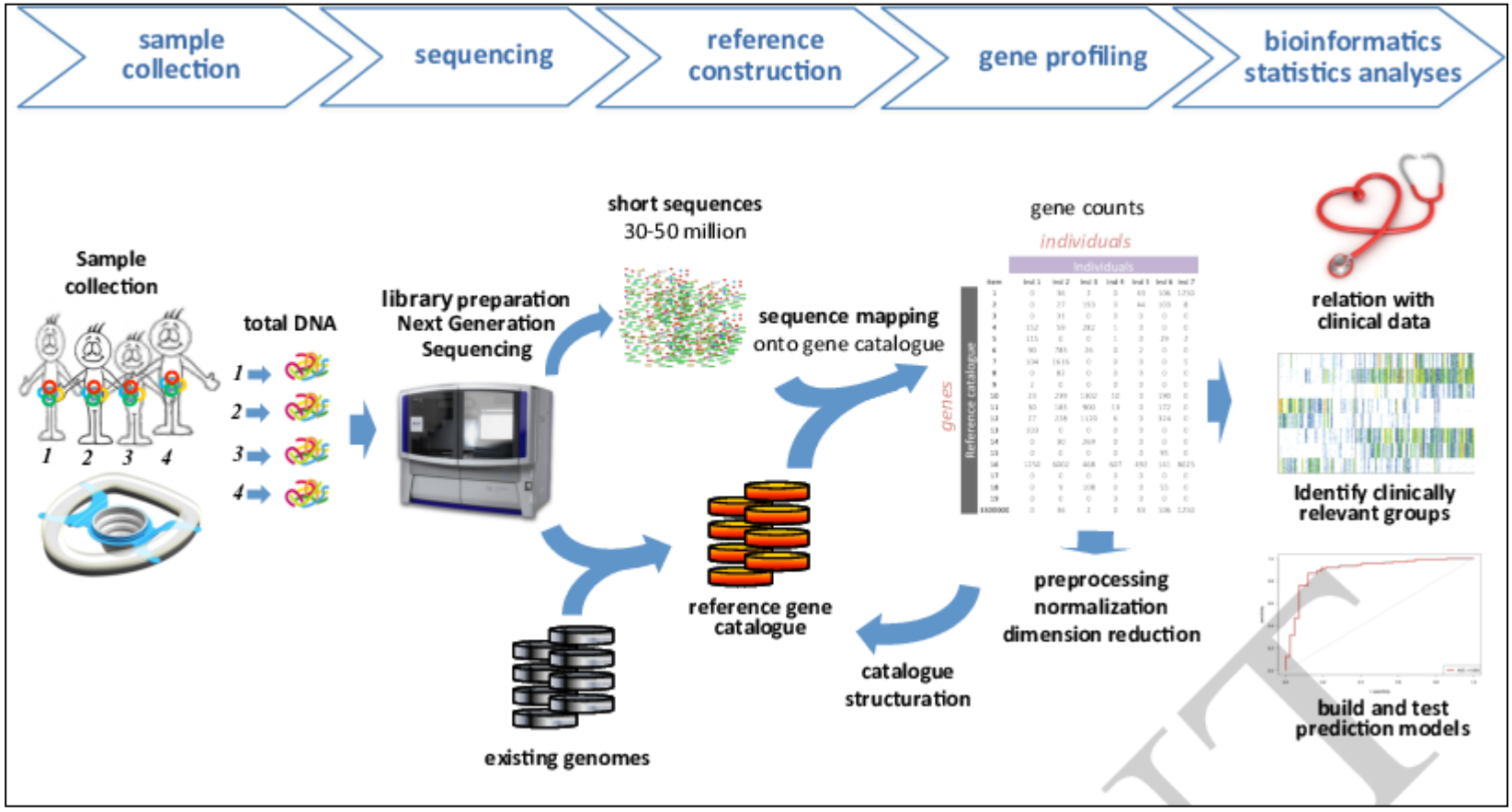
Metagenomics and next-generation sequencing (NGS)



Plant microbiomes

How do we assess microbiomes?

Metagenomics and next-generation sequencing (NGS)

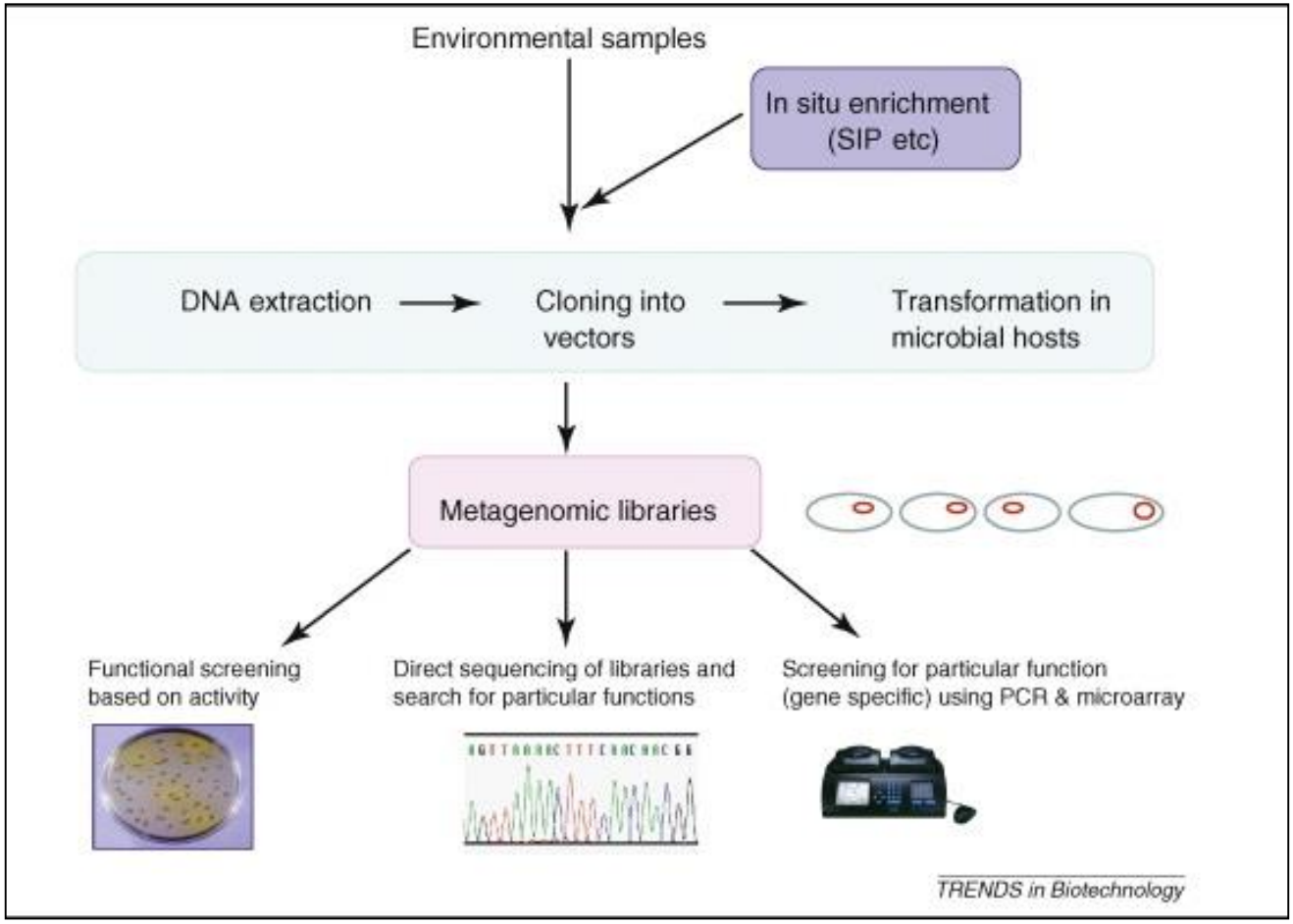


Overview of a whole-metagenome-sequencing project from sample collection to hypotheses generation (after N. Pons & E. Le Chatelier).

Plant microbiomes

How do we assess microbiomes?

Metagenomics



Wikipedia,2017

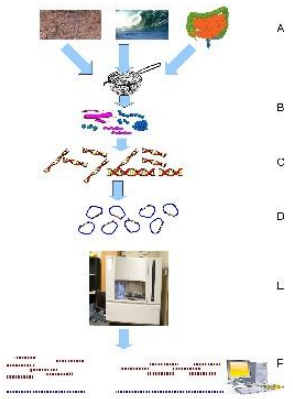
Plant microbiomes

How do we assess microbiomes?

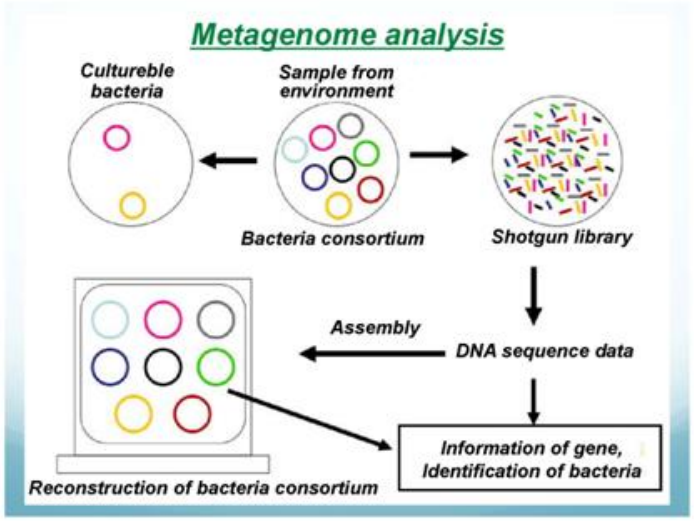
Metagenomics and Metaproteomics

Shotgun metagenomics

- Collect samples;
- Extract DNA;
- Feed into sequencer;
- Computationally analyze.



Wikipedia: Environmental shotgun sequencing.png

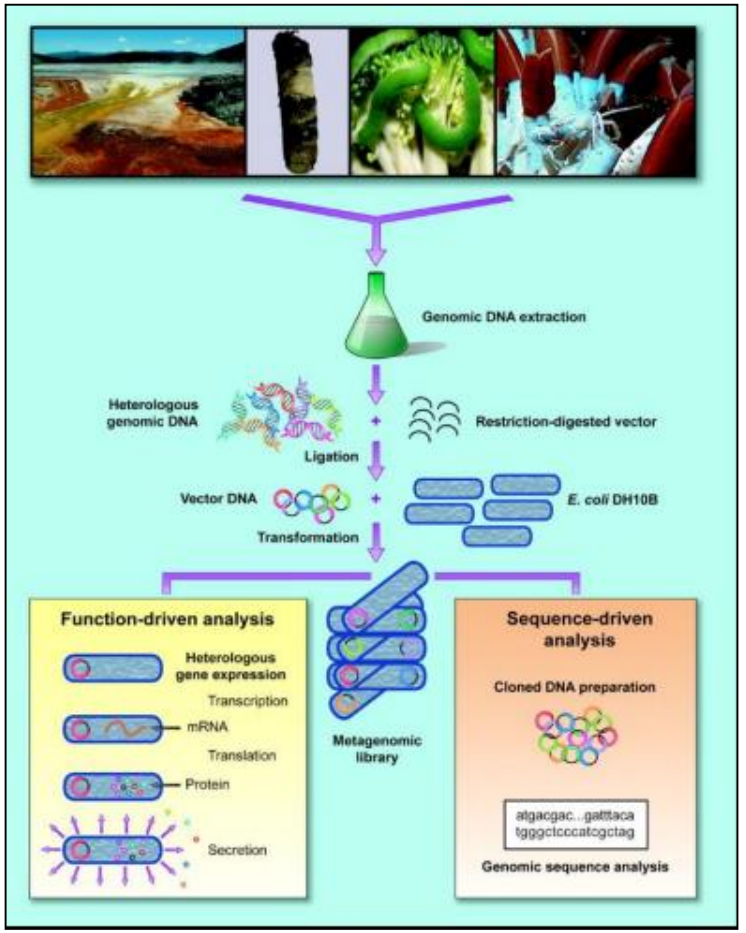


Shotgun metaproteomics is a relatively new technology in its' application to complex and highly diverse microbial communities.

Plant microbiomes

Metagenomics and Metaproteomics

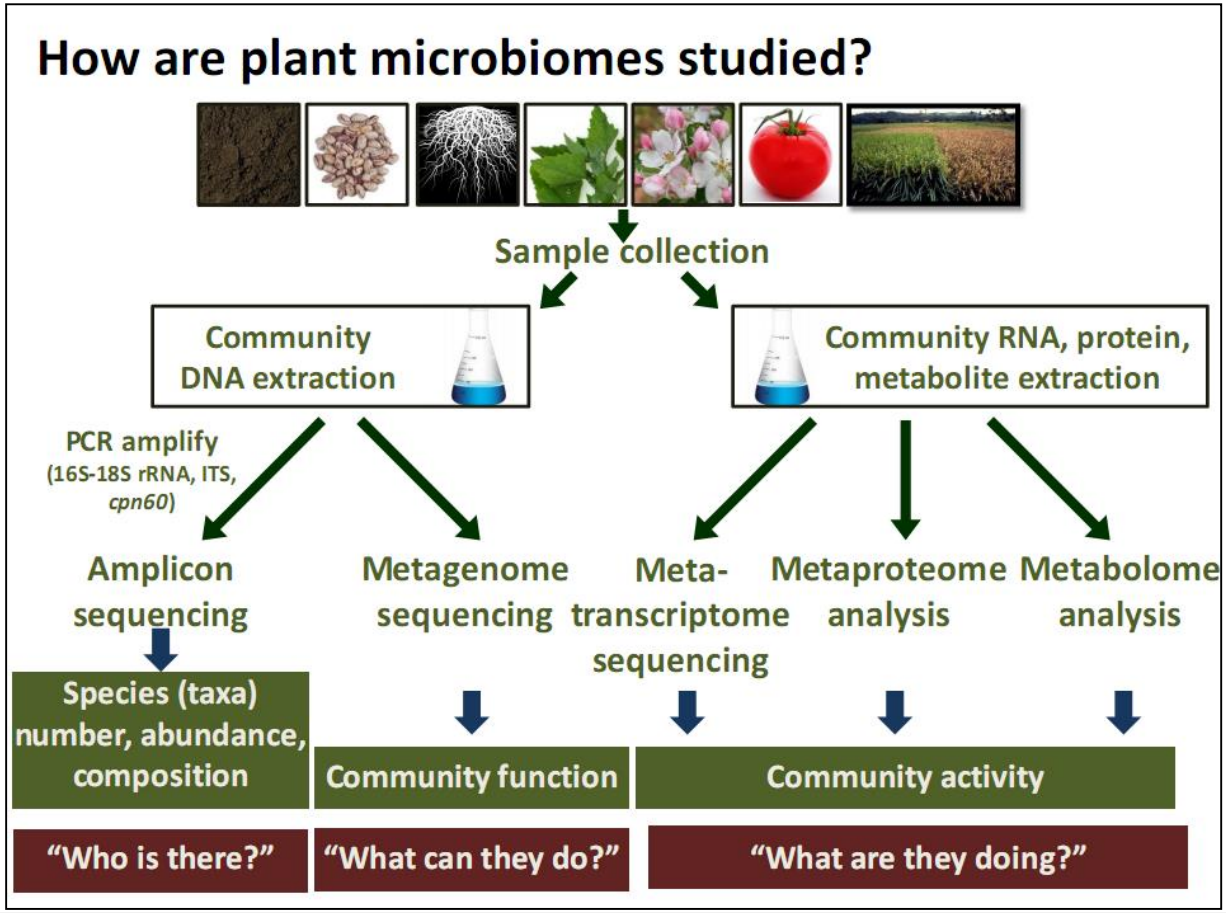
- Genomic study of all organisms:
  - Sequencing 16S rRNA, DNA, or mRNA from environmental samples
- Address questions on:
  - community composition ("Who is there?")
  - function ("What can they do?")
  - activity ("What are they doing?")



Plant microbiomes

How do we assess microbiomes?

Metagenomic sequencing and metaproteomics



Metaproteomics (also Community Proteomics, Environmental Proteomics, or Community Proteogenomics) is the study of all protein samples recovered directly from environmental sources.
Metabolomics is the large-scale study of small molecules, and their interactions within a biological system.

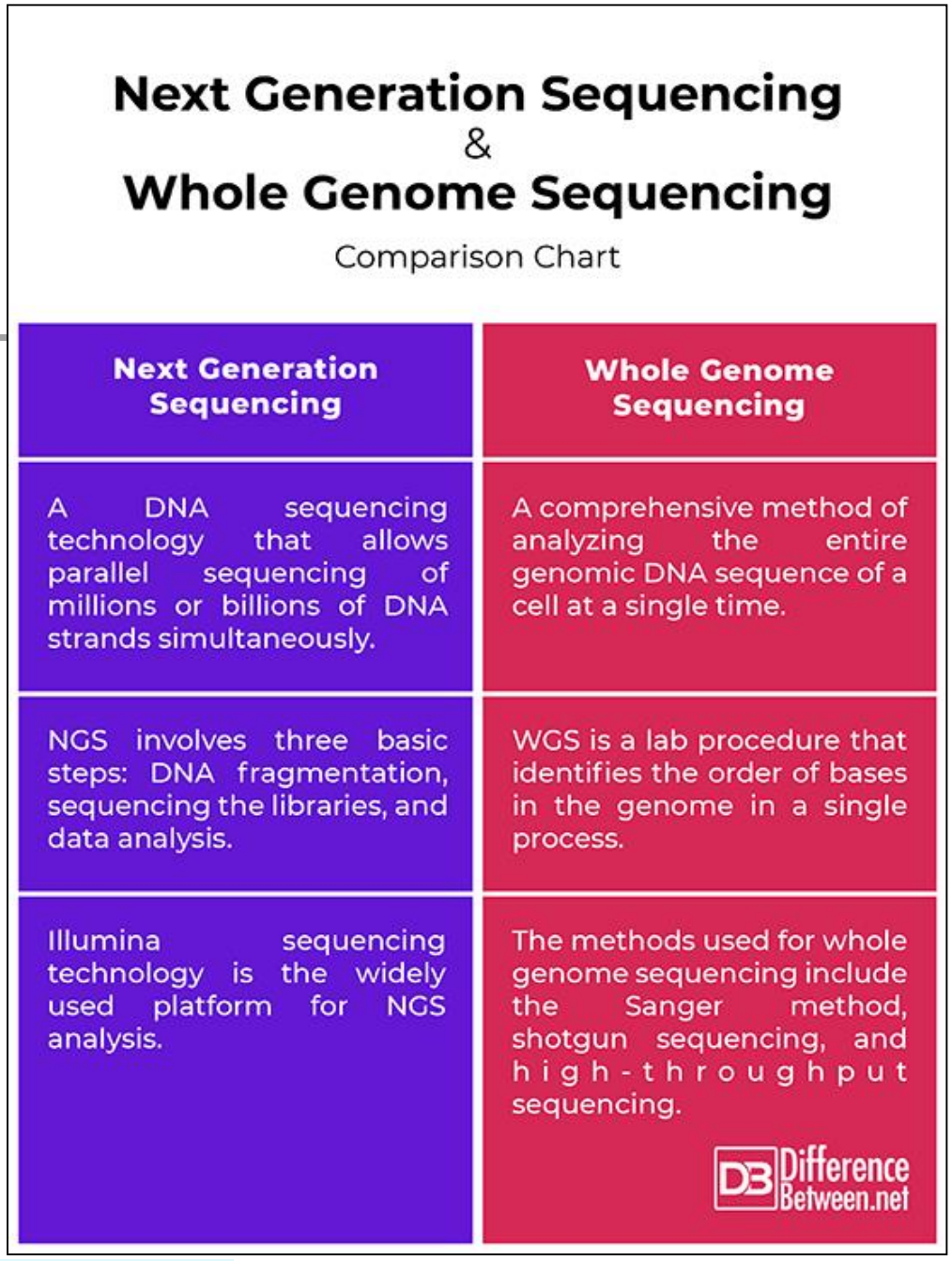
Next-generation sequencing(NGS) Whole genome sequencing(WGS)

- Next-generation sequencing (NGS) is a massively parallel sequencing technology that offers ultra-high throughput, scalability, and speed.
- **It determines the order of nucleotides in entire genomes or targeted regions of DNA or RNA.**

- Whole genome sequencing (WGS) provides the most comprehensive data about a given organism.
- **It determines the entire DNA sequence all at once.**

The conventional Sanger sequencing method which is still considered as the gold standard for sequencing has its limitations.
With the ability to sequence more than a million DNA fragments at a time, the next-generation sequencing (NGS) has revolutionized the ability to generate large volumes of sequence data at an extremely low cost.

Human DNA consists of about 3 billion bases.



Khillar, 2021

Plant microbiomes

Phytopathogens studied by metagenomics

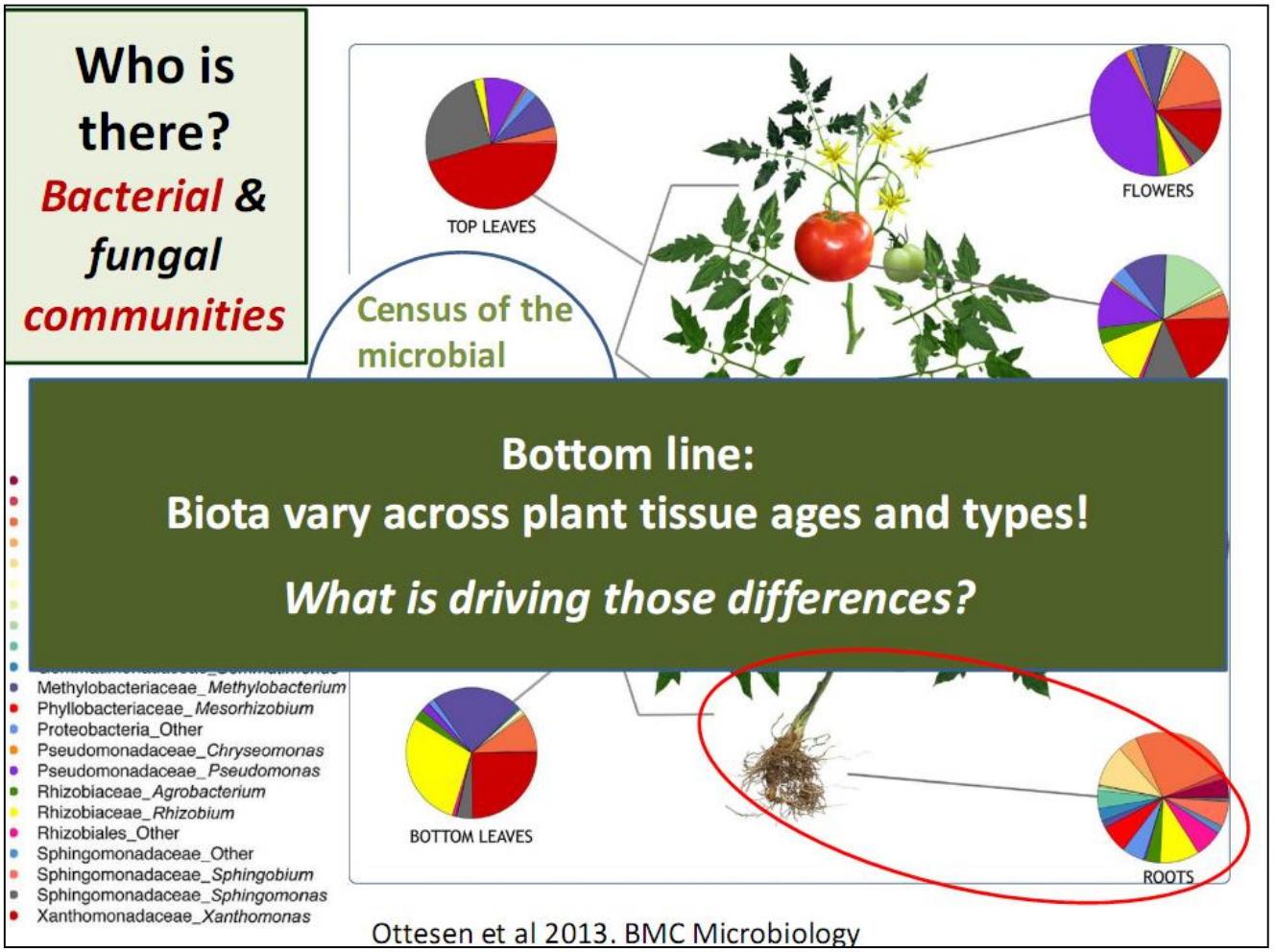
Bacteria

- The phylogenetic information of metagenomic libraries is obtained from 16S rDNA gene.
- The product is first cloned and then the metabolic potential can be explored to identify this group of bacteria.
- It is also important to compare the phylogenetic information with other communities of bacteria.
- With these studies, bacteria have been reclassified into the following taxonomic categories: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Epsilonproteobacteria, Cytophagalike, Actinobacteria, Firmicutes, Bdellovibrio, Verrucomicrobiales, Spirochaetaceae (Cottrell *et al.*, 2005).
- Bacteria make up the most complex and numerous groups of pathogenic organisms.

Lara-Victoriano *et al.*,2011

Tomato plants microbiomes

Bacterial diversity in roots, bottom leaves, stems, tomatoes, flowers and top leaves of tomato plants using 16SrRNA. Bacterial diversity associated with diverse tomato organs (16S)



Aloe vera microbiomes

Endophytic bacteria of *Aloe vera* studied by metagenomics

Next generation sequencing (NGS)

- Next generation sequencing (NGS) enables rapid analysis of the composition and diversity of microbial communities in several habitats.
- We applied the high throughput techniques of NGS to the metagenomics study of endophytic bacteria in *Aloe vera* plant, by assessing its PCR amplicon of 16S rDNA sequences (V3-V4 regions) with the Illumina metagenomics technique used to generate a total of 5,199,102 reads from the samples.

Akinsanya *et al.*,2015

Aloe vera microbiomes

Endophytic bacteria of *Aloe vera*

Next generation sequencing (NGS)

- The analyses revealed Proteobacteria, Firmicutes, Actinobacteria and Bacteriodetes as the predominant genera.
- The roots have the largest composition with 23% not present in other tissues.
- The stems have more of the genus- Pseudomonas and the unclassified Pseudomonadaceae.
- The α-diversity analysis indicated the richness and inverse Simpson diversity index of the bacterial endophyte communities for the leaf, root and stem tissues to be 2.221, 6.603 and 1.491, respectively.

Akinsanya *et al.*,2015

Aloe vera microbiomes

Endophytic bacteria of *Aloe vera*

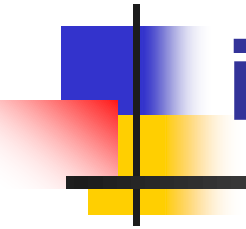
Next generation sequencing (NGS)

- Sequence processed details: merged sequence.
- The raw data forward and reverse reads were merged usingmothur pipeline alignment method.
- These were then filtered and trimmed by removing trailing bases with quality scores lower or equal to 2, maximum number of N allowed = 4, maximum number of homopolymer allowed = 8 and contaminant removed.
- All processing were doneusing mothur pipeline software (http://www.mothur.org/wiki/Download mothur).

Sample reference	Before merge process Number of sequence (total sequence length in bp)	After merge process Number of sequence (total sequence length in bp)
Root	2,528,030 (361,652,861)	1,264,015 (220,836,340)
Stem	1,298,892 (191,468,046)	649,446 (124,733,765)
Leaf	1,372,180 (200,256,446)	686,090 (127,684,140)
Total		2,599,551 (473,254,245)

Sequence input (forward and reverse sequences), quality encoding (Illumine 1.8+and Alignment method (needleman).

Akinsanya *et al.*,2015



Parameters of disease incidence and spread

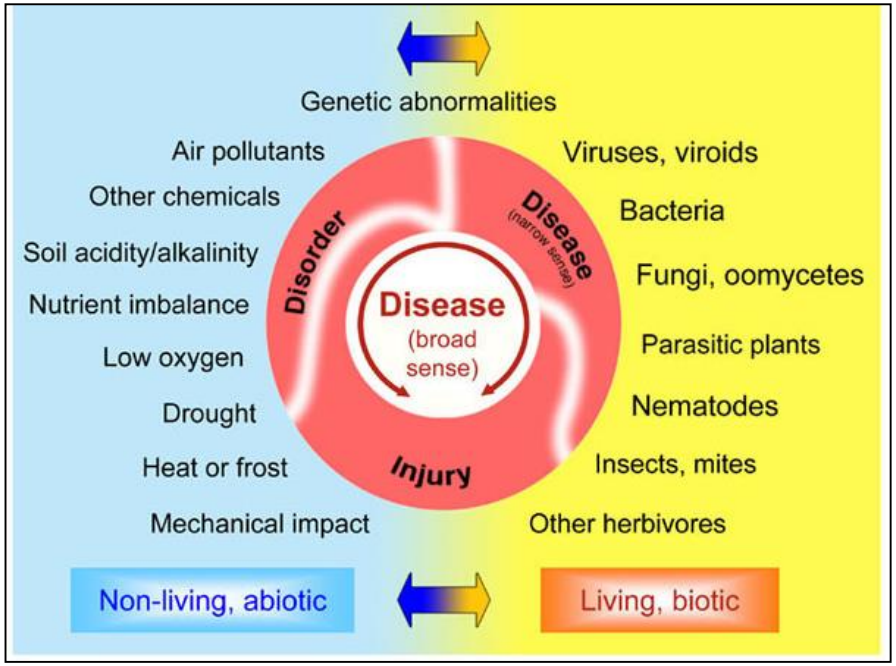
Triangle, tetrahedron, epidemics and forecasting

The disease doughnut

Plant disease factors

Basis for developing disease prediction systems

- The disease doughnut, a graphic for use in teaching about the concepts of disease and pathogen.
- The definition limits "disease largely to the upper right one-third of Figure.
- However, when we wish to diagnose plant problems, we must keep ALL possible causes or incitants in mind, the entire doughnut.



Baudoin,2007 updated 2014

Disease triangle

Plant disease factors

Basis for developing disease prediction systems

- Concerns are increasing every day as crops are continuously under threat by various plant diseases worldwide.
- A sudden epidemic breakout of any plant disease can cause huge economic losses leading towards the famine.
- To cope with this situation, understanding

1. plant disease triangle, and
2. disease epidemic forecasting is very important.

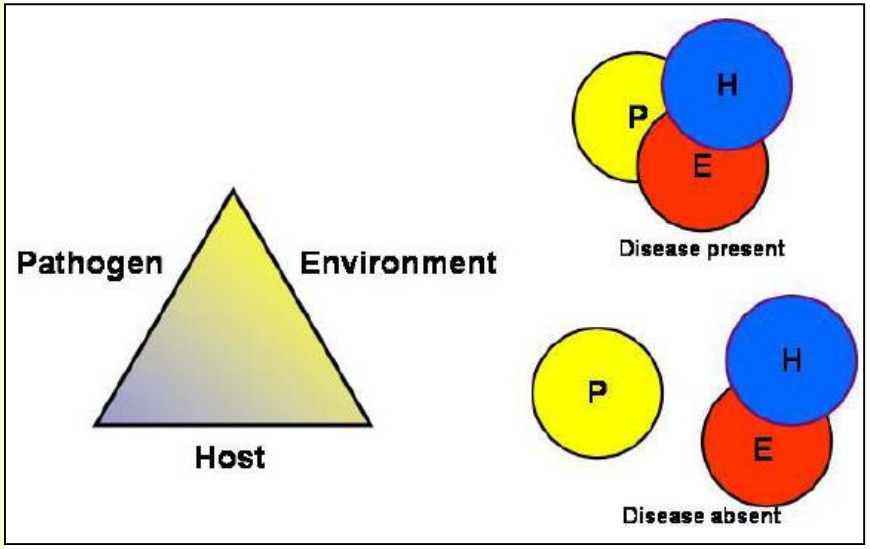
A famine is an extreme shortage, especially of food.

Islam,2018

The disease triangle

Disease develops only when all three factors are favorable

- Three things are required for a disease to occur:
  1. A susceptible plant,
  2. An organism to cause the disease,
  3. A suitable environment.
- If any of these three components is missing or minimized, disease will not occur.

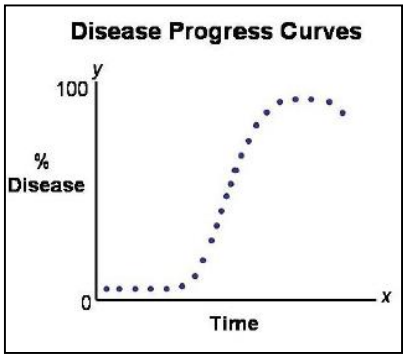


When these three components are present at the same time, a disease will occur.

The Ohio State University,2008,..

Disease triangle

Disease develops over time



- A significant factor not presented by the disease triangle is time.
- A situation may occur where the host, parasite and environment factors occur; but if they don't occur at the right time then disease will not result.
- Diseases are often managed through the use of time:

1. Time of planting,
2. time of harvest,
3. timing of varieties,
4. rotations, etc.

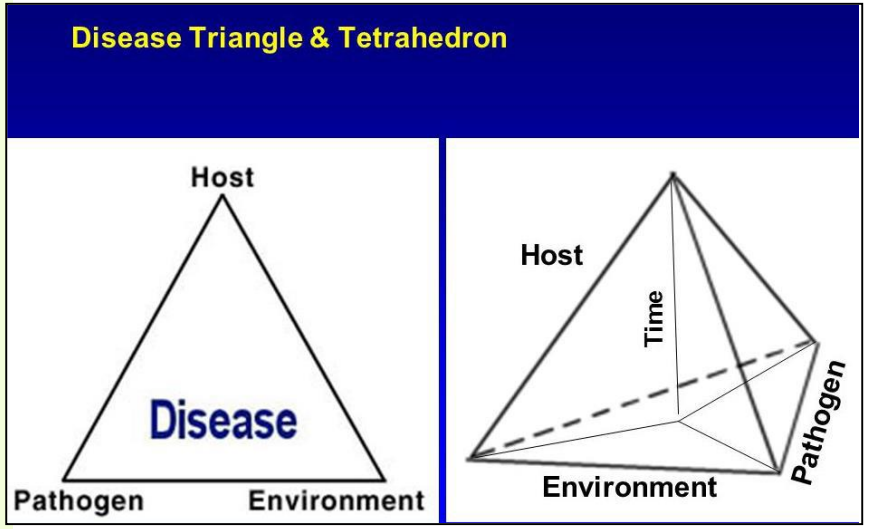
With respect to epidemiology, time (rate) is the central concept.

Partridge,2008; The Ohio State University,2008

Disease triangle and tetrahedron

The stages of the disease cycle form the basis of many plant disease prediction (forecasting) models

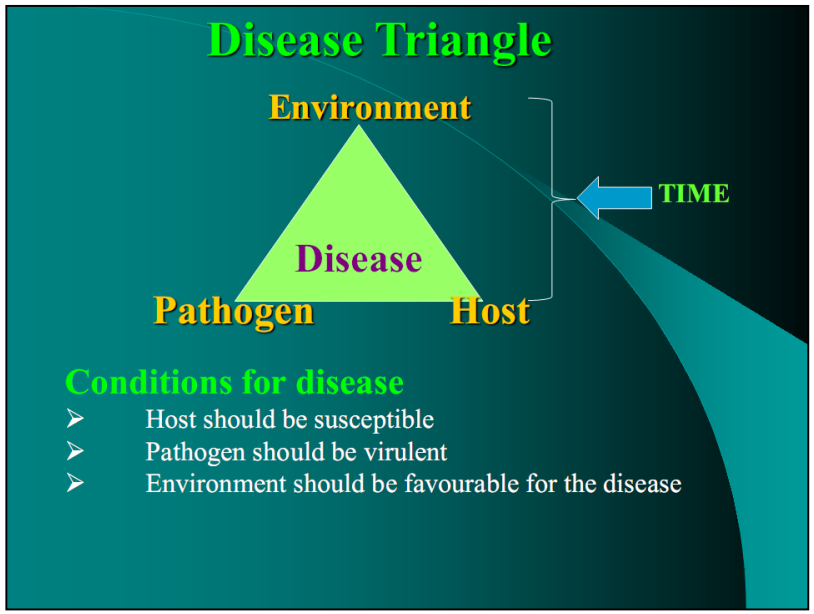
- Understanding the components of the 'disease tetrahedron', the interaction between:
  1. Host,
  2. Inoculum,
  3. Environment, and
  4. Human activity
- is essential for devising suitable forecasting systems.

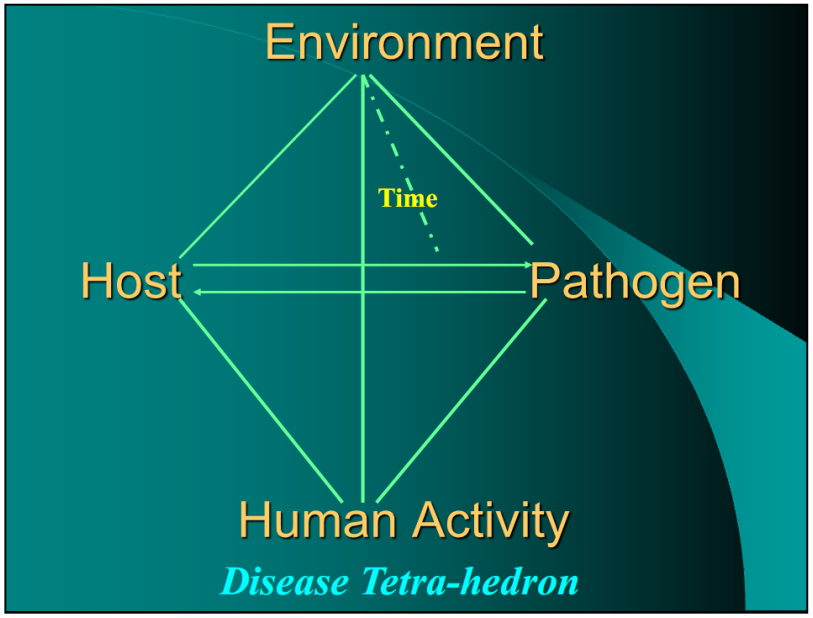


All three factors: 1) virulent pathogen, 2) susceptible cultivar and 3) conducive environment is existing at the same time. These conditions can lead towards PDE(plant disease epidemiology).

Disease triangle and tetrahedron

Disease progress curves are a graphical representation of how a disease develops over time



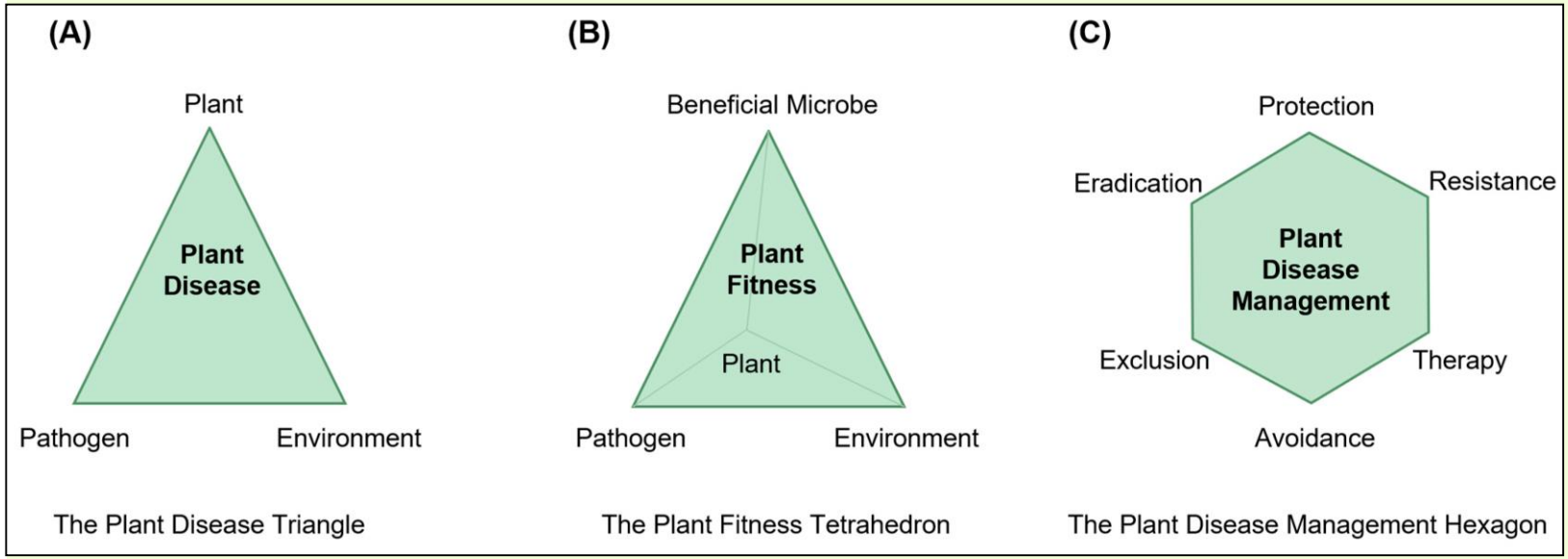


Disease triangle, tetrahedron and Hexagon

Disease progress curves are a graphical representation of how a disease develops over time

The conventional and current plant protection principles:

A. The conventional plant disease triangle model was adapted from reference.
B. The current plant fitness tetrahedron model was adapted from references.
C. The current plant disease management hexagon model was adapted from reference.

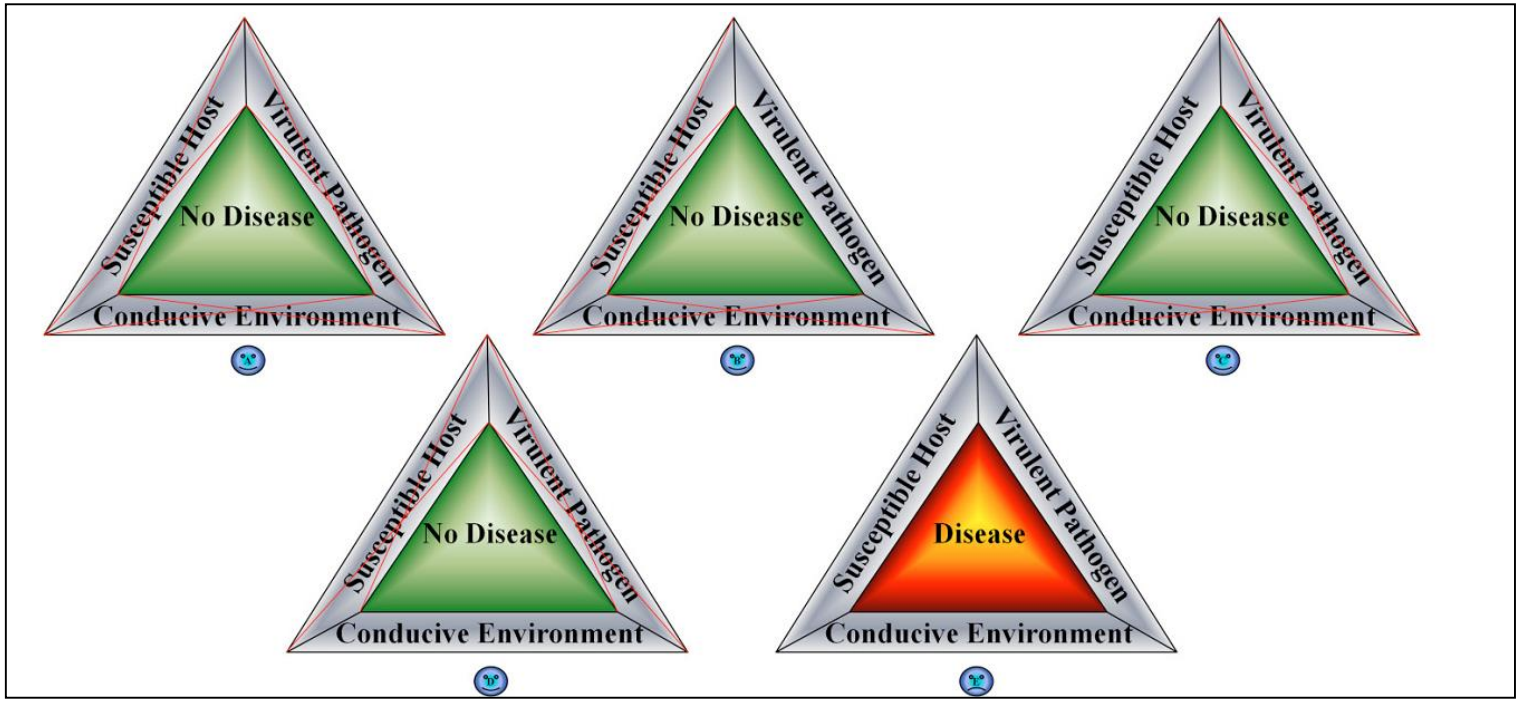


Yang *et al.*,2023

Disease triangle

Plant disease factors

Biotic and abiotic factors

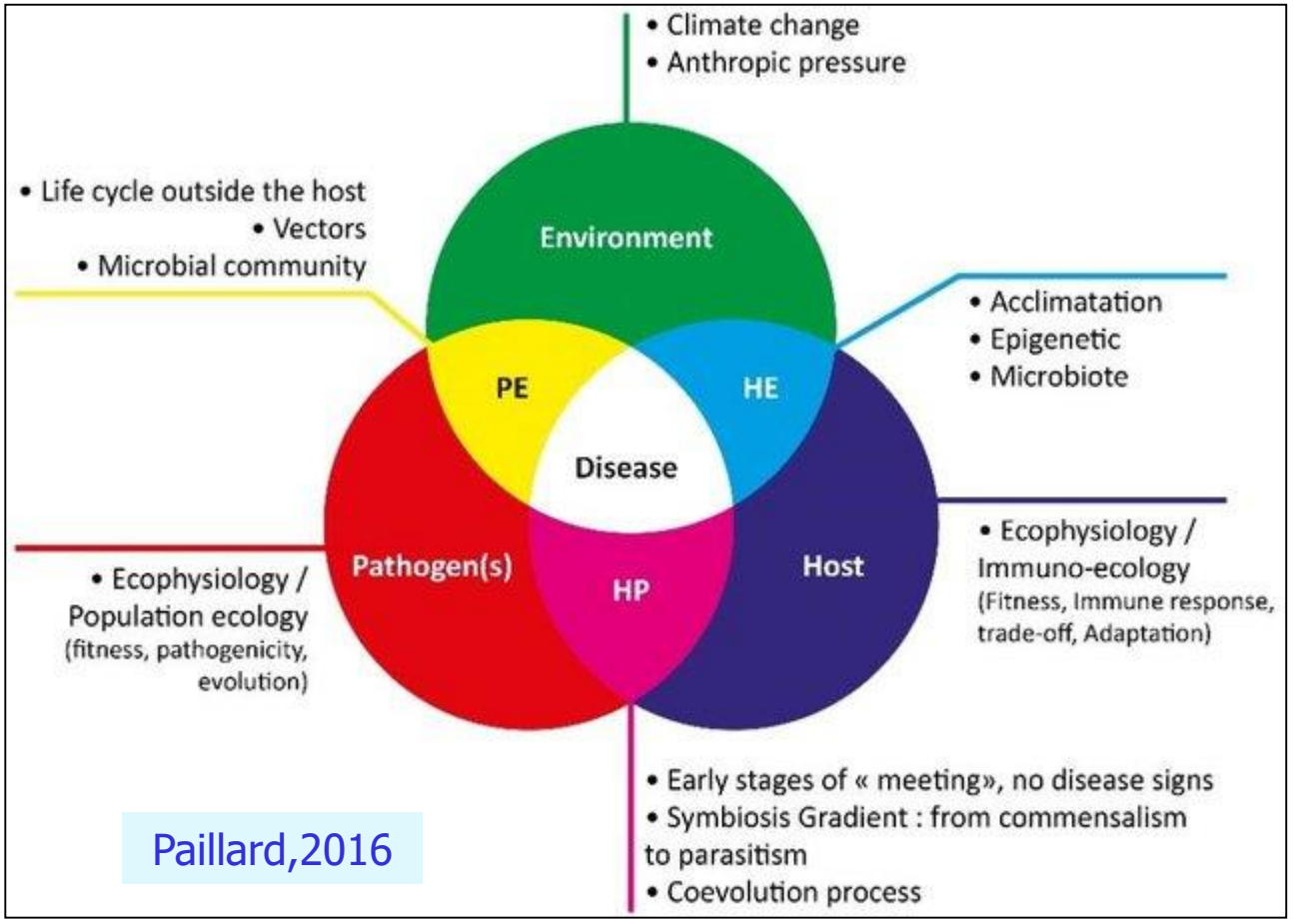


When the three factors of virulent pathogen, susceptible cultivar and conducive environment are existing at the same time, PDE (plant disease epidemiology) will occur.

Islam,2018

The ecological approach of the host-pathogen-environment system

HPE (host, pathogen and environmental interaction)

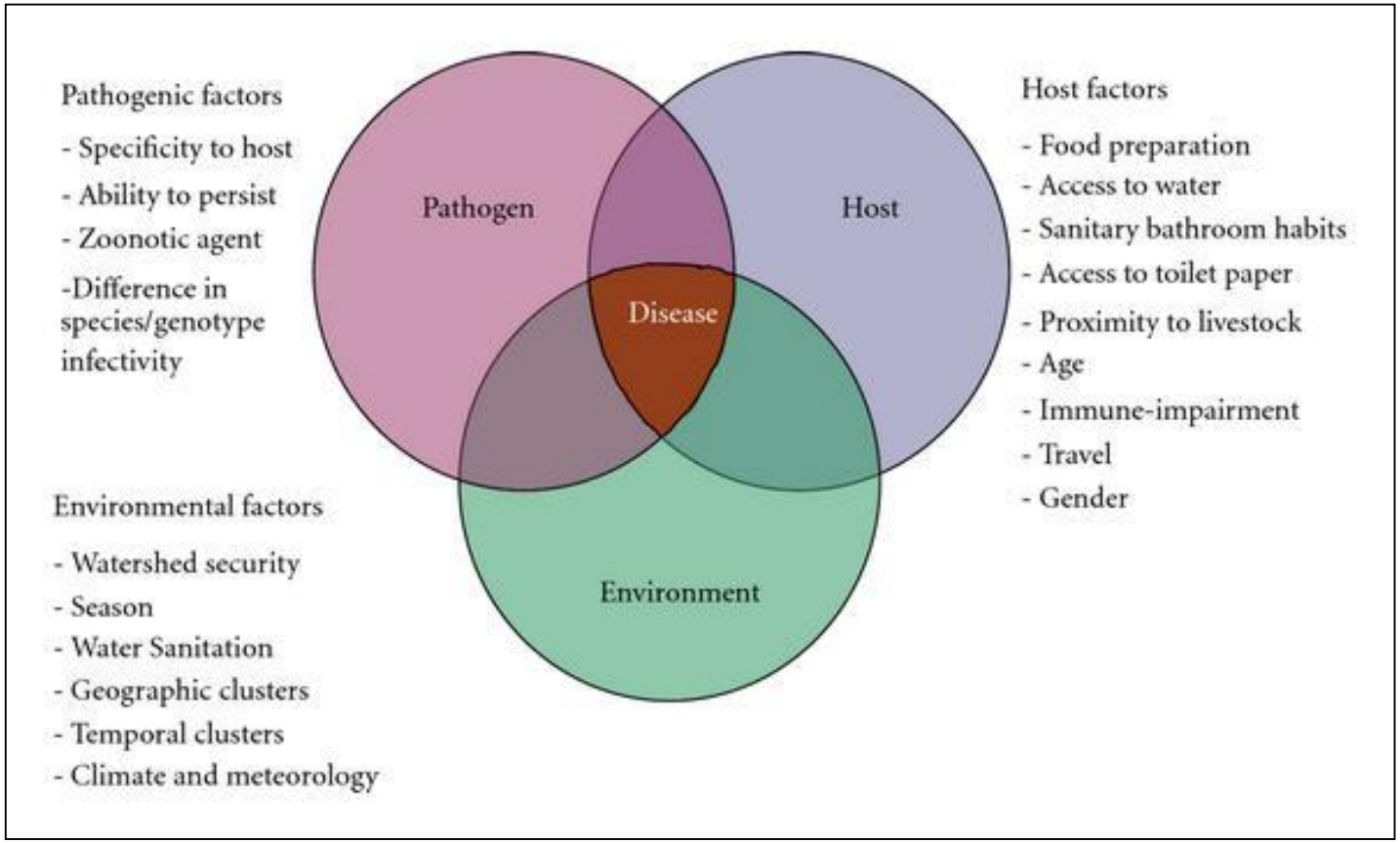


Acclimatization: physiological adjustment by an organism to environmental change.
Epigenetics is the study of how cells control gene activity without changing the DNA sequence.

Disease triangle

Plant disease factors

These variables include genetic diversity, biology and lifecycle of the host plant and pathogen, environmental conditions,…

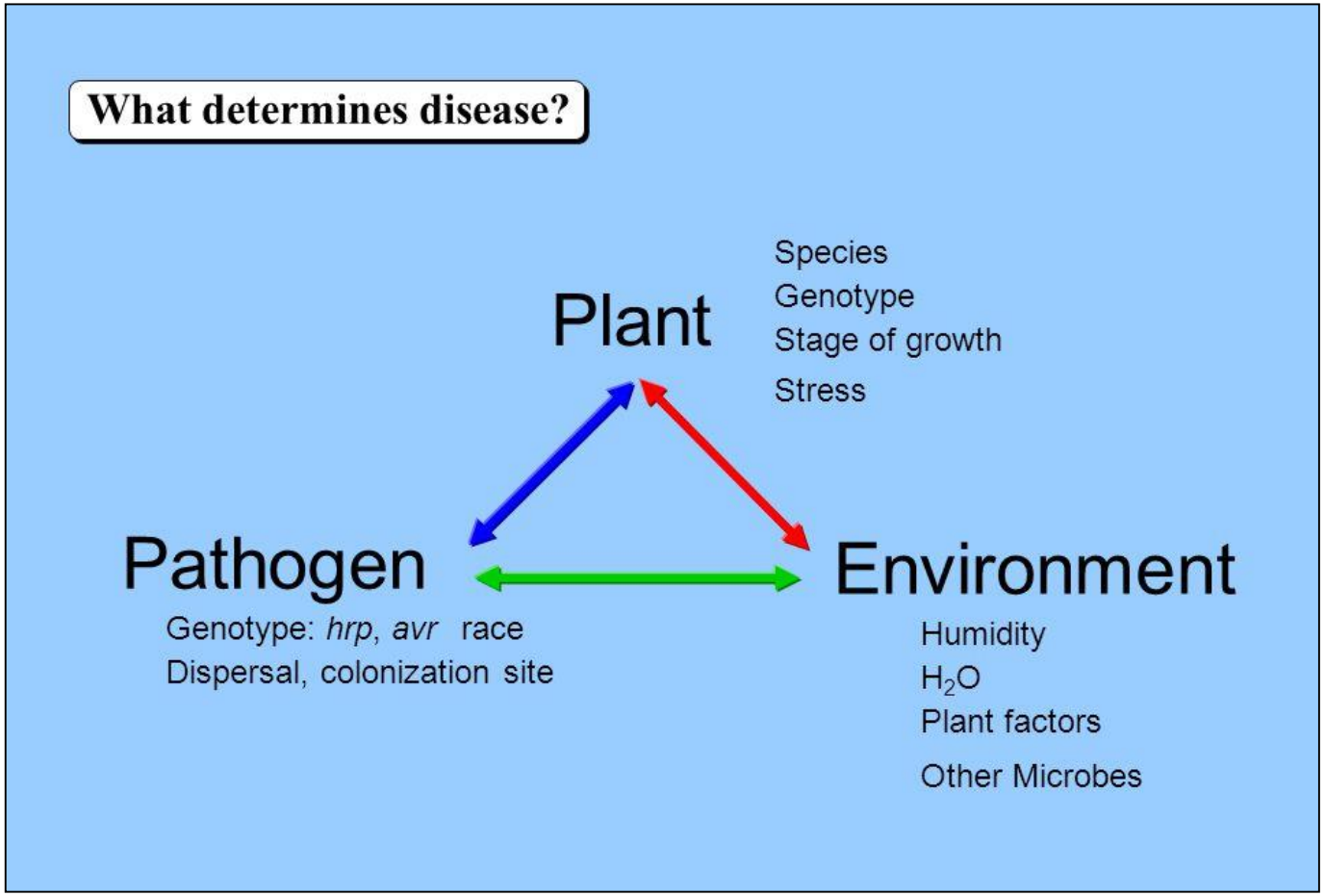


Poignant and Menchella,2010

Disease triangle

Plant disease factors

These variables include genetic diversity, biology and lifecycle of the host plant and pathogen, environmental conditions,…



slideplayer.com;..

Disease triangle

Environmental conditions

Macro- and microclimate conditions

- Pathogens are typically restricted to an area based on the conditions of the macroclimate.
- A microclimate is the prevailing **climatic conditions in a certain geographical area**.
- **Within a macroclimate**, small areas may exist in which the climate may be different than the surrounding areas. This is called a microclimate.
- Each landscape is filled with microclimates that exist because of differences in exposure to:

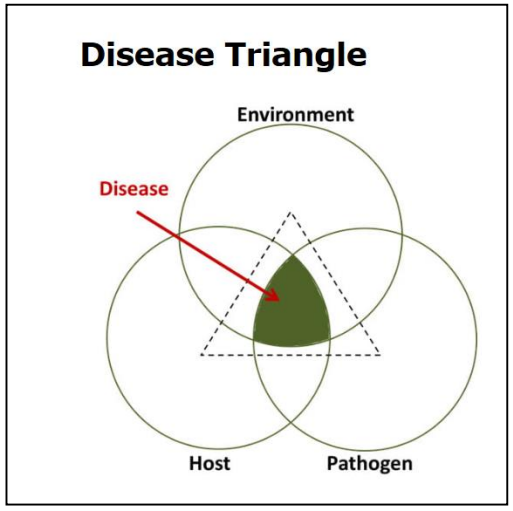
1. sun and wind,
2. soil type, and
3. many other factors.

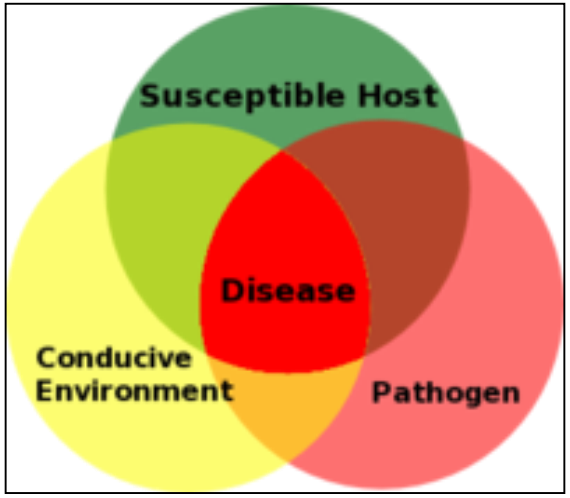
The disease triangle

Disease develops only when all three factors are favorable

Macro- and microclimate conditions

- This concept is represented by the shaded portion of the diagram above.
- When there is a high degree of overlap (as the shaded area becomes larger), there will be a moderate to high amount of disease.





Nebraska Soybean Board; Wikipedia,2022

Disease triangle

Plant disease cycles

Plant disease prediction systems

- Information technology has fueled tremendous innovations in methods used to deploy plant disease prediction models.
- If plant pathologists can keep pace(running) with these technological developments by establishing multi-disciplinary teams with meteorologists and computer information technology specialists, the future of plant disease prediction will remain bright.

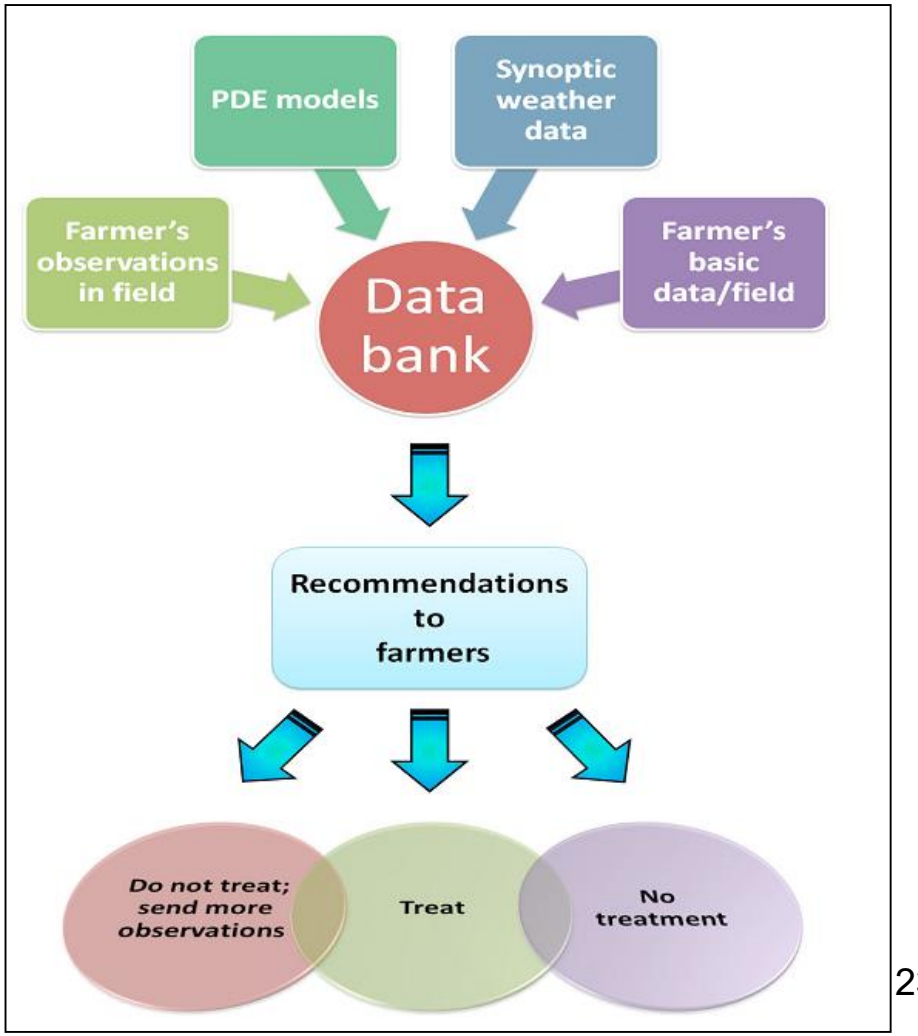
Disease triangle

Plant disease factors

Basis for developing disease prediction systems

Plant disease epidemiology(PDE) involves integrated strategy via incorporation of:

1. agronomical,
2. biological,
3. Ecological, and
4. Statistical tools.



Islam,2018

Disease triangle

Publications on plant disease prediction models

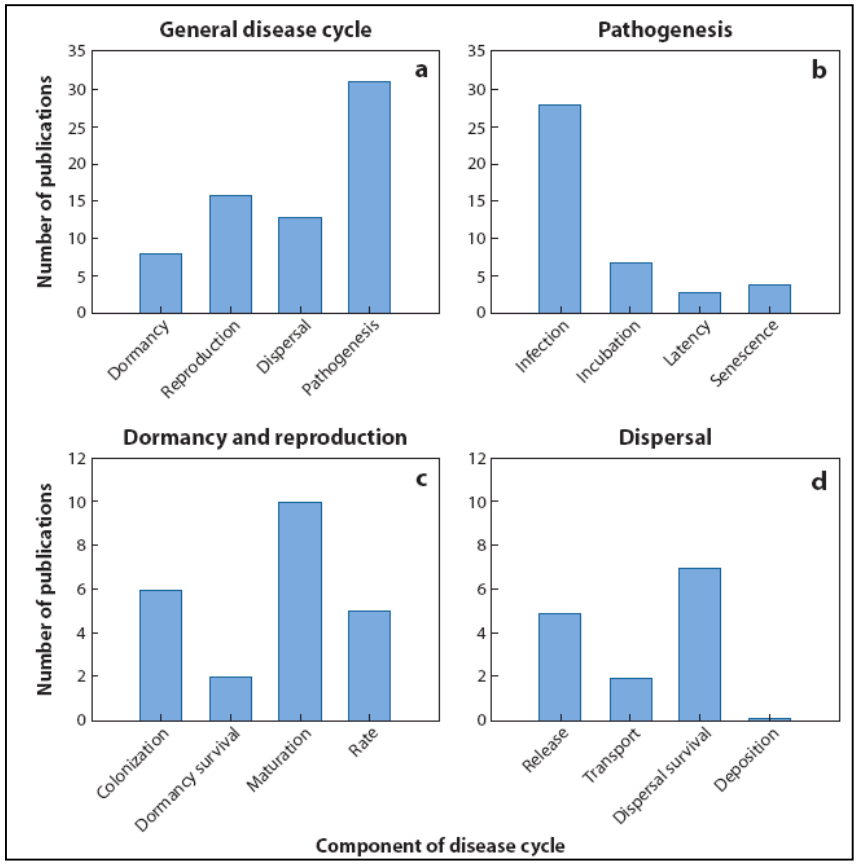
- Plant disease prediction models developed and published from 1994-2006.

a. Models that consider the general stages a disease cycle

b. Pathogenesis;

c. Dormancy, reproduction,

d. dispersal and their substages.



Islam,2018

Bacterial survivals

Short-term survival (hours to days)
Long-term survival (months or years)

To design effective control measures it is essential to know where/how the plant pathogenic bacterium survives.

Disease triangle

Survival mechanisms of plant pathogens and disease management

1. Survival by means of specialized resting structures;
2. Survival as saprophytes;
3. Survival in vital association with living plants;
4. Survival in association with nematodes and fungi;
5. Survival in association with insects;
6. Survival on agricultural materials;
7. Survival on surface water.

Suwaneththiya,2015

Survival mechanisms of plant pathogenic bacteria

- Management of bacterial plant diseases could be solved with a better understanding different aspects of plant bacteriology including survival mechanisms of plant pathogenic bacteria.

Survival mechanisms of plant pathogenic bacteria

- Bacteria are one celled organisms.
- They are the second most important biotic plant disease agent.
- Bacteria are able to reproduce every 20 to 60 minutes.
- One bacteria can result in 17,000,000 bacteria in one day.

La Favre and La Favre,?

Survival mechanisms of plant pathogenic bacteria

Bacterial growth requirements

Physical:

- Moisture and desiccation
- Temperature
- pH
- Osmotic pressure

Chemical:

- Carbon source
- Nitrogen,
- Sulfur
- Phosphorus
- Oxygen

Survival mechanisms of plant pathogenic bacteria

Environmental factors

- Environmental factors, extremes in:

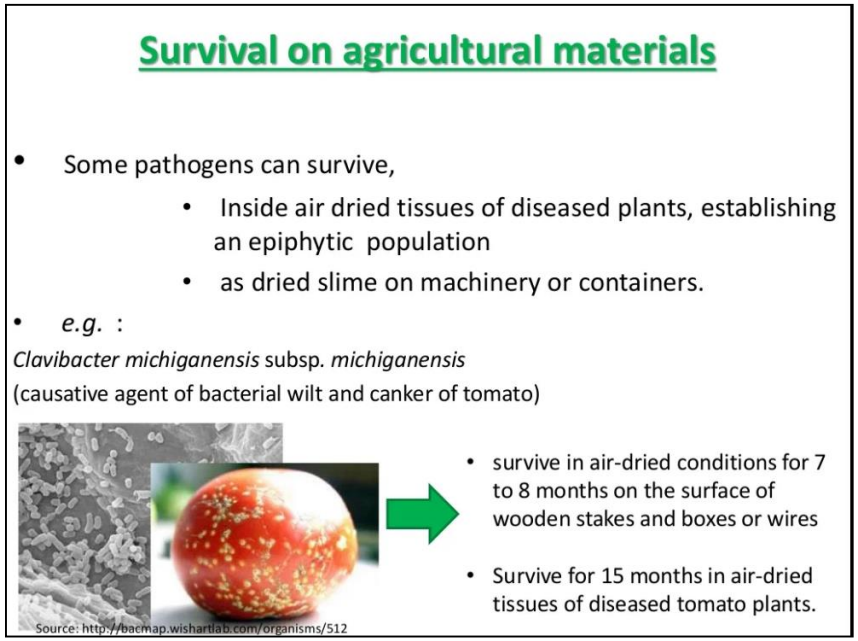
1. Temperature
2. Moisture
3. Light
4. Nutrients (mineral elements)
5. pH

Survival on agricultural materials and diseased tomato plants

Clavibacter michiganensis subsp. *michiganensis*

- *Clavibacter michiganensis* subsp. *michiganensis* (causative agent of bacterial wilt and canker of tomato)

1. survive in air-dried conditions for 7 to 8 months on the surface of wooden stakes and boxes or wires,
2. Survive for 15 months in air-dried tissues of diseased tomato plants.



Suwaneththiya,2015

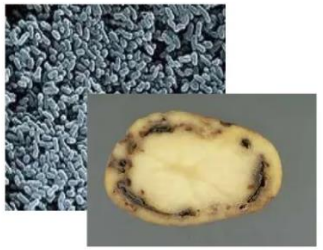
Survival on agricultural materials and diseased potato plants

Clavibacter michiganensis subsp. *sepedonicus*

- *Clavibacter michiganensis* subsp. *sepedonicus* (causative agent of potato ring rot)

1. survive and remain infectious on potato bags, barn walls, machinery and other equipment.
2. Survives inside of an infected tubers.

Clavibacter michiganensis subsp. *Sepedonicus*
(causative agent of potato ring rot)



- survive and remain infectious on potato bags, barn walls, machinery and other equipment.
- Survives inside of an infected tubers

Source: http:// www.pinterest.com/pin/515169644851653467

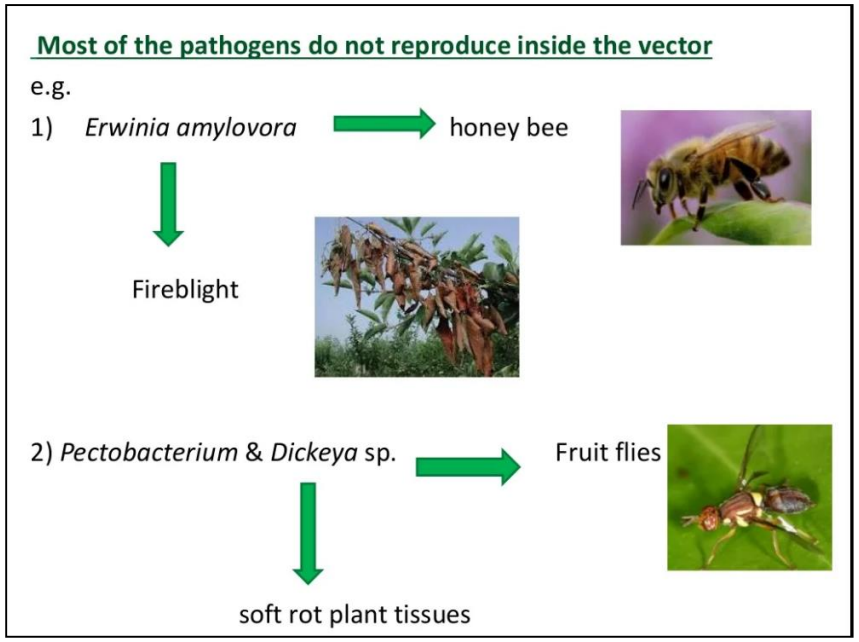
Suwaneththiya,2015

Survival in a vector

Erwinia amylovora and *Pectobacterium* and *Dickeya* sp.

- Most of the pathogens do not reproduce inside the vector. e.g.

1. *Erwinia amylovora* honey bee Fireblight
2. *Pectobacterium* and *Dickeya* sp. Fruit flies soft rot plant tissues.

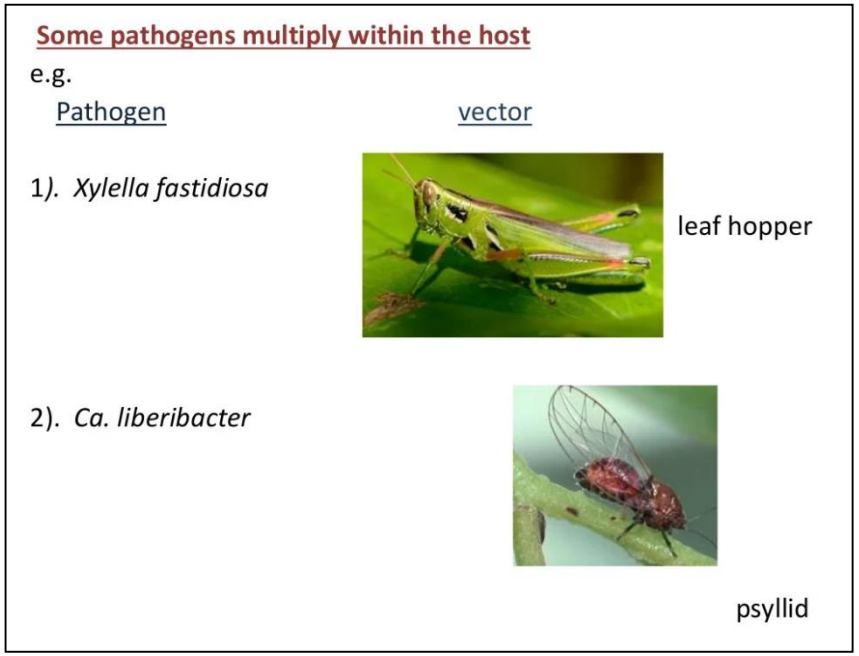


Survival in a vector

Xylella fastidiosa and *Candidatus liberibacter*

- Some pathogens multiply within the host. e.g.
- Pathogen vector

1. *Xylella fastidiosa* leaf hopper,
2. *Ca*. liberibacter psyllid.



Suwaneththiya,2015

Survival mechanisms of plant pathogenic bacteria

Agricultural cropping systems

- Bacterial pathogens have developed diverse survival mechanisms.
- Agricultural cropping systems have major impact on survival capabilities.

1. Cropping is almost continuous;
2. Cropping is discontinuous.

- Growth of plant pathogens is discontinuous.

1. Seasonal effect upon pathogen (temperature, moisture)
2. Growth on host plant is interrupted.

- Successful pathogens must be able to bridge gaps between successive crops and seasons.

Short-term survival

Inanimate (physical) factors

1. Moisture and desiccation

Inanimate (physical) factors which affect short-term survival:

1. Free water

- Probably necessary for multiplication of bacteria.
- Metabolically active bacteria are most sensitive to drying.
- Bacteria in the stationary phase are most tolerant of quick drying.

2. Relative humidity

- During drying low RH limits multiplication.
- High RH favors epiphytic growth.

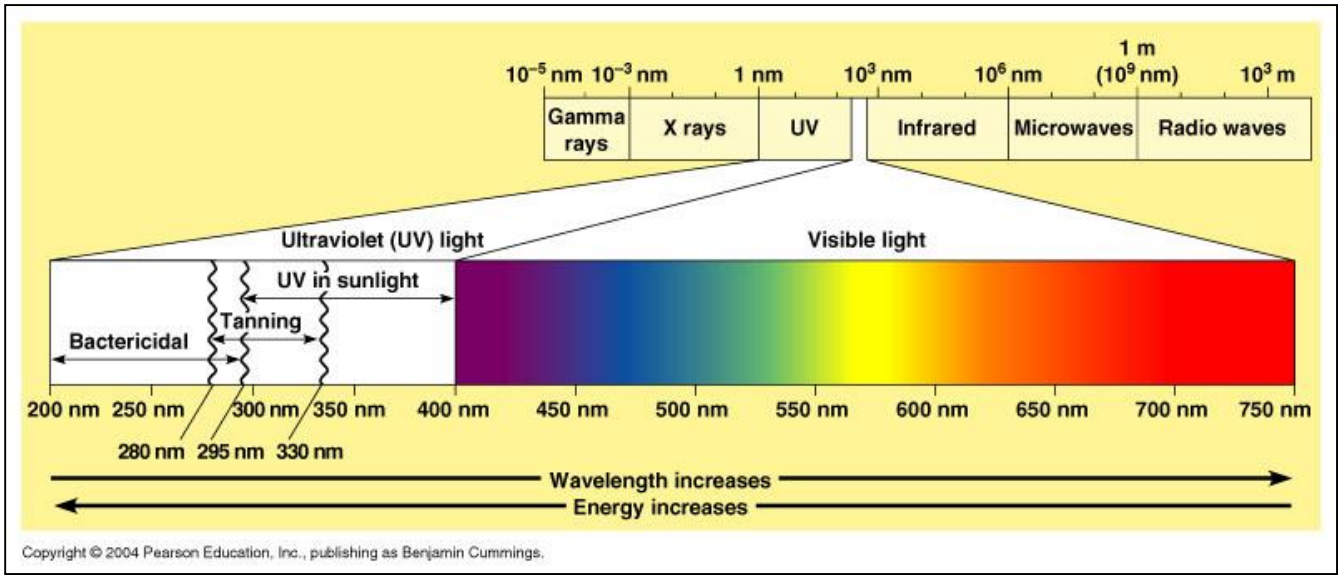
1. Gram+ve *Clavibacter michiganensis* subsp. *michiganensis* most resistant to desiccation,
2. Gram-ve *X. phaseoli* 20-50 times less resistant and *P. carotovorum* and *P. s.* pv. *glycinea* 1000 times less resistant.

Short-term survival

Inanimate (physical) factors

2. Lethal ultraviolet radiation

- Ultraviolet radiation (nonionizing radiation) excites electrons to a higher energy level.
- DNA molecules are good absorbers of ultraviolet light, especially that with wavelengths in the 260 to 280 nm range.

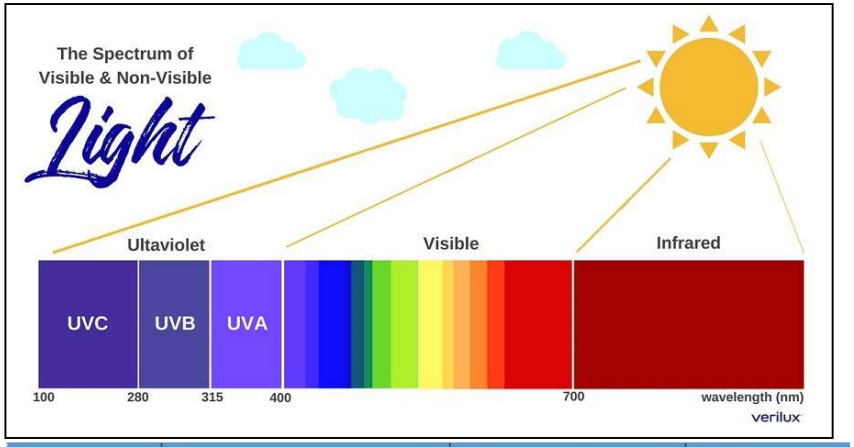


Short-term survival

Inanimate (physical) factors

Lethal ultraviolet rays

- There are many types of ultraviolet rays.
- Common ultraviolet rays are divided into three types: UVA: Longer wavelength, between 320~400 nanometers;
- UVB: The wavelength is in the middle, the wavelength is between 280~320 nanometers;
- UVC: The wavelength is the shortest wavelength between 100~280 nanometers.

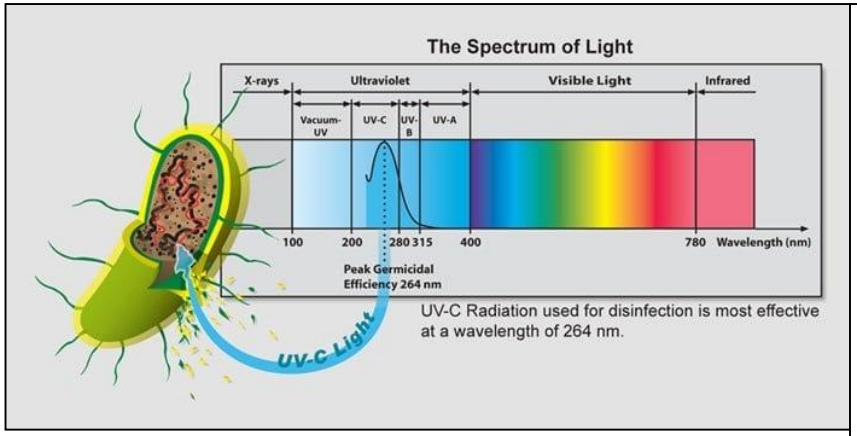


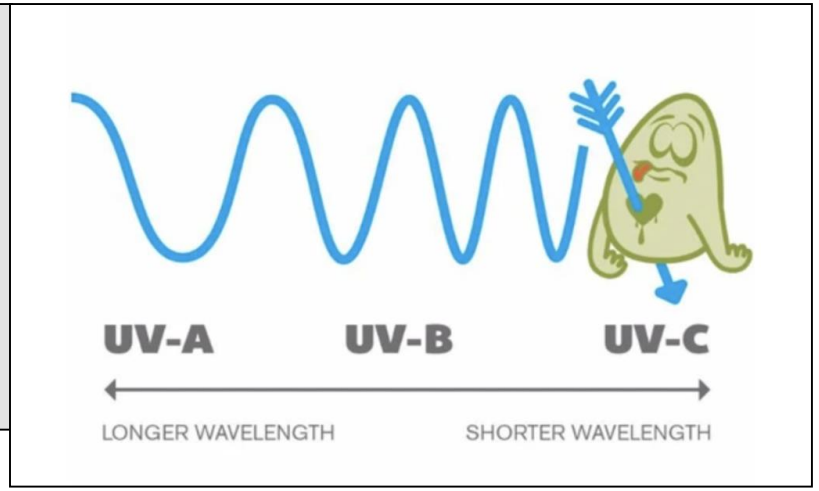
Project	UVA	UVB	UVC
wavelength	315-400nm	280-315nm	200-280nm
application	1.Fluorescence, 2.curing 3.Mosquito trap 4.Anti-counterfeiting detection	1.stimulation of plant trichome growth; 2.reptile growth in synthesizing vitamin d3	air/water/suface strtilization
Can be absorbed by the ozone layer	NO	Partly absorbed	All absorbed
Is it harmful to the human body	NO	NO	Causes eye diseases and skin cancer

Short-term survival

Inanimate (physical) factors

Lethal ultraviolet rays





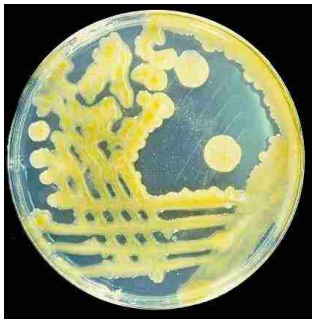
Short-term survival

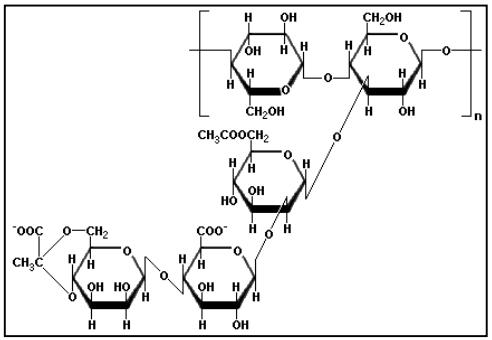
Inanimate (physical) factors

Tolerance to UV-B radiation in different species

- UVB: The wavelength is in the middle, the wavelength is between 280~320 nanometers.
- Bacteria Tolerance factors for side-effects of UV radiation:

1. *recA* gene: involves in DNA repair but also was shown to contribute to UV tolerance in *P. syringae*.
2. EPS: may also play a role in UV tolerance since crude EPS (xanthan) exudate from *X. phaseoli* was more efficient than extracted exudate in absorbing UV.



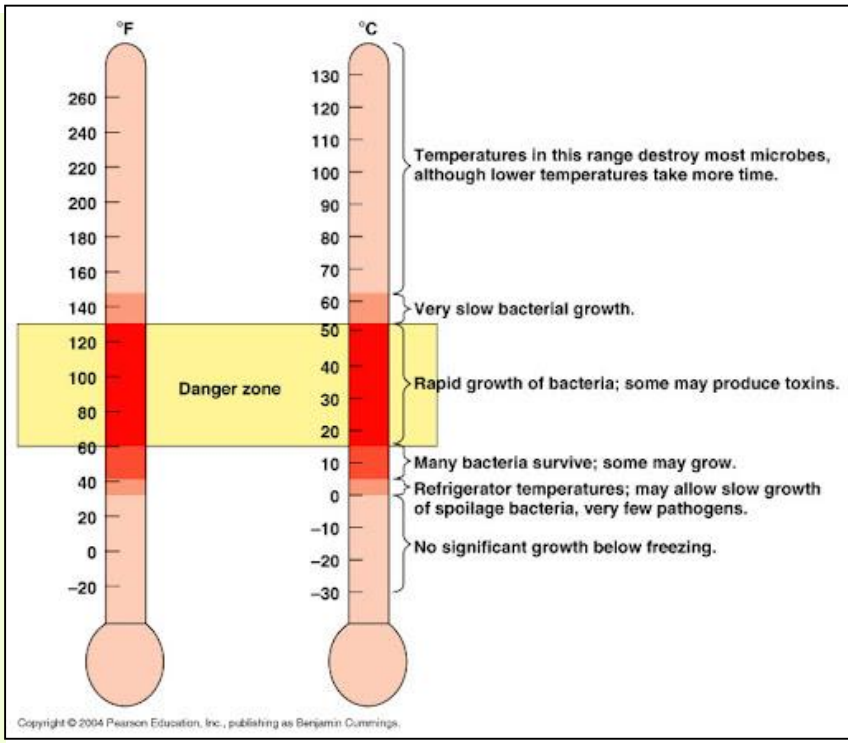


Short-term survival

Inanimate (physical) factors

3. Temperature

1. Psychrophiles (cold loving):True psychrophiles (optimum growth at 15°C); Psychrotophs (optimum growth at 20-30°C).
2. Mesophiles (moderate temperature loving);
3. Thermophiles (heat loving);
4. Hyperthermophiles (tolerate extreme temperatures).



Most pathogenic bacteria are mesophiles (middle loving).

Short-term survival

Inanimate (physical) factors

Temperature

- On laboratory media, plant pathogens usually grow more slowly than non-pathogenic bacteria isolated from plants, with optimal temperatures of 20-30°C.

1. *Pseudomonas phaseolicola* causes disease below 22°C (72°F)
2. *Xanthomonas phaseoli*, above 22°C on dry bean (*Phaseolus vulgaris*).
3. *Burkholderia cepacia* grow at 37°C or higher.
4. Ice nucleation-active (INA) bacteria have competitive advantage over non-INA strains in mild freezing environments.

- Thermal death point (for plant pathogenic bacteria usually 50-55°C, when kept for 10 minutes at this temperature in liquid medium).

Short-term survival

Inanimate (physical) factors

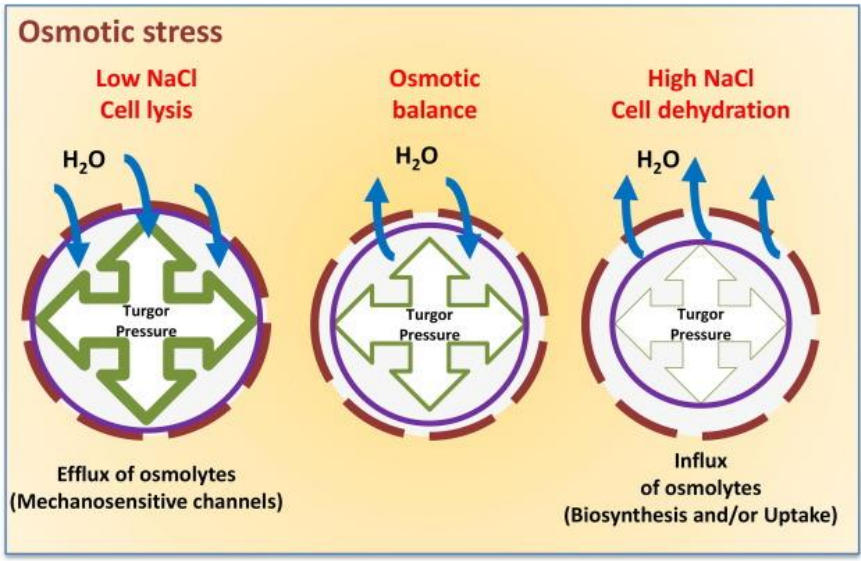
4. Osmotic pressure

- For survival and growth, bacteria require a positive turgor pressure.
- When bacteria experience water activity(a_w) stress, the cells lose water due to osmosis, which results in the shrinkage of the cell and sometimes plasmolysis (shrinking of protoplasm away from the cell wall).

Gregory and Boyd,2021

Short-term survival
Inanimate (physical) factors
Osmotic pressure

- In low NaCl conditions (hypotonic solution) water flows into cell causing increased turgor pressure, which is counteracted by removing osmolytes.
- In high NaCl conditions (hypertonic solution) water flows out of cell and this is counteracted by accumulating osmolytes.



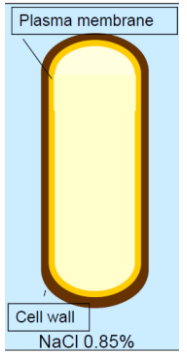
Hypertonic solutions are used for antimicrobial control.

Gregory and Boyd,2021

Short-term survival

Inanimate (physical) factors

Osmotic pressure



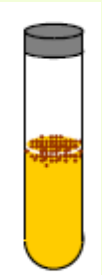
- Bacteria are more tolerant to osmotic variations because of the **mechanical strength of the cell wall**.
- Bacteria don't tolerate well a very low ionic strength medium like water.
- *Ralstonia solanacearum* is inhibited in culture by low concentrations (2%) of sodium chloride (NaCl).
- *Rathayibacter caricis* (phyllosphere of *Carex* sp.) shows weak growth with 5% (w/v) NaCl.
- The maximum NaCl tolerance value of *Xanthomonas fragariae* is 0.5-1.0%.

Short-term survival

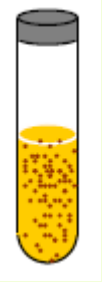
Inanimate (physical) factors

5. Oxygen

- **Obligate aerobes**
- Only aerobic growth, oxygen required (most plant pathogens). E.g. *Pseudomons*, *Xanthomons*



- **Facultative anaerobes**
- Greater growth in presence of oxygen (some plant pathogens). E.g. *Erwinia*



- **Obligate anaerobes**
- Only anaerobic growth, cease with oxygen(few plant pathogens). E.g. *Clostridium* spp.

Long-term survival
Months or years/Season to season

- **Three generalizations concerning survival of plant pathogenic bacteria:**

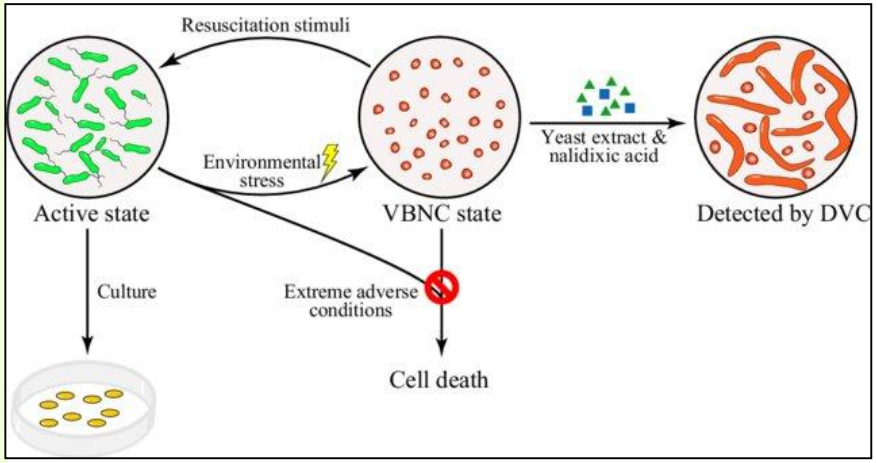
1. Long-term survival for the most part takes place in association with living or dead tissue. It allows the pathogen to survive in the face of recurrent or occasional stresses.
2. Long-term survival is more likely if cells of the pathogen are in aggregates (biofilm formation) or if associated with living plant tissues in protected positions.
3. Pathogens in state of reduced metabolism (hypobiosis/viable but non-culturablem VBNC forms) more likely to survive than are active cells.

Hypobiosis

Persistence in phytopathogenic bacteria

The life cycle of VBNC cells

- VBNC refers to a physiological state where bacteria are metabolically active, but are no longer culturable on conventional growth media.
- It is a survival strategy adopted by many bacteria in response to harsh environmental conditions, and the VBNC cells may return to culturable state under favorable conditions.



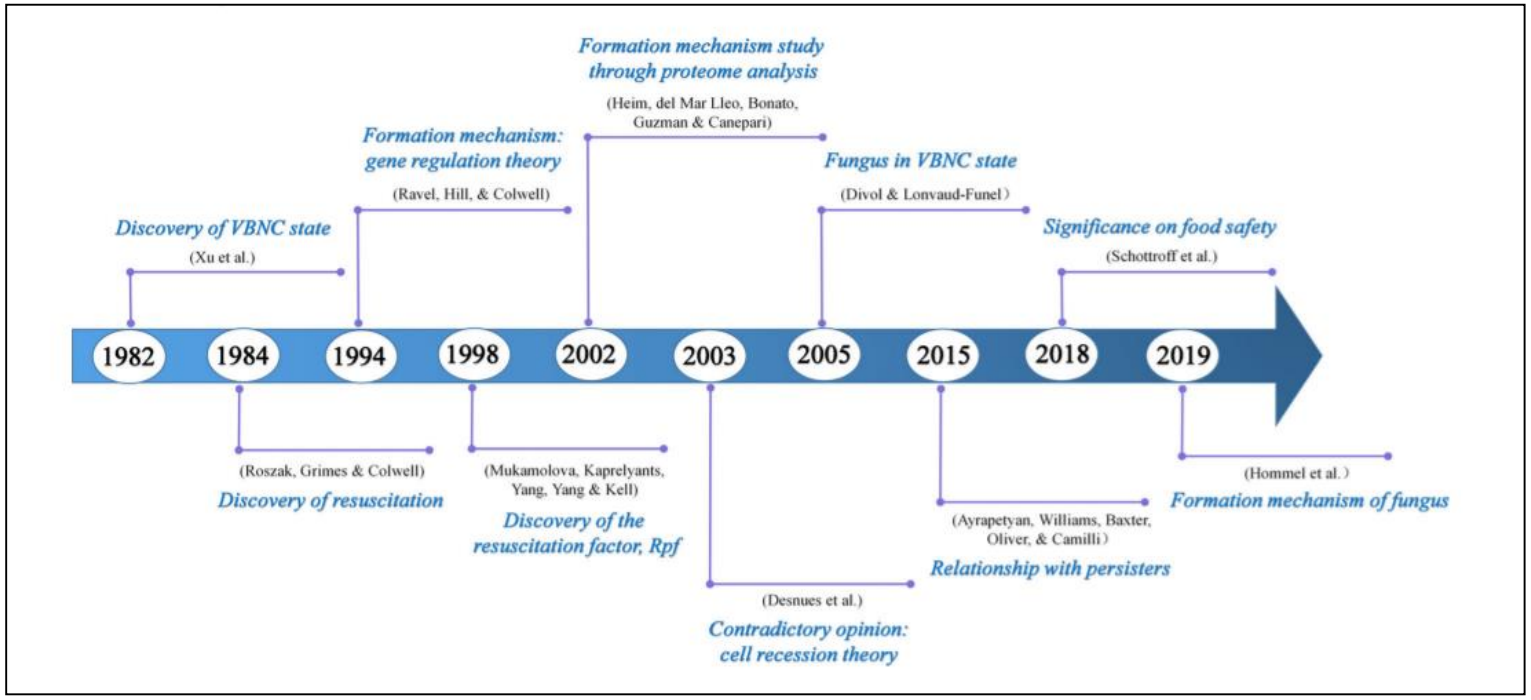
Resuscitation: restoration of culturability.
DVC: direct viable count procedure.
Note: extreme adverse condition ends cell death.

Zhang *et al.*,2020

Long-term survival

Persistence in phytopathogenic bacteria

Timeline of research progress on the VBNC state



Bacterial L-forms, which may arise when normal bacteria (mainly Gram negative bacteria) are subjected to an unfavorable environments. Two types of L-forms: Class I (unstable L-forms or spheroplasts) can revert in the absence of bactericidal; Class II: cannot revert. These are also known as stable L-forms or protoplasts.

Dong *et al.*,2019

VBNC or VNC

Persistence in phytopathogenic bacteria

What induces this state in bacteria?

- Cells enter the VBNC state as a response to some form of natural stress, such as:

1. starvation,
2. incubation outside the temperature range of growth,
3. elevated osmotic concentrations (e.g. seawater),
4. oxygen concentration, or
5. exposure to white light (Oliver, 2000c).

- Cells can remain VBNC for more than a year.

Visible range of the spectrum, appearing white to the eye is called white light.

Oliver,2005

VBNC or VNC

What induces this state in bacteria?

Low temperatures

- Low temperatures also constitute a type of stress that induces persister formation.
- There is evidence that *R. solanacearum* cells can enter in an unculturable state in water bodies during winter, which is of special interest in temperate countries.
- A seasonal oscillation(fluctuation) of *R. solanacearum* in water flows, consistent with the entry of the fully active cells in summer into a persister state during winter, was reported in the Netherlands.
- This may be the reason why this pathogen remains undetected during the coldest months of the year but is still able to induce symptoms in tomato plants when contaminated water is used in irrigation.

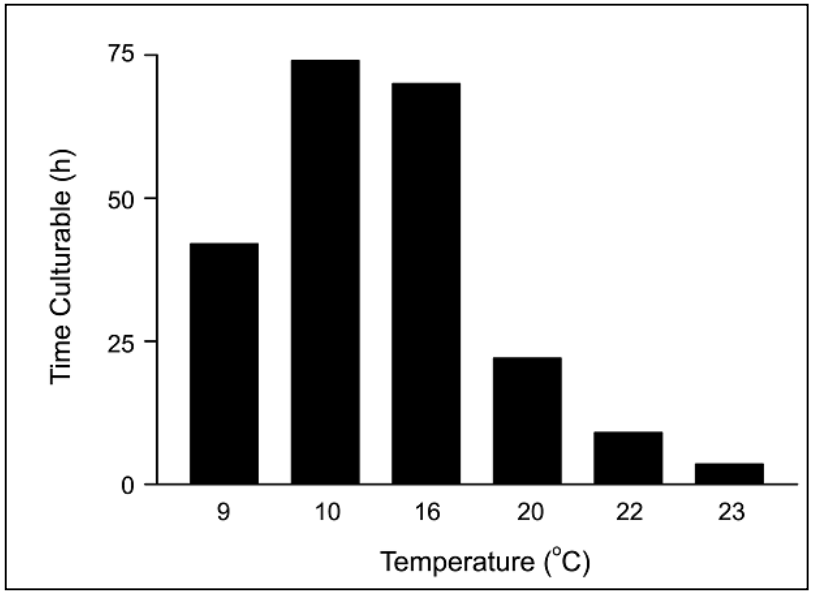
Martins *et al.*,2018

VBNC or VNC

What induces this state in bacteria?

Low temperatures

- The time required for *Helicobacter pylori* cells to lose culturability at various water temperatures.
- Taken from Adams *et al.*, 2003.



Helicobacter pylori is a type of bacteria that is known to be a major cause of peptic ulcer disease.

Oliver,2005

VBNC or VNC

What induces this state in bacteria?

Multidrug tolerance

- One mechanism used by bacteria to survive under stress conditions is the formation of persister cells.
- Persisters are a small fraction of phenotypic variants within an isogenic population(population with essentially identical genes) that exhibits multidrug tolerance without undergoing genetic changes.
- They are dormant cells that survive treatment with antimicrobials by inactivating the metabolic functions that are disrupted by these compounds.

Martins *et al.*,2018

VBNC or VNC

What induces this state in bacteria?

Sterile soil and copper-supplemented soil

- It case of *Ralstonia solanacearum* it was shown the this wilt pathogen enters the persister state in sterile soil, while retaining its virulent potential.
- Grey and Steck, 2001 also showed that:

1. in sterile soil, an initial inoculum (10^{11} cells kg^{-1} soil) is undetected by culturing after 3 days, and
2. in copper-supplemented soil, the culturability threshold is less than 2 days.

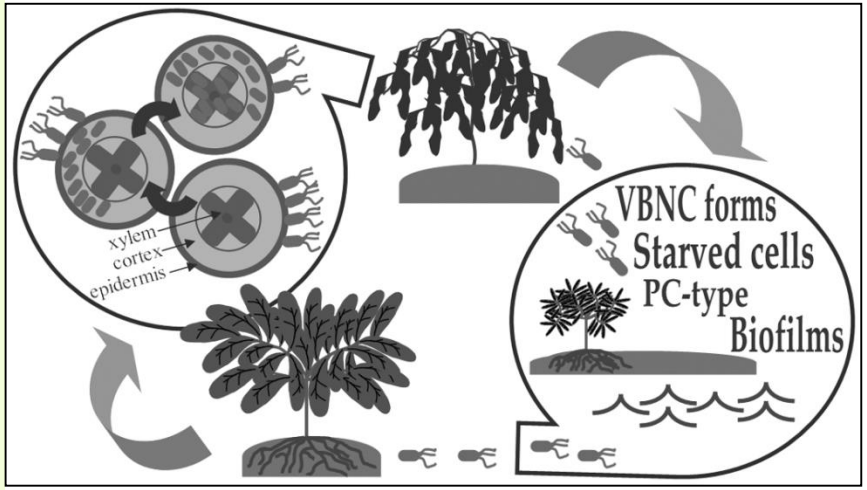
Martins *et al.*,2018

VBNC or VNC

Life cycle of *R. solanacearum*: life inside and outside the host

- There are several forms of resistance of *Ralstonia solanacearum* by which the pathogen can survive in non-favourable environmental conditions:

1. viable but non-culturable (VBNC) forms,
2. starved cells,
3. PC-type(the physiological characteristics e.g. having high motility), and
4. biofilms.



Alvarez *et al.*,2010

VBNC or VNC

What induces this state in bacteria?

Nutritional shortage

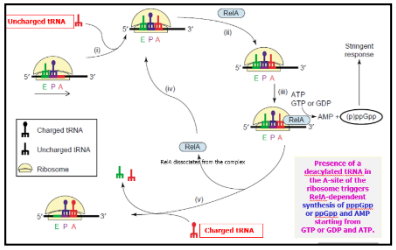
- It is accepted that during its overwintering, *E. amylovora* faces a nutritional shortage, and starvation stress responses may be triggered to enhance its chances to survive.
- One of the major regulators for famine(extreme scarcity of food) in bacteria is the RpoS sigma factor, which is involved in many other stress responses and is widely present throughout the prokaryotes.
- In *E. amylovora*, *rpoS* deletion mutants (*rpoS*) entered into the persister state faster than wild-type cells.

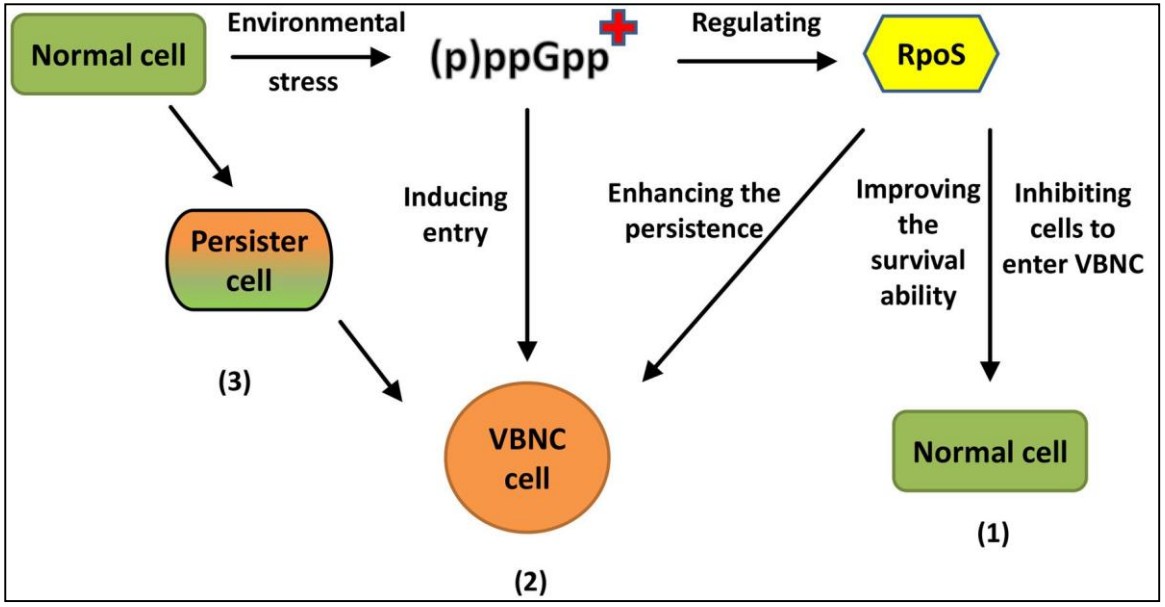
Martins *et al.*,2018

Long-term survival

Persistence in phytopathogenic bacteria

The genes and pathways involved in the formation of VBNC cells





Several known proteins or systems have been shown to play a significant role in VBNC cell formation, including RNA polymerase sigma S (RpoS), (p)ppGpp global regulator(ppGpp) and guanosine pentaphosphate (pppGpp) – collectively known as (p)ppGpp, a nucleoside consisting of guanine and ribose. It is a component of RNA. These are effector molecules, accumulated rapidly when bacterial cells encounter with nutritional stress(starvation) conditions such as amino acids; other cellular stresses, including deprivation of phosphorus, iron, carbon source or fatty acids.

Zaho *et al.*,2017

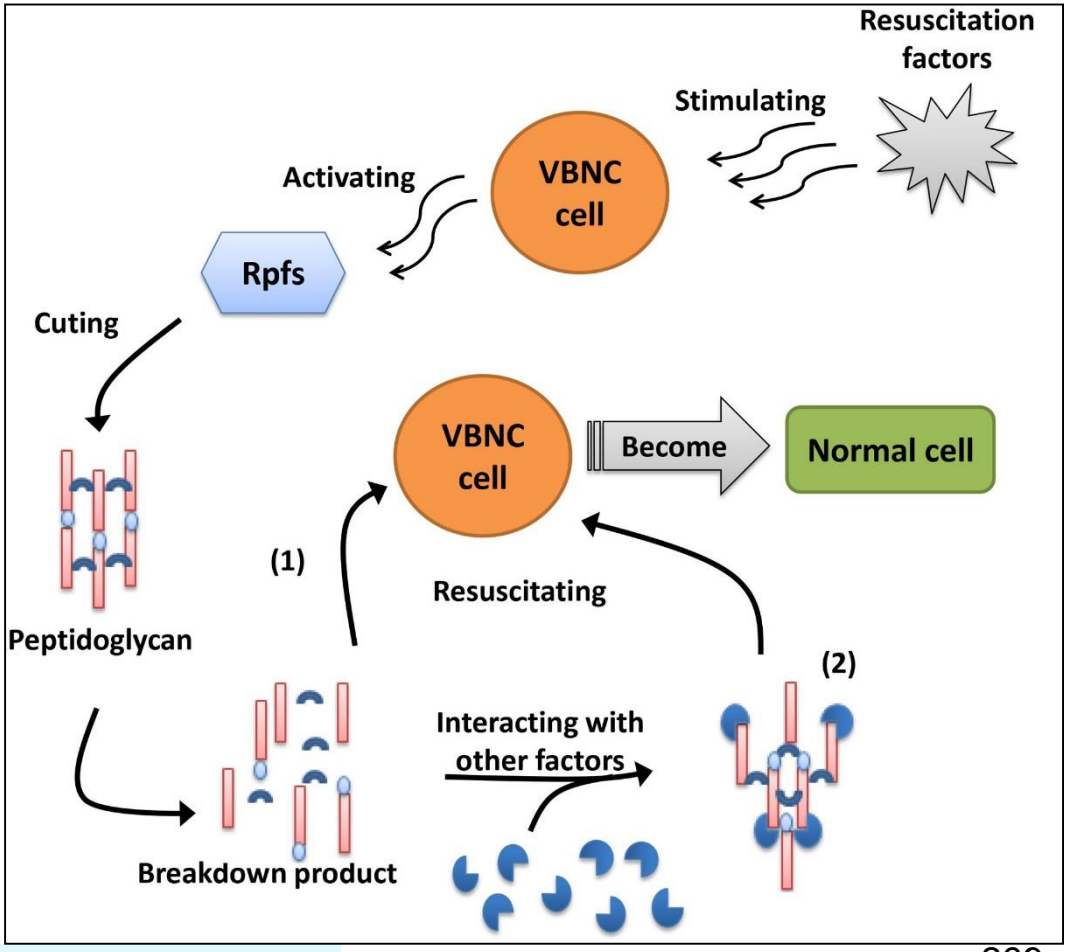
Long-term survival

Resuscitation mechanism of VBNC cells

Resuscitation promoting factor (Rpf)

Two viewpoints about the mechanism of Rpfs. Resuscitation promoting factor (Rpf), a highly conserved protein composed of 220 amino acids that is directly related to the resuscitation of VBNC cells, has been demonstrated to restore the growth and reproductive ability of VBNC cells

1. Rpfs are required to cleave the peptidoglycans with inhibitory properties distributed in specific area of dormant cell wall and thus promote cell division and growth again.
2. The breakdown product(s) of peptidoglycan divided by Rpfs may interact with other factors and function as "second messengers" to stimulate the resuscitation and growth of VBNC cells.

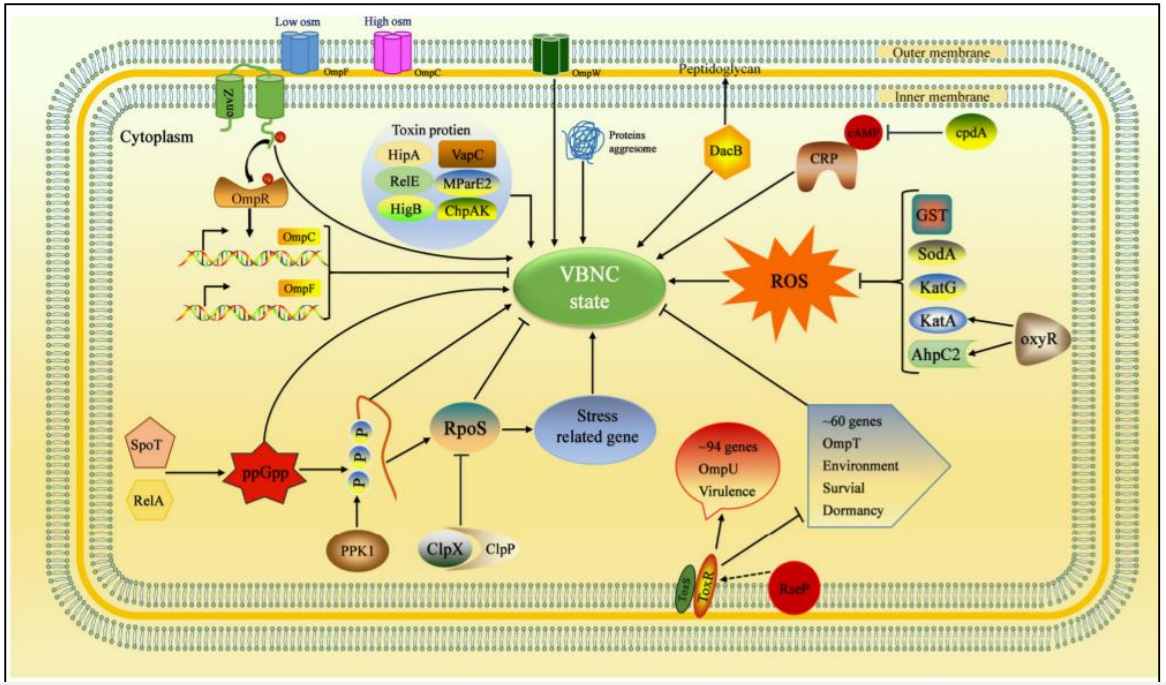


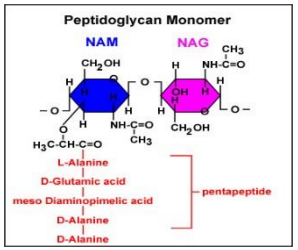
Zaho *et al.*,2017

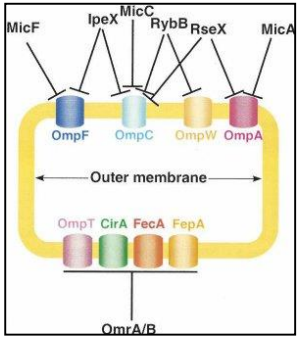
Long-term survival

Persistence in phytopathogenic bacteria

The genes and pathways involved in the formation of VBNC cells





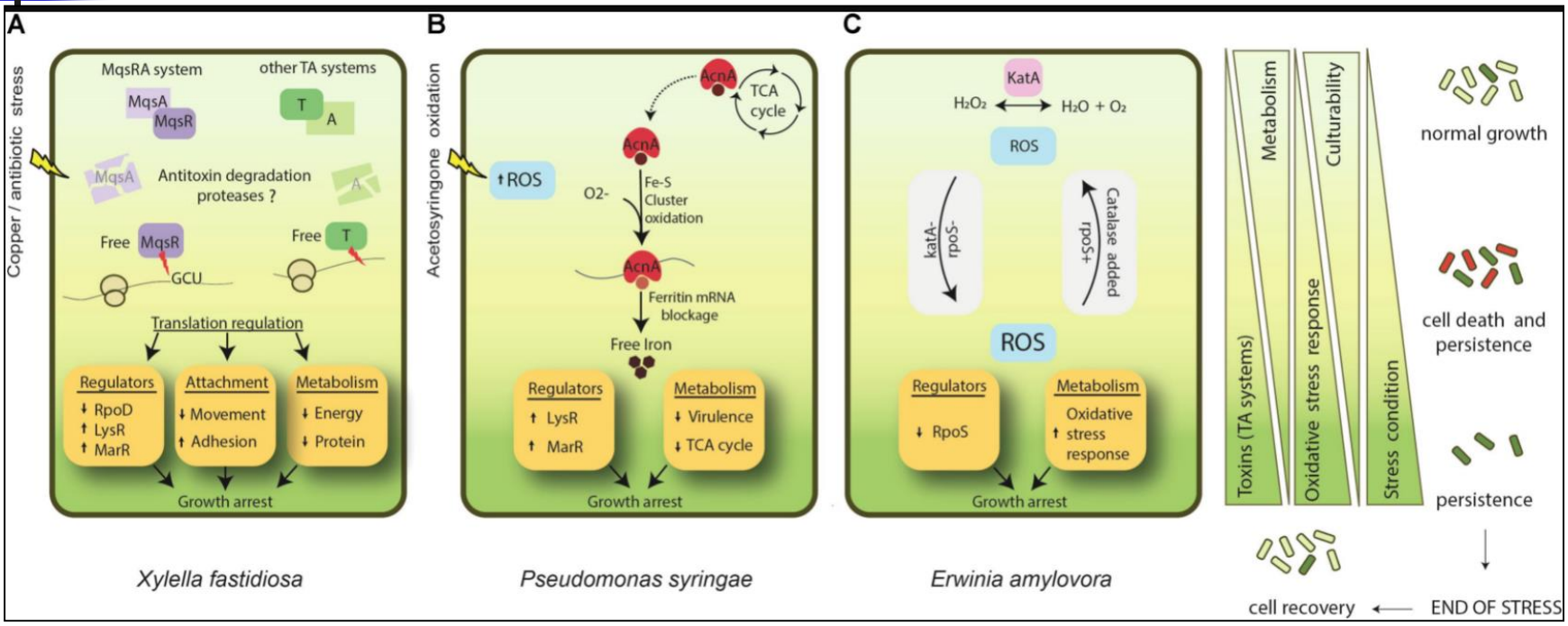


Several known proteins or systems have been shown to play a significant role in VBNC cell formation, including RNA polymerase sigma S (RpoS), a LysR-type transcriptional regulator (OxyR), alkyl hydroperoxide reductase subunit C (AhpC), glutathione S-transferase (GST), catalases KatA and KatG, superoxide dismutase (SodA), sensory histidine kinase (EnvZ), outer membrane proteins (OmpF, OmpC, and OmpW), polyphosphate kinase 1 (PPK1), toxinantitoxin (TA) systems, protease ClpX, toxin transcriptional activator (ToxR), cyclic adenosine monophosphate receptor protein (cAMP-CRP), D-alanyl-alanine carboxypeptidase (DacB), and protein aggresome.

Dong *et al.*,2019;..

VBNC or VNC

Known mechanisms of persister formation in phytopathogenic bacteria



In brief:
In *X. fastidiosa*, under copper/antibiotic stress, presents induction of MqsRA and other TA systems.
In *P. syringae* acetosyringone oxidation leads to an increase in ROS formation. In parallel, aconitase (*acnA*) involved in the tricarboxylic acid (TCA) cycle is up-regulated.
In *E. amylovora* studies on persister cells are based on *katA* (catalase) and *rpoS* (sigma factor 38) mutant phenotypes.

Martins *et al.*,2018

Hypobiosis

Persistence in phytopathogenic bacteria

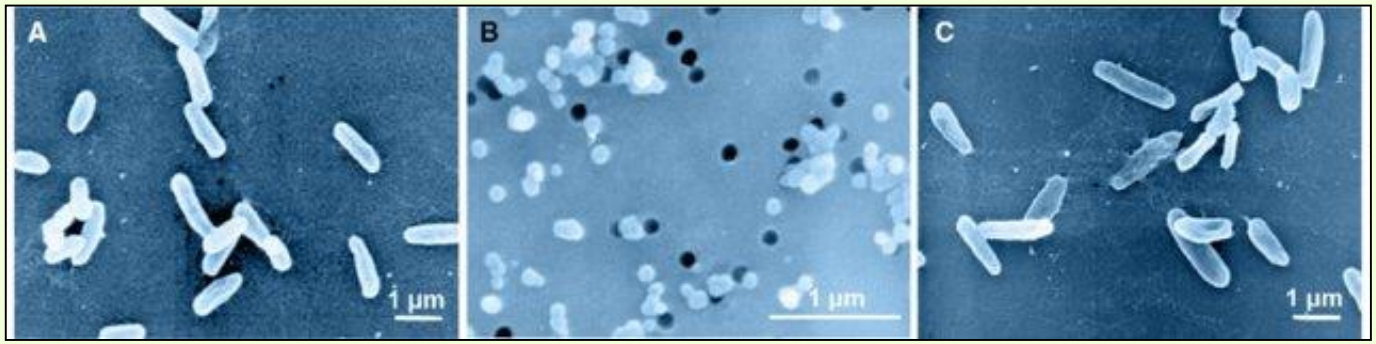
VBNC or VNC

- Morphological characteristics of *Vibrio harveyi* SF1 analyzed with a scanning electron microscope.

A. Normal cells;

B. VBNC cells;

C. Resuscitated cells (restored culturability).



Zhang *et al.*,2020

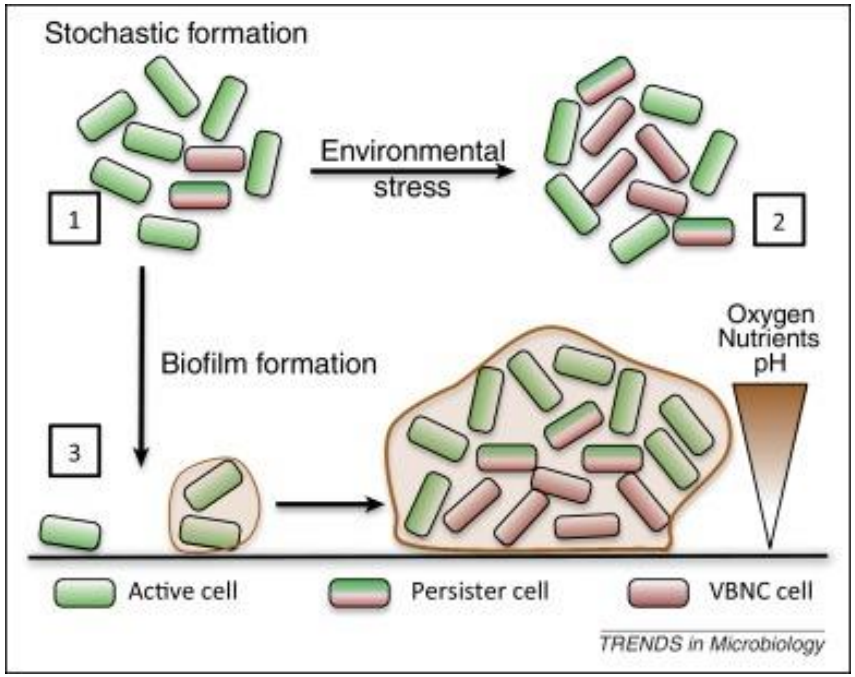
VBNC or VNC

Two dormancy states: Persisters and VBNC

Two closely related phenomena

- Persisters and viable but non-culturable (VBNC) are closely related phenomena.

1. Persisters and VBNC cells are both able to tolerate high-dose antibiotics.
2. Persisters and VBNC cells are induced by common environmental cues.
3. Persisters and VBNC cells share molecular mechanisms that control dormancy.



Ayrapetyan *et al.*,2014

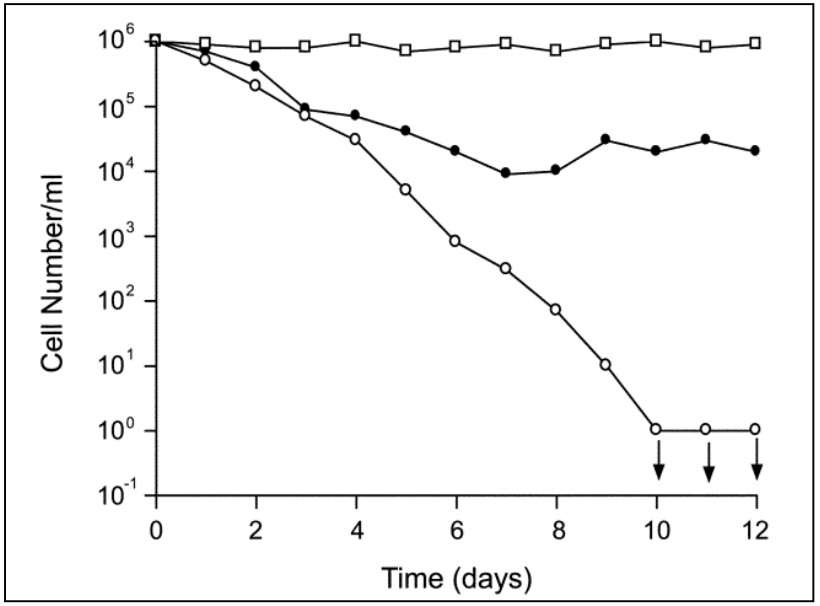
Hypobiosis

Persistence in phytopathogenic bacteria

VBNC or VNC

- Entry of *Vibro vulnificus* causes severe wound infections in human into the VBNC state on incubation at 5°C.
- Shown are:

1. total cell counts (□),
2. culturable counts (○), and
3. viable counts (●).



Culturable curve (○) shows during this period of decline(VBNC), total cell counts generally remain fairly constant.

Oliver,2005

VBNC or VNC

In Gram-positive and negative bacteria

- It is now abundantly evident that numerous bacteria:

1. both gram-positive and negative,
2. both pathogens and nonpathogens, are capable of entering into the VBNC state.

- While the importance of VBNC cells in the initiation of human infection is not yet fully clear, it appears that cells in this state retain virulence, and should be considered by those investigators and government regulators involved in the public health.

Oliver,2005

The species of human pathogens with a proven VBNC state

Aeromonas salmonicida	*Lactobacillus plantarum*	*Serratia marcescens*
Agrobacterium tumefaciens	*Lactococcus lactis*	*Shigella dysenteriae*
Alcaligenes eutrophus	*Legionella pneumophila*	*S. flexneri*
Aquaspirillum sp.	*Listeria monocytogenes*	*S. sonnei*
Burkholderia cepacia	*Micrococcus flavus*	*Sinorhizobium meliloti*
B. pseudomallei	*M. luteus*	*Streptococcus faecalis*
Campylobacter coli	*M. varians*	*Tenacibaculum sp.*
C. jejuni	*Mycobacterium tuberculosis*	*Vibrio anguillarum*
	M. smegmatis	
C. lari	*Pasteurella piscida*	*V. campbellii*
Cytophaga allerginae	*Pseudomonas aeruginosa*	*V. cholerae*
Enterobacter aerogenes	*P. fluorescens*	*V. fischeri*
E. cloacae	*P. putida*	*V. harveyi*
Enterococcus faecalis	*P. syringae*	*V. mimicus*
E. hirae	*Ralstonia solanacearum*	*V. natriegens*
E. faecium	*Rhizobium leguminosarum*	*V. parahaemolyticus*
Escherichia coli (including EHEC)	*R. meliloti*	*V. proteolytica*
Francisella tularensis	*Rhodococcus rhodochrous*	*V. shiloi*
Helicobacter pylori	*Salmonella enteritidis*	*V. vulnificus (types 1&2)*
Klebsiella aerogenes	*S. typhi*	*Xanthomonas campestris*
K. pneumoniae	*S. typhimurium*	
K. planticola		

Oliver,2005

VBNC or VNC

In yeasts

- The Viable But Non Culturable (VBNC) state has been thoroughly studied in bacteria.
- In contrast, it has received much less attention in other microorganisms.
- However, it has been suggested that various yeast species occurring in wine may enter in VBNC following sulfite stress.
- The existence of a VBNC state in yeasts comparable to that described in bacteria.
- E.g. yeast *Saccharomyces cerevisiae* (commonly known as baker's yeast).

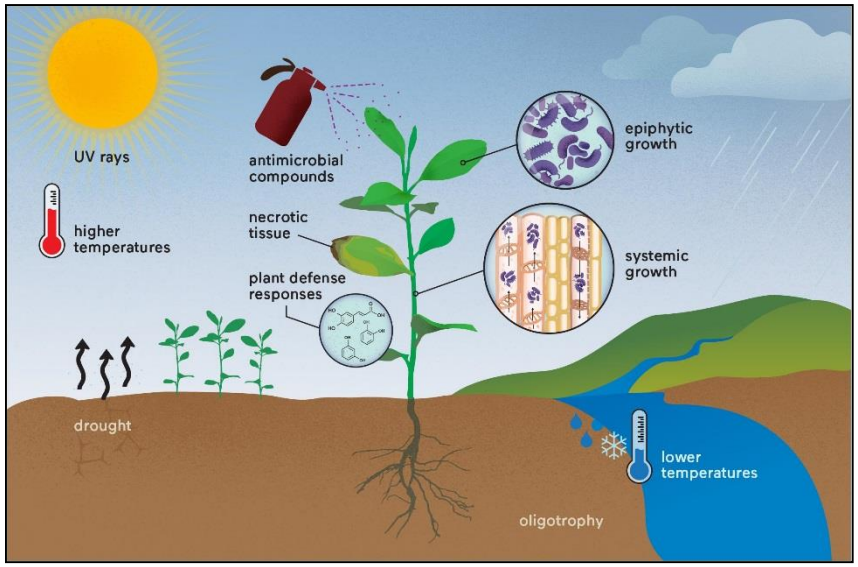
Salma *et al.*,2013

VBNC or VNC

What induces this state in bacteria?

Occurrence of super-phytopathogenic bacteria

- Super-phytopathogenic" bacteria occurrence in the field.
- Different stress conditions are already known to affect phytopathogens that could induce resistance and/or persister cell formation.
- The recurrence of disease outbreaks may result from these genetic and physiological responses, which are still underestimated in both research and crop management.
- Parallels could be made with the human superbugs.



Superbugs are strains of bacteria, viruses, parasites, and fungi that are resistant to most of the antibiotics.

Martins *et al.*,2018

VBNC

Survival strategy of *E. amylovora* against copper

Method

- Some phytopathogenic bacteria enter into the viable-but-nonculturable(VBNC) state in the presence of copper.
- To determine whether copper kills *E. amylovora* or induces the VBNC state:
- A mineral medium without copper or supplemented with 0.005, 0.01, or 0.05 mM Cu^{2+} was inoculated with 10^7 CFU/ml of this bacterium and monitored over 9 months.

Ordax *et al.*,2006

VBNC

Survival strategy of *E. amylovora* against copper

Method

- *Erwinia amylovora* entered into the VBNC state at all three copper concentrations assayed, much faster when the copper concentration increased.
- The addition of different agents which complex copper allowed the resuscitation (restoration of culturability) of copper-induced VBNC cells.

1. Copper-induced VBNC cells were virulent only for the first 5 days,
2. While resuscitated cells always regained their pathogenicity on immature fruits over 9 months.

- These results have shown, for the first time, the induction of the VBNC state in *E. amylovora* as a survival strategy against copper.

Ordax *et al.*,2006

Detection of VBNC or VNC

Method

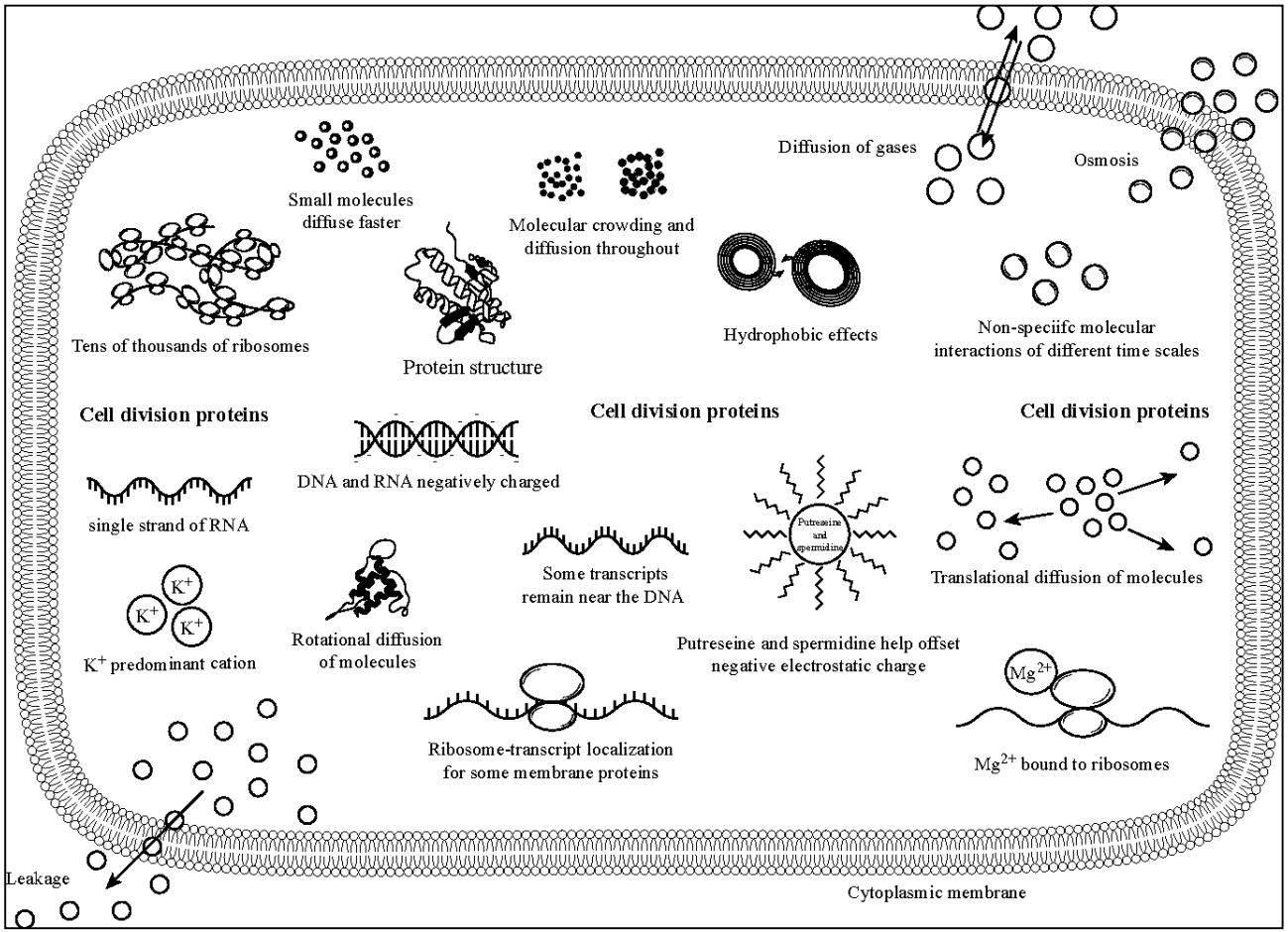
1. Total and viable cell counts were determined by epifluorescence microscopy using the LIVE/DEAD kit and by flow cytometry with 5-cyano-2,3-ditolyl tetrazolium chloride and SYTO 13.
2. Culturable cells were counted on King's B nonselective solid medium.
3. Changes in the bacterial morphology in the presence of copper were observed by scanning electron microscopy.

Epifluorescence microscopy: Specific wavelengths of lights are used to excite the specimen and produce fluorescence. It allows visualization of cell morphology, cellular/subcellular compartments as well as cellular markers of disease (e.g. cancer cells).

Flow cytometry, a technique adapted to the analysis of viability, metabolic state, and antigenic markers of bacteria. In particular, flow cytometry can be readily applied to the enumeration of viable bacteria in a sample.

VBNC or VNC

Organization and localization in the crowded cytoplasm of actively growing bacterial cells



Trevors *et al.*,2013

VBNC or VNC

Comparison of cytoplasm in actively growing/dividing bacterial cells to VBNC cells

Actively growing/dividing	**VBNC physiological state**
Molecularly crowded cytoplasm	Less molecular crowding
Optimal diffusion	Minimal diffusion
Higher total protein concentration	Lower total protein concentration
More organization of molecules such as cell division proteins	Less molecular organization such as cell division proteins
Optimal protein oscillations	Fewer to no protein oscillations
High ribosome numbers	Fewer ribosomes
Optimal gene expression	Minimal to no gene expression
Higher number of transcripts	Minimal transcripts
High tRNA content	Minimal tRNA
Optimal cytoplasmic membrane fluidity	Cytoplasmic membrane may be less fluid with leakage from cytoplasm (e.g., K^+)
Optimal ATP pool	Minimal ATP pool
More nonspecific molecule interactions	Fewer nonspecific molecule interactions
Optimal cytoplasm volume just before cell division	Minimal cytoplasm volume
Replicating DNA	Condensed DNA
Optimal Mg^{2+}	Less Mg^{2+}

Trevors *et al.*,2013

Ecology of plant pathogens

Contribution of pathogen life cycle phases to survival

1. Pathogenic phase

- Large increase in numbers of pathogen cells and production of symptoms contributes most of the cells entering survival period.
- The larger the population entering the period, the greater the chances for survival.

2. Resident phase

- Multiplication on the surface parts of the healthy shoot system.
- Resident is a member of microflora multiplying on surface of aerial parts or roots of healthy plant.

3. Saprophytic phase

- For the most part plant pathogenic bacteria do not have a true saprophytic stage in nature.

Ecology of plant pathogens

Contribution of pathogen life cycle phases to survival

- **In plant residues:**
- Surface infected plant residue bacterium can survive for months (depending on whether tissue is exposed to overwintering or oversummering).
- *Pseudomonas syringae* pv. *syringae* is considered to be the major resident phytopathogenic bacterium.
- *X. c.* pv. *campestris* survives up to 244 days in infected plant debris.
- *Pectobacterium carotovorum* (soft rot bacterium) multiply usually in association with decaying plant materials.
- **In surface water:**
- *P. c.* subsp. *carotovorum* detected in water ditches, streams, rivers and lakes throughout year.

Ecology of plant pathogens

Contribution of pathogen life cycle phases to survival

- **In soil:**
- *Ralstonia solanacearum* and *Agrobacterium* are well known for ability to survive in soil.
- *R. solanacearum* recovered from soil after 4-month fallow period. Reported to survive 4-6 years under bare fallow.
- *Xanthomonas* pv*. campestris*, causal agent of black rot of cabbage survived 42 days in winter in soil free of plant tissue.
- *Pseudomonas syringae* pv. *tomato* recovered from infested soil 7 days after infesting the soil(McCarter *et al.*,1983).

Ecology of plant pathogens

Contribution of pathogen life cycle phases to survival

- **In phylloplane:**
- The aerial portion of vascular plants (stem, leaves, fruit, flowers, etc.) collectively known as the phylloplane.
- These parts of plants are normally colonized by a variety of bacteria, yeasts, and fungi and these inhabitants are called epiphytes.
- **Phyllosphere or phyllobacteria:**
- Phyllobacteria (phyllospheric bacteria) are by far the most abundant inhabitants of the phyllosphere.

Lindow and Brandl,2003;..

Ecology of plant pathogens

Contribution of pathogen life cycle phases to survival

- **Phyllosphere or phyllobacteria:**
- Different phyllospheric bacterial genera have the ability to colonize aerial plant surfaces that includes:
- *Burkholderia*, *Acinetobacter*, *Bacillus*, *Paenibacillus*, *Pantoea*, *Xanthomonas*, *Photobacterium* and *Pseudomonas.*
- Phyllosphere bacteria can promote plant growth by:

1. Suppressing the colonization and infection of tissues by plant pathogens;
2. Production of different metabolites such as siderophore, auxin, etc.

Ecology of plant pathogens

Contribution of pathogen life cycle phases to survival

- **Epiphytic bacteria:**
- Fungi and bacteria with diverse lifestyles including epiphytes, saprophytes, and pathogens(Baker *et al*.,2010).
- Henis & Bashan (1989) stated that "epiphytic bacteria can be either pathogenic or saprophytic. This statement suggests that saprophytes usually do not grow endophytically.
- Two types of epiphytic bacteria are known so far:

1. epiphytic non-pathogenic bacteria, and
2. epiphytic pathogenic bacteria.

Ecology of plant pathogens

Contribution of pathogen life cycle phases to survival

- **Epiphytic bacteria:**
- Bacterial plant pathogens also shown to survive epiphytically include:

1. *Erwinia amylovora*
2. *Pseudomonas syringae* pv. *syringae*
3. *Pseudomonas syringae* pv. *tomato*
4. *Xanthomonas vesicatoria*

- e.g. *Pseudomonas syringae* pv. *tomato*, survived on weeds and served as inoculum source for tomato crop.

Ecology of plant pathogens

Contribution of pathogen life cycle phases to survival

- **Endophytic bacteria:**
- Bacteria that inhabit, for at least one period of their life cycle, the interior of a plant:
- There is possibility that to some extent some endophytes might have a pathogenic association with their host.
- They might for example reside latent within plant tissue and only act as pathogen when the conditions are favourable (e.g. at low temperature).
- In this regard, endophytes and pathogens might not be completely opposed and the two terms not totally incompatible.

Nejad,2005

Ecology of plant pathogens

Contribution of pathogen life cycle phases to survival

- **Endophytic bacteria:**
- Endophytic plant pathogens colonize:

1. Epidermal cells (e.g. *Streptomyces scabies*, causal agent of scab of potato).
2. Apoplast (free diffusional space outside the plasma membrane/space outside living protoplasts) including cell walls and free space (e.g., *Pseudomonas syringae* pv. *phaseolicola*, causal agent of halo blight of bean).
3. Xylem vessels (e.g., *Ralstonia solanacearum*, causal agent of Granville (tobacco) wilt.
4. Phloem (e.g., *Spiroplasma citri*, causing citrus stubborn disease).

Trigiano *et al.*,2006;..

Endophytic *Burkholderia* spp. and their natural plant hosts

Species	Plant hosts
B. cepacia	*Citrus sinensis* (L.) Osbeck *Oryza sativa* L
B. cenocepacia	*Triticum aestivum* L *Lupinus* sp. *Zea mays* L.
B. gladioli	*Coffea* sp. Glycine max (L.)
B. phytofirmans	*Allium cepa* L. *Oryza sativa* L. *Shagnum* spp.
B. pyrrocinia	*Pinus contorta* Dougl.
B. silvatlantica	*Saccharum officinarum* L.
B. tropica	*Ananas comosus* (L.) Merr. *Saccharum officinarum* L. *Zea mays* L.
B. unamae	*Saccharum officinarum* L. Zea mays L.
B. vietamiensis	*Zea mays* L.

Compant *et al.*,2008

Endophytic bacterial communities
Tomato cultivars

- *Sphingomonas yanoikuyae*
- *Pseudomonas pseudoalcaligenes*
- *Serratia marcescens*
- *Bacillus megaterium*
- *Paenibacillus polymyxa*
- *Bacillus pumilus*
- *Bacillus cereus*
- *Pseudomonas fluorescens*
- *Arthrobacter globiformis*

Feng *et al.*,2013

Endophytes
Latent infections

- Further, important plant pathogens like *Ralstonia solanacearum*, *Liberibacter asiaticus*, *X. fastidiosa*, and *Clavibacter sepidonicus* cause long-term latent infections, effectively functioning as endophytes.
- What biological signals or conditions tip the balance and cause an innocuous(harmless) endophyte to become a destructive pathogen?

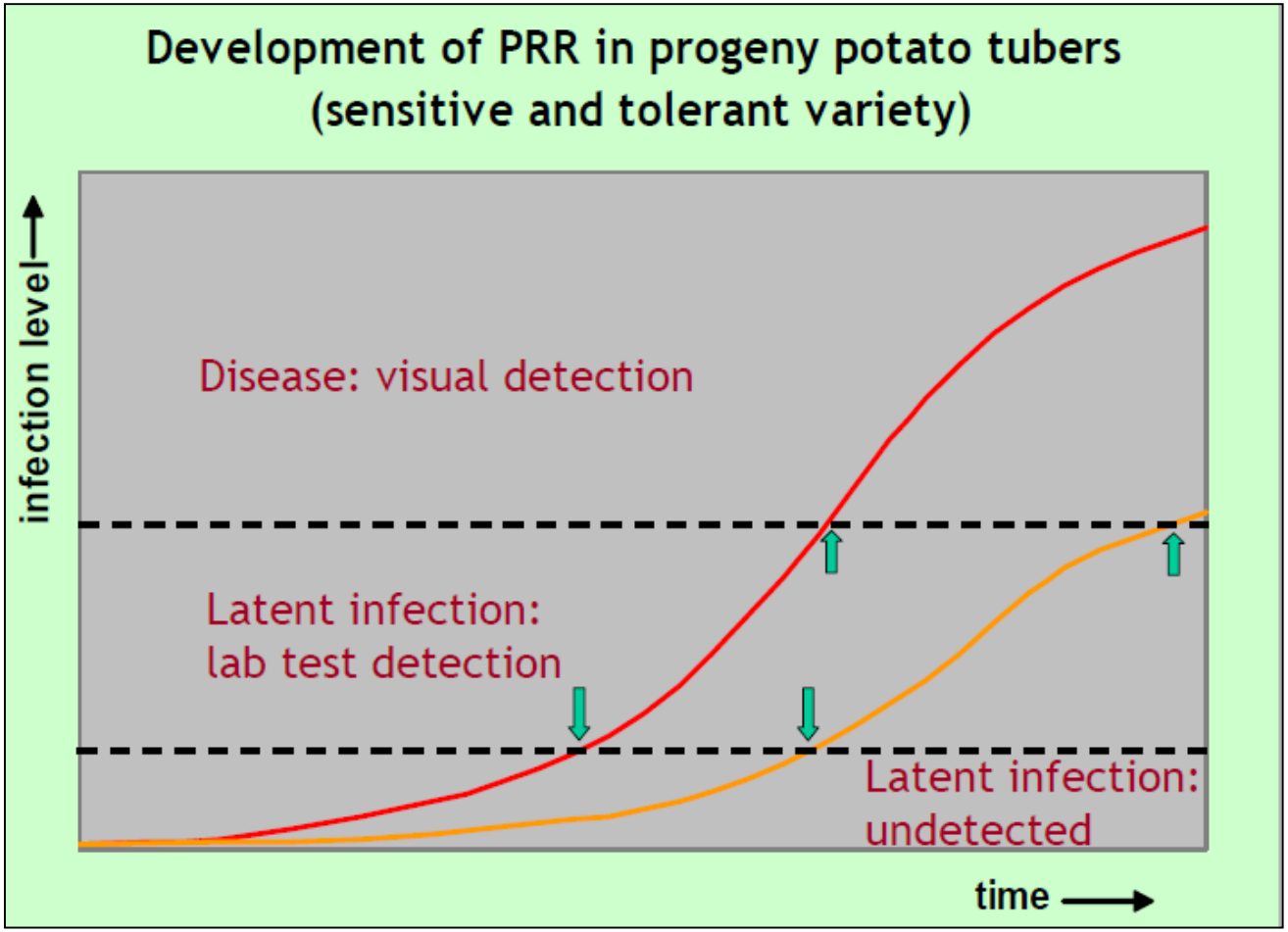
Note: it appears that the 'latent' or the 'dormant' phase of *Mycobacterium tuberculosis* infections represents the VBNC state in this pathogen.

Allen *et al.*,2009

Latent infections

Potato ring rot (PRR)

Clavibacter michiganensis subsp. *sepedonicus*



Vaerenbergh,2006

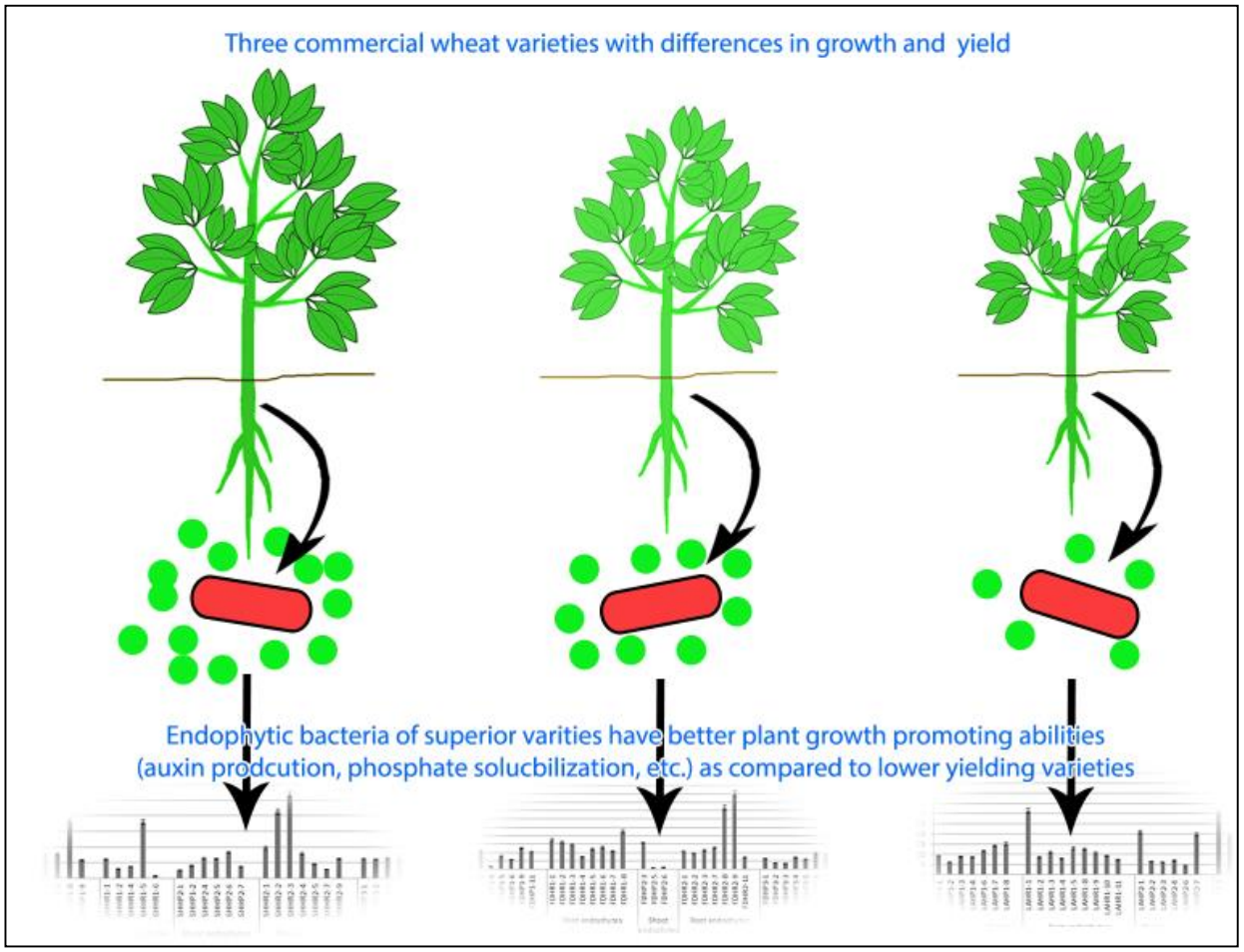
Endophytic bacteria
Functions

- A large number of plant endophytic bacteria reside in plants which establish harmonious and close relationships with their hosts resulting from co-evolutionary processes.
- Endophytes offer a wide range of benefits to plants such as:

1. Promoting growth,
2. Reducing disease severity inducing plant defense mechanisms inducing plant defense mechanisms,
3. Producing anti-herbivory products,
4. Biologically fixing nitrogen, and
5. Increasing plant mineral uptake.

Feng *et al.*,2013

Endophytic bacteria
Functions



Yousaf *et al.*,20170

Endophytic bacteria
Functions

- **Biological control of *Ralstonia solanacearum* with antagonistic endophytic bacteria in pot experiments.**

Antagonistic endophytic bacteria	Disease incidence (%)	Index of disease	Control effect (%)
X-6	50.0	24.2±0.6b	50.0
X-3	20.0	7.5±0.5c	84.5
CK (check)	73.3	48.3±0.3a	

Two antagonistic isolates, X-3 and X-6, isolated from resistant cultivar of tomato Xiahong-1.

Biocontrol efficacy = [(Disease incidence of control- disease incidence of treatment) / Disease incidence of control] × 100

Feng *et al.*,2013; Tariq *et al.*,2009

Ecology of plant pathogens

Contribution of pathogen life cycle phases to survival

- **In Gemmisphere:**
- Favorable site(bud habitat) because protected considerable from outer environment (e.g. *P. syringae* pv. *lachrymans*; *X. glycinea*).
- **Rhizosphere:**
- Plant pathogenic bacteria can survive saprophytically or multiply on healthy host and nonhost material.
- Nutrients secreted by the roots may enhance the ability to compete with other microorganisms.
- e.g.

1. *Xanthomonas vesicatoria* (tomato pathogen) on wheat roots (Diachun and Valleau,1946), and
2. *P. syringae* pv.*tomato* on weed roots.

Some seed-borne and seed-transmitted plant pathogenic bacteria

Bacterium	Main host(s)	Disease
Acidovorax avenae pv. *avenae*	oat, rice	bacterial blight and brown stripe
Acidovorax avenae subsp. *citrulli*	watermelon (*Citrullus lanatus*)	bacterial fruit blotch
Burkholderia glumae	rice	bacterial grain rot of rice
Clavibacter michiganensis subsp. *michiganensis*	tomato	bacterial canker
C. m. subsp. *insidiosus*	alfalfa	bacterial wilt
C. m. subsp. *nebraskensis*	corn	bacterial wilt and blight
Curtobacterium flaccumfaciens pv. *flaccumfaciens*	bean (*Phaseolus, Vigna*)	bacterial wilt
Pantoea stewartii subsp. *stewartii*	maize	stewart's disease, bacterial wilt
Pantoea ananatis	onion	centre rot
Pseudomonas syringae pv. *atrofaciens*	cereals	leaf spot, basal glume rot
P. s. pv. *glycinea*	soybean	bacterial blight of soybean
P. s. pv. *lachrymans*	cucumber, gherkin	angular leaf spot
P. s. pv. *phaseolicola*	bean	halo blight of bean
P. s. pv. *pisi*	pea	bacterial blight of pea
P. s. pv. *tomato*	tomato	bacterial speck
Xanthomonas axonopodis pv. *phaseoli*	bean (*Phaseolus, Vigna*)	common blight of bean
X. a. pv. *phaseoli* var. *fuscans*	bean (*Phaseolus, Vigna*)	common blight of bean
X. a. pv. *malvacearum*	cotton	bacterial blight of cotton
X. a. pv. *vesicatoria*	pepper	bacterial spot
X. a. pv. *vitians*	lettuce	bacterial leaf spot
Xanthomonas campestris pv. *campestris*	cabbage	black rot of crucifers
X. c. pv. *carotae*	carrot	bacterial blight
X. translucens	cereals	bacterial leaf streak, black chaff
X. oryzae pv. *oryzae*	rice	bacterial leaf blight
X. oryzae pv. *oryzicola*	rice	bacterial leaf streak
X. vesicatoria	tomato	bacterial spot
Xylella fastidiosa	orange (*Citrus sinensis*)	citrus variegated chlorosis

Janse,2006

Seed-Inhabiting Bacteria

Crop	Bacteria
Brassica (Crucifers)	*Pseudomonas syringae* pv. *maculicola*
	Xanthomonas campestris pv. *campestris*
Capsicum (Pepper)	*Burkholderia solanacearum*
	Xanthomonas campestris pv. *vesicatoria*
Maize	*Erwinia zeae*
	E. stewartii
	Psedomonas syringae pv. *syringae*
Peanut (groundnut)	*Burkholderia solanacearum*
Rice	*Pseudomonas fuscovaginae*
	P. glumae
	Xanthomonas oryzae pv. *oryzae*
	X. oryzae pv. *oryzicola*
Sorghum	*Pseudomonas syringae* pv. *syringae*
Soybean	*Burkholderia solanacearum*
	Pseuomonas syringae pv. *glycinea*
	P. syringae pv. *tabaci*
	Xanthomonas campestris pv. *glycines*
Tomato	*Clavibater michiganensis* subsp. *michiganensis*
	Pseudomonas corrugata
	P. syringae pv. *tomato*
Wheat	*Bacillus megaterium* pv. *ceralis*
	Clavibacter tritici
	Pseudomonas fuscovaginae
	P. syringae pv. syringae

Narayanasamy,2006

How bacteria gain entry into plants
Dissemination of bacteria

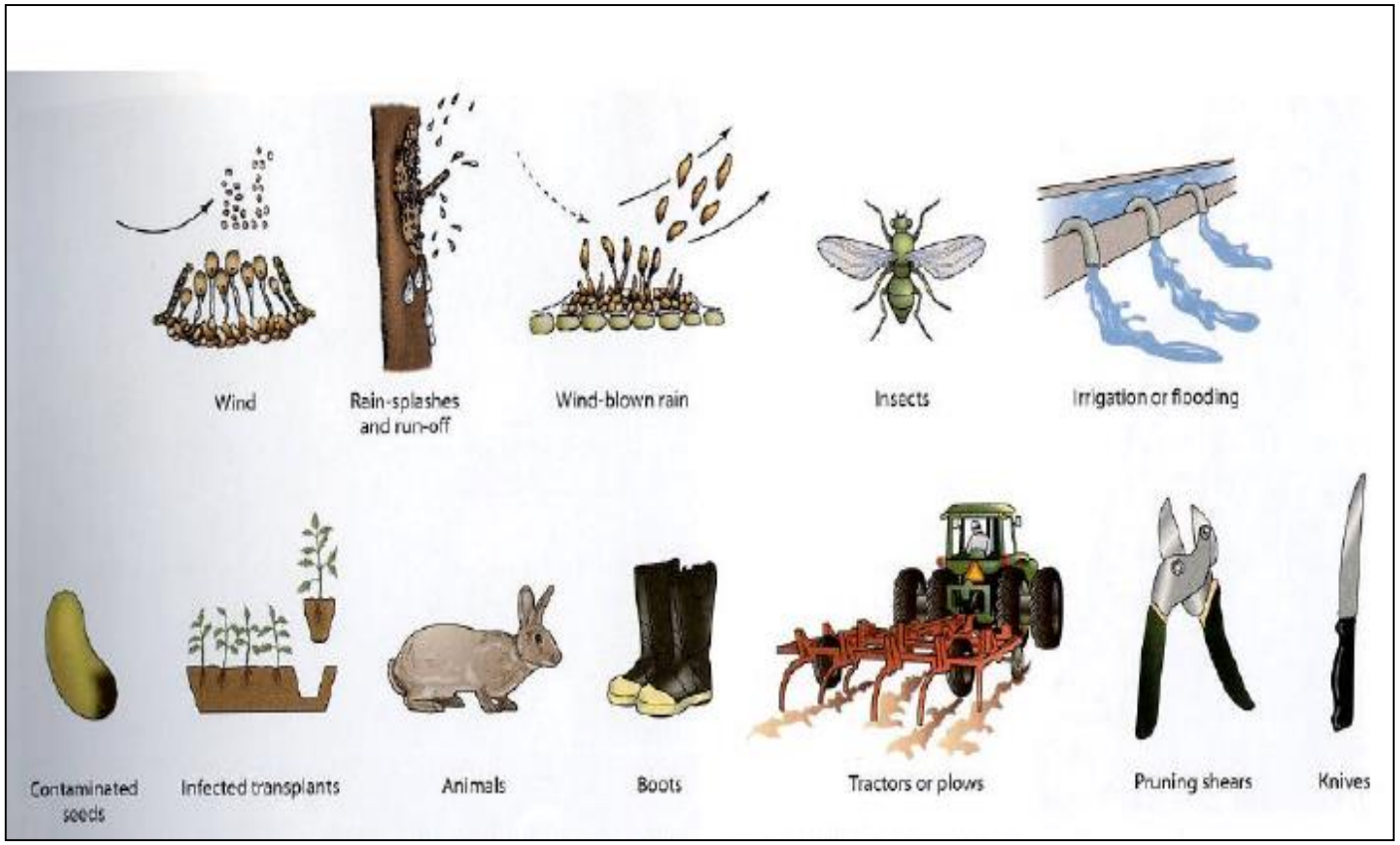
- Plant pathogenic bacteria do not make spores;
- Bacteria cannot penetrate plant tissue directly;
- Bacteria usually enter plant tissue by means of:

1. Wounds
2. Natural openings on plant leaves or stems:
3. Lenticels, hydathodes, etc.;
4. Water saturation aids entry through natural openings.
5. Insects or insect larvae are common vectors of bacterial pathogens.
6. Bacteria may "piggy back" and gain entry through cankers (open wounds) caused by fungi.

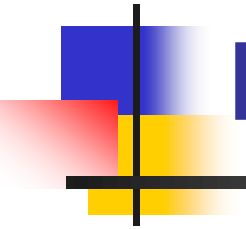
Piggybacking literally refers to carrying someone on one's back or shoulders.

Smith,2011

Dissemination of bacteria



Agrios,2005



Epidemiology

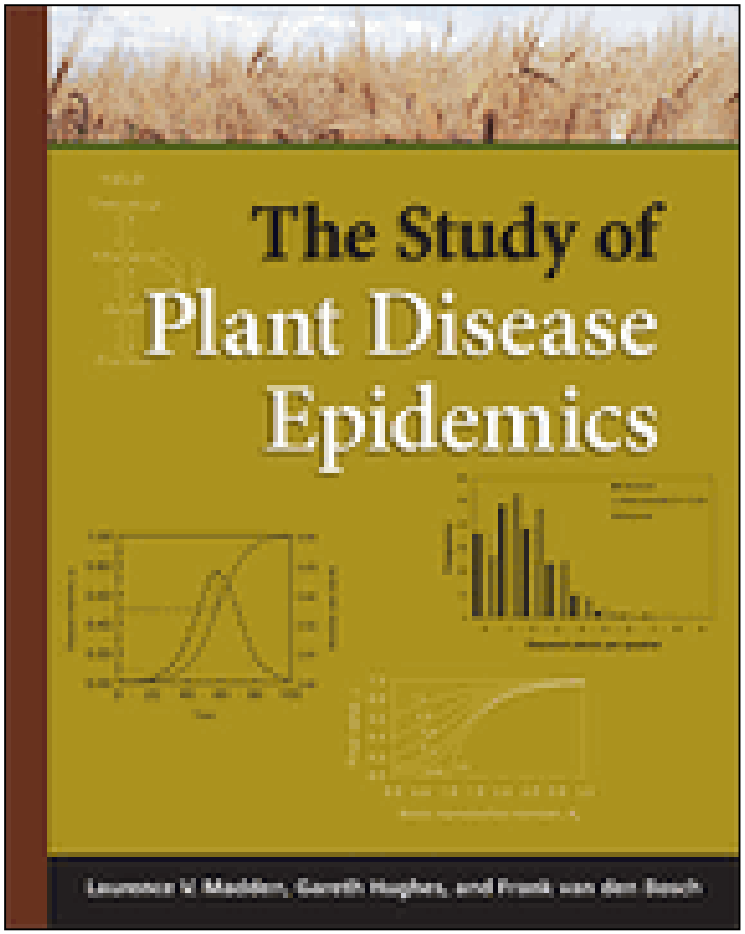
Bacterial disease epidemics

The Epidemiology of Plant Diseases

- **The Epidemiology of Plant Diseases**
- 2nd edition,
- B. M. Cooke, D. G. Jones and B. Kaye
- Publisher: Kluwer Academic
- 2006
- 576 Pages
- Printed in Netherlands.

The Study of Plant Disease Epidemics

- **The Study of Plant Disease Epidemics**
- By Laurence V. Madden, Gareth Hughes, and Frank van den Bosch
- 2007
- 432 pages.



Plant pathology

Difference between epidemiology and etiology

1. Epidemiology deals with the in-depth study of both known and unknown diseases, their risk factors, and how they may affect a certain area.
2. Etiology deals with the origin, cause, and effect of different phenomena. It investigates the causes and origins of disease or the set of factors that contributes to the occurrence of a disease.
3. Epidemiology has greater scope than etiology because it is an ongoing process.
4. Epidemiology involves the study of both determinants and distribution of disease, while etiology only attempts to explain on the determinants.

Celine,2010

Epidemiology

Difference between endemic (enphytotic) and epiphytotics

- Endemic (enphytotic) diseases occur at:

1. relatively constant levels in the same area each year, and
2. generally cause little concern.

- Epidemic (epiphytotics in plants) affect:

1. a high percentage of the host plant population,
2. sometimes across a wide area.
3. They may be mild or destructive and local or regional in occurrence.

Epidemiology
Comparison of epidemics
Endemic or Enphytotic

- When a disease is more or less constantly occurring year after year in a moderate to severe form in a country or locality then it is called as endemic (enphytotic) disease.

1. Wart disease of potato (*Synchytrium endobioticum*) is endemic in Darjeeling,
2. Citrus canker (*Xanthomonas axonopodis* pv. *citri*)in Asia.

- A endemic is an outbreak that occurs at a predictable rate in a certain area or among a set population.
- Endemics remain at a steady state, but do not disappear from a population.

Epidemiology
Epidemic threshold, rate and pandemic

- Epidemic threshold: Epidemic within a population, known as the epidemic threshold.
- Epidemic rate (epidemic rate of the disease or the rate of growth of the epidemic): The epidemic rate is the increase or decrease per units of time commonly day or week or year in a given plant population.
- Reduction in epidemic rates in mixtures was attributable to the reduction in density of susceptible host units.
- Pandemic: An epidemic of disease that has spread across a large region; for instance multiple continents, or worldwide.

Epidemiology
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Epidemiology

When an epidemic is inevitable?

The elements of disease epidemics

- It's difficult to get an epidemic started:

1. Environment must be right,
2. Crop must be at right growth stage,
3. Pathogen must be easily dispersed, stable, & highly virulent.

- Disease epidemics often occur when genetic diversity of plant populations is eliminated by human intervention.

C. Allen;..

Disease epidemics

Disease epidemics often occur when genetic diversity of plant populations is eliminated by human intervention

Coevolution between plants and pathogens

- There is a relationship between pathogens and genetic diversity in plant populations and species diversity in plant communities.
- Since the pathogens as part of the biotic environment **exert a strong selective force on populations of plants and animals**.

See also boom and bust

Collins Johnson

Disease epidemics

Disease epidemics often occur when genetic diversity of plant populations is eliminated by human intervention

Coevolution between plants and pathogens

- Plant pathogens like other microbial parasites and herbivores may be responsible for maintaining:

1. a high degree of genetic polymorphism in plant populations, and
2. a high degree of species diversity within plant communities.

- In another word, plant pathogens may prevent plant communities from becoming dominated by one or several species (i.e. destabilizing force).

See also boom and bust

Collins Johnson

Disease epidemics

Plant disease cycles

Lifecycle of the host plant and pathogen

1. Host plants may be resistant to pathogens at one stage of development but not at another.
2. In a similar manner, some pathogens must be at a critical life stage in order to cause infection.

- Within one species of host plant there may be an incredible range of genetic diversity that greatly influences susceptibility to any particular species of pathogen.
- If the host is resistant to a pathogen, even when the pathogen is present under favorable environmental conditions, a disease will not occur.

Plant disease cycles

Primary versus secondary infection

Monocyclic vs. polycyclic epidemics

- Epidemiologically, there are two main types of diseases:

1. monocyclic, those that have but a single infection cycle (with the rare possibility of a second or even third cycle) per crop season; and
2. polycyclic, those that have many, overlapping, concatenated cycles of infection per crop season.

- For both epidemiological types, the increase of disease slows as the proportion of disease approaches saturation or 100%.

https://www.thefreedictionary.com/

Plant disease cycles

Primary versus secondary infection

Monocyclic vs. polycyclic epidemics

1. Fungi are considered as monocyclic and polycyclic pathogens. E.g. vascular wilt fungi *Fusarium oxysporum* (e.g. F. *oxysporum* f. sp. *cubense*) and *Verticillium dahliae* wilt of cotton.
2. Most plant diseases caused by bacteria are polycyclic, and
3. Many plant viruses, with the aid of their insect vectors, also can produce repeated cycles of infection in one season(polycyclic).

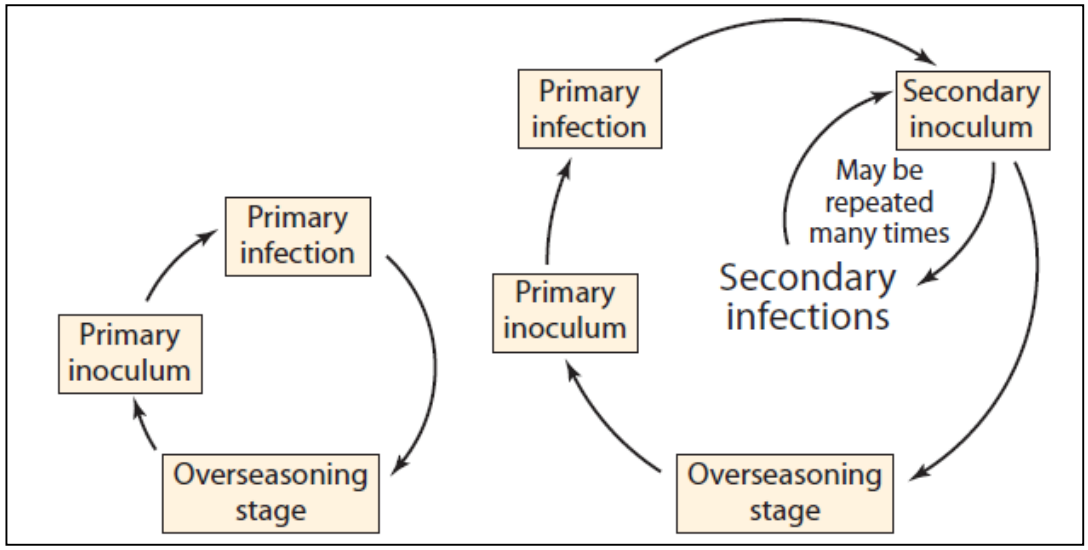
APS,2020;..

Plant disease cycles

Primary versus secondary infection

Monocyclic vs. polycyclic epidemics

- Diagrams of (left) monocyclic and (right) polycyclic plant diseases.
- Monocyclic diseases lack secondary inoculum and secondary infections during the same year.



Agrios,2005

Plant disease cycles

Primary versus secondary infection

Monocyclic and polycyclic epidemics

- **Primary infections:**
- Result from contact between host plants and inoculum produced elsewhere, or in a different epidemic.
- **Monocyclic epidemics consist only of primary infections**
- **Secondary infections:**
- Any infections that ultimately result from the primary infections in the current epidemic.
- Infections resulting from inoculum produced during the current epidemic.
- **Secondary infections in fungi and bacteria occur only in monocyclic epidemics.**

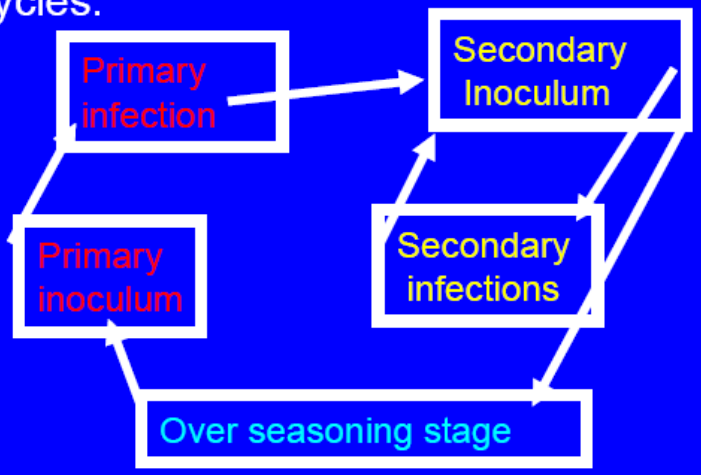
Plant disease cycles

Primary versus secondary infection

Monocyclic vs. polycyclic epidemics

Disease Cycles

Polycyclic (multiple cycles)- Pathogen goes through more than one generation per growing season.
2-30 cycles.



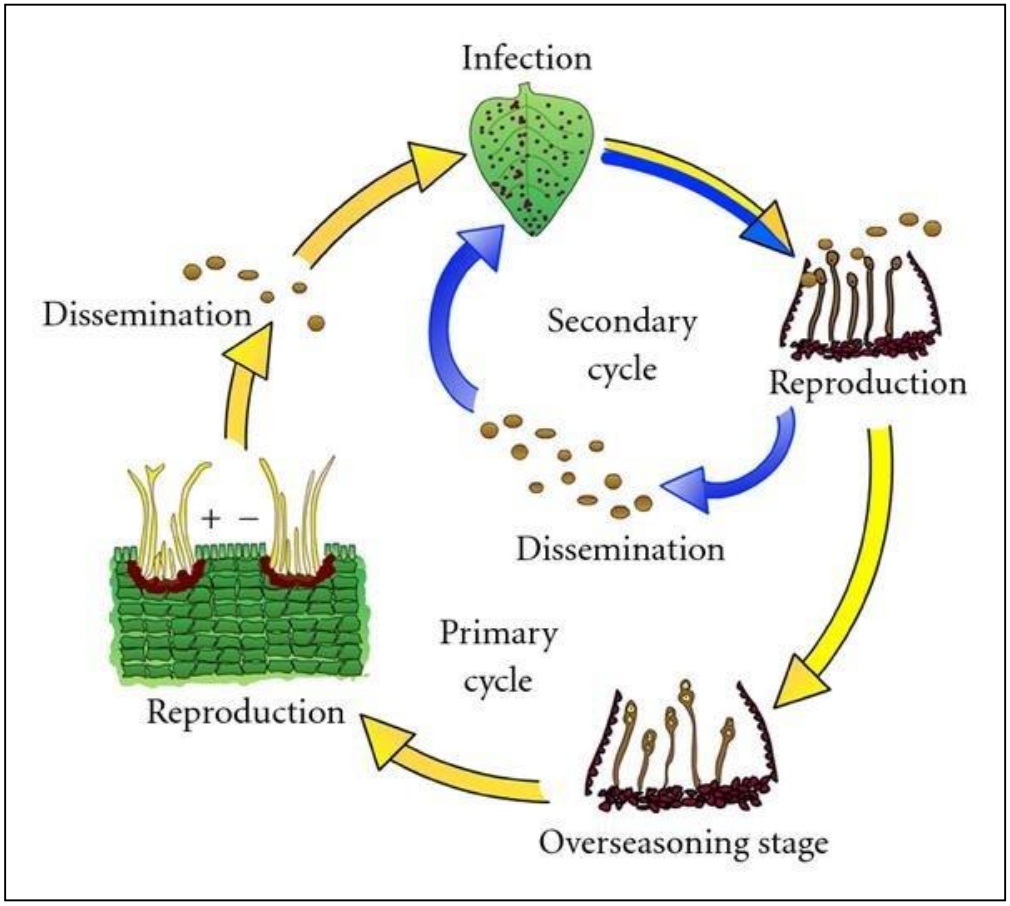
Polycyclic pathogens are disseminated primarily by air or air borne vectors/ insects, or water.
Responsible for the most explosive epidemics- late blight of potato, grain rust, insect-born viruses, powdery mildews, spots and blights. 36

Allen,2009

Plant disease cycles

Primary versus secondary infection

Monocyclic vs. polycyclic epidemics



González-Fernández *et al.*,2010

Disease epidemics

Lifecycle of the pathogen

Monocyclic vs. polycyclic epidemics

- Generally in temperate climates there is only one crop cycle per year.
- In tropical or subtropical climates, however, there can be more than one crop cycle per year.
- In perennial plants (forages, pastures, lawns, orchards, forests, etc.) the inoculum produced in one growing season carries over to the next, and there could actually be a buildup of inoculum over a period of years.

Disease epidemics

Lifecycle of the pathogen

Primary versus secondary infection

- **Monocyclic Epidemics:**
- In general, there are three types of plant diseases that tend to produce only one infection cycle per host cycle:

1. postharvest diseases,
2. diseases caused by soil-borne plant pathogens, and
3. rusts without a urediniospore stage.

Disease epidemics

Lifecycle of the pathogen

Monocyclic vs. polycyclic epidemics

- **Polycyclic Epidemics:**
- Pathogens that produce more than one infection cycle per crop cycle.
- This led to repeated complete infection cycles, pathogen development, new inoculum production, dispersal to new susceptible sites, and new infections, all within a single crop cycle.
- A good example is potato late blight (*Phytophthora infestans*), where a single cycle of infection, lesion development, sporulation, sporangium dispersal, and new infection can occur in as little as five days, and many overlapping cycles occur simultaneously during periods of favorable weather.

APS,2020

Disease epidemics

Polyetic epidemics

Polyetic or mean velocity during successive cropping seasons

- **Polyetic Epidemics:**
- Polyetic diseases, also known as multiyear diseases.
- Can be caused by both monocyclic and polycyclic pathogens.
- In these epidemics, the inoculum builds up from one year to the next, and the epidemic is usually polyetic, i.e., it develops over several years.

BharathM64; Tibayrenc,2007

Disease epidemics

Polyetic epidemics

Can be caused by both monocyclic and polycyclic pathogens

- **Polyetic Epidemics:**
- Some pathogens take several years to produce the inoculum.

1. May not cause new infections in a year.
2. Amount of inoculum does not increase greatly within a year.
3. Inoculum may increase steadily from year to year and cause severe outbreaks.

BharathM64; Agrios,2005

Plant disease cycles

Monocyclic diseases/pathogens

Polyetic epidemics

- Examples of such diseases are:

1. Dutch elm disease,
2. white pine blister rust, and
3. citrus tristeza.

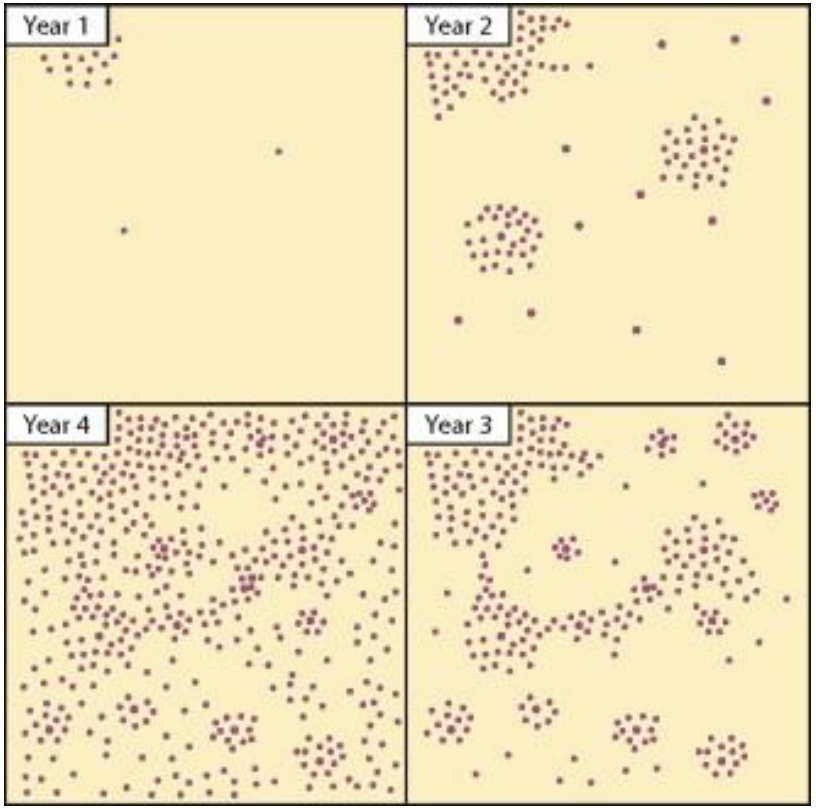
Agrios,2005

Disease epidemics

Polyetic epidemics

Polyetic or mean velocity during successive cropping seasons

- Schematic representation of a polyetic epidemic caused in a crop in a field by a soil pathogen over a 4-year period.



Agrios,2005

Plant disease cycles

Polycyclic diseases/pathogens

Polyetic epidemics

- Cedar apple rust,
- Apple powdery mildew(*Podosphaera leucotricha*) are two example of polyetic epidemics caused by a polycyclic pathogens.
- Huanglongbing (HLB) is a polyetic, i.e., multiyear, disease, it has been difficult to conduct quantitative epidemiological studies on HLB.
- The disease is associated with three bacteria:

1. *Candidatus* Liberibacter asiaticus (Las),
2. *Candidatus* Liberibacter africanus (Laf), and
3. *Candidatus* Liberibacter americanus (Lam).

Gottwald;2010 BharathM64

Disease epidemics

Polyetic epidemics

A polyetic modelling framework for plant disease emergence

- A polyetic process-based model is developed to analyse conditions of disease emergence.
- This model simulates:

1. polycyclic epidemics during successive growing seasons,
2. the yield losses they cause, and
3. the pathogen survival between growing seasons.

- This framework considers one immigrant strain coming in a single event into a system where a resident strain is already established.

Willocquet *et al.*,2020

Disease epidemics

Polyetic epidemics

A polyetic modelling framework for plant disease emergence

- Outcomes are formulated as:

1. probability of emergence,
2. time to emergence, and
3. yield loss, resulting from deterministic and stochastic simulations.

- Analyses focus on the effects of two fitness parameters on emergence:

1. the relative rate of reproduction (epidemic speed), and
2. the relative rate of mortality (decay of population between seasons).

Willocquet *et al.*,2020

Disease epidemics

Polyetic epidemics

A polyetic modelling framework for plant disease emergence

- Analyses revealed that stochasticity is a critical feature of disease emergence.
- The simulations suggest that:

1. emergence may require a series of independent immigration events before a successful invasion takes place;
2. an explosion in the population size of the new pathogen (or strain) may be preceded by many successive growing seasons of cryptic presence following an immigration event; and
3. survival between growing seasons is as important as reproduction during the growing season in determining disease emergence.

Willocquet *et al.*,2020

Disease epidemics

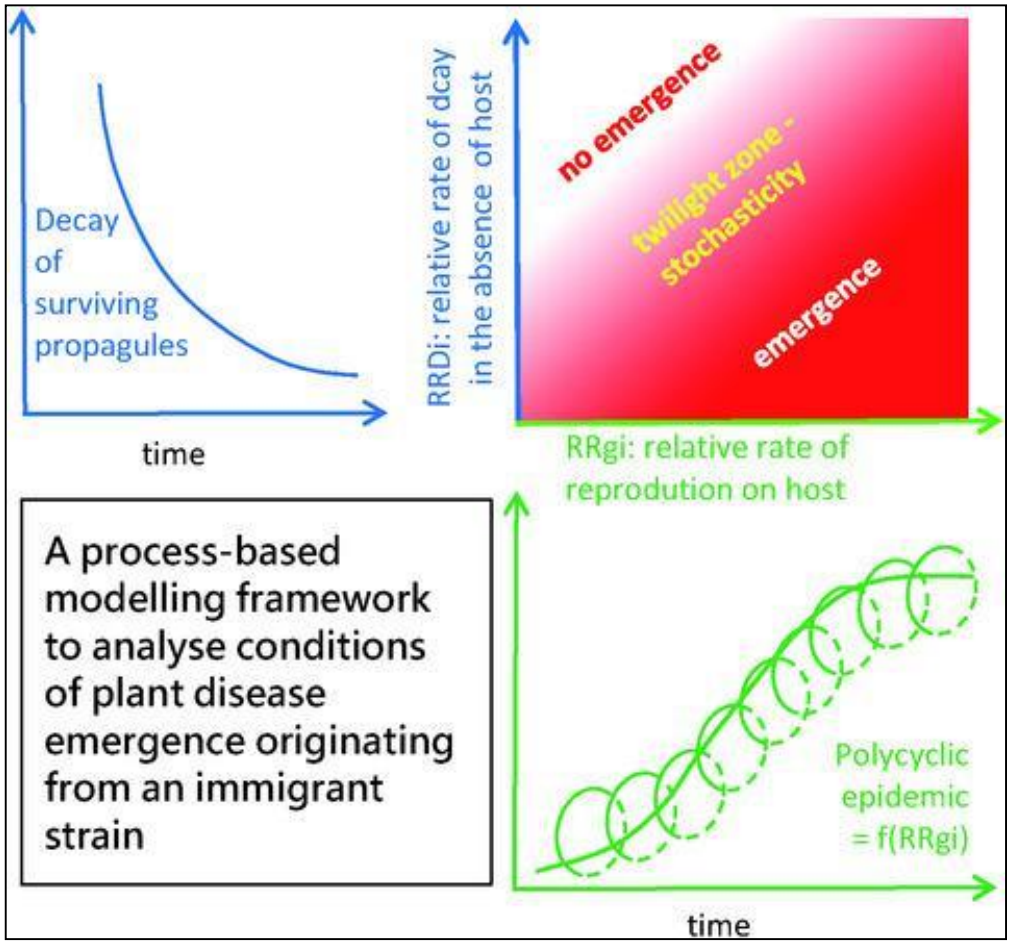
Polyetic epidemics

A polyetic modelling framework for plant disease emergence

Analyses revealed that stochasticity is a critical feature of disease emergence.

Twilight: evening time when sky begins to get dark; light from sky at twilight.

Stochastic: having a random probability distribution or pattern that may be analysed statistically but may not be predicted precisely.



Willocquet *et al.*,2020

Plant disease cycles
Sporadic epidemics

- **Sporadic epidemics:**
- Diseases which occur at irregular intervals over limited areas or locations are called sporadic.
- They occur relatively in few instances. e.g:

1. Fusarium wilt of cotton (*Fusarium oxysporum* f.sp. *vasiinfectum*);
2. grain smut of sorghum (*Sporisorium sorghi*);
3. loose smut of wheat (*Ustilago nuda*);
4. Bacterial leaf streak (BLS) of wheat (*Xanthomonas translucens*).

Sporadic: occurring at irregular intervals or only in a few places; scattered or isolated

Duveiller *et al.*,2002

Plant disease cycles

Sporadic epidemics

Bacterial leaf streak of wheat

- Bacterial leaf streak is a sporadic but widespread disease of wheat that can cause significant losses.
- The major problem is that the disease is seed-borne.
- **Although zero tolerance of bacteria in the seed is not required** due to its **low transmission rate**, there is a very real possibility that primary inoculum may increase during seed multiplication.
- The risk of disease is variable in many wheat-growing areas of the world, but the possibility of it occurring in areas where it is not usually found should not be overlooked.

Duveiller *et al.*,2002

Plant disease cycles

Sporadic epidemics

Bacterial leaf streak(BLS) of wheat

- Fortunately, a specific succession of events is necessary to induce an epidemic. If one of the events required for disease development does not occur, the epidemic may not materialize.
- Epiphytic populations may be important for understanding the etiology of BLS and discovering why the disease is sporadic.
- In Mexico, it was possible to monitor a *Xanthomonas translucens* pv. *undulosa* population in plots of symptomless genotypes contrasting in their field resistance to the pathogen (Duveiller, 1994a).

Duveiller *et al.*,2002

Plant disease cycles

Sporadic epidemics

Bacterial leaf streak (BLS) of wheat

- Moisture facilitates the pathogen's release from the seed and contributes to leaf colonization and invasion of leaf tissue.
- Free water allows the pathogen to spread in the field and to disperse on the leaf, thus increasing the number of lesions.
- Bacteria enter through the stomata and multiply in large masses in the parenchyma.
- This causes elongated streaks limited by the veins, which act as barriers.
- Later milky or yellow exudates form on the surface of lesions.

University of Minnesota Extension,2018

Bacterial leaf streak of wheat

Sporadic epidemics

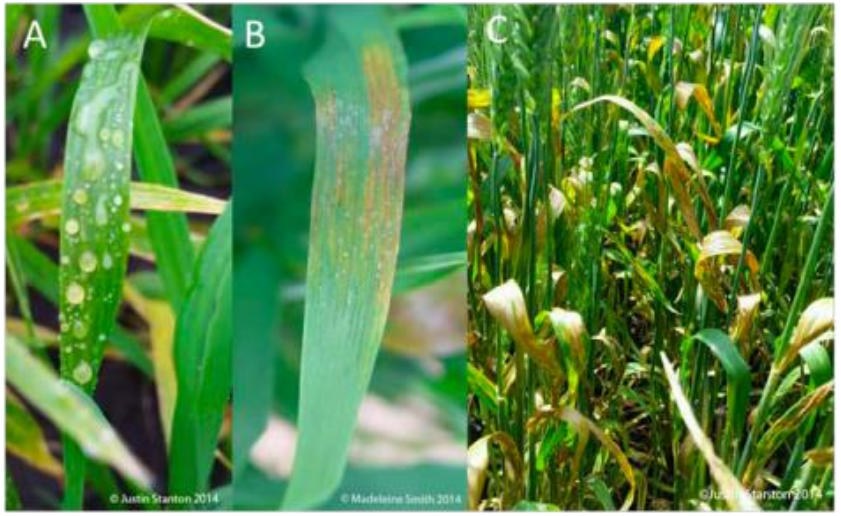
Xanthomonas translucens pv. *translucens*

- Stages of bacterial leaf streak infection:

A. Early symptoms of bacterial oozing,

B. dried bacterial ooze, and

C. advanced necrotic. symptoms.



University of Minnesota Extension,2018

Comparison of epidemics

The patterns of epidemic rates

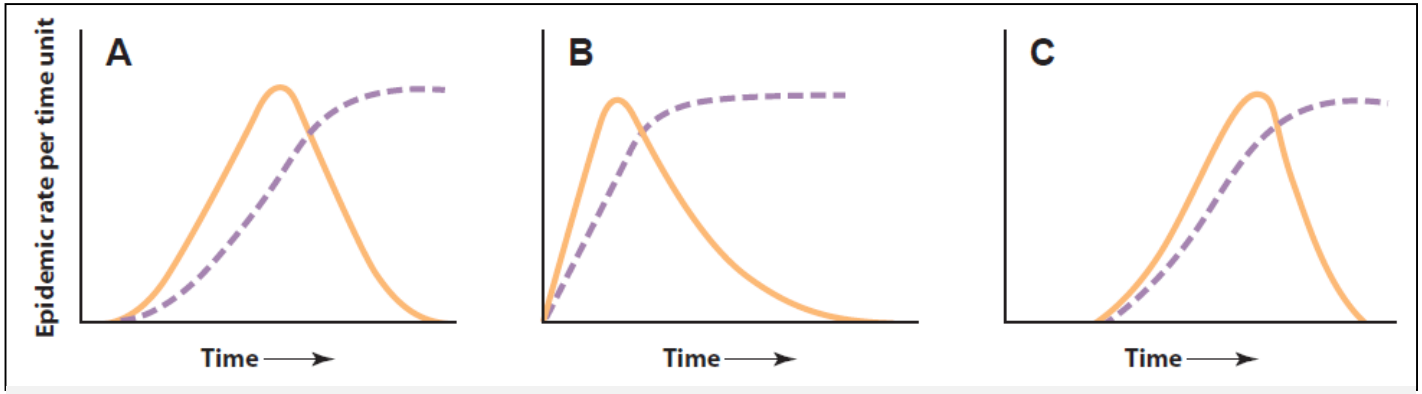
- Control of plant disease is defined as the maintenance of disease severity below a certain threshold, which is determined by economic losses.

1. Diseases may be high in incidence but low in severity, or
2. low in incidence but high in severity, and are kept in check by preventing the development of epidemics.

https://www.thefreedictionary.com/

Comparison of epidemics
The patterns of epidemic rates

- The patterns of epidemic rates are given by curves called rate curves, and these curves are different for various groups of diseases.
- In some diseases, e.g., the late blight of potato (*Phytophthora infestans*), the rate curves are symmetrical (bell shaped) (A).

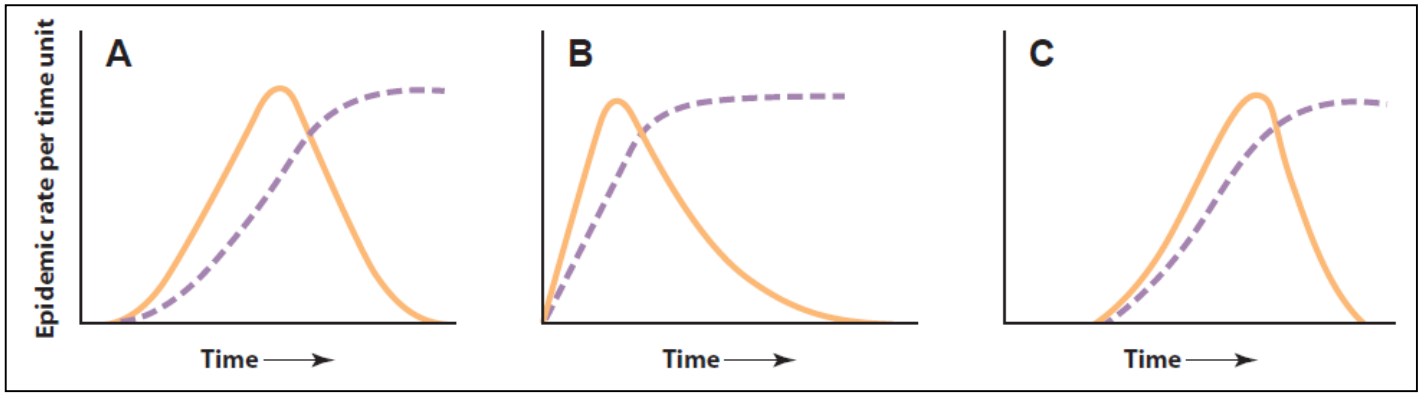


Dashed curves indicate possible disease-progress curves that may be produced in each case from the accumulated epidemic rate curves.

Comparison of epidemics

The patterns of epidemic rates

- In some diseases, e.g., in apple scab(*Venturia inaequalis*) and most downy mildews and powdery mildews, the rate curves are asymmetrical, with the epidemic rate being greater early in the season (B) because of the greater susceptibility of young leaves.

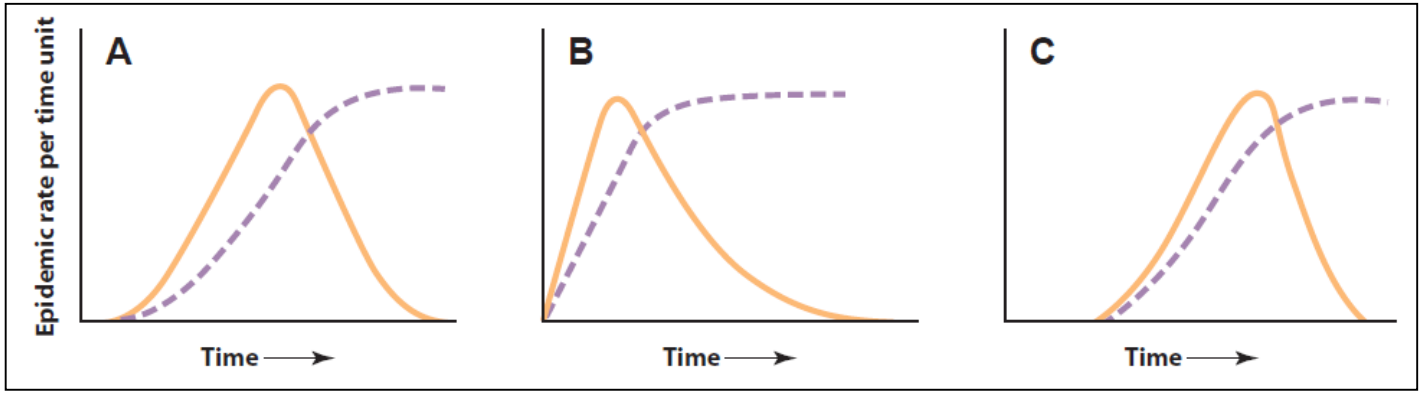


Agrois,2005

Comparison of epidemics

The patterns of epidemic rates

- In still other diseases, the rate curves are asymmetrical, with the epidemic rate being greater late in the season (C). This is observed in the many diseases, e.g., *Alternaria* leaf blights and *Verticillium* wilts, that start slowly but accelerate markedly as host susceptibility increases late in the season.



Agrois,2005

Epidemic rate

Combination of the number of new infection cases (unit) and the amount of time(day, week..)

- In epidemiology, a rate is a measure of the frequency with which an event (disease infections) occurs in a defined population over a specified period of time.
- Because rates put disease frequency in the perspective of the size of the population, rates are particularly useful for comparing disease frequency in different locations, at different times, or among different groups of persons with potentially different sized populations; that is, a rate is a measure of risk.

www.cdc.gov/csels/dsepd/ss1978/lesson3

Epidemic rate
R value

- The epidemic rate is the increase or decrease per unit or time (day, week or year) in a given plant population.
- The effective reproductive number (R) is the average number of secondary cases per infectious case in a population made up of both susceptible and non-susceptible hosts.

1. If R>1, the number of cases will increase, such as at the start of an epidemic.
2. Where R=1, the disease is endemic, and
3. Where R<1 there will be a decline in the number of cases.

Barratt and Kirwan, 2009; Shantikumar,2018

Epidemiology

Difference between endemic, epidemic and pandemic

Epidemic rate: R>1, R=1 and R<1

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2. Where R=1, the disease is endemic, and
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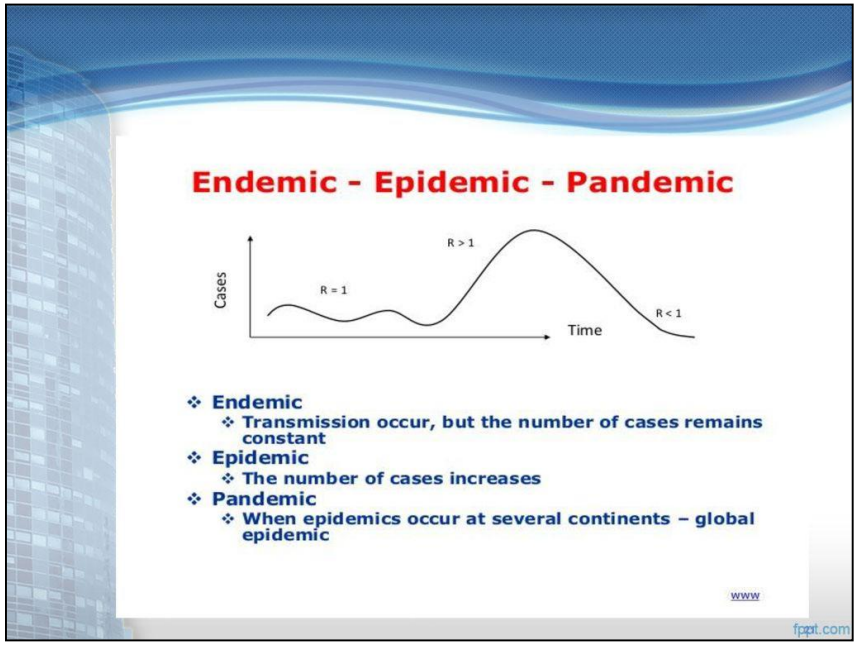


Araz,2018

Epidemic rate

R_0 value

R_0 and the COVID-19 Pandemic

These few slides were prepared because the subject matter of the lesson coincided with the Covid-19 epidemic.

- R_0, or the basic reproduction number/rate, refers to the contagiousness and transmissibility of infectious pathogens.
- **How is R_0 Calculated?**
- R_0 is normally calculated based on 3 parameters:

1. duration of contagiousness after infection,
2. the likelihood of infection between the affected individual and susceptible individual,
3. contact rate.

A disease that is contagious(kuhn·tay·juhs) can be caught by touching people or things that are infected with it.

Shabir,2020

Epidemic rate

R_0 value

R_0 and the COVID-19 Pandemic

- R_0 in an estimate of the speed at which a particular infectious disease can currently spread through a given population.
- Specifically, it refers to the number of people that one person can transmit on average.

1. If the average R_0 in the population is greater than 1, the infection will spread exponentially(rapidly).
2. If R_0 is less than 1, the infection will spread only slowly, and it will eventually die out.
3. The higher the value of R_0, the faster an epidemic will progress.

Shabir,2020;Aronson *et al.*,2020

Epidemic rate

R_0 value

R_0 and the COVID-19 Pandemic

- Specifically, it refers to the number of people that one person can transmit on average.

1. If R_0 >1, then the disease can spread to a wider population (exponentially) from one single person, thus potentially creating an epidemic or pandemic.
2. If R_0 is 1, then 1 person is capable of spreading to 1 other person on average.
3. Typically, the R_0 varies between <1 if the disease is controlled or not spreading too quickly.

Epidemic rate

R_0 value

R_0 and the COVID-19 Pandemic

- If R_0 is greater than 1 where 1 person can infect more than 1 person;
- R_0=2, then 1 person infects 2 people, and those 2 people infect 2 people each, thus 4 people, and the rate exponentially increases) leading to an epidemic – and if not controlled, a global pandemic.

Epidemic rate

R_0 value

R_0 and the COVID-19 Pandemic

- Estimates for the R_0 for COVID-19 vary considerably, but values range between 2.2-2.7, although some estimates place the R_0 at around 5.7.
- This value was based on the assumption that:

1. the virus incubation period was around 4.2 days (time from exposure to symptoms), and
2. a disease doubling time of 2-3 days.

Shabir,2020

Epidemic rate

r or r_I value

Late blight of potato (*Phytophthora infestans*)

- r (or r_I): This denotes the infection rate and is largely what epidemiology is about.
- **r is expressed as X per Unit per Time Period.**
- Late blight of potato (*Phytophthora infestans*) increased in a field of potatoes in the Netherlands at a rate of r =0.42 per unit per day.
- This r value indicates that:

1. The parasite/pathogen is virulent,
2. The host (potato) is susceptible, and
3. The environment is not limiting to the disease.

Partridge,2008

Theories of epidemic development

- Fry (1982) has summarized the three factors, host, pathogen and environment which need to operate over a period of time in an equation as follows:

$$D_t = \Sigma_{i=0}^{t} f(p_i,\ h_i,\ e_i)$$

Σ(sigma symbol) means sum up"

- Where D_t is a measure of disease at time *t*.
- *pi*, *hi* and *ei* are all the pathogen, host and environmental factors, respectively, that contribute to **an increase in disease**.
- f is a factor that relates the interaction of p, h and e over the period *i* =0 to *t* to the amount of disease at time *t*.

Time is represented by t. Can be measured in units of days (common), weeks, months, years. For modeling purposes, t is considered to be continuous. All time values are possible between the beginning (often *t* = 0) and end of the epidemic.

Theories of epidemic development
Polycyclic pathogens

- *Pseudomonas tabaci*, spread through tobacco fields in Virginia so rapidly that the disease it causes was given the name wildfire!
- The inoculum of polycyclic pathogens, unlike that of monocyclic pathogens, increases during the season.
- An equation that takes this into account is as follows:

$$\frac{dx}{dt} = xr(1-x)$$

dy/dt is a continuous function (because of continuous time).

- Where, as before,
- x is the amount of disease on a scale of 0-1,
- r is the exponential rate of disease increase, and
- t is the time under consideration during which host and pathogen have interacted.

Models for disease progress, starting with exponential

Strange,2003

New techniques in epidemiology

Modern techniques

- Disease can be caused by a variety of complex plant pathogens including fungi, bacteria, viruses and nematodes.
- Their management requires the use of techniques in transgenic technology, biochemistry and genetics.
- A comprehensive review is needed of:

1. recent developments in modern techniques, and
2. the understanding of how pathogens cause disease with epidemic potential.

New techniques in epidemiology

- The study of plant disease epidemiology has been facilitated greatly by new methods and new equipment that make possible studies of aspects of plant disease that were impossible or very difficult to study earlier.

New techniques in epidemiology

- Some of the methods and other equipment that have been used to great advantage in plant disease epidemiology include the following:
  - Molecular Tools (PCR, DNA probes etc.)
  - Geographic Information System(GIS)
  - Global Positioning System
  - Geostatistics
  - Remote Sensing
  - Image Analysis
  - Information Technology

Weather monitoring equipments for plant disease forecast/control

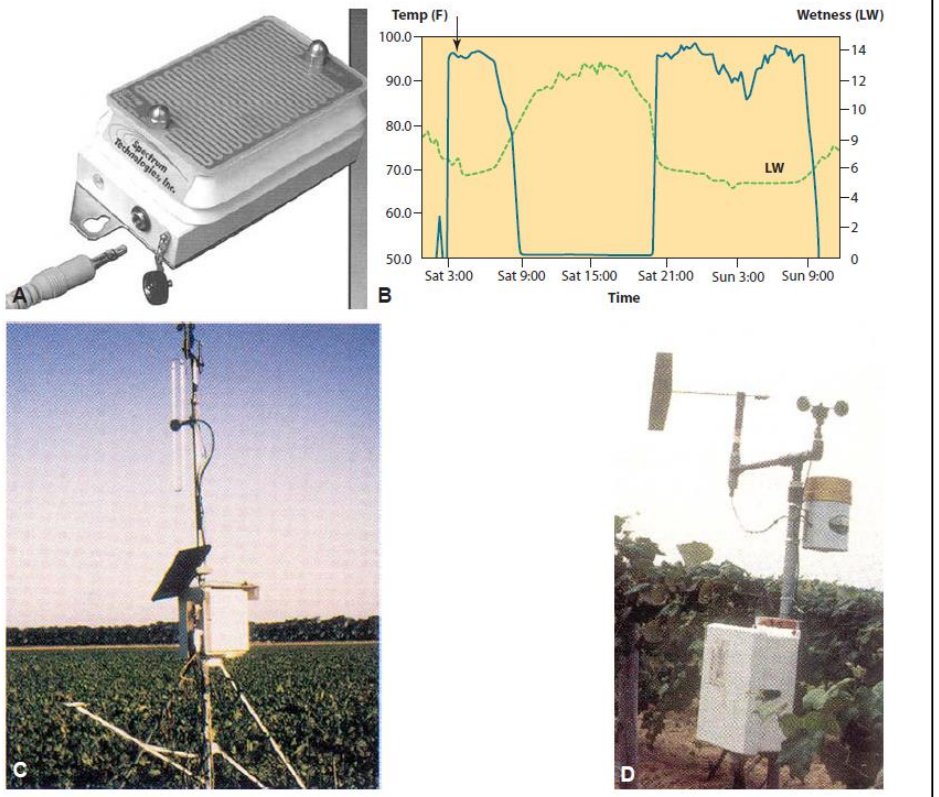


FIGURE 9-28 Weather monitoring equipment for plant disease control. (A) Temperature and surface wetness monitor. (B) A typical graph of the relationship between temperature and surface wetness during a 30-hour period. (C and D) Two types of complete weather monitoring systems (C: From Windels et al. 1998, Plant Dis., 82: 716–726.).

Agrios,2005

Geographic Information System
A computer system

- The geographic information system (GIS) is a computer system, adaptable to operations of any size, and data can be used at any scale from a single field to an agricultural region.
- It is used to better understand and manage the environment, including the understanding and management of plant disease epidemics.
- GIS techniques allow one to make connections between events based on geographic proximity, connections that are essential to the understanding and management of epidemics but which often go unrecognized without GIS.

Geographic Information System
A computer system

- GIS techniques can even incorporate disease forecasting systems, although the time and cost for it may be prohibitive.
- However, as high-resolution weather forecast data are often available, the development of plant disease epidemics can be predicted by knowing:

1. their dependency on some critical weather variable, and
2. from estimated geographic distribution of the pathogen inoculum within a GIS framework.

- GIS is often used for the spatial and temporal analysis of disease development over relatively large geographic areas and helps.

Temporal scale is habitat lifespan relative to the generation time of the organism, and spatial scale is the distance between habitat patches relative to the dispersal distance of the organism.

Spatial and temporal distribution of the pathogen/disease epidemics

- Emerging and re-emerging diseases with pandemic potential continue to challenge fragile health systems in Africa, creating enormous human and economic toll.
- To develop such deployment strategies, knowledge of spatial and temporal distribution of the pathogen is needed.

Spatial and temporal distribution of the pathogen/disease epidemics

- In 2007 and 2008 more than 100 million dollars of fresh market tomatoes were grown in Virginia, with the majority of production occurring on the Eastern Shore of Virginia (ESV), according to the National Agricultural Statistics Service.
- Bacterial wilt of tomato, caused by *Ralstonia solanacearum* (Smith) and Yabucchi et al., is the most devastating disease of tomato on the ESV.
- Four â observational trials' were conducted on the ESV over three growing seasons to determine the temporal and spatial distribution of this disease in commercial tomato fields.

Wimer,2009

Spatial and temporal distribution of the pathogen/disease epidemics

- Plants were assessed at approximately one-week intervals throughout the growing seasons and the incidence of bacterial wilt for each individual plant was recorded.
- A steady increase in both disease incidence and clustered distribution of the disease within rows was observed as the growing season progressed.
- Positive correlations between disease incidence and percentage of rows exhibiting a significant clustered distribution occurred in all trials, which indicated an increase in clustered distribution as disease incidence increased.

Wimer,2009

Spatial patterns of epidemics

- The progress of an epidemic in space, in terms of changes in the number of lesions, the amount of diseased tissue, and the number of diseased plants as it spreads over distance, is called its spatial pattern, i.e., the arrangement of disease entities relative to each other and to the area of cultivation of the crop.
- Spatial patterns of epidemics are influenced by the dispersal of the pathogen, i.e., the process of movement of individuals of the pathogen in and out of the host population or population area, and is given by a curve that is called the dispersal or disease-gradient curve.

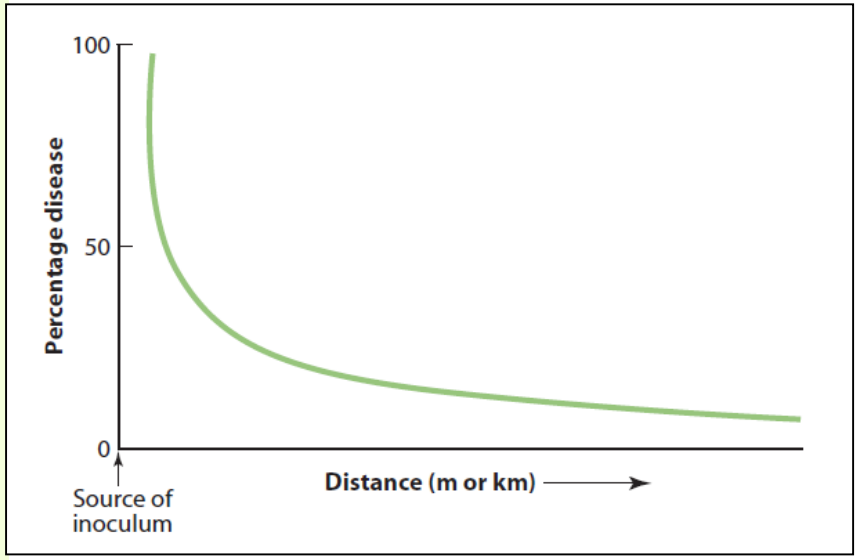
Agrios,2005

Spatial patterns of epidemics

Schematic diagram of a disease-gradient curve

- The percentage of disease and the scale for distance vary with:

1. the type of pathogen or its method of dispersal,
2. being small for soilborne pathogens or vectors and larger for airborne pathogens.



Agrios,2005

Methods of disease assessment

1. **Disease incidence and disease severity;**
2. **AHP: Analytic Hierarchy Process (assessment of the health and economic impact of the diseases)**

Methods of disease assessment

Yield losses

- Yield was divided into:
- Attainable yield- when crops were grown under optimum conditions;
- Primitive yield- when no disease control was applied;
- Economic yield- highest net return on expenditure;
- Actual yield-obtained using disease management programmes;
- Theoretical yield- obtained using calculations based on crop physiology or crop growth simulation models.
- The difference between actual and attainable yield was the method used by the Food and Agriculture Organization (FAO) to report crop losses.
- Most disease management programmes aim to close the gap between these two yield concepts.

Methods of disease assessment
Measurement of yield loss

- Yields of plant products are generally non-controversial and are usually recorded in terms of weight or number.
- Cereal yields, for example, are usually measured in terms of 1000-grain weight, spikelets per tiller, numbers of tillers and kg or metric tonne per hectare.
- For example, Adhikari and co-workers (1999) measured losses of rice caused by *Xanthomonas oryzae* pv. *oryzae* as reductions in the number of tillers, grains per panicle and 1000-grain weight.
- Quality is highly prized and so, unfortunately, is uniformity.

Methods of disease assessment

Yield losses

Disease incidence and disease severity formula

- Disease can be measured by:
- Direct methods, i.e. measuring disease on the plant, or by
- Indirect methods, e.g. monitoring the pathogen population.
- Direct methods have been more widely used because they are better correlated with losses in production than the indirect methods, which are rather laborious and time-consuming (James and Teng,1979).
- Direct methods measure disease as incidence or severity, as defined below.
- The term disease intensity is often used to denote either incidence or severity.

$$\text{Disease incidence } (I) \text{ (Frequency)} = \frac{\text{Number of infected plant units}}{\text{Total number (healthy and infected) of units assessed}} \times 100$$

$$\text{Disease severity } (S) \text{ (Area)} = \frac{\text{Area of plant tissue affected by disease}}{\text{Total area}} \times 100$$

Plant Pathologist's Pocketbook,2002

Methods of disease assessment

Yield losses

Disease incidence and disease severity formula

- Disease incidence(DI): No. of infected plants x100/Total no. of plant assessed.
- Disease severity(DS): is the percentage of relevant host tissues or organ covered by symptom or lesion or damaged by the disease. Severity results from the number and size of the lesions.

Plant Pathologist's Pocketbook,2002

Methods of disease assessment

Yield losses

Disease incidence and disease severity formula

- The disease severity is estimated by a rater as a value on the interval scale and has been used to determine a disease severity index (DSI) on a percentage basis, where DSI (%) = [sum (class frequency × score of rating class)] / [(total number of plants) × (maximal disease index)] × 100.
- Severity of symptoms on individual plants was rated on a scale from 0 to 4 according to percentage of foliage with yellowing or necrosis in acropetal progression: 0 = 0%, 1 = 1 to 33%, 2= 34 to 66%, 3 = 67 to 100%, and 4 = dead plant.

Methods of disease assessment

Yield losses

Assessment of yield loss

- The assessment of yield loss was carried out mainly based on yield comparisons between infected and healthy plants or between plants with different disease severities using field plots, micro plots (hill plots), single plants or tillers; between resistant and susceptible varieties; between infected plants and plants treated with fungicides; or between healthy plants and plants where disease damage has been simulated by the removal of essential plant organs, such as the flag leaf on a cereal plant (Cooke, 2006).
- Percent yield loss (%YL) in terms of grain weight was calculated as follows (Mousanejad *et al.*,2010).

$$\%\ YL = \frac{\text{Yield in intensive protected plot - Yield in particular treatment}\quad X100}{\text{Yield in intensive protected plot}}$$

Gashaw *et al.*,2013

Methods of disease assessment

Yield losses

Disease incidence and disease severity formula

- Incidence of coffee bacterial blight (*Pseudomonas syringae* pv. *garcae*) was assessed by counting the number of diseased plants per total number of plants inspected and expressed as percentage of total plants as described by CABI, (2006). Per cent disease incidence was competed according to the following equation.

$$\text{Disease incidence } \% = \frac{\text{Number of diseased plants}}{\text{Total number of plants inspected}} \text{x100}$$

- The number of infected leaves per branch, number of infected branches and/or twigs per tree were used to rate the percentage of disease severity.

$$\text{Disease severity } \% = \frac{\text{Number of diseased twigs, leaves, priymery branches}}{\text{Total plant part}} \text{x100}$$

Hinkosa *et al.*,2017

Plant disease ratings

Pictorial/visual disease assessment keys available for measuring disease severity on a range of hosts

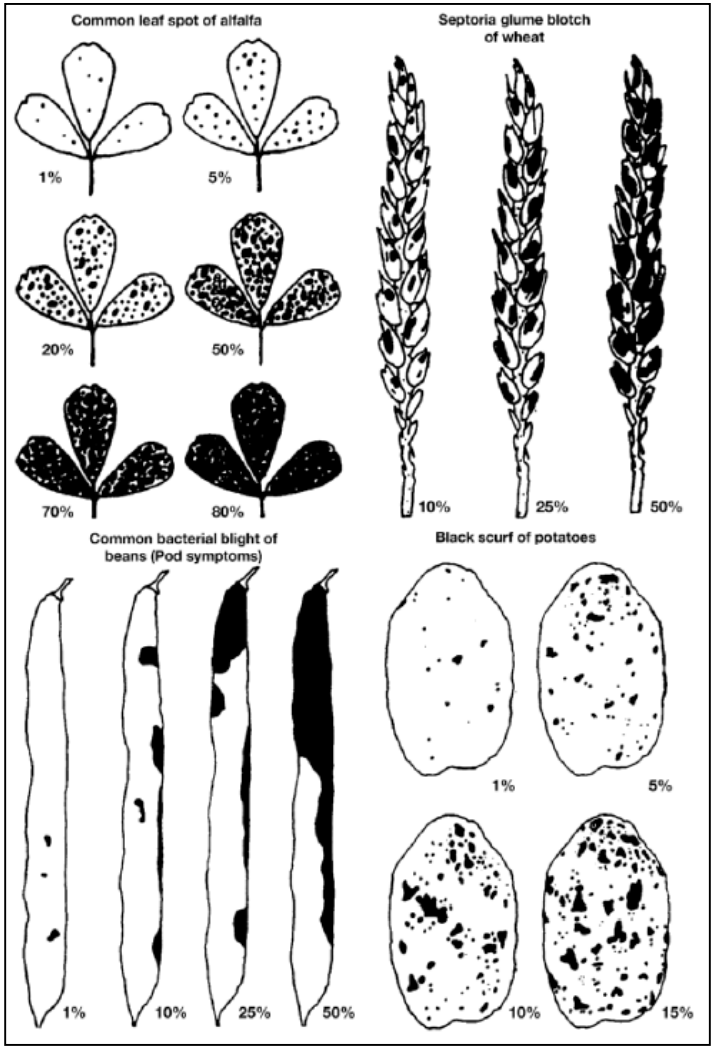
- The pictorial disease assessment key uses standard area diagrams that illustrate the developmental stages of a disease:

1. On small simple units (leaves, fruits) or
2. On large composite units such as branches or whole plants.

Disease ratings

Pictorial disease assessment keys available for measuring disease severity on a range of hosts

- Examples of pictorial/visual assessment keys for estimating disease severity (after James,1971).



COOKE, B.M.; D. GARETH JONES and B. KAYE (Eds.).2006.

Cucurbit Bacterial Fruit Blotch

Symptoms severity scale

Acidovorax avenue subsp. *citrulli*

- Disease rating was on a 0-9 scale when the disease was uniformly distributed across the field:
- 0= no symptoms,
- 1-2 = trace,
- 3-4 = slight,
- 5-6 =moderate,
- 7-8 = severe
- 9 = dead.

Ma *et al.*,2014

Cucurbit Bacterial Fruit Blotch

Rating scale for bacterial leaf blotch of watermelon *Acidovorax avenue* subsp. *citrulli*

- Five leaflets were selected at random among the 150 plant stands rated.
- Visual observation of the selected leaflets was carried out and the severity recorded.
- Results represent the mean rating.

Scale	Description	Inference
0	No symptoms on leaves	No Infection
1	1 - 25% leaf area covered with lesions	Mild Infection
2	26 - 50% leaf area covered with lesions	Moderate Infection
3	51 - 75% leaf area covered with lesions	Severe Infection
4	76% and above	Very Severe/Devastating

Amadi *et al.*,2009

USDA Fire Blight Scoring System
Erwinia amylovora

- **The scale is a descending rating from 10 to 1:**
- 10 = no blight;
- 9 = 1-3%, current season wood only;
- 8 = 4-6%, 1 - to 2-year-old wood;
- 7 = 7-12%, 1- to 3-year-old wood in upper 1/8 of tree;
- 6 = 13-25%, 2- to 3-year-old or older wood and in upper 1/4 of tree;
- 5 = 26-50%, 3-year-old or older wood and in upper 1/2 of tree;
- 4 = 51-75%, older wood in lower 1/2 of tree;
- 3 = 76-88%, old wood in lower 1/4 of tree;
- 2 = 89-99%, base of trunk and
- 1 = 100%, tree dead.

Quamme,1997

Blossom infection severity scale
Erwinia amylovora

- Blossom infection severity scale based on tissue infected:
- 0 = no infection;
- 1 = receptacle;
- 2 = pedicle;
- 3 = basal tissue of cluster;
- 4 = spur of 1-year old wood;
- 5 = spur-bearing or 2-year old wood;
- 6 = wood 3-year old or older.

USDA,2013

Qualitative measurement of symptoms *Pectobacterium carotovorum*

- Disease incidence: To calculate disease incidence (%),

$$\frac{number\ of\ diseased\ plant}{total\ number\ of\ plants} \times 100$$

- Plants were randomly picked and 3 plants were selected for sampling from each treatment. All plants it stem rots and tubers showing signs and symptoms of soft rot disease were regarded as diseased plants.
- Stem rot severity:
- Disease severity was assessed on a scale of 0-3 as reported by Wright *et al.*,2005 where:
- 0 no disease symptoms on plant
- 1 less than 50% of the plant has disease symptoms
- 2 more than 50% of the plant has disease symptoms
- 3 plant totally dead.
- Plants were randomly picked from the plot for sampling and were assigned to the scale accordingly

Tuhwe,2015

Qualitative measurement of symptoms *Pectobacterium atrosepticum*

- Disease incidence: On the appearance of first symptom of the disease, incidence was recorded as given by James (1969):

$$\text{Disease incidence} = \frac{\text{No. of affected plants/unit area}}{\text{Total no. of plants/unit area}} \times 100$$

- Disease severity: was assessed by visual rating scale (0-7) based on parent plant, tuber surface showing symptoms (Ahmad *et al*.,1995): 1, No symptoms; 2, 1 to 10% plant/leaf area affected; 3, 11 to 20% plant/leaf area affected; 4, 21 to 30% plant/leaf area affected; 5, 31 to 40% plant/leaf area affected; 6, 41 to 50% plant/leaf area affected; 7, 51% or more area affected.
- The susceptible and resistant varieties were screened against blackleg disease of potato by the above mentioned scale.

Rashid *et al*.,2012

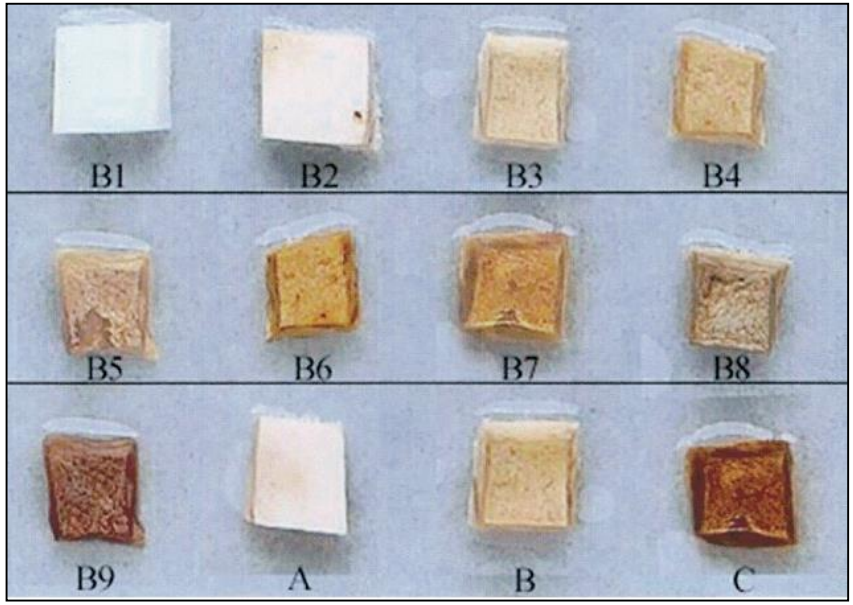
Qualitative measurement of symptoms
Cube pathogenicity bioassay
Pseudomonas tolaasii

- 1-day-old *A. bisporus* cubes (1 cm^3) of cap tissue were excised with sterile scalpel blades and placed into a sterile petri dish containing a 50-mm-pore-size paper filter dampened with 800 ml of sterile double-distilled water.
- Four cubes were placed 2 cm apart to eliminate cross-contamination by motile pseudomonads.
- Bacterial strains were cultured in KB medium to a density of 10^9 CFU/ml^{-1}, and a 50-µl aliquot of cells was placed onto three cubes.
- The fourth cube was inoculated with a 50-µl control of uninoculated KB.
- Petri dishes were sealed with parafilm and incubated under ambient conditions for 24 h.
- Mushroom caps incubated with bacterial isolates were scored for the degree of blotch discoloration on a scale of B1 to B9 (where B5 blotch).

Godfrey *et al.*,2001

Qualitative measurement of symptoms
Cube pathogenicity bioassay
Pseudomonas tolaasii

- Bioassays to determine the capability of bacterial isolates in inducing discoloration of *A. bisporus* tissue to varying degrees.
- Pictured are cubes within the assigned color scale, B1 through B9. B= Blotch
- B1, cube inoculated with KB alone (control).
- B2, 3.1%[n=3], B3, 36.8%[n=35], B4, 10.5%[n=10], B5, 11.6%[n=11], B6, 11.6%[n=11], B8, 2.1%[n=2] and B9, 4.2%[n=4].



The following reference strains are included for comparison: A, *P. reactans* NCPPB 1311 (B2); B, *P. gingeri* NCPPB 3147T (B5); C, *P. tolaasii* NCPPB 2192T (B9).

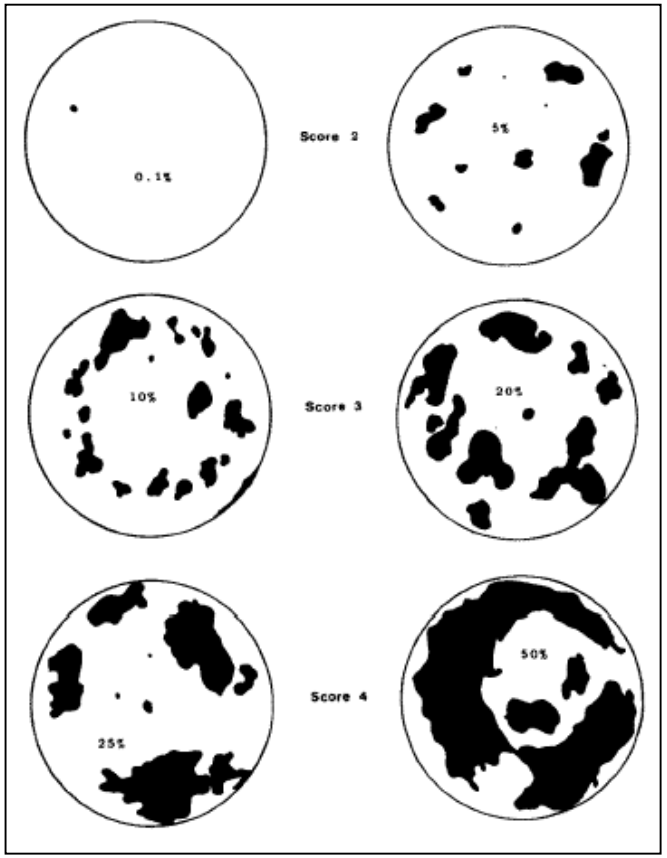
Godfrey *et al.*,2001

Assessment of disease severity

Scaled severity score

Pseudomonas tolaasii

- **Area of mushroom caps covered by brown lesions.**
- Six disease symptom area diagrams, drawn from diseased cultivated mushrooms and selected as standard diagrams, with 0.1%, 5%, 10%, 20%, 25% and 50% of the cap surface affected by bacterial blotch lesions.
- In any experimental treatment, or sample of a mushroom crop, each mushroom examined was given a score of 1,2,3 or 4 according to whether the extent of disease on the mushroom, compared with the standard diagrams, was 0, 0.1-5%, 10-20%, or 25% or above.
- Overall blotch disease severity was evaluated and symptoms severity scale was determined.



Wong and Preece,1982

Assessment of disease severity

Scaled severity score

Pseudomonas tolaasii

- Disease severity for the two flushes of fruiting bodies was assessed according to the affected area based on the modified method of Wong et al. (Wong and Preece, 1982).
- Each of the mushroom fruiting bodies examined in this study was given a score of 0, 1, 2, 3 according to the size of the blotch:
- 0=no symptom,
- 1=slight symptom development, with few small spots on the pileus (0.1-1 % area covered by blotch),
- 2=moderate symptom development, with many small spots on the pileus (1-5% area covered by blotch),
- 3=severe symptom development, with many spots or large blotches on the pileus (5-10% area covered by blotch).
- The average disease severity of each strain was calculated.

Wong and Preece,1982

Assessment of disease severity

Disease severity was determined according to the size of the blotches

Pseudomonas tolaasii

Strains	Origin names	Pileus color[a]	Inoculation on caps		Inoculation on substrates		
			Disease severity	The color of blotch	Disease severity[b]	The color of blotch	Resistance[c]
ACCC50618	Yefeng 118	Grey	2	Brown	0.45±0.51a	Brown	R
ACCC50236	Tebai 1	White	2	Yellow	1.15±0.75b	Yellow	MS
ACCC50075	1112	Pale grey	2	Brown	2.35±0.81c	Brown	S
ACCC50116	8010	Grey	2	Brown	2.35±0.81c	Brown	S
ACCC50150	Nongda 11	Grey	2	Brown	2.35±0.81c	Brown	S
ACCC50168	PI-27	Grey	2	Brown	2.35±0.88c	Brown	S
ACCC50495	Jinong 11	Grey	2	Brown	2.35±0.75c	Brown	S
ACCC51550	Jiangdu 5178	Black	2	Brown	2.35±0.88c	Brown	S
ACCC50020	DP02	Pale brown	2	Brown	2.40±0.82c	Brown	S
ACCC50476	Yaguang 1	Brown	2	Brown	2.40±0.82c	Brown	S
ACCC52305	Shiji 3	Brown	2	Brown	2.40±0.88c	Brown	S
ACCC50060	ZM5.23	Grey	2	Brown	2.45±0.83c	Brown	S
ACCC50122	Yunnanbai	Brown	2	Brown	2.45±0.76c	Brown	S
ACCC50123	Ping 2	Brown	2	Brown	2.45±0.83c	Brown	S
ACCC50838	99	Black	2	Brown	2.45±0.83c	Brown	S
ACCC51604	650	Grey	2	Brown	2.45±0.83c	Brown	S
ACCC50165	Zhongshu 10	Pale grey	2	Brown	2.50±0.76c	Brown	S
ACCC50948	Nongke 5	Black	2	Brown	2.50±0.76c	Brown	S
ACCC51123	Qingdaohei	Grey	2	Brown	2.50±0.76c	Brown	S
ACCC51371	Xiuzhengu	Brown	2	Brown	2.50±0.83c	Brown	S
ACCC51557	Jiyin2005	Grey	2	Brown	2.50±0.83c	Brown	S
ACCC51568	Guanping 1	Pale grey	2	Brown	2.50±0.83c	Brown	S
ACCC50121	EA38	Grey	2	Brown	2.55±0.76c	Brown	S
ACCC50596	Xide 89	Grey	2	Brown	2.55±0.76c	Brown	S
ACCC50865	Nanjing 1	Brown	2	Brown	2.55±0.76c	Brown	S
ACCC51372	pg2	Brown	2	Brown	2.55±0.76c	Brown	S
ACCC51652	39	Grey	2	Brown	2.55±0.76c	Brown	S
ACCC51933	Guangdong	Grey	2	Brown	2.55±0.83c	Brown	S
ACCC50050	Ce813	Brown	2	Brown	2.60±0.68c	Brown	S
ACCC51340	Jiangdu2026	Grey	2	Brown	2.60±0.68c	Brown	S
ACCC51553	Kangbing 2	Grey	2	Brown	2.60±0.75c	Brown	S
ACCC51570	Daxing	Grey	2	Brown	2.60±0.75c	Brown	S
ACCC51601	615	Brown	2	Brown	2.60±0.75c	Brown	S
ACCC51942	Shuangkang	Grey	2	Brown	2.60±0.75c	Brown	S
ACCC51423	Heixiuzhegu	Brown	2	Brown	2.65±0.75c	Brown	S
ACCC51556			2	Brown	2.75±0.55c	Brown	S
ACCC51599			2	Brown	2.75±0.55c	Brown	S

[a]The colour of the pileus fruiting and growing at 16 °C. The pileus colour of *P. ostreatus* varied at different temperature. The temperature was lower, the pileus color was darker for the oyster mushroom

Zhang *et al.*,2013

Qualitative measurement of symptoms *Pseudomonas syringae* pv. *apii*

- Carborundum, an abrasive powder used to make minute wounds in leaf tissue, was added to the culture.
- Ten- to 12-wk-old celery plants (*Apium* sp.) were inoculated by dipping sterile cotton swabs into the culture and rubbing the swabs onto leaves that had expanded one-half to three-quarters.
- For negative controls, plants were inoculated with nutrient broth plus carborundum; as a positive control, the *P. syringae* pv. *apii* strain was used.
- Plants were maintained in a greenhouse, and 7 to 10 days after inoculation, we rated disease severity on inoculated leaves on the following scale:
- 0 = no disease reaction
- + = localized necrosis or chlorosis around area of inoculation
- ++ = water-soaked brown lesions developing at and around the point of inoculation
- +++ = large expanding brown water-soaked lesions with entire area becoming necrotic.

Koike *et al.*,1994

Olive knot

Interaction between *Pseudomonas savastanoi* pv. *savastanoi* and *Pantoea agglomerans* in olive knots

- Symptoms were classified on a 6-point scale from 0 to 5:
- 0, healed wound – a thin layer of plant tissue covering the entire wound surface;
- 1, wound margins slightly sunken, the centre of the wound not covered with newly formed plant tissue;
- 2, wound margins slightly sunken, the centre of the wound covered with new tissue;
- 3, the centre of the wound surrounded by an irregular mound of new tissue;
- 4, entire wound covered with an irregular mound of new tissue;
- 5, large raised knot.

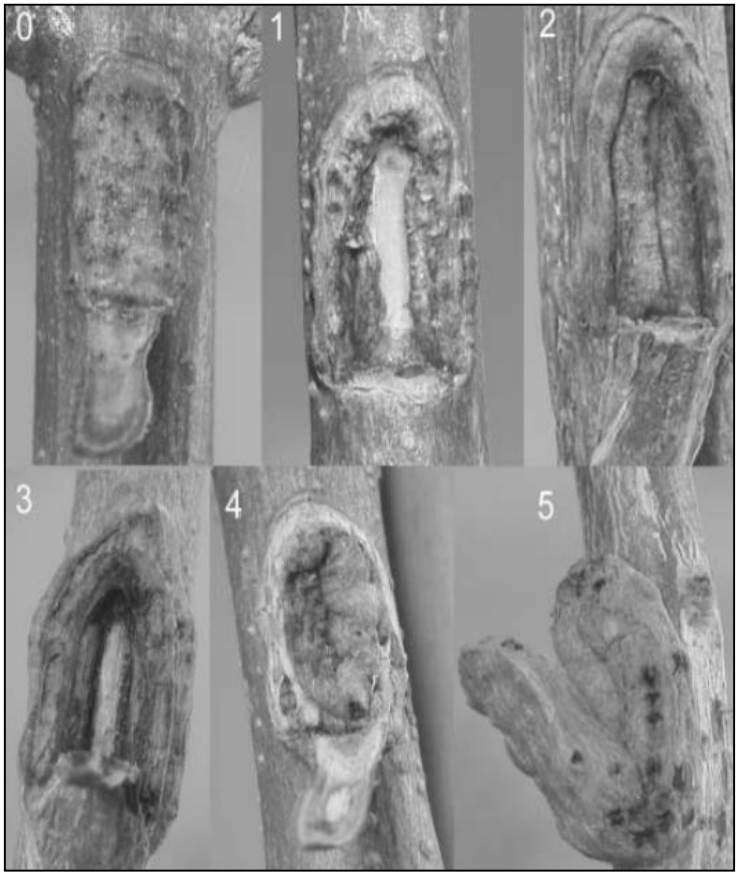
Marchi *et al.*,2006

Olive knot

Interaction between *Pseudomonas savastanoi* pv. *savastanoi* and *Pantoea agglomerans* in olive knots

- Symptoms on 1-year-old olive stems 60 days after inoculation

1. With *Pantoea agglomerans*
2. With *Pseudomonas savastanoi* or
3. With a suspension of those bacteria mixed in a ratio of 1:1.

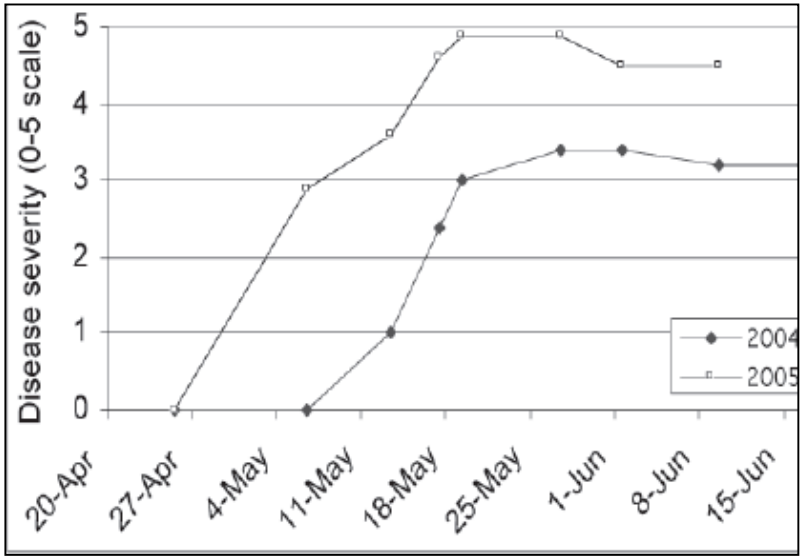


Marchi *et al.*,2006

Bacterial blight of Cornelian cherry

Pseudomonas syringae

- Development of Bacterial leaf blight in *Cornus mas* under 50% shadecloth.
- Disease severity was evaluated on a scale of 0 to 5 in which:
- 0= no infection,
- 1=1% to 10%,
- 2= 11% to 25%,
- 3= 26% to 50%,
- 4= 51% to 75%,
- 5= 76% to 100% of foliage showing disease symptoms.



Mmbaga and Nnosu,2006

Disease index

Ralstonia solanacearum

- The disease index (DI) was determined periodically according to the key proposed by Winstead and Kelman (1952) describing the wilt symptoms in the plant as follow:
- 0= no symptoms; 1= one or 2 leaves wilted; 2= three leaves wilted; 3= four or more leaves wilted and 4= plant died.
- Disease index (DI) was calculated by the following formula:

$$\text{Disease index (DI)} = \frac{\sum R\,T \quad X\,100}{4N}$$

- Where, R= disease severity scale (0, 1, 2, 3 and 4); T= number of wilted plants in each category and N= total number of tested plants.

Mikhail *et al.*,2012

Rate of disease severity

Ralstonia solanacearum

- Wilt severity was determined by calculating the proportion of wilted leaves in each tomato plant as follows:

$$\frac{\text{No. of wilted leaves per plant}}{\text{Total No. of leaves per plant}} \times 100$$

Isolates	Disease severity (%)		
	7	15	25 days
1F[a]	33.3	62.0	100.0
2F	17.2	40.0	66.2
3F	22.1	48.2	70.5
4F	33.3	60.6	75.5
5F	16.6	37.5	50.6
6B[b]	33.3	62.6	77.5
7B	50.0	77.6	100.0
8B	37.4	50.2	83.2
9B	16.6	40.9	66.7
10F	16.6	35.2	61.2
11F	33.3	70.5	82.1
12F	17.2	40.0	66.2
13B	25.0	50.8	69.1
14F	16.6	38.2	65.5
15F	17.2	41.5	62.1
16B	37.4	50.2	80.2
17F	33.3	62.6	77.5
18F	16.6	48.6	82.5
19B	50.0	79.2	100.0
Control (uninoculated)	0.0	0.0	0.0

Source of isolates: [a]F: Minufia; [b]B: Behera

Tawfik *et al.*,2008

Maceration rating disease scale
On detached onion bulb scales
Burkholderia cepacia complex

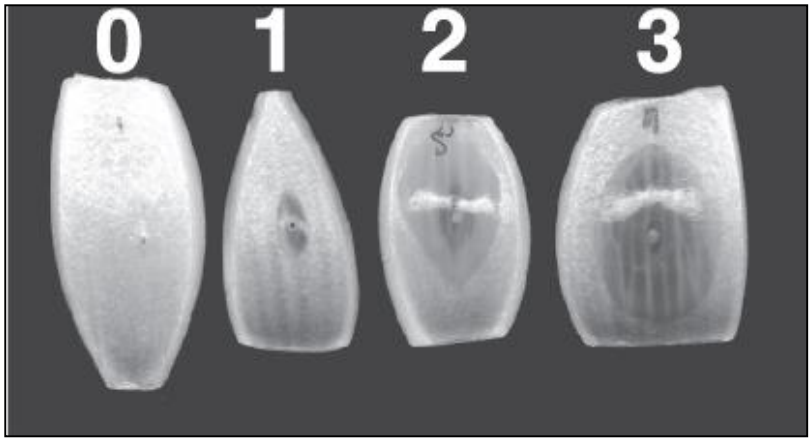
- Individual onion scales were wounded on the inner surface with a sterile pipette tip (1- to 200 µl volume), and 5 µl of bacterial culture (10^7 CFU/ml) was inoculated into the wound.
- The onion scales were incubated at 30°C for 48 h.
- The degree of maceration was estimated by probing with a toothpick (toothpick method).
- A rating scale of 0 to 3 was used to indicate the degree of tissue maceration.
- A rating of 0 indicated no maceration,
- 1 indicated 1 to 33% macerated tissue area,
- 2 indicated 34% to 66% macerated tissue area, and
- 3 indicated 67% to 100% macerated tissue area.

Jacobs *et al.*,2008

Maceration rating disease scale

On detached onion bulb scales

Burkholderia cepacia complex



Species	No. of isolates	No. of isolates with an onion pathogenicity rating of[a]:			
		0	1	2	3
B. cepacia	160	0	23	52	85
B. cenocepacia	480	0	46	174	260
B. ambifaria	623	4	143	223	253
B. pyrrocinia	27	10	13	3	1

A rating of 0 indicated no maceration, 1 indicated 1 to 33% macerated tissue area, 2 indicated 34% to 66% macerated tissue area, and 3 indicated 67% to 100% macerated tissue area.

Jacobs *et al.*,2008

Qualitative measurement of symptoms

Visual disease severity assessment citrus of canker

Xanthomonas citri pv. *citri*

- The severity of canker in each 5-tree block was rated visually on the following scale:
- 0 = no symptoms,
- 1 = isolated leaf lesions,
- 2 = lesions restricted to one side of the canopy,
- 3 = lesions distributed over the entire canopy, and
- 4 = greater occurrence of leaf lesions than in 3.

Agostini *et al.*,1985

Qualitative measurement of symptoms
Bacterial Blight of Caladium
X. axonopodis pv. *dieffenbachiae*

- The leaves of all plants, both abaxial and adaxial surfaces, were sprayed with inoculum, except that the three strains were mixed in equal proportions after their concentrations were adjusted to $O.D._{590nm}$= 0.1 to ensure that results will apply to the wide range of Xad strains encountered in the field.
- Inoculated plants were maintained in the greenhouse for 6 weeks(Greenhouse evaluation).
- Disease severity ratings (DSRs) were taken at 3 and 6 weeks postinoculation (WPI) using the 0 to 11 Horsfall-Barrett scale for area of leaf infection, in which:
- 0 = 0%, 1 = 0% to 3%, 2 = 3% to 6%, 3 = 6% to 12%, 4 = 12% to 25%, 5 = 25% to 50%, 6 = 50% to 75%, 7 = 75% to 88%, 8 = 88% to 94%, 9 = 94% to 97%, 10 = 97% to 100%, and 11 = mortality (Horsfall and Cowling,1978).

Seijo and Peres,2010

Qualitative measurement of symptoms
Bacterial Blight of Caladium
Xanthomonas axonopodis pv. *dieffenbachiae*

- Evaluation of commercial cultivars in the field:
- Disease severity ratings (DSRs) were taken on 25 Sept. 2007 using a 0 to 5 scale:
- 0 = no BB lesion,
- 1 = one to five BB lesions per 30-plant plot,
- 2 = lesions present on less than 50% of leaves,
- 3 = lesions on 50% to 90% of leaves,
- 4 = lesions on greater than 90% of leaves, and
- 5 = lesions on greater than 90% of leaves plus significant defoliation observed).

Seijo and Peres,2010

Rating scale used for evaluating cotton lines against *Xanthomonas malvacearum*

- 10 leaves, 4 from bottom,4 for middle and 2 from top were collected per plant and scored on the 7-grade system(Santhanam,1967) and the average grade point per plant determined(instead of grading the plant by the best grade noticed on each plant).
- The average grade point so obtained was rated as follows:

Average grade point	Disease incidence	Rating
4 and above	Severe	Highly susceptible
3.9 to 2.0	Moderate	Susceptible
1.9 to 0.1	Traces	Resistant
0	No disease	Immune

Verma and Singh,1970

Disease severity index (DSI)

Determine a disease severity index (DSI) on a percentage basis

- DSI is a metric that analysts use to determine the efficiency of sales.
- Disease severity first was assessed by visual rating scale (0-??) based on disease symptoms.
- Then, disease severity index (DSI) in percent was calculated as fallows:
- DSI (%) = [sum (class frequency × score of rating class)] / [(total number of plants) × (maximal disease index)] × 100
- Example of DSI(%):

$$DH(\%) = \frac{\text{No. of infected leaves} \times \text{scale1} + \text{No. of infected leaves} \times \text{scale2} + \text{No. of infected leaves} \times \text{scale3}}{\text{Total number of leaves} \times \text{Maximal scale}} \times 100$$

Disease severity index (DSI)

Determine a disease severity index (DSI) on a percentage basis

- Disease severity first was assessed by visual rating scale (0-??) based on disease symptoms.
- Then, severity (dimensions of lesions on attacked leaves) was calculated using the following formula:
- Disease Severity of Index (DSI): DSI = $\{(a_1N_1 + a_2N_2 +...+ a_nN_n)/(\text{number of plants scored} \times 9)\} \times 100$ where:
- a is the score of each plant,
- N is the of plants with a certain score, and
- 9 is the maximal score of the most infected plants among different treatments.

Hastuti and Saraswati,2012; Sarra *et al.*,2010

Disease severity index (DSI)

Disease grade scale

Xanthomonas malvacearum

- Five leaves each at bottom, middle and top were observed and scored using the 0-4 scale prescribed by Sheo Raj, 1988 as given below:

Grade point	Per cent of leaf area infected	Reaction
0	Completely free from foliar diseases	Immune
1	1-10% infection	Highly resistant
2	11-20% infection	Moderately resistant
3	21-40% infection	Moderately susceptible
4	>40% infection	Highly susceptible

- The percentage disease intensity/severity (DSI (%) were recorded on experimental plot and calculated by using formula:

$$\text{Per cent disease intensity} = \frac{\text{Sum of all disease rating scale}}{\text{No. of rating x maximum disease grade}} \times 100$$

Kharat,2018

Disease severity index (DSI)

Disease grade scale

Disease grade	Total rating	No. of ratings
0	5	0
1	5	5
3	8	24
5	4	20
7	8	56
9	4	36
	34	186

Sum of all ratings = 186; total ratings = 34

Max. Disease grade = 9

Dis. Severity = {186/ 34 x 9} X 100

= 60%

Maximal disease index in this case is 9.

P. N. Sharma

Qualitative measurement of symptoms
Visual disease severity assessments
Bacterial blight caused by *X. malvacearum*

- Infection of plants by pathogens gives rise to a variety of symptoms which also vary according to the severity of the attack.
- Several scales have been proposed for visual disease severity assessments.
- One of these, a 1-4 scale, is given for bacterial blight caused by *Xanthomonas axonopodis* pv. *malvacearum*.

Ratings in the 1-4 range:

- Rate of progression of symptoms:
- The disease grade scale was based on the sizes of the macroscopically visible water-soaked areas:
- 0 = no water-soaking;
- 1 = pinpoint-sized dots;
- 2 = small, round speckles (≈ 0.3 mm);
- 3 = merged angular patches; and
- 4 = confluent areas.
- Intermediate grades between each of the established grades, e.g. 1.3, 2.7, and 3.3, were sometimes recorded (Essenberg *et al.*,2002).

Qualitative measurement of symptoms Bacterial blight in susceptible varieties *Xanthomonas malvacearum*

1. Seedlings: Disease incidence can be assessed by inspecting at least 20 randomly selected sets of ten plants –carefully checking the undersurface of cotyledons and leaves for the presence or absence of bacterial blight.
2. Leaf symptoms: Disease severity can be assessed on the basis of 'percentage leaf area infected' using a pictorial assessment key.

- Either assess every leaf on ten randomly selected plants or assess disease severity on the lowest one, two or three mainstem leaves on each of 20 randomly selected plants.

3. Bolls: The percentage of bolls with blight can be estimated by inspecting all bolls on at least ten randomly selected plants.

- It is important to peel back the calyx crown when checking each boll.

Allen *et al.*,2000. The Australian Cotton Cooperative Research Centre

Qualitative measurement of symptoms
Xanthomonas malvacearum

Seedling diseases (Seedling mortality) – Count plants /metre at at least 20 randomly selected sites across field

Plants/m																			Mean =

Divide by seed rate to calculate Seedling mortality.

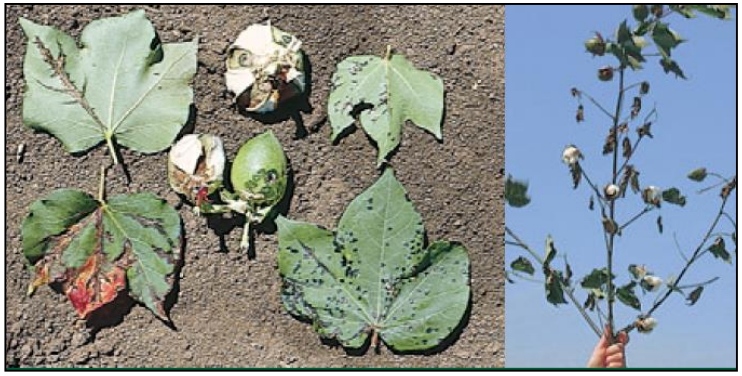
Bacterial blight (incidence) – Count affected plants in at least 10 groups of 10 plants

Plants/10																			Mean =

Bacterial blight of bolls (incidence) - Inspect all the bolls on at least 10 randomly selected plants

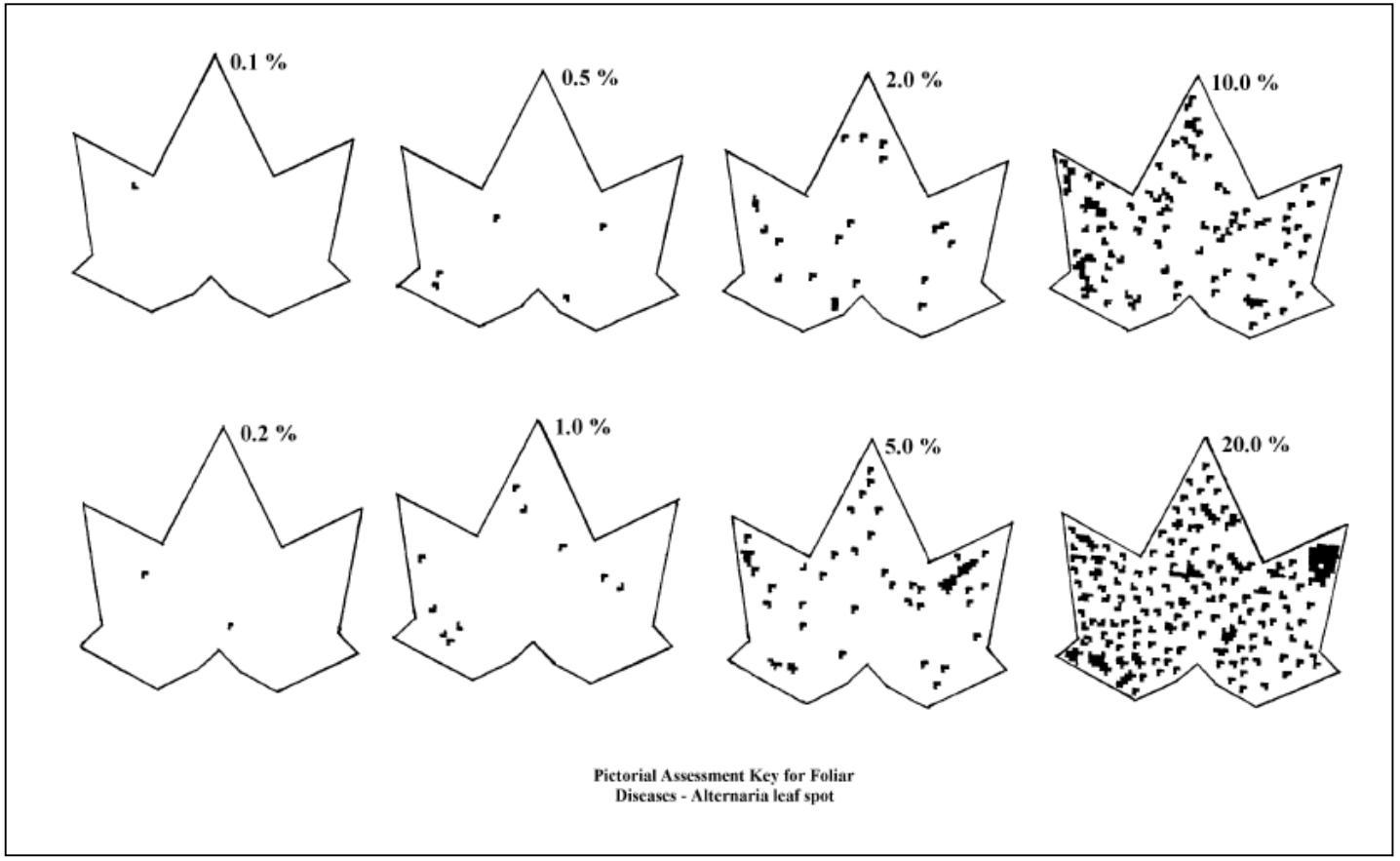
Blighted bolls																			Mean =
Total bolls																			Mean =

Calculate percentage of blighted bolls over total bolls



Allen *et al.*,2002. Integrated Disease Management. The Australian Cotton Cooperative Research Centre.

Qualitative measurement of symptoms
Pictorial disease assessment
Xanthomonas malvacearum



Allen *et al.*,2002. Integrated Disease Management. The Australian Cotton Cooperative Research Centre.

Qualitative measurement of symptoms Banana *Xanthomonas* wilt(BXW) *Xanthomonas vasicola* pv.*musacearum*

- From the center of each field, we made two diagonal lines, and five banana mats from each line were selected to assess the incidence and severity of *Xanthomonas* wilt.
- The incidence was calculated for each field as the percentage of symptomatic mats of the total number of surveyed mats.
- The severity was recorded for each surveyed mat based on a 1–5 severity scale transformed into percent wilting, where scale
- 1 = 0% wilting, 2 = 20% wilting, 3 = 50% wilting, 4 = 75% wilting, and 5 = 100% wilting.
- The average wilting percentage for surveyed mats per field gave the disease severity for that field.

Uwamahoro *et al.*,2019

Qualitative measurement of symptoms
Bacterial leaf blight of rice
Xanthomonas oryzae pv. *oryzae*

- The severity varied from 1 to 7.
- The severity of the attacks on the leaves of each variety was marked every week following a scale of 0 to 9:
- 0 = no traces;
- 1 = traces;
- 3 = 1/4 of the leaf;
- 5 = ½ of the leaf;
- 7 = ¾ of the leaf, and
- 9 = all the leaf.
- These severity marks were used to classify lines and varieties as:
- immune (0 = IM); resistant (1 = R); moderately resistant (3 = MR); moderately susceptible (5 = MS); susceptible (7 = S) and highly susceptible (9 = TS).

Sarra *et al.*,2010

Qualitative measurement of symptoms
Leaf scorch in almond (ALS)
Xylella fastidiosa

- Incidence and severity of *Xf* symptoms were visually assessed by plant pathologists in 1426 almond trees distributed over 20 orchards naturally infected by *Xf* (subsp. *multiplex*), in 9 municipalities of Alicante province, Spain.
- The assessment was carried out between 7 and 11 July 2018.
- *Xf* disease severity (DS) assessments consisted of visual inspection of *Xf* foliar symptoms, rating each almond tree on a **0-4 scale** based on the fraction of the crown canopy with disease symptoms (DS), where zero corresponds to no visual symptoms (i.e., asymptomatic), one, two and three correspond to trees with visual *Xf* symptoms in between 1 and 25%, 25-50% and 50–75% of the tree-crown, respectively, and four corresponds to a tree with mostly dead branches (≥75% of the crown canopy; with leaf collapse or leaf scorch).
- Of the inspected trees, 46% were asymptomatic (DS_0) and 54% showed *Xf* disease symptoms (sample sizes: $n_{DS0} = 657$, $n_{DS1} = 359$, $n_{DS2} = 214$, $n_{DS3} = 142$, $n_{DS4} = 54$).

Camino *et al.*,2021

Qualitative measurement of symptoms Huanglongbing disease, Citrus greening *Candidatus Liberibacter asiaticus*

- Percentage of disease severity was defined based on the symptom existed. The grading system as follow:
- 0) No symptom (no symptom observed on plant canopy);
- 1) Mild (from 1 to 30% of the canopy);
- 2) Moderate (from 31 to 50% of the canopy);
- 3) Severe (more than 50 % of the canopy).
- The below formula was adopted to calculate percentage of disease severity:

$$\% \quad \text{disease severity} = \frac{X_1 + X_2 + \ldots + Xn}{Y \times \text{Maximum rating scale}} \times 100$$

- Whereby:
- X= sum score of disease severity of each citrus plant;
- Y= total number of plants at the same experiment.

Ahmad *et al.*,2011

Methods of disease assessment

- However, recent methods involving:
- Remote sensing and detection of crop stress due to disease are likely to increase the accuracy of indirect disease measurements.
- Direct methods are concerned with both the quantitative and qualitative estimations of disease.

Remote sensing
Indirect disease measurements

- The use of aerial photography and photogrammetry using infrared film or colour filter combinations to enhance the differentiation between healthy and diseased tissue, represent a separate approach to disease assessment.
- Remote sensing now relies on digital image processing and image analysis, including advanced nuclear magnetic resonance imaging (NMRI), for the interpretation and quantification of non-destructive disease measurements in crops.

Remote sensing

Indirect disease measurements

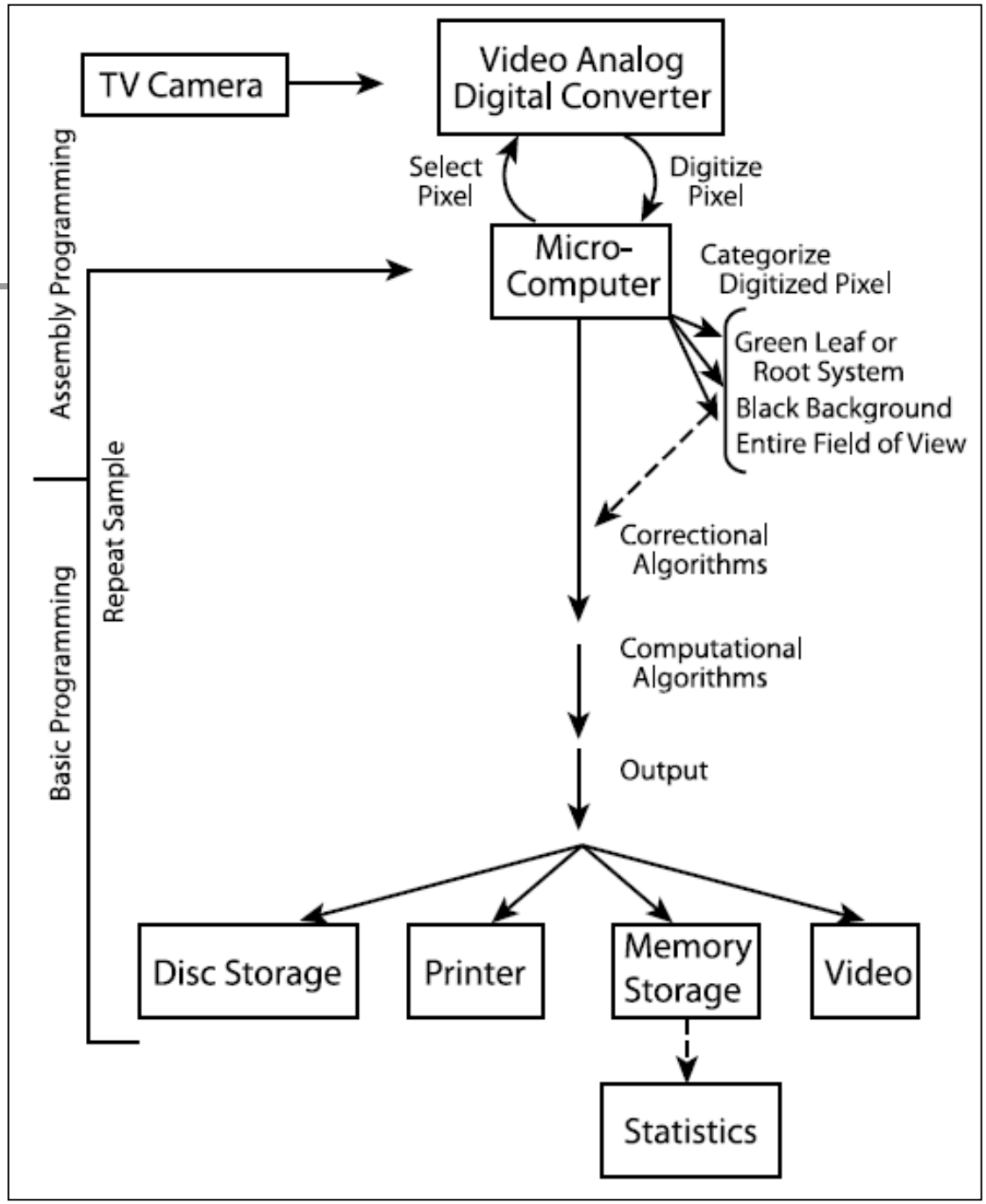
- Remote sensing for detecting and estimating severity of plant diseases is used at three altitudes or levels above the crop canopy.

1. At the lowest altitude, within 1.5-2.0 m above crop height, hand-held multispectral radiometers or multiple waveband video cameras are used.
2. At 75-1500 m, aerial photography is used.
3. At the highest altitude, satellite imagery is employed utilizing satellites orbiting at 650-850 km above the earth's surface.

Remote sensing
Indirect disease measurements

- Video image analysis systems, which uses a video camera interfaced through a digitizer to a microcomputer and display monitor, can be used under laboratory conditions for measuring diseased or damaged tissue at close quarters.
- Systems such as the Delta-T Devices WinDIAS true-color Windows based system are able to differentiate the primary colors of diseased and healthy tissue (brown, yellow and green) in order to analyze percentage diseased leaf area automatically.

Video image analysis system for measuring diseased or damaged plant tissue (Lindow and Wenn, 1983).



Cooke *et al.*,2006

Remote sensing of plant diseases
Thermal and spectral remote sensing

- Thermal and spectral remote sensing can be used to diagnose and monitor effects of environmental stresses on plants.

Jones and Schofield,2008

Remote sensing of plant diseases
Thermal sensing

- Thermal sensing is primarily used to study plant water relations, and specifically stomatal conductance, because a major determinant of leaf temperature is the rate of evaporation or transpiration from the leaf.
- Infrared thermography (IRT) assesses plant temperature and is correlated with:

1. plant water status,
2. the microclimate in crop stands, and
3. with changes in transpiration due to early infections by plant pathogens.

Mahlein,2016

Remote sensing of plant diseases
Thermal sensing

- Emitted infrared radiation in the thermal infrared range from 8 to 12 μm can be detected by thermographic and infrared cameras and is illustrated in false color images, where each image pixel contains the temperature value of the measured object.

Mahlein,2016

Remote sensing of plant diseases
Thermal sensing

- The leaf temperature shows a close correlation to the plant transpiration, which is affected by a diversity of pathogens in different ways.

1. Whereas many foliar pathogens, such as leaf spots or rusts, induce local and well-defined changes, impairment by root pathogens (e.g., *Rhizoctonia solani* or *Pythium* spp.), or
2. systemic infections (e.g., *Fusarium* spp.) often influences the transpiration rate and the water flow of the entire plant or plant organs.

Mahlein,2016

Remote sensing of plant diseases
Fluorescence imaging

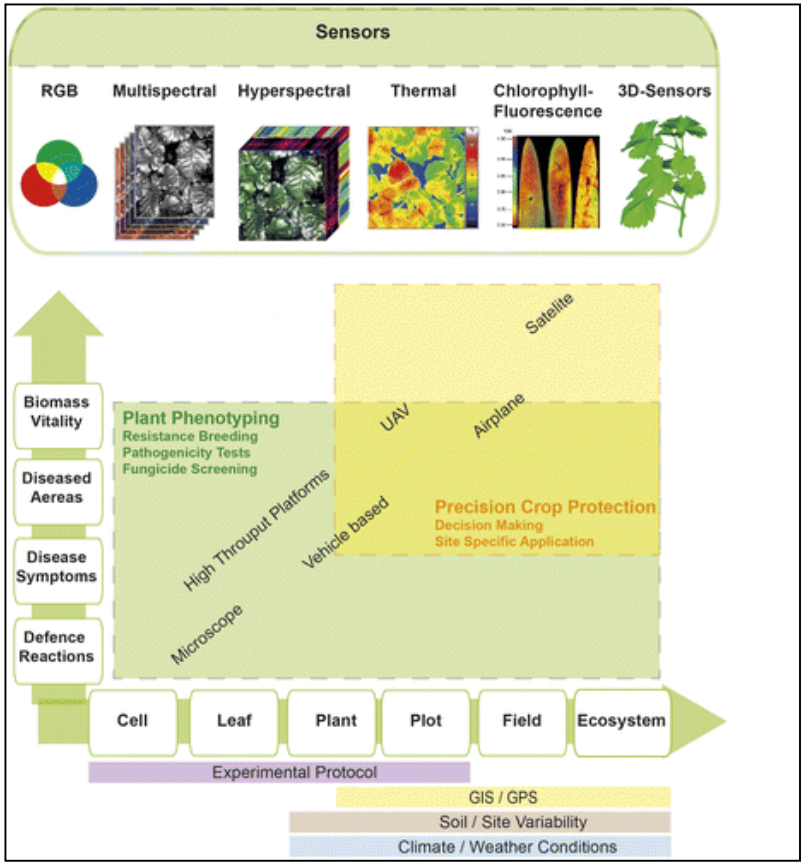
- Even more information about the stress responses of a leaf may be obtained from the fluorescence emission.
- The main wavebands involved in the fluorescence emission from a green leaf when excited by UV-A radiation are:
- in the blue at 440 nm,
- in the green at 520 nm,
- in the red at 690 nm, and
- in the far red at 740 nm.

Jones and Schofield,2008

Remote sensing of plant diseases

Overview of current sensor technologies used for the automated detection and identification of host-plant interactions

- These sensors can be implemented in precision agriculture applications and plant phenotyping on different scales from single cells to entire ecosystems.
- Depending on the scale, different platforms can be operated and consequentially different plant parameters can be observed (Oerke *et al.*, 2014, modified).



Mahlein,2016

Examples of plant pathosystems and plant diseases assessed by optical sensors

Sensor	Crop	Disease / Pathogen	Reference
RGB	Cotton	Bacterial angular (*Xanthomonas campestris*) Ascochyta blight (*Ascochyta gossypii*)	Camargo and Smith (2009)
	Sugar beet	Cercospora leaf spot (*Cercospora beticola*), Sugar beet rust (*Uromyces betae*), Ramularia leaf spot (*Ramularia beticola*), Phoma leaf spot (*Phoma betae*), bacterial leaf spot (*Pseudomonas syringae* pv. *Aptata*)	Neumann et al. (2014)
	Grapefruit	Citrus canker (*X. axonopodis*)	Bock et al. (2008)
	Tabaco	Anthracnose (*Colletotrichum destructivum*)	Wijekoon et al. (2008)
	Apple	Apple scab (*Venturia inaequalis*)	Wijekoon et al. (2008)
	Canadian goldenrod	Rust (*Coleosporium asterum*)	Wijekoon et al. (2008)
Spectral sensors	Barley	Net blotch (*Pyrenophora teres*), Brown rust (*Puccinia hordei*), Powdery mildew (*Blumeria graminis hordei*)	Kuska et al. (2015); Wahabzada et al. (2015a)
	Wheat	Head blight (*Fusarium graminearum*) Yellow rust (*Puccinia striiformis* f. sp. *tritici*)	Bauriegel et al. (2011); Bravo et al. (2003); Huang et al. (2007); Moshou et al. (2004)
	Sugar beet	Cercospora leaf spot (*C. beticola*), Sugar beet rust (*U. betae*), Powdery mildew (*Erysiphe betae*), Root rot (*Rhizoctonia solani*), Rhizomania (*Beet necrotic yellow vein virus*)	Bergsträsser et al. (2015); Hillnhütter et al. (2011); Mahlein et al. (2010, 2012, 2013); Rumpf et al. (2010); Steddom et al. (2003, 2005)
	Tomato	Late blight (*Phytophthora infestans*)	Wang et al. (2008)
	Apple	Apple scab (*V. inaequalis*)	Delalieux et a. (2007)
	Tulip	*Tulip breaking virus* (TBV)	Polder et al. (2014)
	Sugar cane	Orange rust (*Puccinia kuehnii*)	Apan et al. (2004)
Thermal sensors	Sugar beet	Cercospora leaf spot (*C. beticola*)	Chaerle et al. (2004)
	Cucumber	Downy mildew (*Pseudoperonospora cubensis*), Powdery mildew (*Podosphaera xanthii*)	Berdugo et al. (2014); Oerke et al. (2006)
	Apple	Apple scab (*V. inaequalis*)	Oerke et al. (2011)
	Rosa	Downy mildew (*Peronospora sparsa*)	Gomez (2014)
Fluorescence imaging	Wheat	Leaf rust (*Puccinia triticina*) Powdery mildew (*Blumeria graminis* f. sp. *tritici*)	Bürling et al. (2011)
	Sugar beet	Cercospora leaf spot (*C. beticola*)	Chaerle et al. (2004, 2007); Konanz et al. (2014)
	Bean	Common Bacterial Blight (*Xanthomonas fuscans* subsp. *fuscans*)	Rousseau et al. (2013)
	Lettuce	Downy mildew (*Bremia lactucae*)	Bauriegel et al. (2014); Brabandt et al. (2014)

Mahlein,2016

Remote sensing of plant diseases
Bacterial diseases

1. Bacterial angular(*Xanthomonas campestris*)
2. Bacterial leaf spot (*P. syringae*)
3. Citrus canker (*X. axoonopodis*)
4. Common bacterial blight (*Xanthomonas fuscans* subsp. *fuscans*)

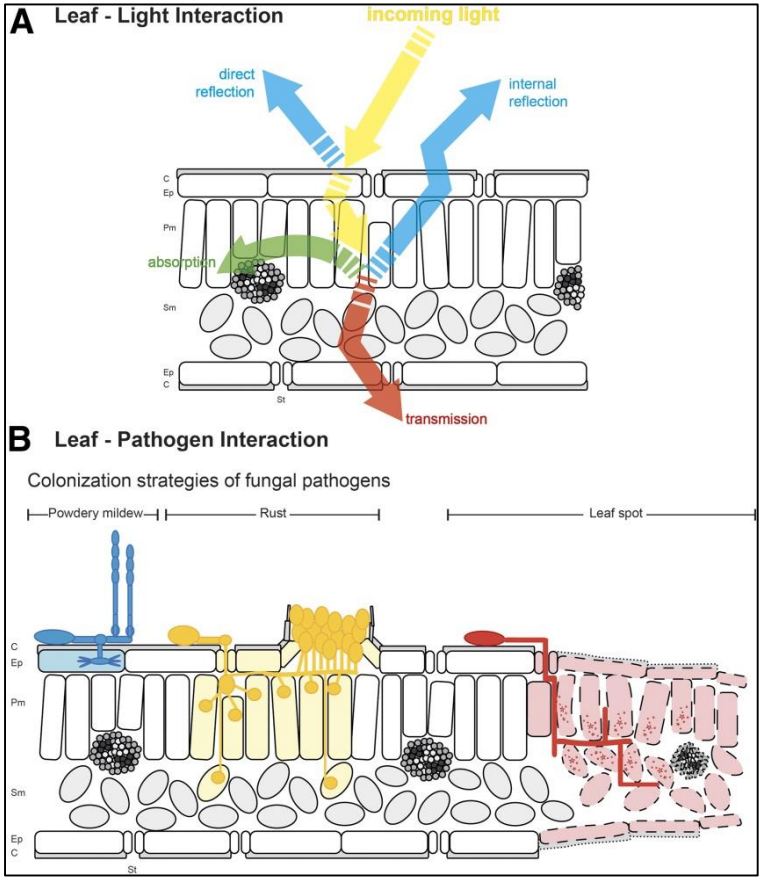
Mahlein,2016

Remote sensing of plant disease

A. The interaction of leaf tissue with light depends on:

- structural, and
- leaf chemical properties.

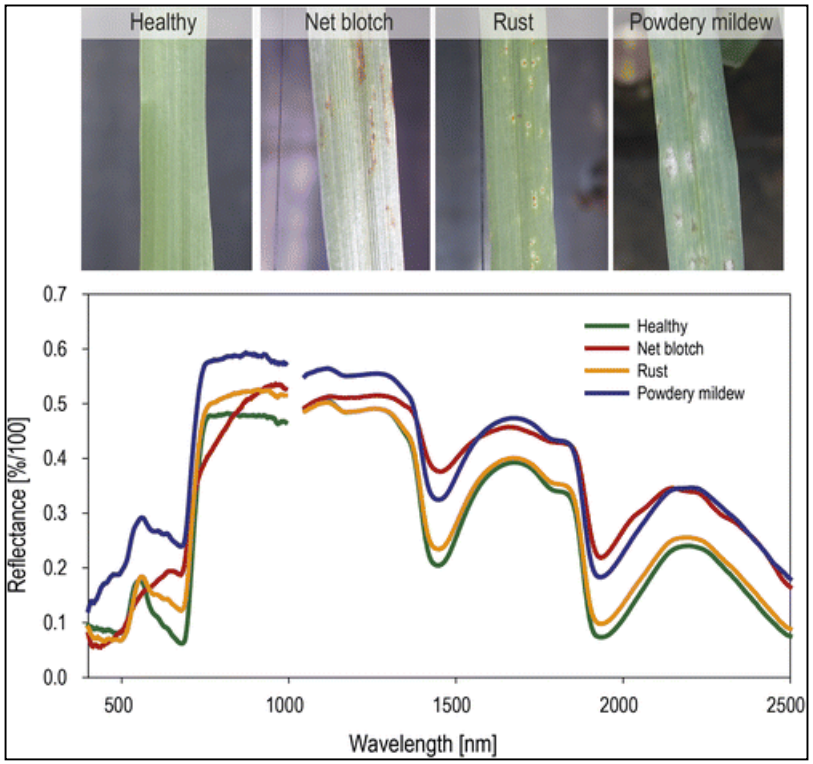
A. During pathogenesis, leaf pathogens influence leaf structural and chemical properties, and by this the leaf optics are altered.



Mahlein,2016

Remote sensing of plant diseases

- Characteristic spectral signatures of barley leaves diseased with net blotch, rust, and powdery mildew, respectively.
- The spectral reflectance of the different disease symptoms were estimated using SMA and the least squares method.
- The reflectance of different disease symptoms in the 450~1000 nm were studied carefully using the Fisher function.

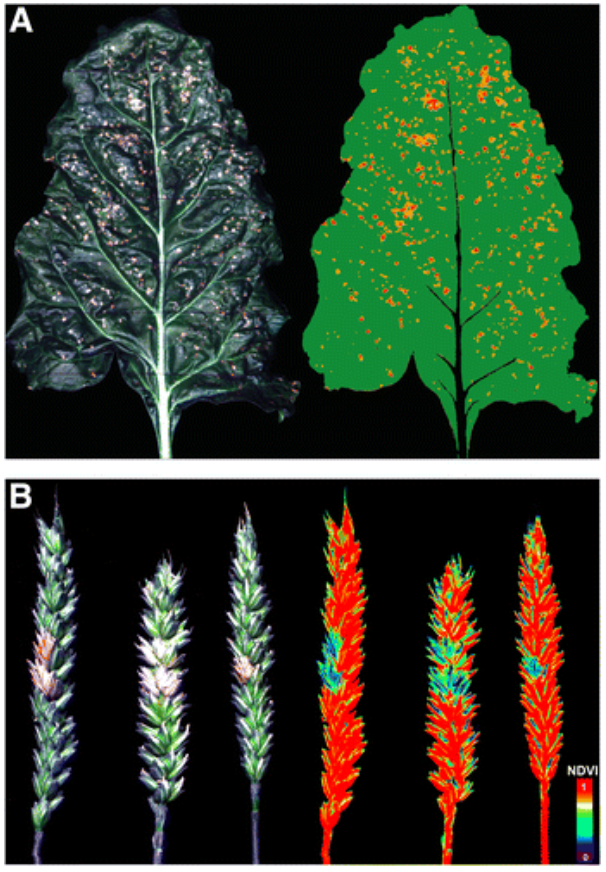


Ashourloo *et al.*,2014; Mahlein,2016

Remote sensing of plant diseases

Disease detection of fungal plant diseases based on hyperspectral images

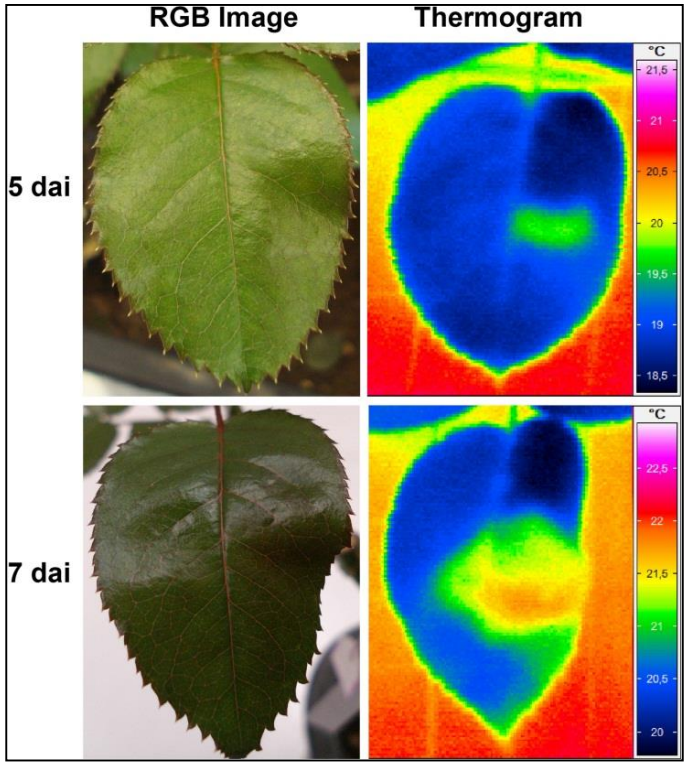
- A, Supervised classification (spectral angle mapper) of *Cercospora* leaf spot on sugar beet.
- The green color denotes healthy leaf tissue,
- The yellow color the border of *Cercospora* leaf spot, and
- The red color the necrotic center of *Cercospora* leaf spot.
- B, Spikelets, diseased by *Fusarium* head blight, can be visualized by calculation of the normalized difference vegetation index.



Mahlein,2016

Remote sensing of plant diseases
Thermal sensors

- Monitoring of rose leaf colonization by *Peronospora sparsa* and symptom development of downy mildew in early stages (5 and 7 days after inoculation) of the disease by thermographic imaging.



Digital photographic images are important tools in plant pathology for assessing plant health. Digital cameras are easy to handle and are a simple source of RGB (red, green, and blue) digital images for disease detection, identification, and quantification.

Remote sensing of plant diseases

- Cost and availability of imaging spectroscopy data could be improved using an Unmanned Aerial Vehicle (UAV) remote sensing system.
- The md4-1000 UAV used by Torres-Sánchez *et al.*,2013 can carry any sensor weighing less than 1.25 kg.
- For evaluation of weed infestation, it was equipped with a still point-and-shoot camera and a six band multispectral camera.

Martinelli *et al.*,2016

Analytic Hierarchy Process

Security risk assessment

Pair-wise Comparison

- Analytic Hierarchy Process (AHP) is one of Multi Criteria decision making method that was originally developed by Prof. Thomas L. Saaty,1990.
- In short, it is a method to derive ratio scales from paired comparisons.
- The input can be obtained from:

1. actual measurement such as price, weight etc., or from
2. subjective opinion such as satisfaction feelings and preference.

web.cjcu.edu.tw/~lcc/Courses/TUTORIAL/AHP%20Tutorial.doc

Analytic Hierarchy Process

Security risk assessment

Pair-wise comparison

- Increasing complexity of risk management requires the use of more flexible approaches to measure information security risk.
- Adapting complex risk analysis tools in today's information systems is a very difficult task due to the shortage of reliable data.
- Analytic Hierarchy Process group decision making (AHP-GDM) offers a technical support for risk analysis by taking the judgments of managers and systematically calculating the relative risk values.

Eren-Dogu and Celikoglu,2012

Analytic Hierarchy Process

Four stages of AHP

Pair-wise comparison

- The AHP comprises of four stages:

1. Modeling,
2. Valuation,
3. Prioritization, and
4. Synthesis.

Eren-Dogu and Celikoglu,2012

Analytic Hierarchy Process

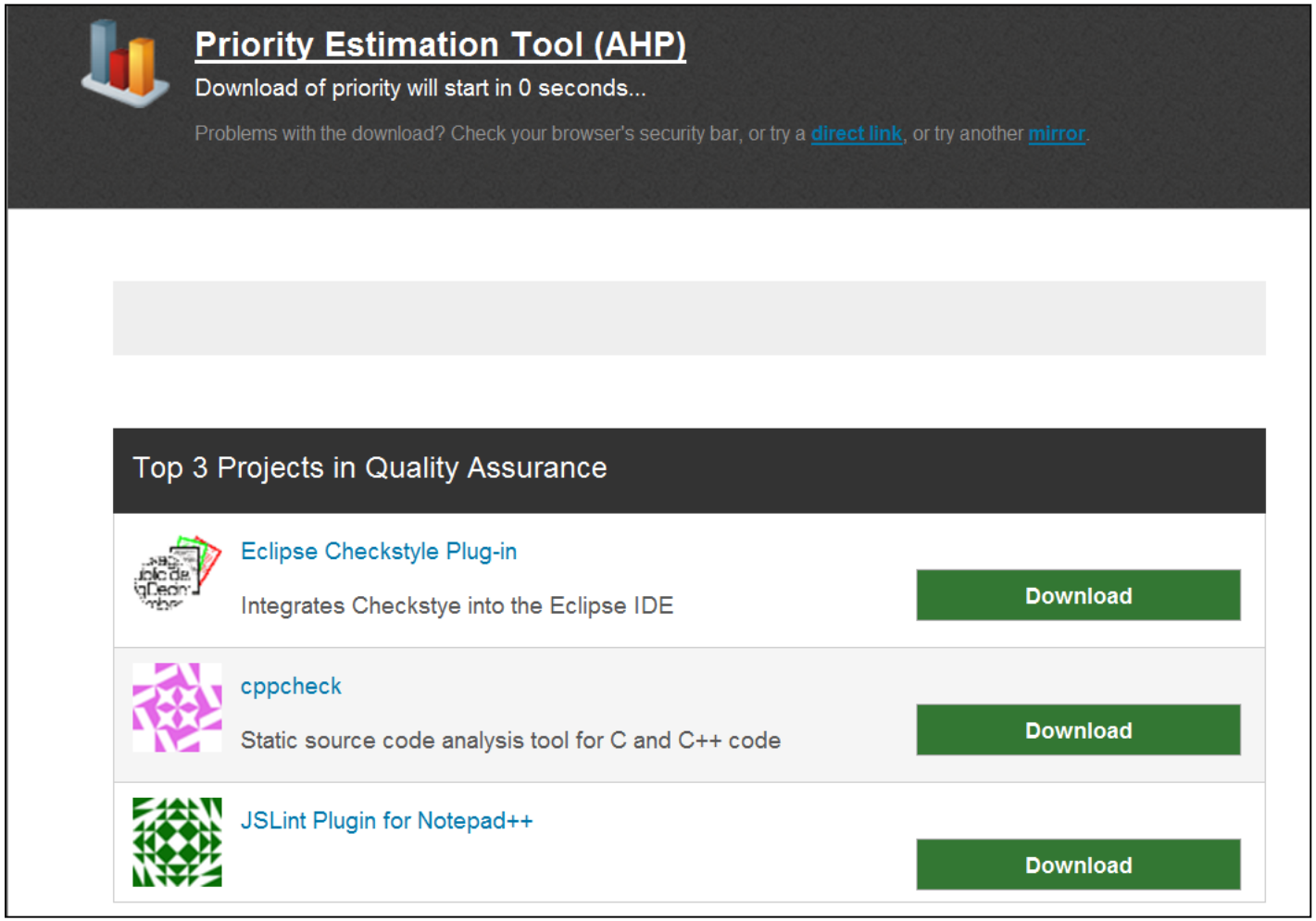
Four stages of AHP

Pair-wise comparison

1. In the modeling stage, a hierarchy which describes the problem is constructed.
2. In the evaluation stage, decision makers compare (pairwise comparison) all the criteria with regard to goal and then all the alternatives with respect to each criterion.
3. In the prioritization stage, the local priorities are derived.
4. In the synthesis stage, the global priorities for each alternative are synthesized in order to get their total priorities.

Eren-Dogu and Celikoglu,2012

AHP software



Analytic Hierarchy Process

An Assessment model for rating high-threat crop pathogens

- Natural, accidental, and deliberate introductions of nonindigenous crop pathogens have become increasingly recognized as threats to the U.S. economy.
- Given the large number of pathogens that could be introduced, development of rapid detection methods and control strategies for every potential agent would be extremely difficult and costly.
- Thus, to ensure the most effective direction of resources a list of high-threat pathogens is needed.
- We address development of a pathogen threat assessment model based on the analytic hierarchy process (AHP) that can be applied world worldwide.

Schaad *et al.*,2006

Analytic Hierarchy Process

An Assessment model for rating high-threat crop pathogens

- Previously, the AHP has been shown to work well for strategic planning and risk assessment.
- Using the collective knowledge of subject matter expert panels incorporated into commercial decision-making software, 17 biological and economic criteria were determined and given weights for assessing the threat of accidental or deliberately introduced pathogens.
- The rating model can be applied by experts on particular crops to develop threat lists, especially those of high priority, based on the current knowledge of individual diseases.

Schaad *et al.*,2006

Analytic Hierarchy Process

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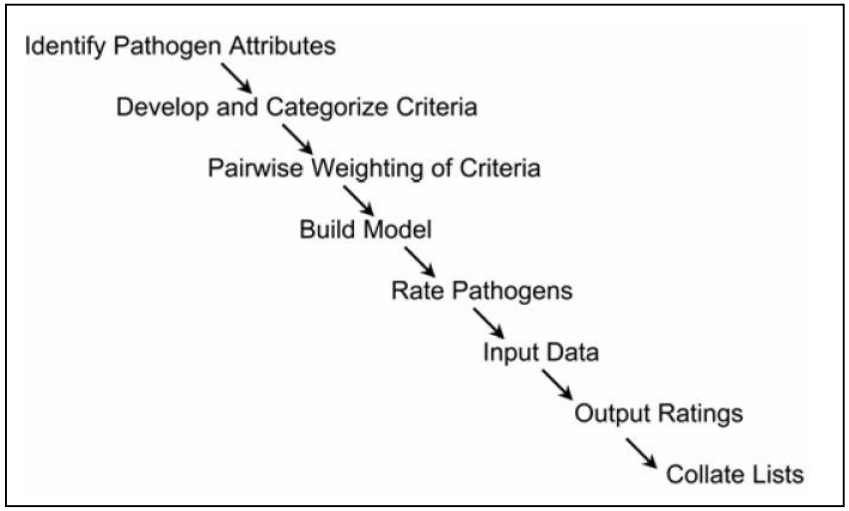
- The rating model can be applied by experts on particular crops to develop threat lists, especially those of high priority, based on the current knowledge of individual diseases.

Schaad *et al.*,2006

Analytic Hierarchy Process

An Assessment model for rating high-threat crop pathogens

- Application of the analytical hierarchy process to:
- Develop, and weight criteria, and rate high consequence pathogens.



Schaad *et al.*,2006

Analytic Hierarchy Process

Master list of groups of criteria developed for rating threats from deliberate plant pathogen introductions

- **Pathogen properties**
- 1. Pathogen survives easily for long periods under field conditions
- 2. Organism produces toxin or other compound in planta toxic to animals/humans
- 3. Organism is easily manipulated genetically
- 4. Organism targets multiple hosts
- 5. Organism is easily disseminated or transmitted in nature
- 6. Affects yield
- 7. Virulence of pathogen is high
- **Production and dissemination**
- 8. Pathogen is easily fermented or grown
- 9. Organism is easily introduced and not dependent upon weather conditions
- 10. Organism is seed-transmitted and breeder seed is often produced abroad
- **Detection**
- 11. Organism is difficult to detect, often latent, escaping detection
- 12. Attributes of organism make it difficult to trace
- **Controls**
- 13. No chemical controls available
- 14. No resistance available
- **Impact**
- 15. Presence of organism would result in a negative psychological impact
- 16. Pathogen is of quarantine significance and affects trade
- 17. Presence of organism or product could greatly affect economics

Schaad *et al.*,2006

Analytic Hierarchy Process

Results of potato pathogens scored under a deliberate introduction scenario with the assessment model for high-threat crop pathogens

a. The 17 criteria were applied to selected potato pathogens, under a deliberate introduction scenario.

b. Pathogens were rated by subject matter experts against all criteria using a simple scale with three levels (low [L], medium [M], and high [H]), represented in the model by numerical values.

	Criteria[a]																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Pathogen	Score[b]																	Total
Phytophthora infestans	M	L	M	L	H	H	M	M	L	L	L	H	L	L	M	L	M	45.1
Rhizoctonia solani	H	L	M	L	M	L	H	M	H	L	L	L	L	L	L	L	L	29.0
Heterodera rostochiensis	H	L	L	M	H	L	M	M	H	L	M	M	M	M	H	H	H	71.7
Ralstonia solanacearum race 3 biovar 2	H	L	H	L	M	H	M	H	H	L	H	M	H	M	H	H	H	84.4
Clavibacter michiganensis subsp. *sepedonicus*	M	L	M	M	M	M	M	H	M	L	M	M	H	M	H	H	H	74.6
Erwinia chrysanthemi	M	L	H	M	H	M	L	H	M	L	M	M	H	M	L	L	L	48.2
Potato leafroll virus	L	L	H	M	H	M	L	L	L	L	H	H	H	L	L	L	M	38.1
Potato spindle tuber viroid	L	L	H	M	M	M	L	L	L	L	M	M	H	L	L	L	L	29.2

Schaad *et al.*,2006

Analytic Hierarchy Process
AHP Prioritized Pest List

- The AHP model prioritizes pests based on risk factors such as introduction potential and pest impact.
- The end result is a prioritize pest list that ranks the top fifty pests predicted to cause damage to agricultural and / or natural resources if introduced into the United States.

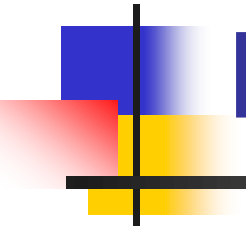
Colorado plant pest and disease emergency response plan,2010

Analytic Hierarchy Process

AHP Prioritized Pest List

Analytic Hierarchy Process Prioritized Pest List Bacterial disease list		
Rank	**Scientific Name**	**Common Name**
12	*Ralstonia solanacearum* race 3 biovar 2	Bacterial wilt of potato
38	*Xanthomonas oryzae*	Bacterial leaf streak, bacterial blight
39	*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*	Dry bean bacterial wilt
71	*Pantoea stewartii*	Stewart's wilt disease

Colorado plant pest and disease emergency response plan,2010



Part I

Principles of plant diseases managements

Diseases management principles

1. Non-plant-based control: Physical, Biological and Chemical.
2. Plant-based control: Breeding and Transgenic.

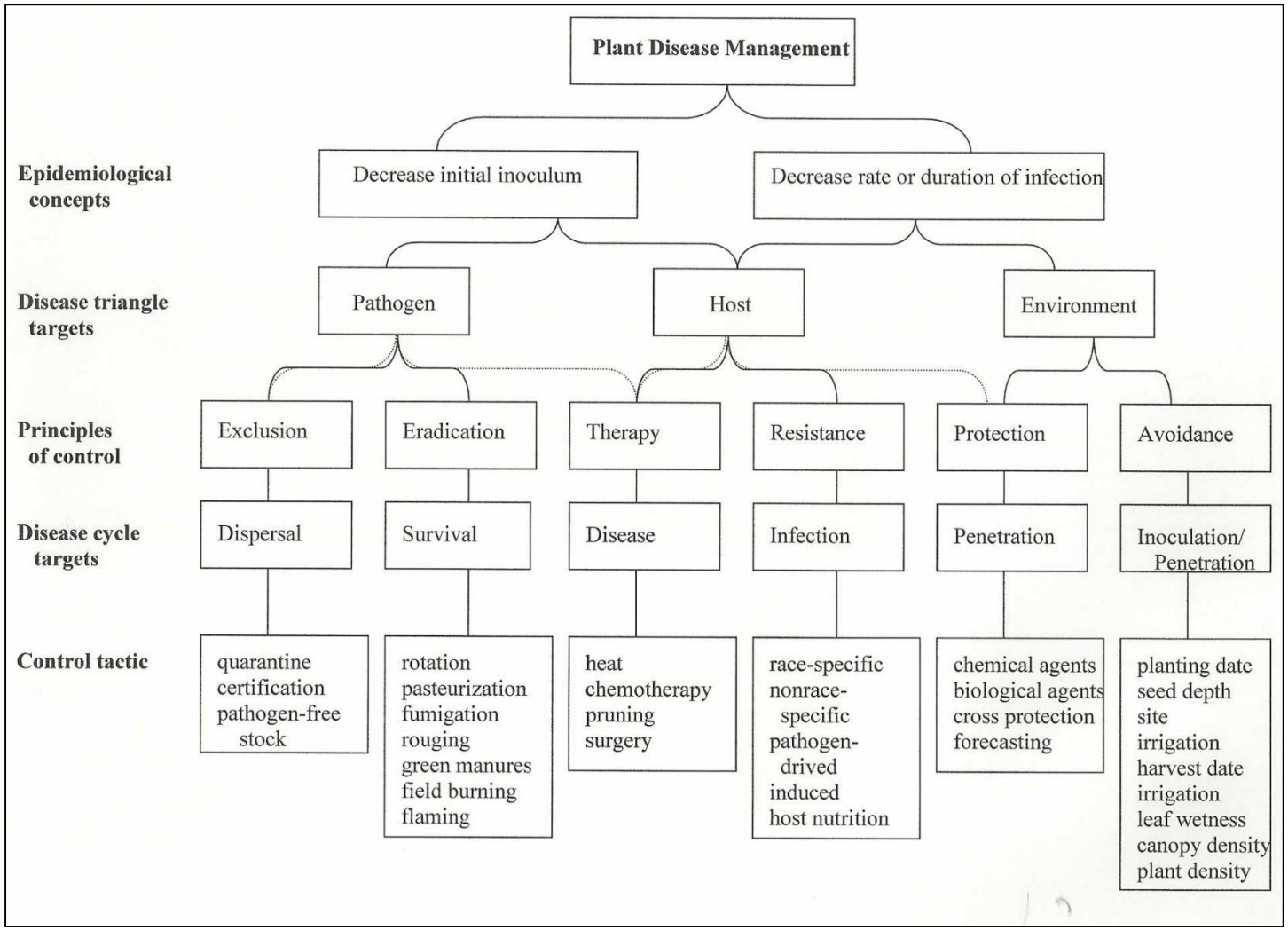
Disease management methods

Major principles of plant disease management

1. Avoid pathogen - planting time
2. Exclude inoculum - clean seed
3. Eradicate pathogen - clean inoculum
4. Fungicide
5. Induced resistance
6. Breed for resistance

Major principles of plant disease management

Exclusion; Eradication; Protection/heat therapy; modifying cultural practices to manage plant diseases; host resistance



Disease management methods

Major principles of plant disease management

Induced resistance

- Induced resistance:

1. Good for fungi, bacteria and virus
2. Several mechanisms - stable
3. Systemic and persistent
4. Safe for humans and environment
5. Extract chemical - seed
6. Sprayed with yeast derived resistance elicitors 24 hours before inoculation of powder mildew - three different elicitors.

Disease management methods

Major principles of plant disease management

Plasma-treated methods

- Plasma can be applied directly or indirectly (plasma-treated water or media) to plants.
- Many plasma factors such as ROS, RNS, electric field, electromagnetic rays, active ions, and UV can be involved in disease control in direct plasma treatment.
- Whereas ROS and RNS from plasma are major players in indirect plasma treatment.
- Plasma (direct and indirect treatment) can inactivate pathogens associated with plants and seeds by causing membrane lipid peroxidation and DNA damage.
- In addition, it can be possible that plasma (direct and indirect treatment) induces plant immune responses by causing oxidative burst and continuously activating defense signaling, leading to the expression of defense genes.

Disease management methods

Major principles of plant disease management

Plasma-treated methods

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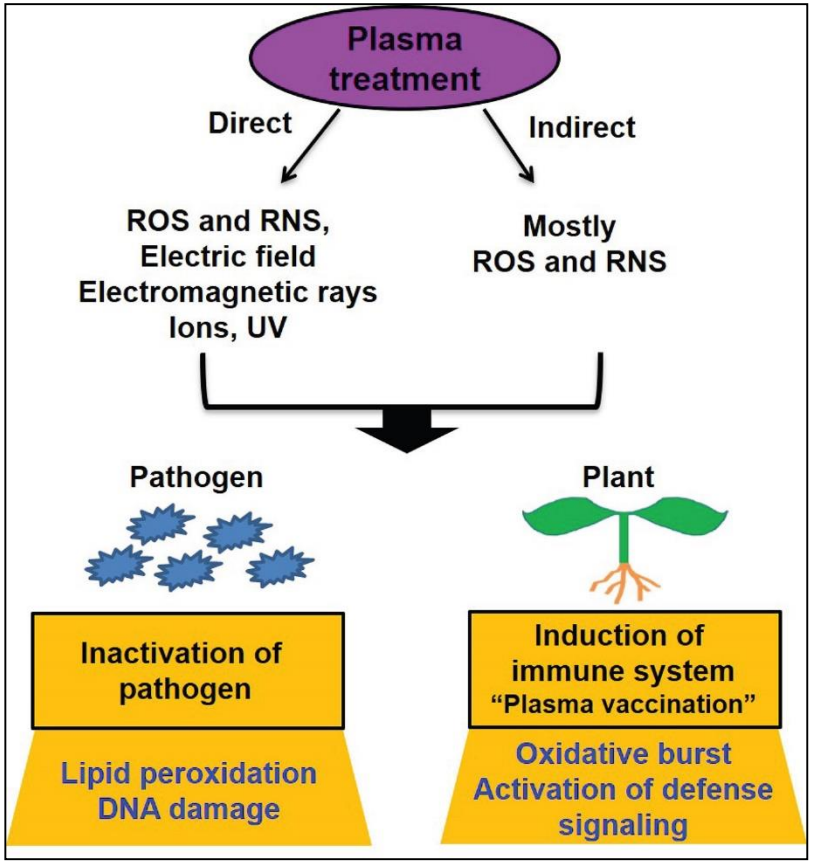


Plasma-derived reactive oxygen and nitrogen species (ROS/RNS)

Adhikari *et al.*,2020

Disease management methods

Exclude inoculum - clean seed

Indexing

- Indexing involves laboratory or greenhouse tests to determine infection by pathogens in vegetatively propagated plants such as potatoes and fruit trees.
- Only the healthy materials are saved for further increase.

Indexing: Testing the plants or seeds or propagative plant materials for the presence of microbial pathogens by biological and/molecular techniques.

Heat therapy or thermal therapy

Thermotherapy to free plant materials from pathogens, especially seeds from bacteria

- Treating planting materials with heat is a one-century-old method of disease control that has proved to be efficient against various pathogenic microorganisms.
- When no efficient chemicals are known to control a disease, treating seeds by heat may be of great interest.

Eli Rogosa Kaufman; Grondeau *et al.*,2011

Heat therapy or thermal therapy

Physical cleaning and eradicating pathogens

- Other common seed treatments (e.g., fungicide treatments) can also help reduce disease, but typically do not eliminate pathogens that have penetrated the seed coat.

Toporek and Hudelson,2017;..

Heat therapy or thermal therapy

Thermotherapy to free plant materials from pathogens, especially seeds from bacteria

- Surface seed treatments reduce disease-causing fungi and bacteria found on the seed.
- Most bacterial diseases of annual plants are seed-borne.
- Elimination of seed-borne bacteria by:

1. Thermotherapy, and
2. meristem culture.

Eli Rogosa Kaufman; Grondeau *et al.*,2011

Heat therapy
Seed surface treatments

- Hot-water seed treatment is one method that you can use to **eradicate**, or at least reduce the level of pathogens (particularly bacterial pathogens), in vegetable seed.
- Water treatments control many seed-borne diseases by using temperatures hot enough to kill the organism but not quite hot enough to kill the seed.
- It must be carefully and accurately done. Because, a few degrees cooler or hotter than recommended may not control the disease or may kill the seed.

Heat therapy or thermal therapy

Thermotherapy to free plant materials from pathogens, especially seeds from bacteria

- Satisfactory control has been obtained for several bacterial diseases on:
- tomato, tobacco, rice, barley, cucumber, pumpkin, cotton, eggplant, pepper, carrot, spinach, lettuce, celery, cabbage, turnip, radish, and other crucifers, mostly caused by the genera:

1. *Xanthomonas*, and
2. *Pseudomonas*.

Eli Rogosa Kaufman; Grondeau *et al.*,2011

Heat therapy

The seed disinfection unit is used to treat seeds in fluids to eliminate bacterial contamination or seedborne diseases

- Thermotherapy has been applied to a number of bacterial diseases in different plant parts with reasonable success, including:
- True seeds, e.g. Cabbage
- Bulbs, e.g. *Hyacinthus*
- Rhizomes, e.g. Ginger
- Plantlets or cuttings, e.g. sugarcane

Heat therapy
Hot water treatment

- Exceptional cases:
- Hot-water seed treatment works best for small seed.

1. Seeds of cucurbits such as squash, gourds, pumpkins, watermelons, etc. can be severely damaged by hot water and thus should NOT be treated.
2. Also, thermotherapy is more difficult to use with large seeds of legumes, such as pea, bean, or soybean, because a significant decrease of germination is often obtained before the bacteria have been totally killed.

Grondeau et al.,2011; Ohio State University Extension

Heat therapy

The seed disinfection unit is used to treat seeds in fluids to eliminate bacterial contamination or seedborne diseases

- Thermotherapy may be performed by:

1. Hot water treatment, usually 50-54°C for 5-30 min.
2. Aerated steam at 50°C for 1 h.
3. Dry heat at 70°C for 3-7 days.

Heat therapy
Seed surface treatments
Instructions

- The following equipment and supplies are needed to hot water treat organic vegetable seeds:

1. Water bath (preferably two: one for pre-warming and one for treatment);
2. Thermometer;
3. Cotton cloth, cotton bags, or nylon bags;
4. Screen for seed drying.

Heat therapy

How to Hot Water-Treat Seeds

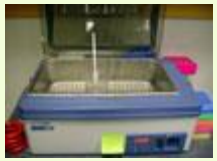
Hot-water treatments can eliminate disease-causing organisms from seed.

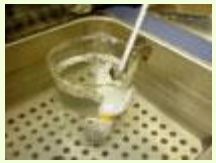
- **Step 1:** Wrap seeds loosely in a woven cotton (such as cheesecloth) or nylon bag.


- **Step 2:** Pre-warm seeds for 10 minutes in 100ºF (37ºC) water.



- **Step 3:** Place pre-warmed seeds in a water bath that will constantly hold the water at the recommended temperature (see table that follows). Length of treatment and temperature of water must be exactly as prescribed. If water is too hot or treatment is too long, seeds may be damaged.





Heat therapy

How to Hot Water-Treat Seeds

- **Step 4:** After treatment, place bags in cold tap water for 5 minutes to stop heating action.

- **Step 5:** Spread seeds in a single, uniform layer on screen to dry.

Hot water treatment of seeds (32°F=0°C)

e.g. 122°F=50°C

Crop	Temp (°F)	Time (min)	Diseases Controlled
Brussels sprouts	122	25	Alternaria leaf spot, bacterial leaf spot, black leg, black rot
Broccoli	122	20	Alternaria leaf spot, bacterial leaf spot, black leg, black rot
Cabbage	122	25	Alternaria leaf spot, bacterial leaf spot, black leg, black rot
Carrot	122	20	Alternaria leaf blight, bacterial leaf blight, cercospora leaf spot, Crater rot/foliar blight
Cauliflower	122	20	Alternaria leaf spot, bacterial leaf spot, black leg, black rot
Celeriac	118	30	Bacterial leaf spot, Cercospora leaf spot, Septoria leaf spot, Phoma crown and root rot
Celery	118	30	Bacterial leaf spot, Cercospora leaf spot, Septoria leaf spot, Phoma crown and root rot
Chinese cabbage	122	20	Alternaria leaf spot, bacterial leaf spot, black leg, black rot
Collards	122	20	Alternaria leaf spot, bacterial leaf spot, black leg, black rot
Coriander	127	30	Bacterial leaf spot
Cress	122	15	Alternaria leaf spot, bacterial leaf spot, black leg, black rot
Eggplant	122	25	Anthracnose, Early blight, Phomopsis, Verticillium wilt
Kale	122	20	Alternaria leaf spot, bacterial leaf spot, black leg, black rot
Kohlrabi	122	20	Alternaria leaf spot, bacterial leaf spot, black leg, black rot
Lettuce	118	30	Anthracnose, Bacterial leaf spot, lettuce mosaic virus, Septoria leaf spot, Verticillium wilt
Mustard	122	15	Alternaria leaf spot, bacterial leaf spot, black leg, black rot

University of Massachusetts Amherst,2019

Hot water treatment of seeds

Crop	Temp (°F)	Time (min)	Diseases Controlled
Onion (seeds)	122	20	Purple blotch, Stemphylium leaf blight
Onion (sets)	115	60	Botrytis, downy mildew, purple blotch, smut, Stemphylium leaf blight
Parsley	122	30	Alternaria leaf blight, Cercospora leaf spot
Pepper	125	30	Anthracnose, bacterial leaf spot, cucumber mosaic virus, pepper mild mosaic virus, tobacco mosaic virus, tomato mosaic virus
Radish	122	15	Alternaria leaf spot, bacterial leaf spot, black leg, black rot
Rutabaga	122	20	Alternaria leaf spot, bacterial leaf spot, black leg, black rot
Shallot	115	60	White rot
Spinach	122	25	Anthracnose, Cladosporium leaf spot, cucumber mosaic virus, downy mildew, Fusarium wilt, Stemphylium leaf spot, Verticillium wilt
Sweet potato (roots)	115	65	Scurf, black rot
(cuttings, sprouts)	120	10	Scurf, black rot
Tomato	122	25	Alfalfa mosaic virus, Anthracnose, bacterial canker, bacterial speck, bacterial spot, cucumber mosaic virus, early blight, Fusarium wilt, leaf mold, Septoria leaf spot, Tomato mosaic virus, Verticillium wilt, double virus streak
Turnip	122	20	Alternaria leaf spot, brown spot, black leg, black rot
Yam (tubers)	112	30	Nematodes

University of Massachusetts Amherst,2019

How to Hot Water-Treat Seeds

How to test for seed germination after hot water treatment

1. Mix seeds thoroughly in each seed lot and count out 100 seeds per seed lot.*
2. Treat 50 of the seeds exactly as described in the fact sheet.
3. After treated seeds have dried, plant the two groups of seeds separately in flats or pots containing planting mix according to standard practice. Label each group as "treated" or "untreated".
4. Allow the seeds to germinate and grow until the first true leaf appears (to allow for differences in germination rates to be observed).
5. Count seedlings in each group separately.
6. Determine the percent germination in each group:

$$\text{percent germination} = \frac{\text{number of seedlings emerged}}{\text{number of seeds planted}} (\text{X } 100)$$

- Compare percent germination in each group: they should be within 5% of each other.
- * If seed supply is limited, use a smaller number (at least 30) of seeds to test germination.

Seed disinfection unit

Dry heat treatment of mainly infected vegetable seeds

- This system is used for dry heat treatment of mainly infected vegetable seeds, like cucumbers, gherkins, melons and peppers.
- Using our seed drum as a carrier the seeds are treated with a certain temperature for a period of time.

Disease management methods
Cultural Disease Control

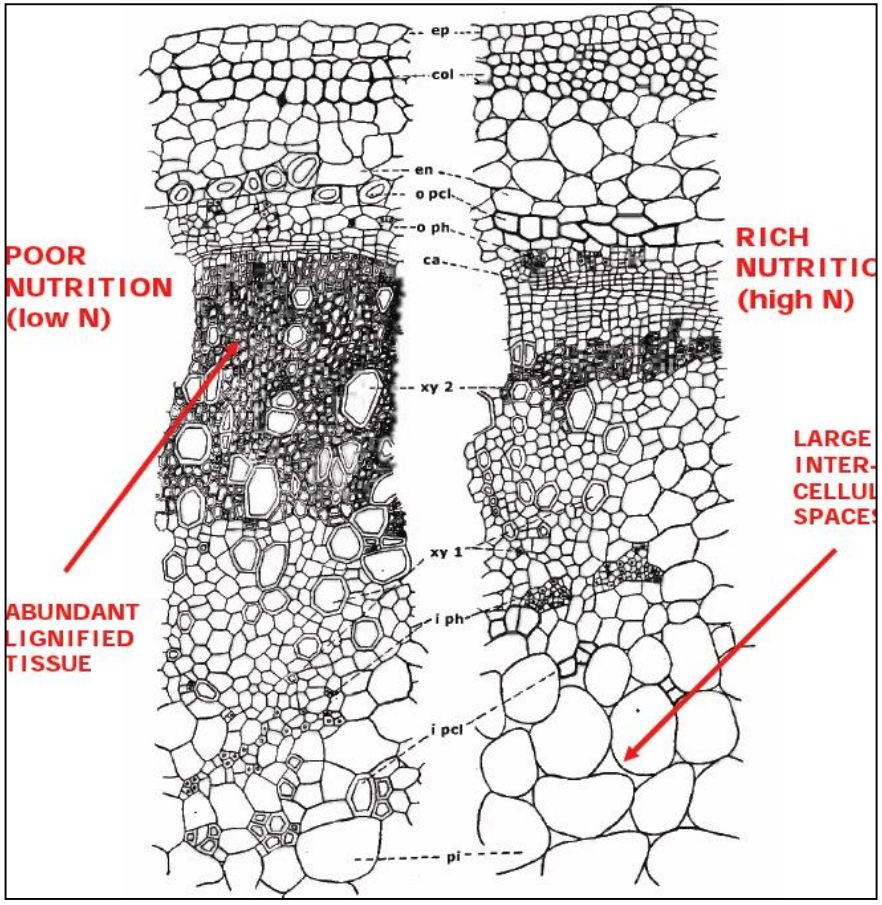
- Develop soil rich in organic matter
- Plant on raised beds
- Plant tolerant or resistant varieties
- Crop rotation
- Plants adapted to area
- Plant at proper depth (below crown or graft)
- Use only thoroughly composted material
- Soil pH adjustment
- Improve air circulation by staking, trellis or pruning
- Remove weeds: carry disease, circulation
- Water in the morning
- Avoid high angle sprinklers
- Do not over fertilize
- Remove diseased plants and destroy

Cultural Disease Control

Effect of nutrition

High N uptake= high susceptibility to diseases

- From this figure it is clear that the highly vegetative plant (right) has large intercellular spaces in the pith and less lignified tissue in cortex and xylem.
- Such plants are in fact weakened and are easily attacked by bacteria, which cause so-called pith necrosis (*Pseudomonas corrugata* and others).
- **Ca** = cambium; **col** = collenchyma; **en** = endodermis cells; **ep** = epidermis; **i** and **o ph** =. inner and outer phloem cells; **i** and **o pcl** = inner and outer pericycle cells; **pi** = pith; **xy 1** and **xy 2** = xylem.



Left: weakly vegetative stem.
Right: highly vegetative stem, due to high N uptake.

Janse,2006

Effect of nutrition

High Ca uptake=less susceptibility to diseases

- Effect of Ca nutrition on brown rot (*Ralstonia solanacearum*) incidence in tomato when treated with different amounts of essential nutrients (P and K constant and optimal).
- **Clear positive effect of Ca (treatment 2 and 6).**
- Mg seems to have a negative effect.
- Nutrient added in grams per kg air-dried soil.

Treatment	CaO	$Ca(NO_3)_2$	NH_4NO_3	MgO	Disease incidence and SD
1	-	-	3.0	-	85 (0.15)
2	2.1	-	3.0	-	17 (0.03)
3	-	-	3.0	1.5	35 (0.35)
4	1.0	4.4	1.5	-	25 (0.25)
5	-	8.8	-	1.5	25 (0.05)
6	-	8.8	-	-	5 (0.05)

SD = standard deviation; - = no dosage.

Janse,2006

Recent discoveries in molecular mechanisms of plant disease resistance responses to pathogen attacks

Bacterial diseases management at the molecular level

Genetic disease control

New control strategies

- The expectation from basic researches such as biochemical and genetic mechanisms of pathogenicity of plant pathogenic bacteria is to develop a new or improved approaches for disease control.

Genetic disease control

New control strategies

- From the earliest days of farming, plant disease and pests have been a critical challenge for farmers. Although mankind has split the atom, travelled to the moon and connected the world, plant pathogens continue to be a significant challenge to food security despite our best efforts to thwart them.
- Estimates of average global losses to diseases and pests range from 11–30% Savary *et al*., 2019).
- Importantly, crop losses are highest in regions that already suffer from food insecurity (Savary *et al*., 2019).

van Esse *et al*.,2020

Genetic disease control

New control strategies

- The disease issues of wheat are not an isolated example, and challenges such as these are becoming more frequent as global warming and increased global trade facilitate the spread of known and emerging pathogens.
- Top of these issues is the fundamental reality that **821 million people do not have enough to eat** (FAO *et al.*, 2018).
- The world population is projected to reach nearly 10 billion in 2050 (United Nations, 2017).
- This forecast brings with it the associated need to increase world food production by at least 60%.

van Esse *et al.*,2020

Genetic disease control

New control strategies

- Losses from diseases would be far worse without past steady advances in agricultural practices, including:

1. cultural controls,
2. agrochemical use, and
3. plant breeding.

- However, we have learned that there are no 'silver bullets'.
- An integrated approach is needed to combat plant diseases, combining the best technologies and practices that are available.

Van Esse *et al.*,2020

Genetic disease control

Antipathogenic approach

New control strategies

- The antipathogenesis approach to disease control involves:
- The identification of weaknesses in a pathogenesis strategy, as targets for the development of effective disease control measures.

Birch,2001

Genetic disease control

New control strategies

Genetic modification (GM) technologies

- We need to increase world food production by at least 60% using the same amount of land, by 2050.
- One of the most effective and sustainable ways to manage plant pathogens is to use genetic modification (GM) and genome editing, expanding the breeder's toolkit.
- For The time to act is now and we cannot afford to ignore the new solutions that GM provides to manage plant pathogens.

van Esse *et al.*,2020

Genetic disease control

New control strategies

Genetic modification (GM) technologies

- Genetic engineering can be used in a variety of ways to protect plants from damaging pests and diseases.
- The three most common traits found in GMO crops are:

1. Resistance to insect damage;
2. Tolerance to herbicides;
3. Resistance to plant viruses.

Genetic disease control

New control strategies

Genetic modification (GM) technologies

- How is genetic engineering used to prevent diseases?
- By Fixing mutated genes.
- Mutated genes that cause disease could be turned off so that they no longer promote disease, or
- healthy genes that help prevent disease could be turned on so that they could inhibit the disease.

Genetic disease control

New control strategies

Genetic modification (GM) technologies

- How is genetic modification used in agriculture?
- Genetic modification of plants involves adding a specific stretch of DNA into the plant's genome, giving it new or different characteristics.
- This could include changing the way the plant grows, or making it resistant to a particular disease.

Gene delivery methods

There are many different methods of gene delivery for various types of cells and tissues

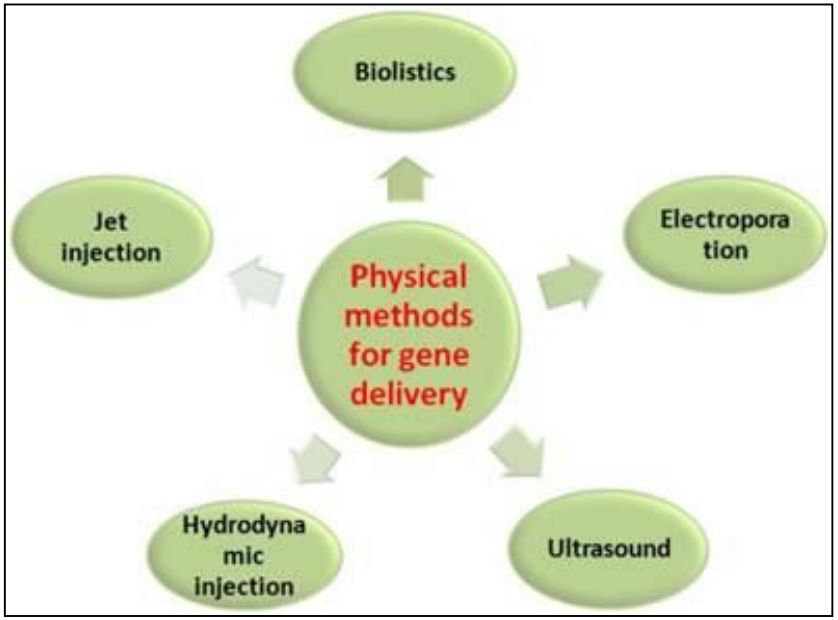
- Electroporation
- Biolistics
- Microinjection
- Sonoporation
- Photoporation
- Magnetofection
- Hydroporation.

Gene delivery methods

There are many different methods of gene delivery for various types of cells and tissues

- Current gene transfection systems contain three major groups:

1. viral (transduction);
2. physical (direct micro injection); and
3. chemical methods.



Jin *et al.*,2014

Genetic disease control

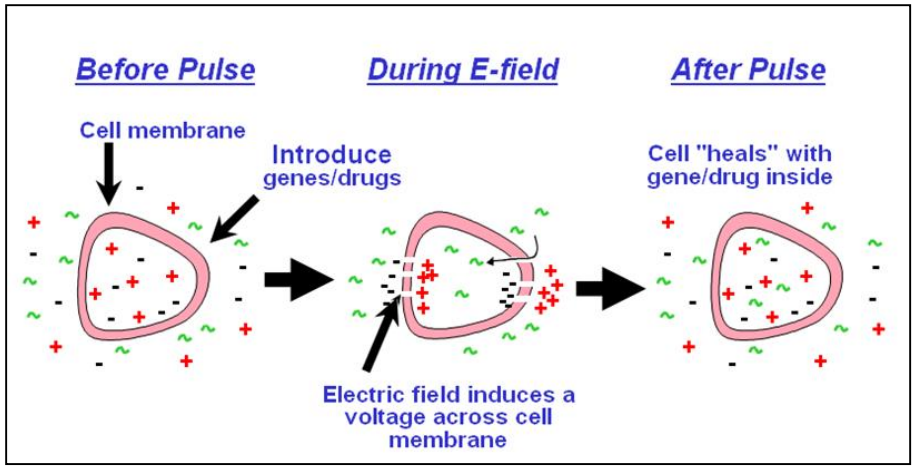
Gene delivery methods

- The three most common DNA delivery systems are:

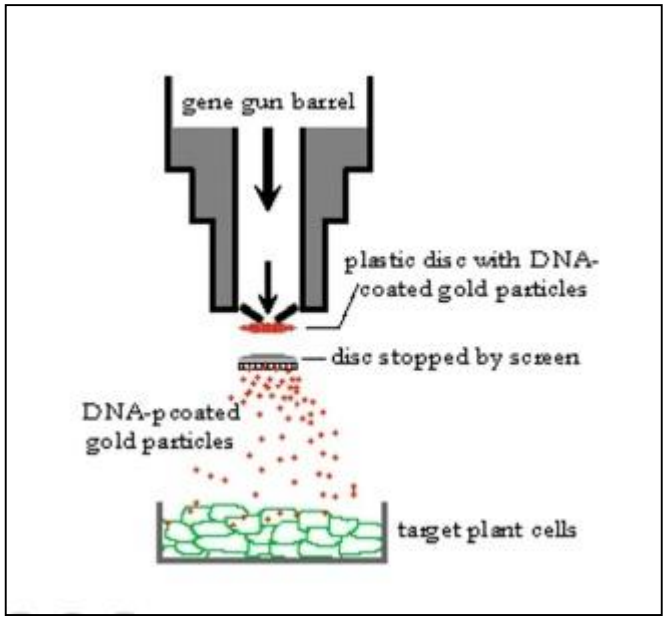
1. Biolistics(gene-gun) delivery: the method of directly shooting DNA fragments into cells using a device called a gene gun.
2. Electroporation: Electroporation is the process of using an electrical current across a cell membrane resulting in temporary pore formation in the cell membrane, allowing the cell to take up DNA sequences.
3. Agrobacterium-mediated genetic transformation: based on the bacterium *Agrobacterium tumefaciens* as the biological vector to transfer exogenous T-DNA into the plant.

Genetic disease control

Gene delivery methods



Electroporation method



Gene Gun method

BTX online;..

Genetic disease control

Gene delivery methods

Candidate genes transformations

- These techniques has opened the door to the rapid incorporation of defense components into plants across species barriers.
- Candidate genes for such transformations are those which encode:

1. Proteins that inhibit pathogen enzymes or degrade their toxins.
2. Those that enhance the concentrations of saponins (phytochemicals), antimicrobial peptides, reactive oxygen species or modify the phytoalexin response, and
3. Those that switch on systemic acquired resistance (SAR).

Strange,2003

Genetic disease control

Candidate genes transformations

- Genes derived from pathogens are also candidates since they, paradoxically, confer resistance (See gene silencing in *Agrobacterium*).
- Resistance genes also be used to broaden the resistance of plants to a greater spectrum of pathogens.

Strange,2003

Examples of genetic disease solutions currently available for bacterial, viral, fungal and oomycete pathogens

Point of intervention	GM technology	Example
Pathogen perception	Interspecies transfer of PRRs	EF-Tu receptor (EFR)
	Interspecies transfer of NLRs	Rpi-Vnt1
		Bs2
	Modification of NLRs	Pikp-1
	NLR protease trap	PBS1 kinase
	NLR resurrection	NRCs (NLR helpers)
Pathogen effector binding	Deletion of effector targets	MAPK3K StVIK1
	Modification of effector binding sites	COI1
	Deletion of effector binding sites	Os11N3/OsSWEET14
	Addition of effector binding sites	Xa27
Defence signalling pathway	Altered expression of signalling components	NPR1
	Altered expression of transcription factors	IPA1/OsSPL14
Recessive resistance alleles	Gene deletion	mlo
	Gene modification	bs5
Dominant plant resistance proteins	Interspecies transfer of signalling components	PFLP
	Transfer of detoxifying enzymes targeting pathogen toxins	Oxalate oxidase

PRRs: pattern-recognition receptors, EF-Tu receptor (EFR): The cell-surface, NLRs: Leucine-rich repeat immune receptors

Examples of genetic disease solutions currently available for bacterial, viral, fungal and oomycete pathogens

Antimicrobial compound production	Transfer of antimicrobials from plants	Rs-AFP defensin
	Transfer of antimicrobials from microorganisms or animals	Virus KP4
	Expression of synthetic antimicrobials	MsrA1
RNAi	Viral gene silencing through RNAi	Coat protein or replicase domain gene from Papaya ringspot virus
		AC1 from bean golden mosaic virus
		Coat protein gene from plum pox virus
		Coat protein gene from potato virus Y[a]
		Putative replicase domain or helicase domain gene from potato leaf roll virus[b]
		Coat protein gene from cucumber mosaic cucumovirus, zucchini yellow mosaic potyvirus and watermelon mosaic potyvirus 2
	Fungal and oomycete gene silencing through RNAi	HAM34 or CES1 gene of *Bremia lactucae*

van Esse *et al.*,2020

The most common resistance

Host and non-host disease resistance

Disease management methods
Host Resistance
The fundamental questions

- The fundamental questions are:

1. why some plants get infected by a particular pathogen and others don´t, and, vice versa.
2. why a given pathogen can only successfully colonize a limited number of plant species, which collectively form its host range.
3. if a resistance is so complete and persists over so many generations, is there some way we could transfer it to susceptible plants like wheat and thereby stop disease?

Plant disease resistance mechanisms
Passive and induced resistance
Vertical and horizontal resistance

- Plants' resistance mechanisms against pathogens are often **chemical in nature**.
- These resistance mechanisms may be:

1. Naturally occurring resistance mechanisms are present in the host plant tissues prior to their contact with pathogens.
2. Induced resistance mechanisms occur only after such contact with the pathogen.
3. The plant pathologist "Vander Plank" introduced the concept of vertical and horizontal resistance in 1963.

Plant disease resistance mechanisms
Three lines of defence
Passive and induced resistance

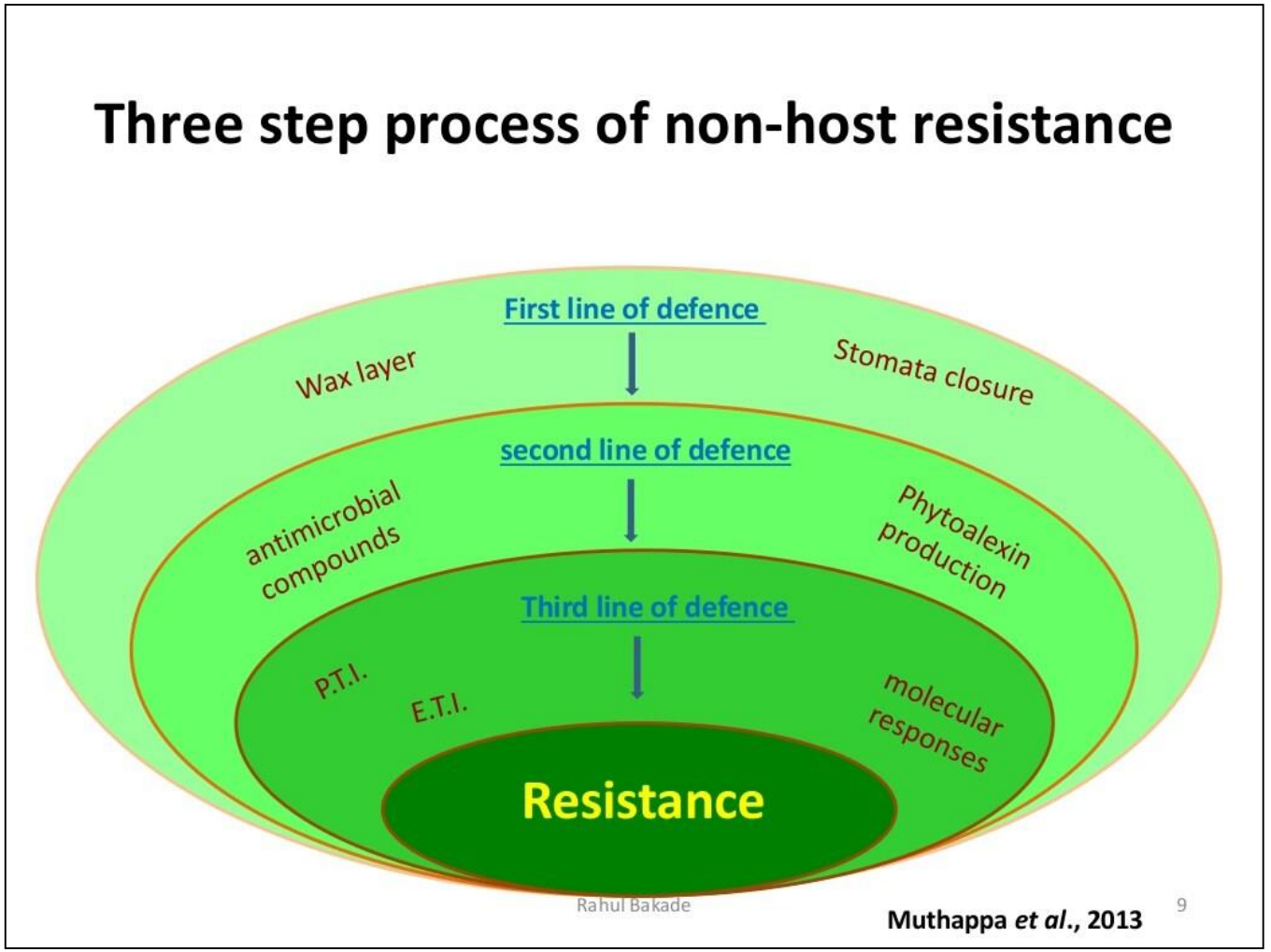
1. **A first line of defence**: Includes the waxy cuticle and the plant cell wall.
2. **The second line of defence**: When specific pathogens are able to evade or break this barrier, either through wounds or stomata, by producing cuticle- or cell wall dissolving enzymes or by mechanical disruption, plants contain as a second line of defence large amounts of so-called preformed antimicrobial compounds aimed at directly inhibiting pathogen growth.
3. **The third line of defence**: Some inducible defence mechanisms are mediated by or activated through the plant signaling molecules, salicylic acid, jasmonic acid and ethylene.

Custers *et al.*,2007; Bakade,2018

Plant disease resistance mechanisms

Three lines of defence

Passive and induced resistance



Passive (Constitutive, Pre-existing, preformed) Defense Mechanisms

The first and second defense lines:

1. **Physical barriers**
2. **Chemical barriers**

Active (Induced, post infectional) Defense Mechanisms

The second defense line:

3. **Oxidative burst, HR, SAR, LAR,..**

For more details see the Plant Bacterial Disease Management-Part 2.

The most common resistance
Resistance mechanisms

- Plant resistance to pathogens and pests can be:

1. Passive(preformed);
2. Active (induced).

- Passive resistance depends on defences that are constitutively expressed in the plant,
- While active resistance relies on defences that are induced after infection or attack.
- Induced resistance can be local or systemic.
- At least two forms of induced resistance, known as:

1. systemic acquired resistance (SAR), and
2. induced systemic resistance (ISR).

Defense mechanisms

Passive defense mechanisms

- **Passive (preformed or constitutive) defense** (= The first lines of defenses that are constantly available).
- Such as cell walls, wax layers and chemical barriers confer broad resistance to a wide variety of pathogens.

Passive(constitutive)defense mechanisms
Physical barriers(defenses)

- Constantly present, whether there is demand or not.
- Cutin, waxes, suberins are made of hydrophobic compounds(having water-repelling properties)
- These compounds are non-polar.
- Fatty acids are one type of hydrophobic compound.

Passive (preformed) defense mechanisms
Physical barriers(defenses)

- Unlike animals, plant cells have walls, which present a formidable barrier to any invading organism.
- Cutin or suberin: Plant cells have walls occur on the outside of the plant and usually covered with cutin or suberin.
- Lignin is often a component of secondary cell walls and confers considerable resistance to microbial decay.
- Bark: Undoubtedly provides physical protection against potential invaders.

Preformed resistance mechanisms
Preformed antimicrobial compounds
Phytoanticipins

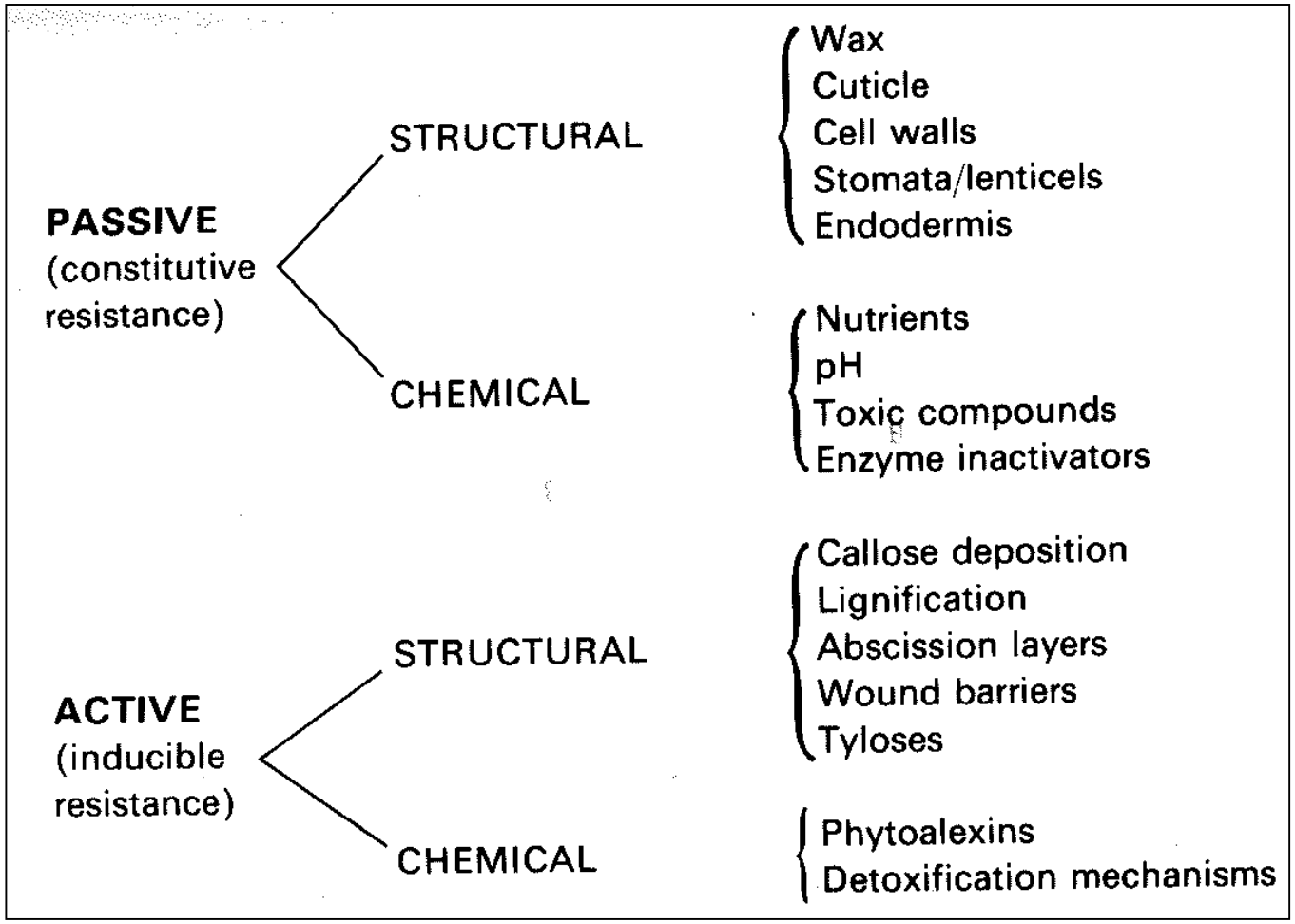
- Plants produce a diverse array of secondary metabolites, some with antimicrobial activity.
- Phytoanticipins are unique LMW defense-related compounds(antimicrobial compounds) present in plants even before the attack by pathogens. e.g. saponins, the natural detergents.
- What is the difference between Phytoalexins and Phytoanticipins?

1. Phytoanticipins are produced and stored constitutively in plant tissue(VanEtten *et al*.,1994), whereas
2. Phytoalexins are synthesized de novo in response to infection are termed phytoalexins (Müller & Börger,1940; Paxton, 1981).

Defense mechanisms

Passive and active defense mechanisms

Structural and chemicals

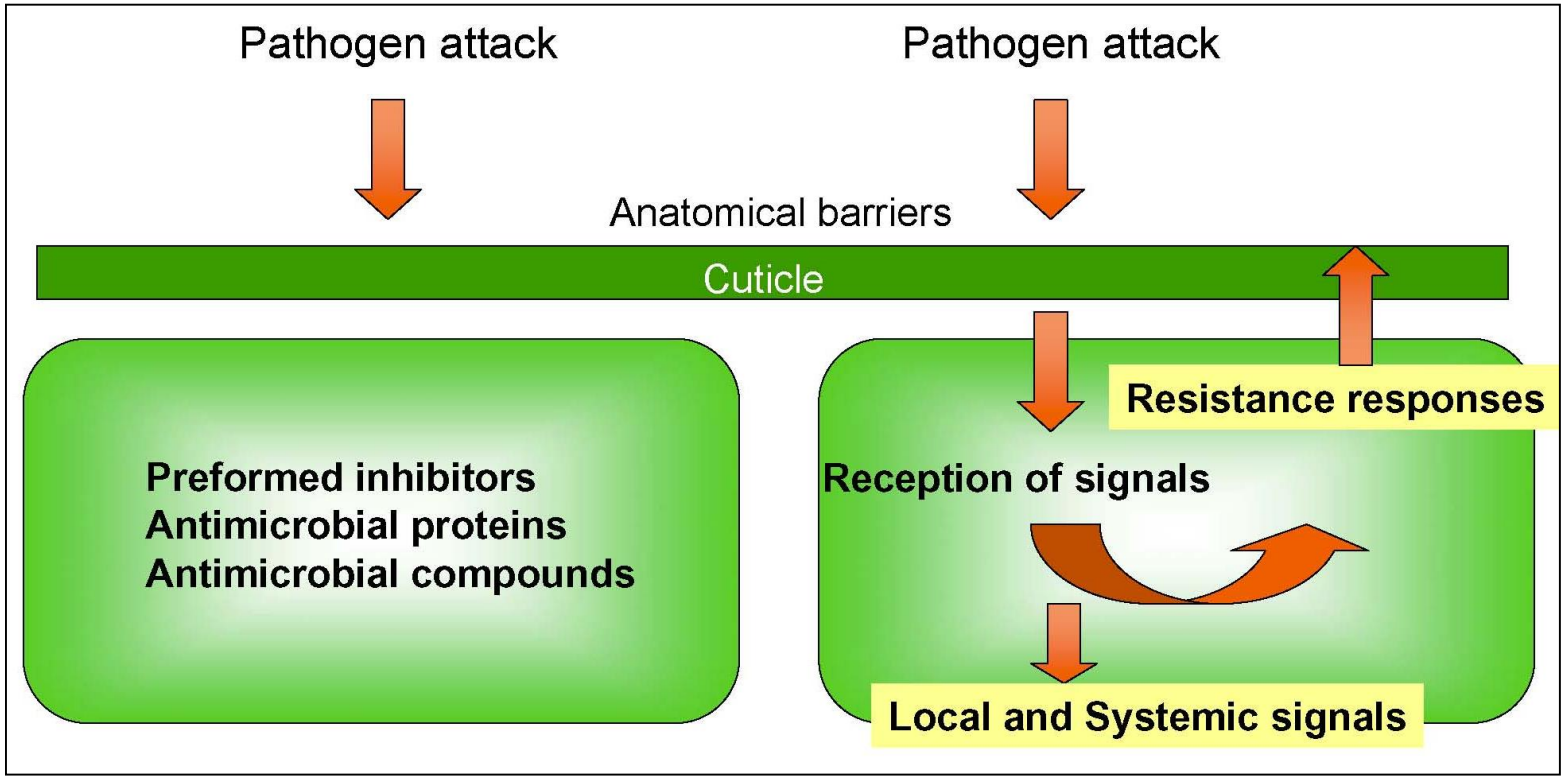


Dickinson & Lucas,1982

Defense mechanisms

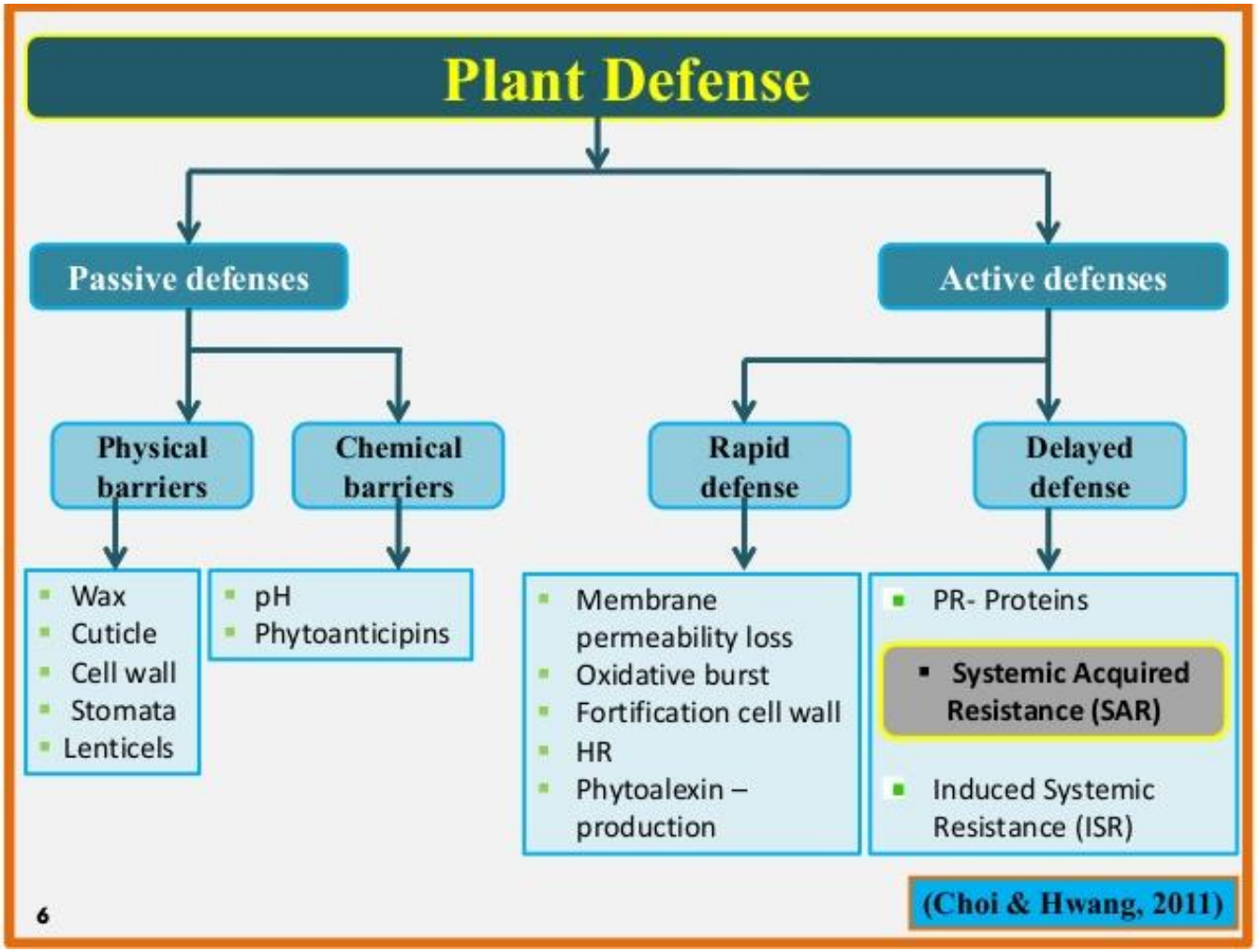
Passive and active defense mechanisms

Passive (constitutive) defense **Active (induced) defense**



The most common resistance

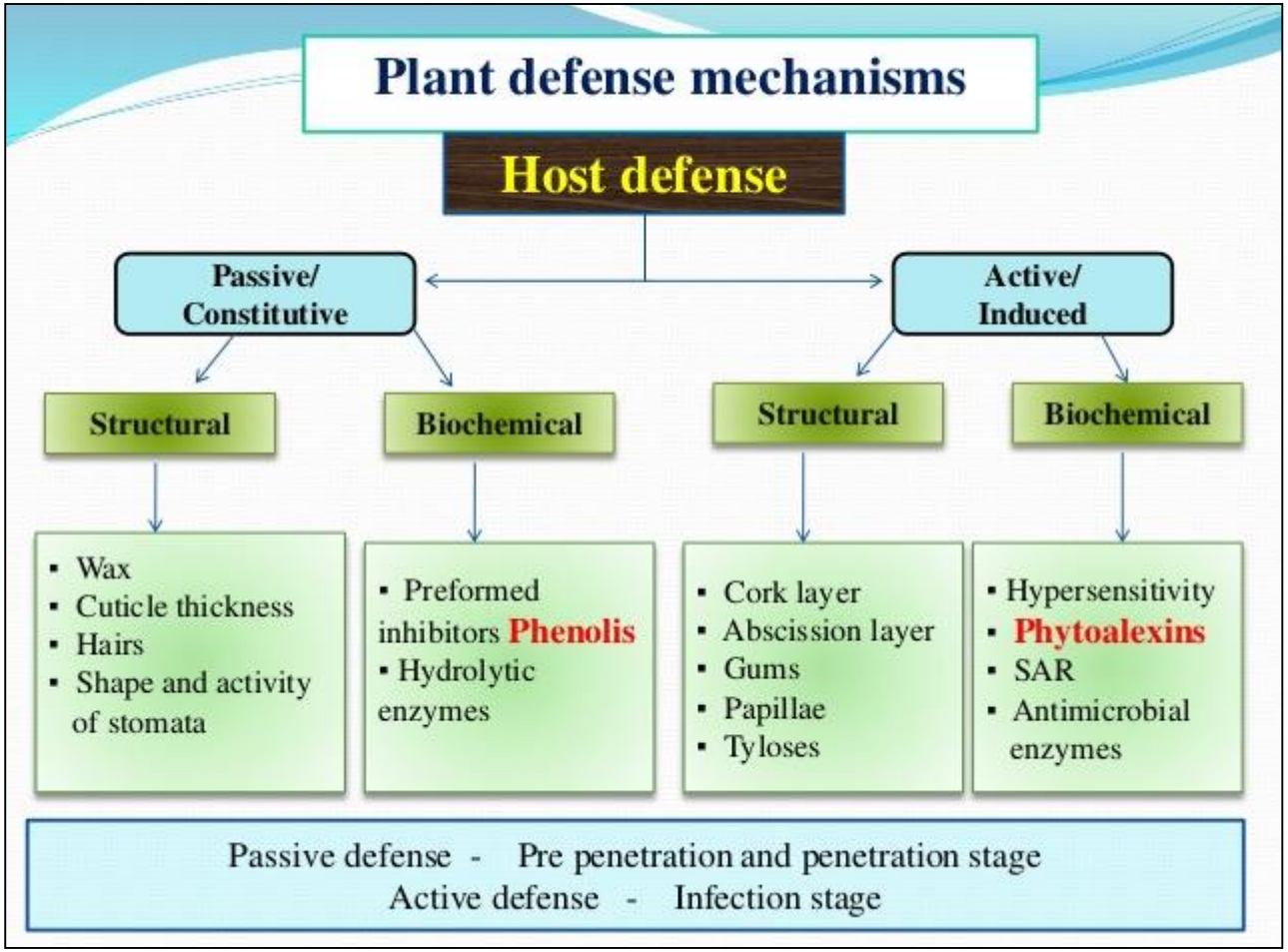
Passive(preformed)and active (induced) resistance



Defence mechanisms

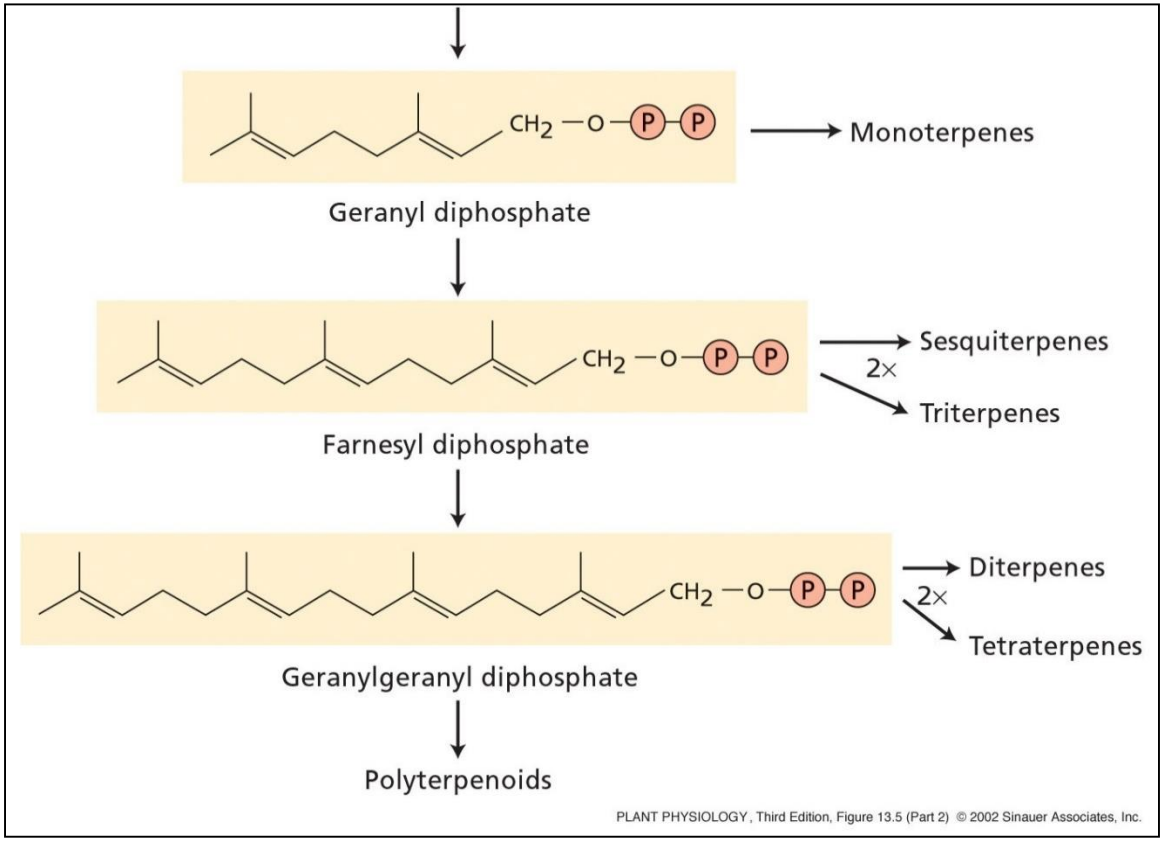
Passive and active defense mechanisms

Structural and chemicals/biochemicals



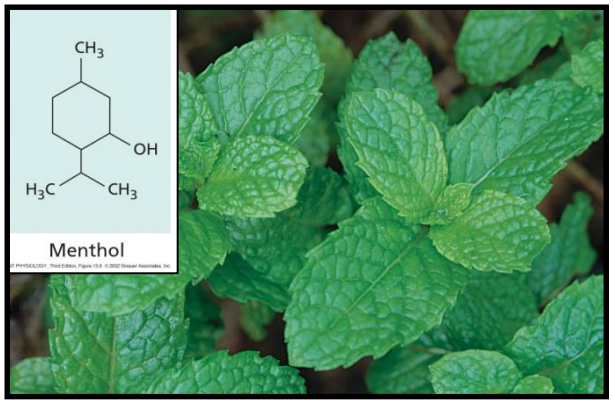
Pawar *et al.*,2017

Passive (constitutive) defense mechanisms
Secondary metabolites
Terpenes: defensive compounds produced from the mevalonic acid pathway

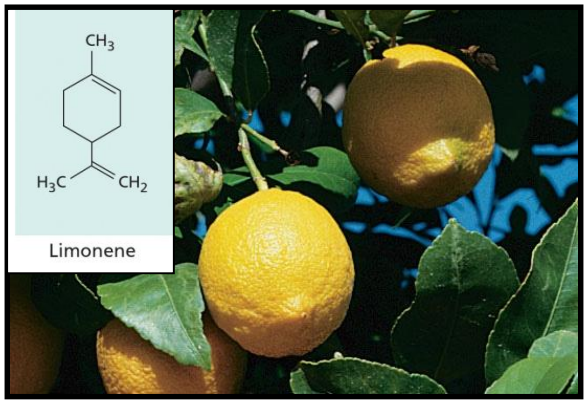


Sesquiterpene lactones (SLs) are a class of sesquiterpenoids that contain a lactone ring. They are most often found in plants of the family Asteraceae (daisies, asters).

Passive (constitutive) defense mechanisms
Secondary metabolites
Terpenes: defensive compounds produced from the mevalonic acid pathway



Volatile terpenes such as menthol broadcast a smell that warns herbivores that the plant is toxic to them before herbivore feeding commences.



Non-volatile terpenes – limonene apparently distasteful to herbivores.

Plant disease resistance mechanisms
Horizontal resistance

- According to J. E. van der plank,1963 when the resistance is evenly spread against all races of pathogen it is called "horizontal" or "lateral" resistance.
- Such resistance is sometimes called:

Horizontal resistance	
1. partial, 2. race non specific, 3. general, 4. quantitative, 5. polygenic, 6. adult-plant, 7.field, 8. additive,	9. durable, 10. stable, 11. non-differential 12. rate-reducing, 13. minor gene, 14. Incomplete, 15. Innate, 16. multigenic (non-host)resistance.

- In the case of horizontal resistance, reproduction rate of pathogen is never zero, but it is less than one, i.e., r>0 but <1.

Plant disease resistance mechanisms
Vertical resistance

- According to Van der plank, 1963 vertical resistance is that kind of resistance in plant varieties that effective against some races of pathogen and not against others.
- Such resistance differentiates clearly between races of pathogen, as it is effective against specific races of pathogen and ineffective against others.
- Such resistance is sometimes called:

Vertical resistance	
1. major gene, 2. race specific, 3. strong, 4. qualitative, 5. monogenic or oligogenic, 6. R-gene (host) resistance,	7. Less durable 8. unstable, 9. differential 10. racial resistance, 11. pathotype- specific 12. hypersensitive resistance 13. complete

Reproduction rate of pathogen is zero or 1(r=0 or 1)

Dhakar,2019

Plant disease resistance mechanisms

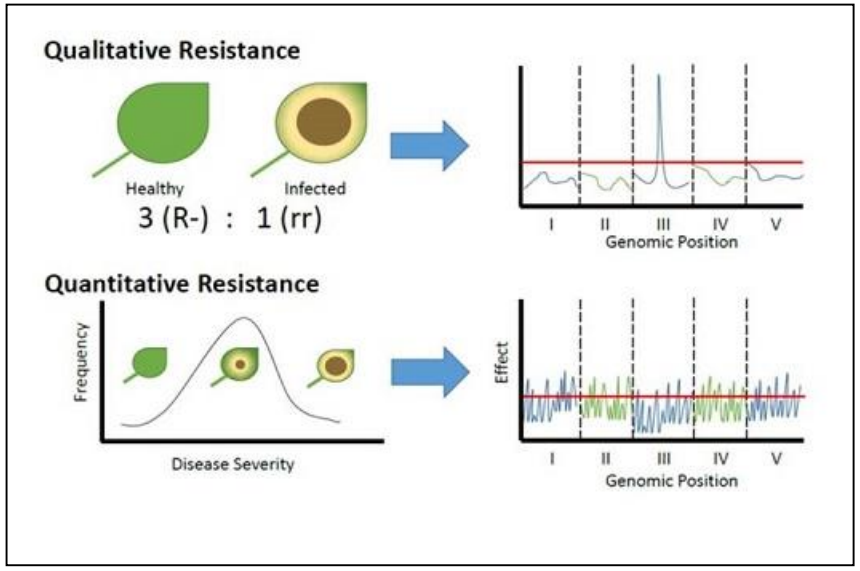
What are the similarities between vertical and horizontal resistance?

1. Both are types of disease resistance in plants.
2. They are very important for plant immunity against pathogens.
3. They emphasize the relationship between plant and pathogen.
4. Both are under genetic control.

Types of host resistance

Comparison between qualitative (vertical) and quantitative (horizontal) resistance

- In qualitative resistance, only a single gene (R) is involved with a major trait of:
- susceptibility, or
- resistance.
- While in quantitative resistance more than one gene is involved with both major and minor effects.



Williams,2017; Tariq,2020

Types of host resistance

Comparison between qualitative (vertical) and quantitative (horizontal) resistance

- In qualitative resistance, only a single major gene (R) is involved.
- R genes block the pathogen at the infection site and ultimately prevents the pathogen from further spread into the host cell.
- This mechanism expresses two discrete traits,

1. the host plant is resistant, or
2. susceptible.

- In quantitative resistance, more than one gene is involved with both major and minor effects.
- It does not block the pathogen at the infection site but decreases:

1. the symptom severity,
2. pathogen colonization & multiplication.

Types of host resistance

Comparison between qualitative (vertical) and quantitative (horizontal) resistance

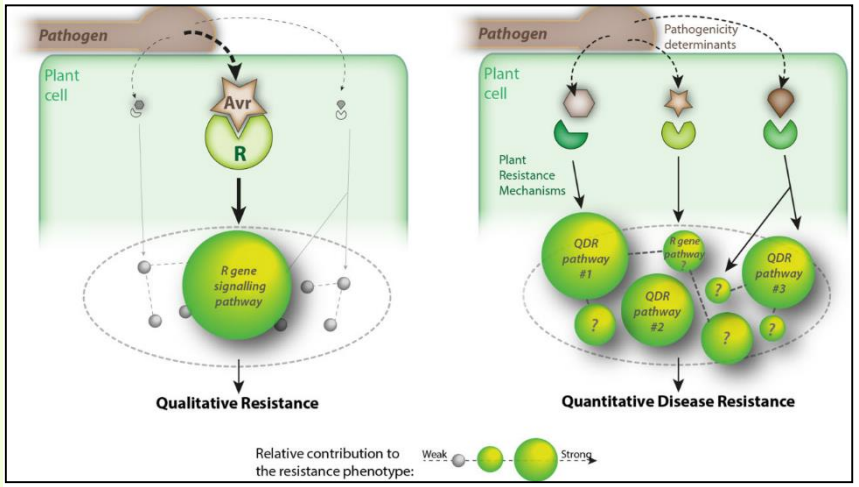
- Quantitative disease resistance(QDR) in which many genes make small contributions to the plant's resistance.
- These quantitative resistance loci(QTL) are lesser known and more difficult to study, but nevertheless govern the outcome of the majority of plant-pathogen interactions.

Williams,2017

Types of host resistance

Comparison between qualitative (vertical) and quantitative (horizontal) resistance

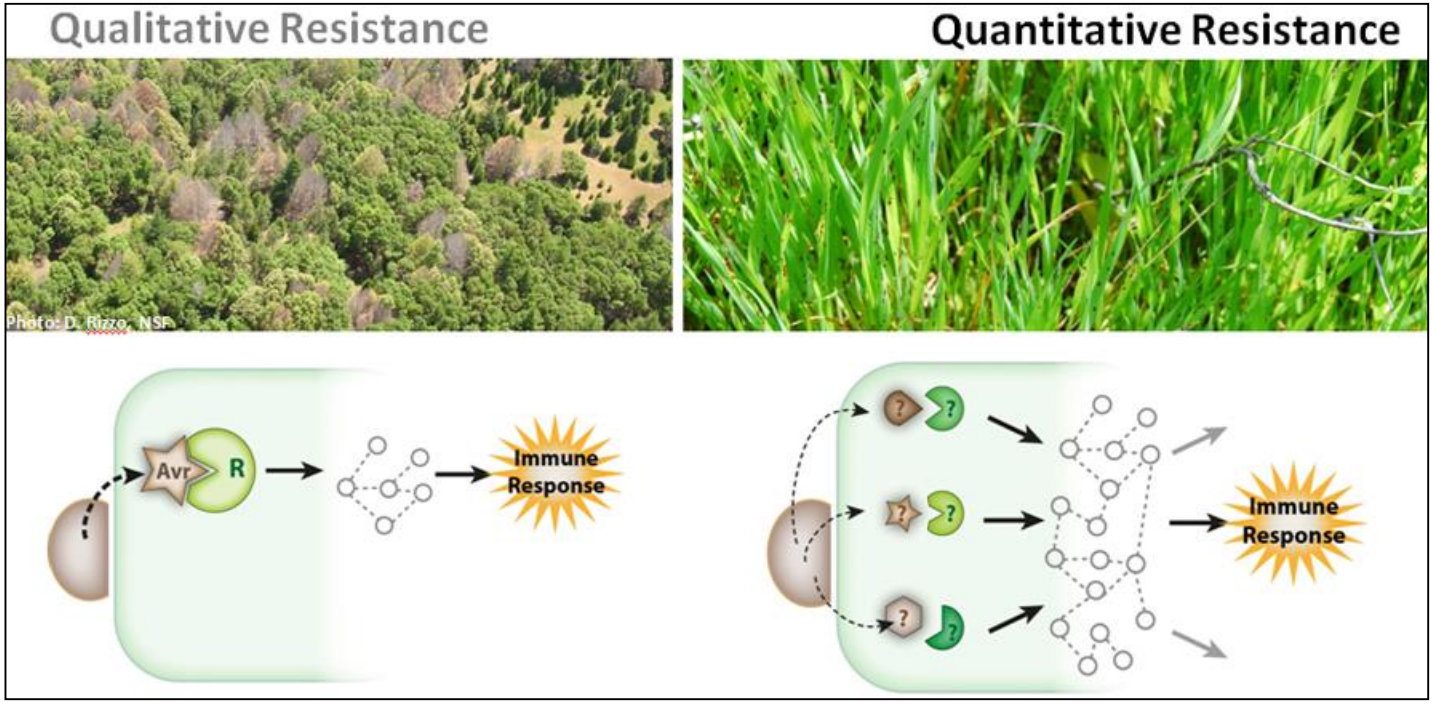
- Qualitative resistance (left panels) results from the perception of a single pathogen effector (Avr) by a plant resistance (R) gene.
- Whereas, quantitative disease resistance(QDR) results from the integration of multiple perception pathways activated simultaneously, each having a relatively minor contribution to the overall resistance phenotype.



Roux *et al.*,2014

Types of host resistance

Comparison between qualitative (vertical) and quantitative (horizontal) resistance

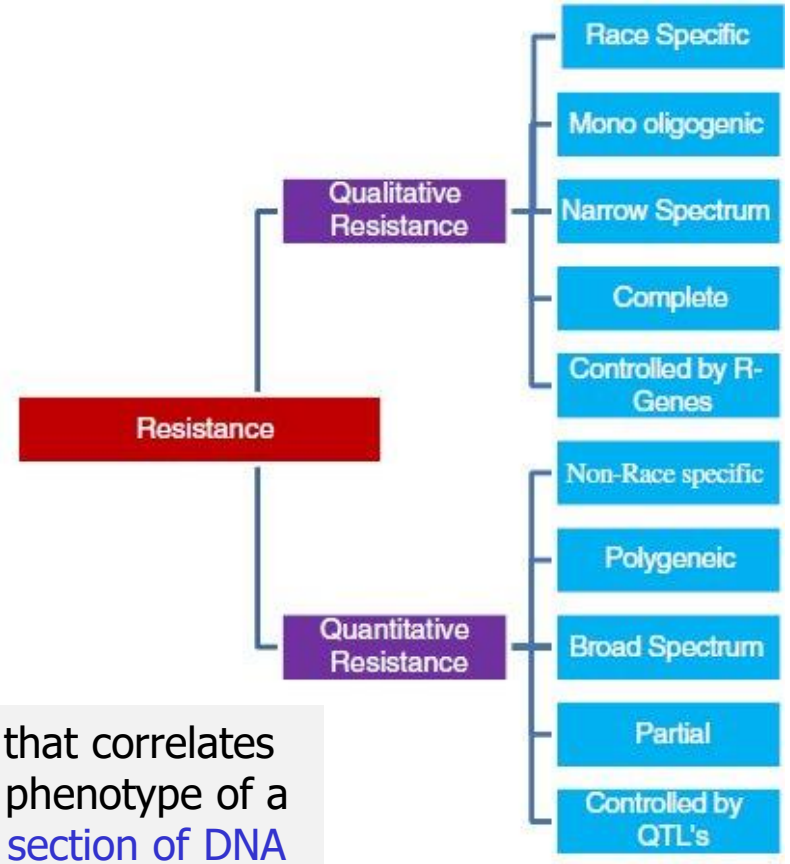


Roby and Raffaele,2011

Types of host resistance

Comparison between qualitative(horizontal) and quantitative(vertical) resistance

1. Qualitative disease resistance(R) increases the durability of qualitative (*R*-gene mediated) resistance.
2. Quantitative disease resistance (QDR) is usually controlled by multiple genetic factors (genes) known as quantitative trait loci or QTL.



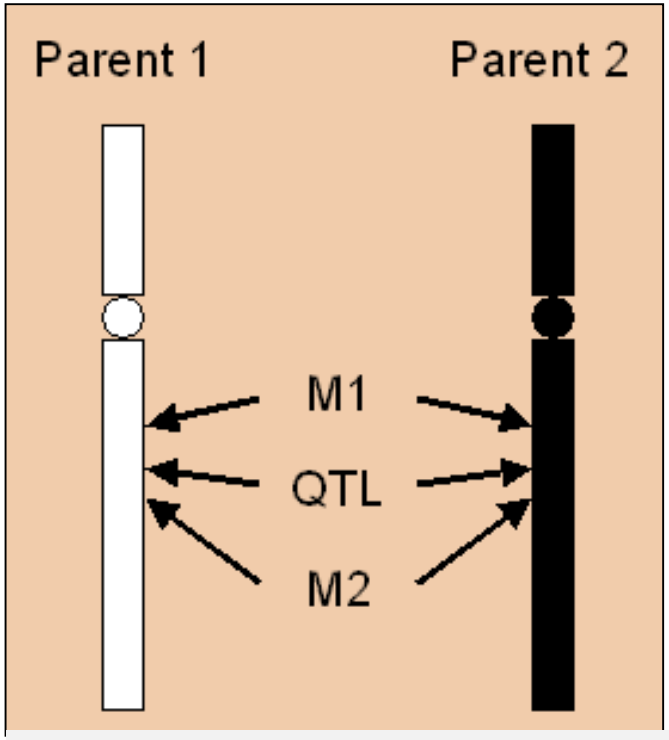
A quantitative trait locus (QTL) is a locus that correlates with variation of a quantitative trait in the phenotype of a population of organisms. A QTL is a small section of DNA on a chromosome thought to influence a specific trait.

Brun *et al.*,2019; Pusadkar,2018..

Quantitative trait locus (QTL)

QTLs are often found on different chromosomes

- Let's assume that the same chromosome region contains three loci.
- M1 and M2 are molecular marker loci that flank a QTL.
- A quantitative trait locus (QTL) is a region of DNA which is associated with a particular phenotypic trait, which varies in degree and which can be attributed to polygenic effects, i.e., the product of two or more genes, and their environment.



A QTL is a small section of DNA on a chromosome thought to influence a specific trait.

Plant disease resistance mechanisms
Vertical vs horizontal resistance

Horizontal resistance	Vertical resistance
Multigenic	Oligogenic
Race nonspecific	Race specific
Durable	Less durable
Quantitative resistance	Qualitative R resistance
Environmentally influenced	Environmentally not influenced
Often effective against necrotrophs	Often effective against biotrophs
Partial (r=0 to 1)	Complete (r=0 or 1)

Plant disease resistance mechanisms

Vertical vs horizontal resistance

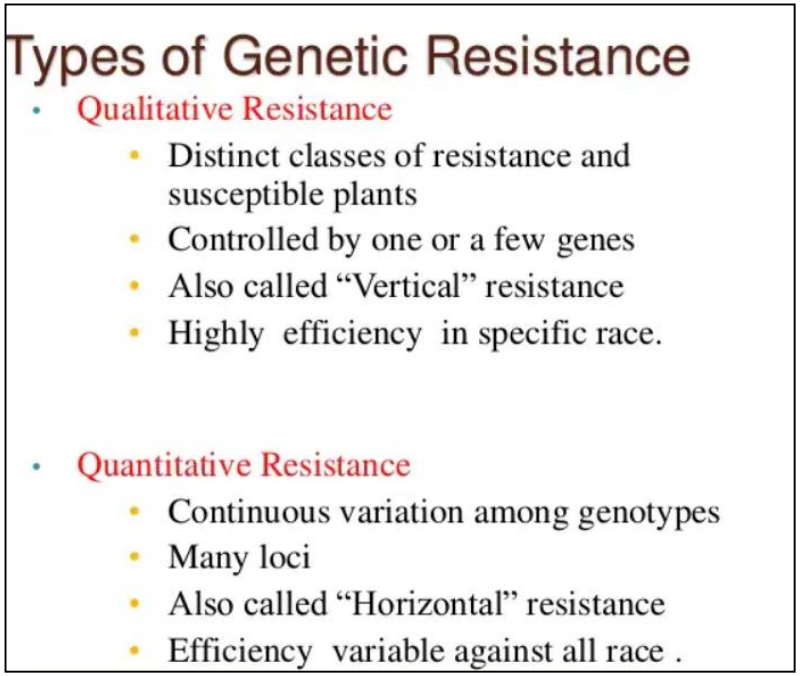
Features	horizontal resistance	Vertical resistance
1. Other Names	Partial Resistance, Polygenic Resistance, Gene non-specific Resistance, field resistanc	Qualitative resistance, R-resistance, Monogenic resistance, gene-specific resistance,
2. Nature Of Gene Action	Polygenic	Monogenic/oligogenic
3. Pathotype Specificity	Non-specific	Specific
4. Efficiency	Against all races	Against specific races
5. Effectivity	Nectrophs	Biotrophs
6. Stage Of Expression	Increases with maturity	Same from seedling to maturity
7. Chance of epidemic	Less/Minimal	Present

Plant disease resistance mechanisms
Comparison between qualitative (vertical) and quantitative (horizontal) resistance

Types of Genetic Resistance:

1. **Qualitative R Resistance:**
- Distinct classes of resistance and susceptible plants;
- Controlled by one or a few genes,
- Also called "Vertical" resistance;
- Highly efficiency in specific race.

2. **Quantitative Resistance:**
- Continuous variation among genotypes;
- Many loci;
- Also called "Horizontal" resistance;
- Efficiency variable against all race.



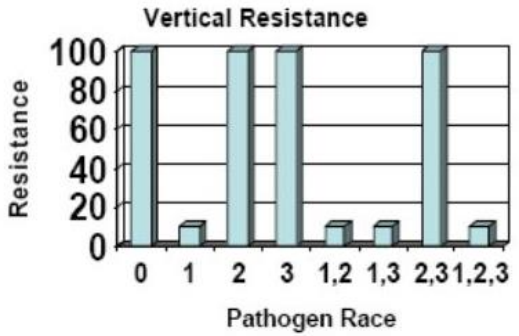
Nagar,2015

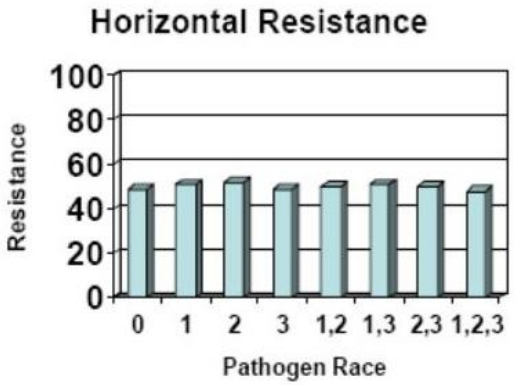
Plant disease resistance mechanisms

Comparison between qualitative (vertical) and quantitative (horizontal) resistance

Types of Plant Resistance

- **Vertical Resistance**
  - Monogenic, single R-gene
  - Hypersensitive response
  - "major gene"
  - "race-specific"
- **Horizontal Resistance**
  - Polygenic, many genes
  - Reduced disease
  - "field resistance"
  - "race-nonspecific"

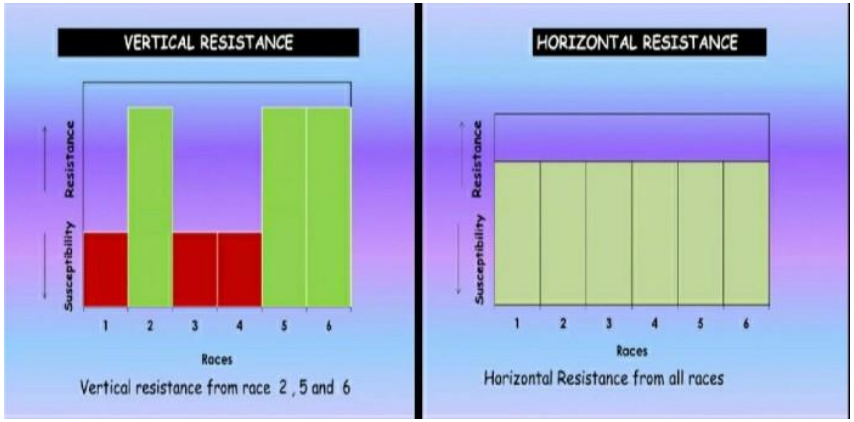




Plant disease resistance mechanisms

Comparison between qualitative (vertical) and quantitative (horizontal) resistance

- The first bar diagram explains: Vertical resistance is shown against the races 2,5 and 6. The resistance shown is complete that means the plants do not loss any production. They are completely healthy. Remaining races become completely susceptible.
-
- The second bar diagram explains:- Horizontal Resistance acts against all the races. But they can't completely oppose the races. They are mimizing the damage caused by pathogens. That's why the bar diagram is light green and height is half.



Prakash

Plant disease resistance mechanisms

Comparison between qualitative (vertical) and quantitative (horizontal) resistance

- Qualitative Resistance (R-gens) is characterized by two distinct phenotypes:
  1. Resistant, or
  2. susceptible.
- Quantitative Resistance (controlled by Quantitative Resistance Loci, QRL) is characterized by continuous phenotypic variation.
- For Quantitative Resistance:
  1. any gene involved in pathogen recognition, or
  2. Defense.

Qualitative vs Quantitative

- Qualitative Resistance is characterized by two distinct phenotypes: resistant or susceptible.
- Quantitative Resistance is characterized by continuous phenotypic variation.
- For Quantitative Resistance any gene involved in pathogen recognition or defense can be involved and can be identified as Quantitative Resistance Loci (QRL)

Amir Moarefi

Two levels of host defenses

Non-specific & specific plant disease resistance

1. Non-specific plant disease resistance (general, non-host or basic, innate resistance):

- Response to all races of a particular pathogen, and occurs in all cultivars of a host plant species.
- In these basal defenses, HR is not elicited (basal defenses).

2. Specific plant disease resistance:

- Dependent upon the presence of:

1. A particular pathogen race,
2. A particular host plant cultivar, or
3. Both.

- In this resistance, HR is elicited (HR defenses).

Types of host resistance

Qualitative disease resistance

Host specific, R gene resistance

1. **Susceptible:** Phenotypic expression related to extensive symptom development and/or pathogen reproduction and accomplished by uninhibited invasion of host by pathogen.
2. **Resistant:** Phenotypic expression related to complete or partial suppression of symptom severity and/or pathogen reproduction and accomplished by arrested or slowed invasion of host by pathogen.
3. **Partial susceptibility or resistance:** Expression of symptoms, but less than full susceptibility or greater than complete resistance.

Plant immune system

Two branches of the plant immune system

- Like animals, plants need to be on a constant lookout to recognize and respond to invasion by microbes.
- Plants have an innate immune system to avoid pathogen infection, and the two major branches of which are:

1. PAMP-triggered immunity (PTI) known as basal resistance or non-specific disease resistance;
2. Effector-triggered immunity (ETI) or specific disease resistance. Because of coevolution of plant resistance (*R*) genes, which specifically recognize pathogen strain- or race-specific factors.

Azevedo *et al.*,2008; Nürnberger,2021

Plant immune system

Two branches of the plant immune system

- Nonhost resistance is a broad-spectrum plant defense that provides immunity to all members of a plant species against all isolates of a microorganism that is pathogenic to other plant species.
- Upon landing on the surface of a nonhost plant species, a potential bacterial pathogen initially encounters preformed and, later, induced plant defenses.

Plant immune system

Two branches of the plant immune system

1. One of the initial/basal defense responses from the plant is pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI). **PAMP-induced defence in susceptible host plants is a weak and insufficient non-specific immune response to stop infection**.
2. Whereas, host plants also have mechanisms to detect host-pathogen effectors and can trigger a defense response referred to as effector-triggered immunity (ETI).

Plant immune system

Two branches of the plant immune system

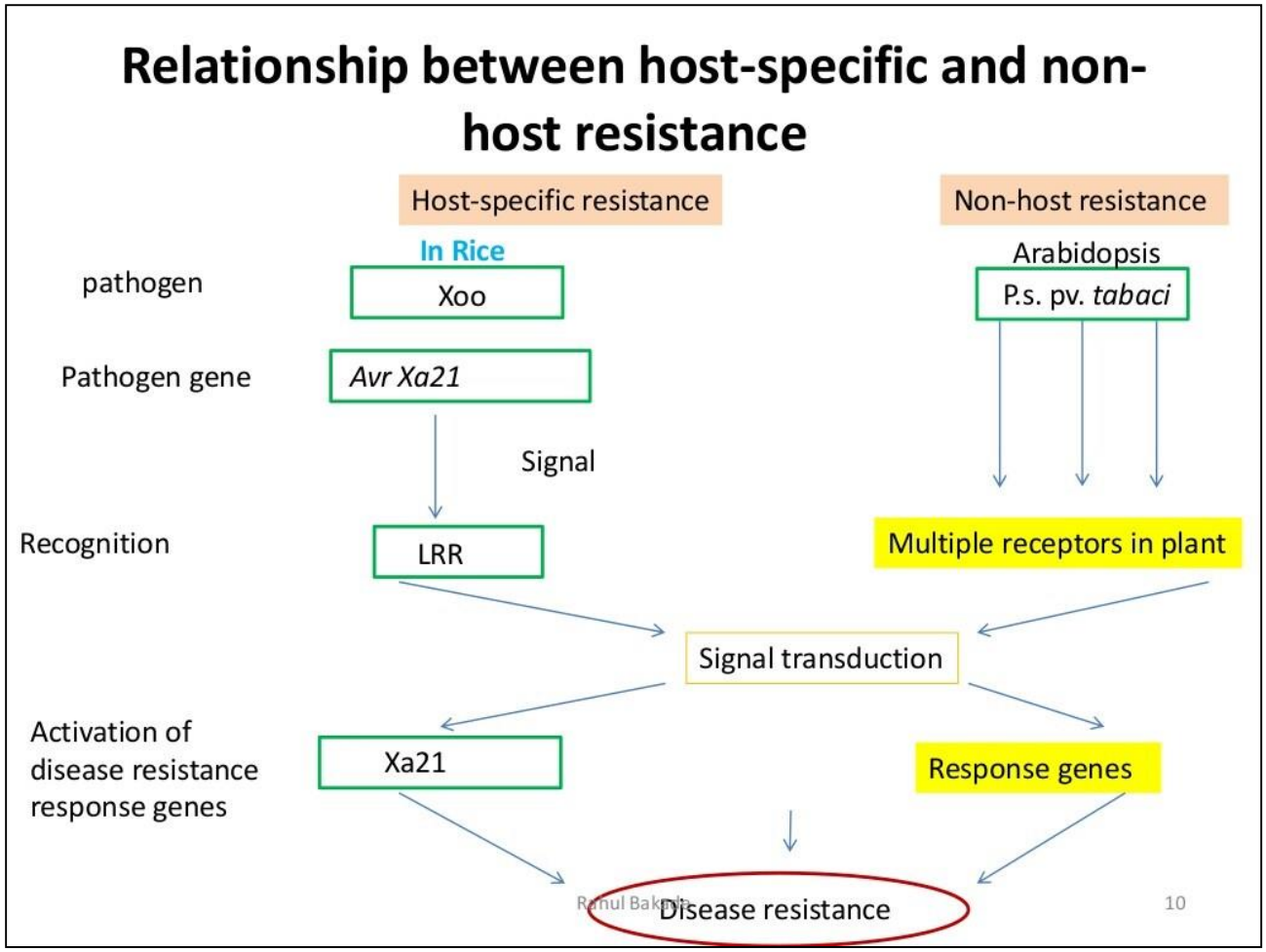
Host and non-host resistance

- **Nonhost resistance:**
- The initial/basal plant defense begins with the detection of invaders and their pathogen-associated molecular patterns (PAMPs) by receptors of plants at the cell surface i.e. transmembrane pattern recognition receptors (PRRs).
- **Host resistance:**
- The second alarm pathway is triggered by receptors within the cell. This was acting largely inside the cell and using resistance genes, coding for NB-LRR proteins(R proteins). In other words, gene recognition mediated by *R* and *avr* genes.

Azevedo *et al.*,2008; Nürnberger,2021

Mechanisms of host and nonhost resistance against fungal and bacterial plant pathogens

Host-specific vs. non-host resistance

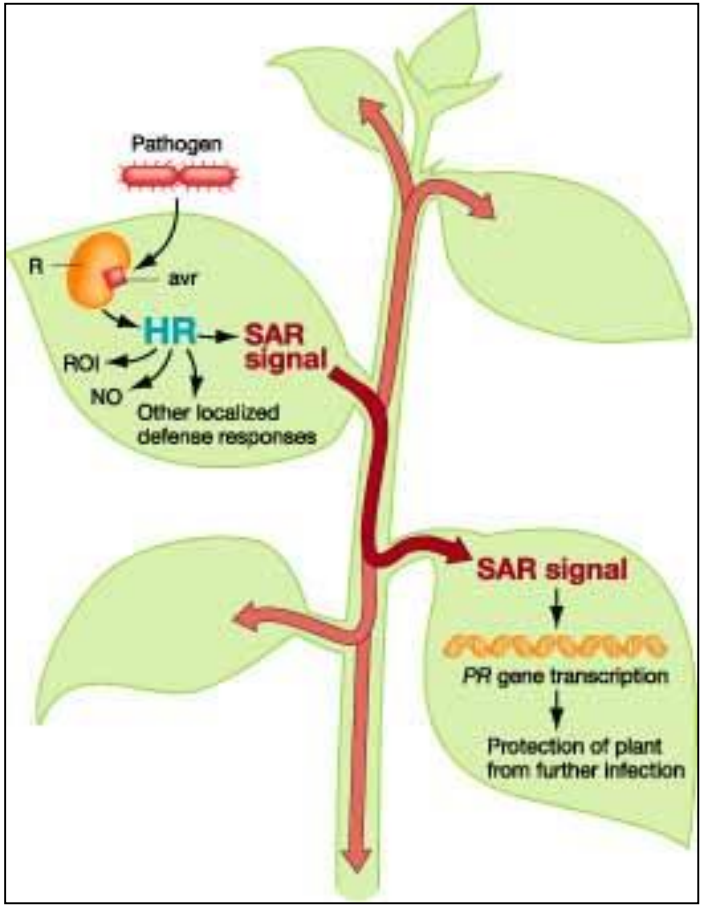


Active Defense Mechanisms

Effector-triggered immunity (ETI)

Induced/systematic resistance

- Systemic acquired resistance (SAR) refers to a distinct signal transduction process, that plays an important role in the ability of plants to defend themselves against pathogens.
- After the formation of a necrotic lesion, either as a part of hypersensitive response (HR) or as a symptom of disease, the SAR process is activated.



Naga Satyasri Ch;Elmore,2015

Mechanisms of host and nonhost resistance against fungal and bacterial plant pathogens

Non-host type-I and type-II resistance

- Non-Host resistance (NHR) is defined as resistance from plants to many incompatible microbial-pathogens (viral, fungal and bacterial).
- As a result of initial response of plant defense, the NHR against the pathogens showed two different types of reactions.

1. The first type (Non-host type-I) does not appear any visible symptoms and called plant-triggered immunity (PTI),
2. The second type of NHR (Non-host II) observes several hypersensitive responses (HR) with necrosis (ETI).

Hafez *et al.*,2020

Mechanisms of host and nonhost resistance against fungal and bacterial plant pathogens

Non-host type-I and type-II resistance

- Based on the type of hypersensitive reaction (HR) triggered, non-host resistance(NHR) was classified into two types, namely

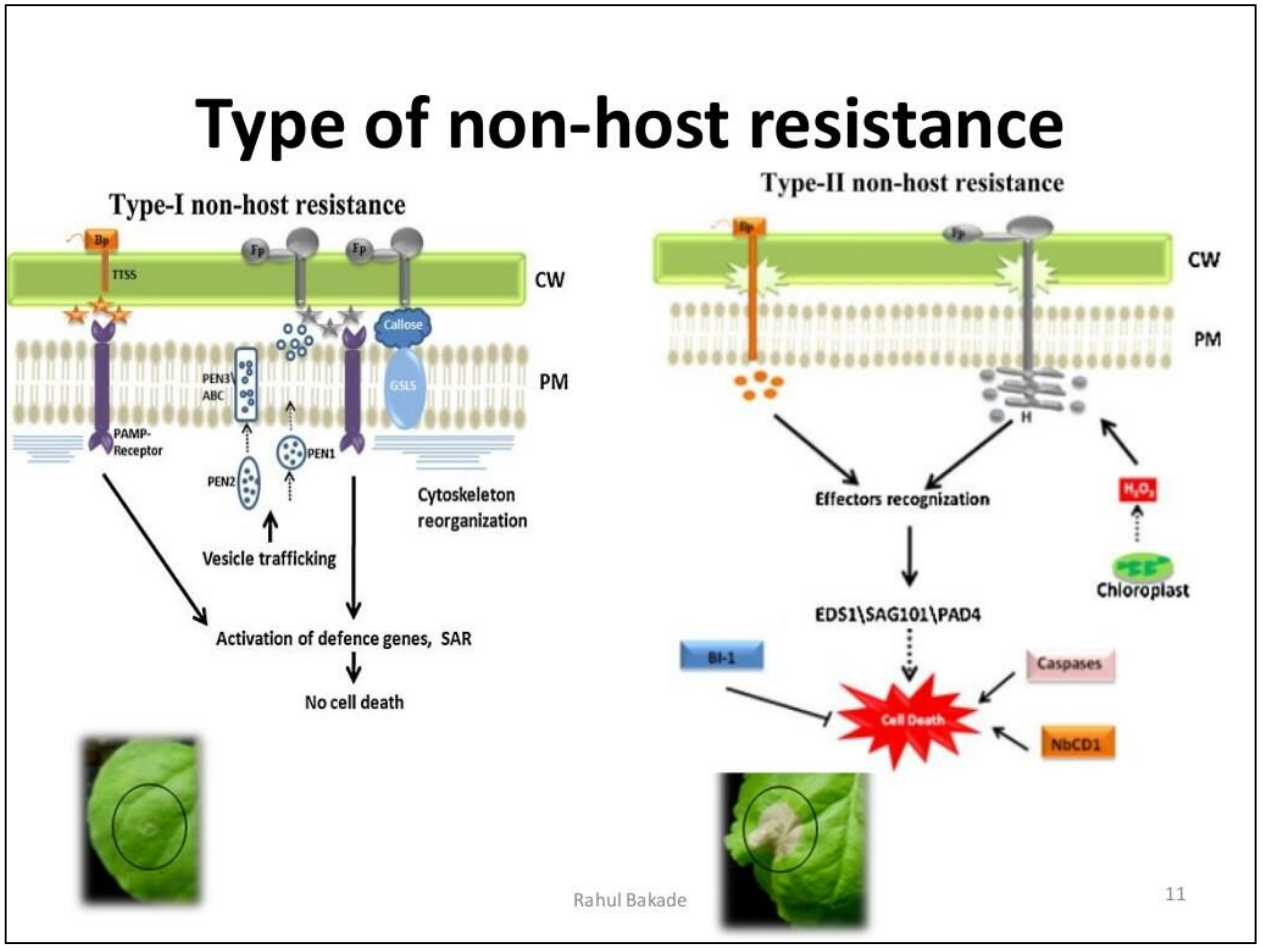
1. type-I, and
2. type-II.

- As might be expected, R-gene mediated resistance(Host-specific) is found to overlap with Non-host resistance(NHR), but the extent to which the genes/pathways are common between these two forms of disease resistance is unknown.

Uma *et al.*,2011

Mechanisms of host and nonhost resistance against fungal and bacterial plant pathogens

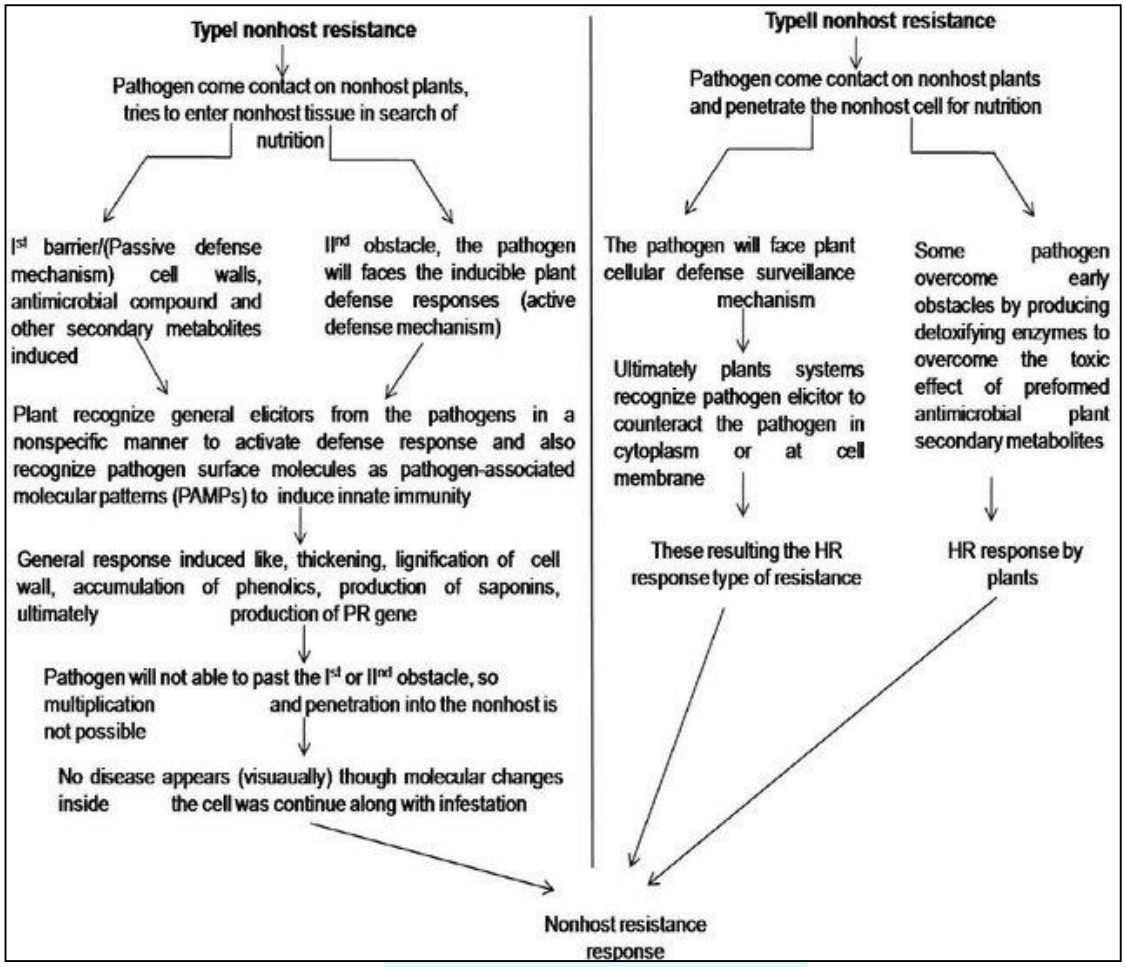
Non-host type-I and type-II resistance



Uma *et al.*,2011

Mechanisms of host and nonhost resistance against fungal and bacterial plant pathogens

Non-host type-I and type-II resistance



Shamim *et al.*,2013

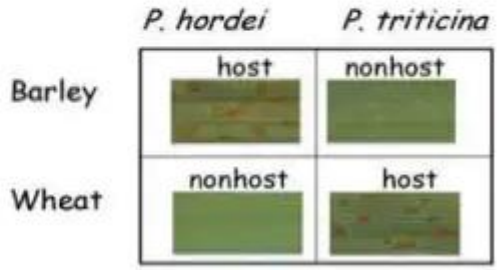
Mechanisms of host and nonhost resistance against fungal and bacterial plant pathogens

Non-host type-I and type-II resistance

Non host resistance

Plant Innate Immunity, Species Resistance, Durable resistance, Basal defense and Nonspecific resistance

- Is a broad spectrum resistance
- Resistance of an entire plant species to all isolates of a microbial species
- Preformed barriers such as cell wall, cuticle, phytoanticipins
- Induced defense responses such as lignin accumulation, production of antimicrobials like phytoalexins, HR response, induction of pathogenesis-related (PR) proteins
- Eg., Barley is typically susceptible to *P. hordei*, to Which wheat is a nonhost. The reverse is true for *P. triticina*



Singanodi,2016

Mechanisms of host and nonhost resistance against fungal and bacterial plant pathogens

Non-host type-I and type-II resistance

- Nonhost resistance exhibited against bacteria, fungi and oomycetes can be of two types:
- **Type I nonhost resistance** does not produce any visible symptoms, plant defense responses include:

1. cell wall thickening,
2. phytoalexin accumulation,
3. other plant secondary metabolites, and
4. papilla formation.

Mysore and Ryu,2004; Künstler *et al.*,2018;..

Mechanisms of host and nonhost resistance against fungal and bacterial plant pathogens

Non-host type-I and type-II resistance

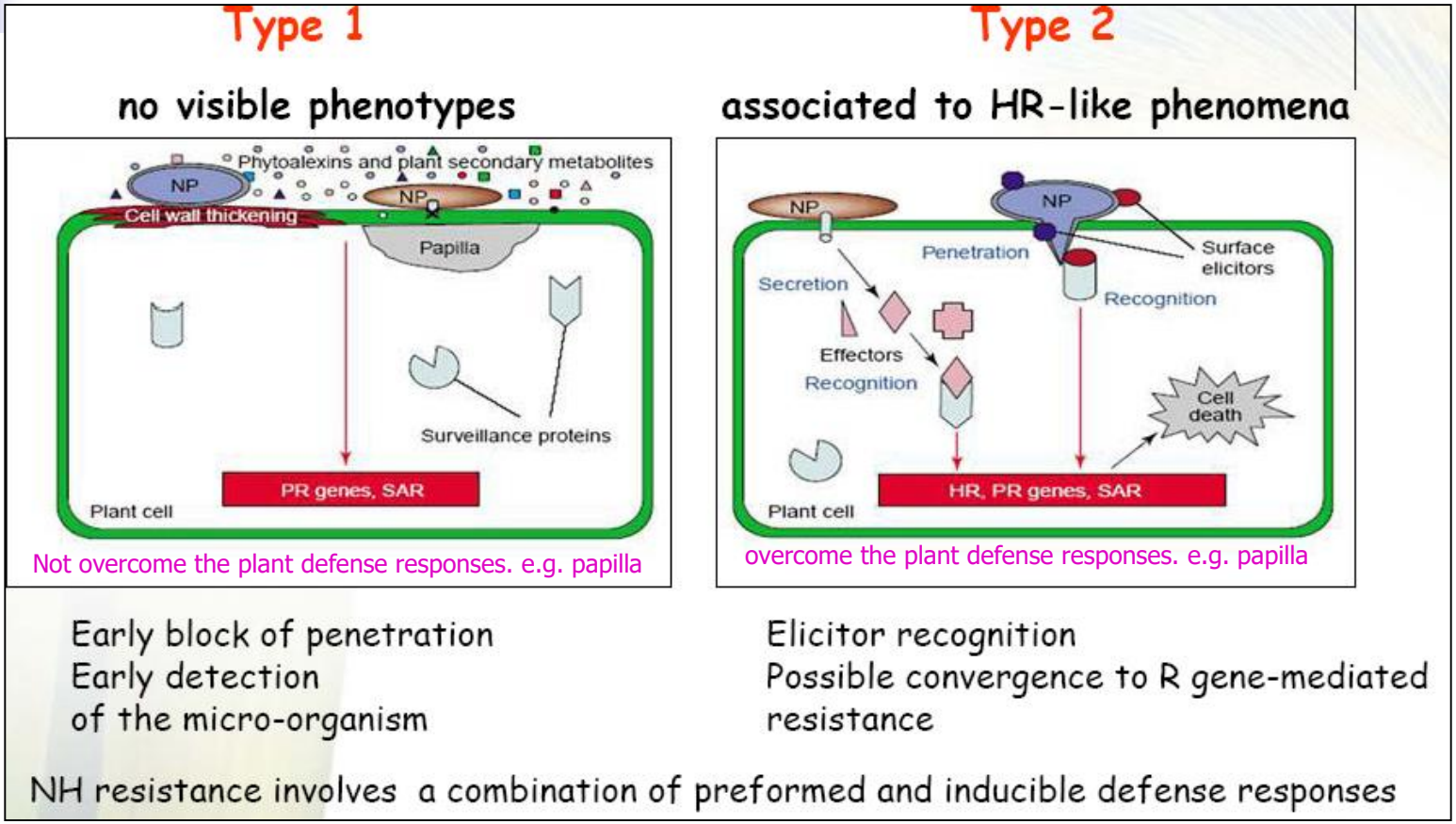
1. **Type II nonhost resistance** results in a rapid hypersensitive response with cell death.

- Specific pathogen elicitors (Avr gene) are then recognized by the plant surveillance system(R gene) and this triggers plant defense leading to a hypersensitive response (HR).
- R gene expression and SAR are also induced during type II nonhost resistance.

Mysore and Ryu,2004

A model for type I and type II non-host resistance

HR is not elicited in type 1



Mysore and Ryu,2004

Blue-colored NPs (nonhost pathogens) represent fungi or oomycetes and brown-colored NPs represent bacteria. The timing/speed of defences is much more rapid during type I nonhost resistance than during type II nonhost and host ("gene-for-gene") resistance

Examples of type I and type II non-host resistance

Pathogen	Strain or isolate	Nonhost plant(s)	Visible symptoms
Type I nonhost resistance			
Pseudomonas syringae pv. phaseolicola	NPS3121	*Arabidopsis*	None
P. s. pv. phaseolicola (at 30 °C)	S2	*Nicotiana tabacum*	None
P. s. pv. syringae	B76	*Arabidopsis*	None
P. s. pv. savastanoi	213-3 (IAA^-)	*Arabidopsis*	None
P. s. pv. delphinii	PDDCC529	*Arabidopsis*	None
P. s. pv. morsprunorum	B60-1	*Arabidopsis*	None
P. s. pv. atrofaciens	B143	*Arabidopsis*	None
P. s. pv. coronafaciens	B142	*Arabidopsis*	None
Xanthomas campestris pv. campestris	8004	*Nicotiana benthamiana*	None
Gaeumannomyces graminis var.*tritici*	T5	*Avena strigosa*	None
Puccinia recondita f. sp.*tritici*	WBR I	Oat	None
Puccinia graminis f. sp.*tritici*	ANZ	Oat	None
Phytophthora infestans	88069	*N. alata* cv. lime green	None
P. infestans	88069	*N. clevelandii*	None
P. infestans	88069	*N. tabacum* cv. *xanthi*	None
Type II nonhost resistance			
Pseudomonas syringae pv.*maculicola*	m2	*Nicotiana benthamiana*	HR
P. s. pv. tomato	DC3000	*N. tabacum*	HR
P. s. pv. phaseolicola	NPS3121	*N. tabacum*	HR
P. s. pv. glycinea	PG4180	*N. tabacum*	HR
P. s. pv. pisi	ATCC # 11055	*N. tabacum*	HR
P. s. pv. syringae	61	*N. tabacum*	HR
P. cichorii	83-1	*Arabidopsis*	HR
Xanthomonas axinopodis pv. vesicatoria	82-8	*N. benthamiana*	HR
X. campestris pv. glycines	8ra	Pepper, tomato	HR
X. citri	3213	Cotton, bean	HR
Erwinia rubrifaciens		*N. tabacum*	HR
Alternaria brassicicola	MUCL20297	*Arabidopsis*	HR
Blumeria graminis f. sp. *tritici*	bgtA95	Barley	HR
Phytophthora infestans		*Arabidopsis*	HR
P. infestans	88069	*N. benthamiana*, *N. rustica*, parsley	HR
P. sojae		*Arabidopsis*	HR
Fusarium solani f. sp. *phaseoli*	W-8	Pea	HR

Mysore and Ryu,2004

Examples of bacterial pathogens
Type I non-host resistance

Pathogen	Nonhost plant(s)	Visible symptoms
Pseudomonas syringae pv. *atrofaciens*	*Arabidopsis*	None
P. s. pv. *coronafaciens*	*Arabidopsis*	None
P. s. pv. *delphinii*	*Arabidopsis*	None
P. s. pv. *morsprunorum*	*Arabidopsis*	None
P. s. pv. *phaseolicola*	*Arabidopsis*	None
P. s. pv. *savastanoi*	*Arabidopsis*	None
Xanthomas campestris pv. *campestris*	*Nicotiana benthamiana*	None

Mysore and Ryu,2004

Examples of bacterial pathogens
Type II non-host resistance

Pathogen	Nonhost plant(s)	Visible symptoms
Pseudomonas syringae pv. *tomato*	*Nicotiana tabacum*	HR
P. s. pv. *maculicola*	*Nicotiana benthamiana*	HR
P. s. pv. *glycinea*	*N. tabacum*	HR
P. s. pv. *syringae*	*N. tabacum*	HR
P. s. pv. *pisi*	*N. tabacum*	HR
P. s. pv. *phaseolicola*	*N. tabacum*	HR
P. s. pv. *cichorii*	*Arabidopsis*	HR
Xanthomas axonopodis pv. *glycines*	Pepper, tomato	HR
Xanthomas axonopodis pv. *vesicatoria*	*Nicotiana benthamiana*	HR
X. citri	Cotton, bean	HR
Erwinia rubrifaciens	*N. tabacum*	HR

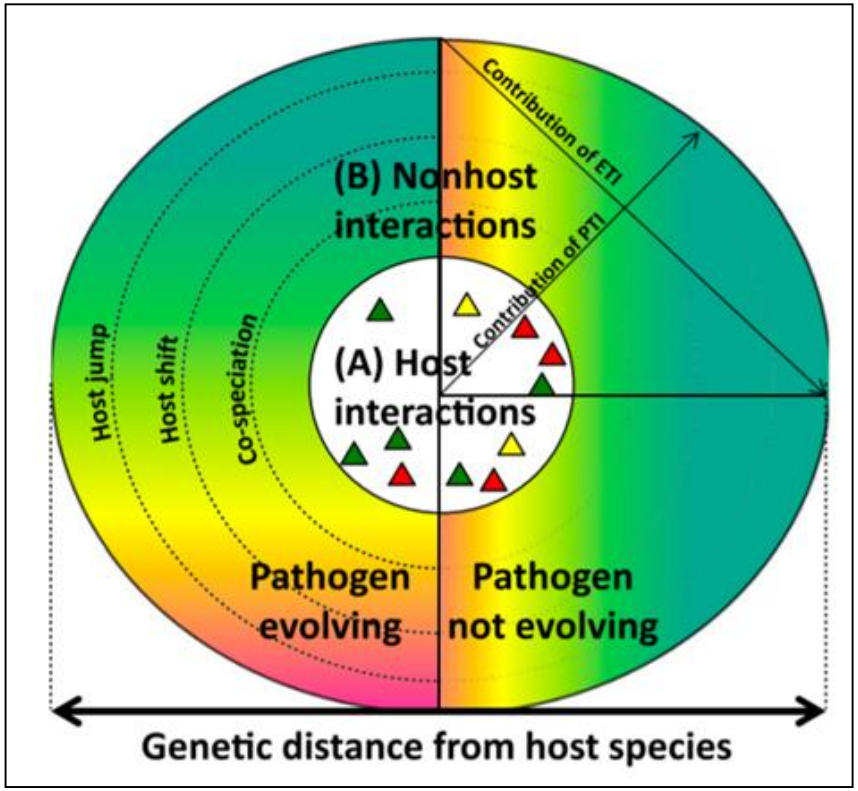
Mysore and Ryu,2004

Pathogen recognition
Evolution of host and non-host resistance

A. **Host resistance is primarily controlled by AVR-R recognition.** A micro-evolution creates diversity within host species for resistance/susceptibility and also within pathogen to develop new races with diverse suite of effectors.

B. **Outcomes of no host interactions vary with genetic distance from host species and the pathogen's ability to evolve.**

- A rapidly evolving pathogen due to co-speciation, host shift and host jump has better capability to adept to new no host species by breaking the nonhost barriers.



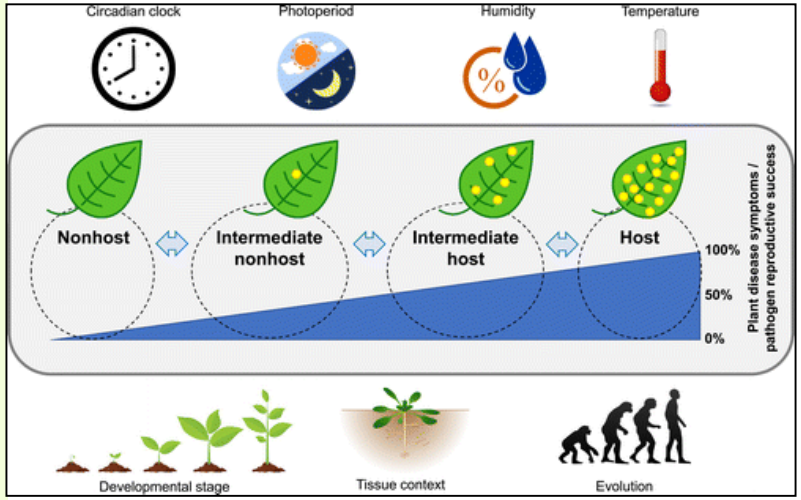
Gill *et al.*,2015

Plant immune system

Non-host resistance

Broad-spectrum resistance

- Nonhost resistance is a **gradual phenomenon** that is modulated by various exogenous factors. The central part of the figure depicts the **continuum between nonhost and host plants**, with several intermediate forms possible.
- Yellow circles on the leaves signify the extent of pathogen colonization.
- Around the center, several factors (circadian clock, photoperiod, humidity, temperature, developmental stage, tissue context, and evolution) are illustrated that may condition a shift from one state to another, as indicated by the light blue double-headed arrows shown in the central part.



Panstruga and Moscou,2020

Antimicrobial peptides

Antimicrobial peptides and plant disease control

Antimicrobial peptides (AMPs) are often the first line of defense against invading pathogens in human, animals and plants and play an important role in innate immunity.

Antimicrobial peptides comprise a host's natural defense against the daily exposure to millions of potential pathogens.

See also Bacterial Pathogenesis PowerPoint Presentation file as well as Genetic Engineering Plants: Antibacterial peptides (AMPs)-mediated resistance section in current file. Note: Bacterial resistance to AMPs has also been reported recently(Hong *et al.*,2016, Abdi *et al.*,2019; Lee *et al.*,2019.

Antimicrobial Peptides

Antimicrobial peptides synthesized from plants and destroy pathogens at multiple targets

- Antimicrobial peptides (AMPs) are the small molecular peptides that play a crucial role in the innate immunity of the host against a broad range of microorganisms, including bacteria, fungi, parasites and viruses.
- Theses compounds found in:

1. animals,
2. Plants, and even
3. Microorganisms.

- In plants, this mechanism is crucial for survival.

Laila *et al.*,2021; Zhang *et al.*,2021

Antimicrobial/antibacterial peptides

The broad spectrum antimicrobial activities of AMPs

- Initially, AMPs were identified as endogenous antibiotics due to their potential to kill various pathogens by disrupting their membranes.
- Antibiotics are often derived from moulds or are made synthetically and are absorbed into the body with the aim of:

1. killing bacteria (bactericidal), or
2. preventing their multiplication (bacteriostatic).

The endogenous antimicrobial peptides of animals are products of single genes and are synthesized as preproproteins. Multistep processing yields the mature peptide, which generally acts by inducing microbial membrane permeabilization.

Antimicrobial/antibacterial peptides

The broad spectrum antimicrobial activities of AMPs

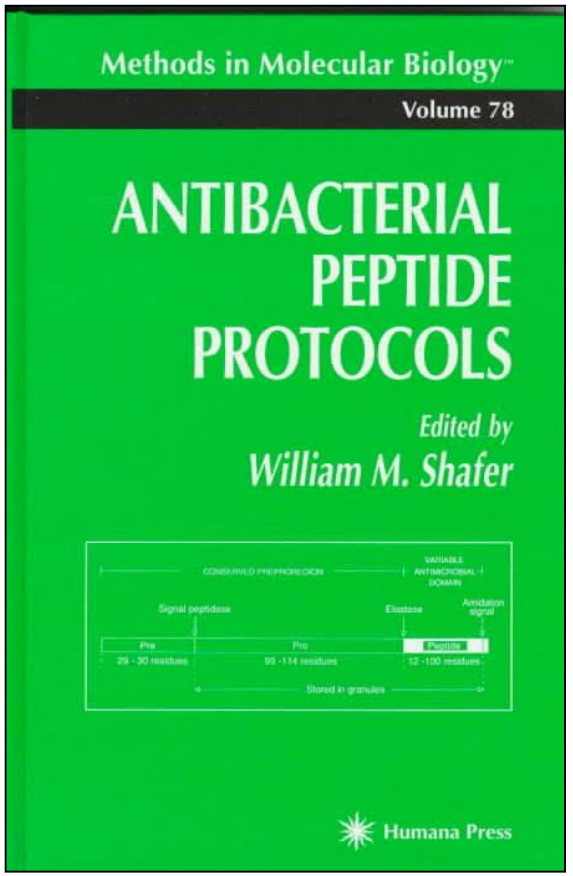
- Unlike traditional antibiotics with only one target, AMPs can destroy pathogens at multiple targets, greatly reducing the emergence of drug-resistant bacteria.
- They have broad-spectrum antibacterial properties and are currently being used in:

1. clinical treatment of pathogen infection,
2. wound healing, and
3. cancer.

Xuan *et al.*,2023

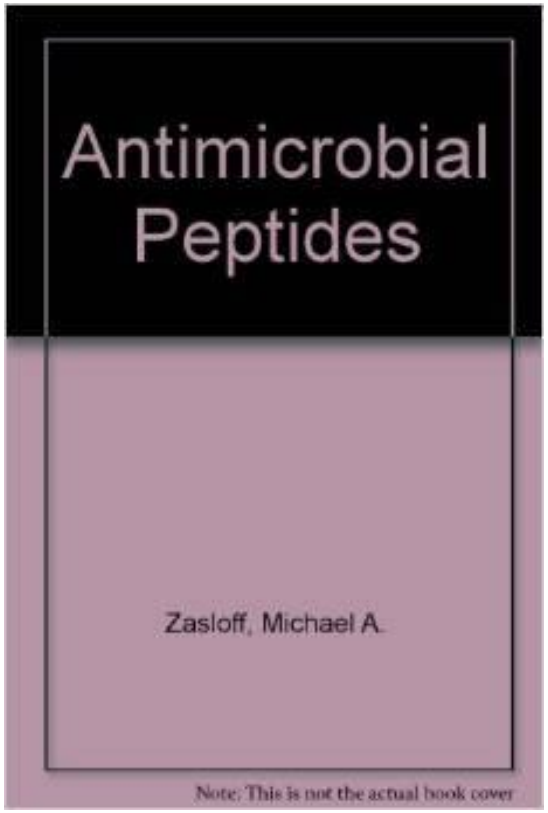
Antimicrobial Peptides

- **Antibacterial Peptide Protocols**
- by William Schaffer (Editor)
- Publisher: Humana Press
- 1997 edition.
- 259 pages



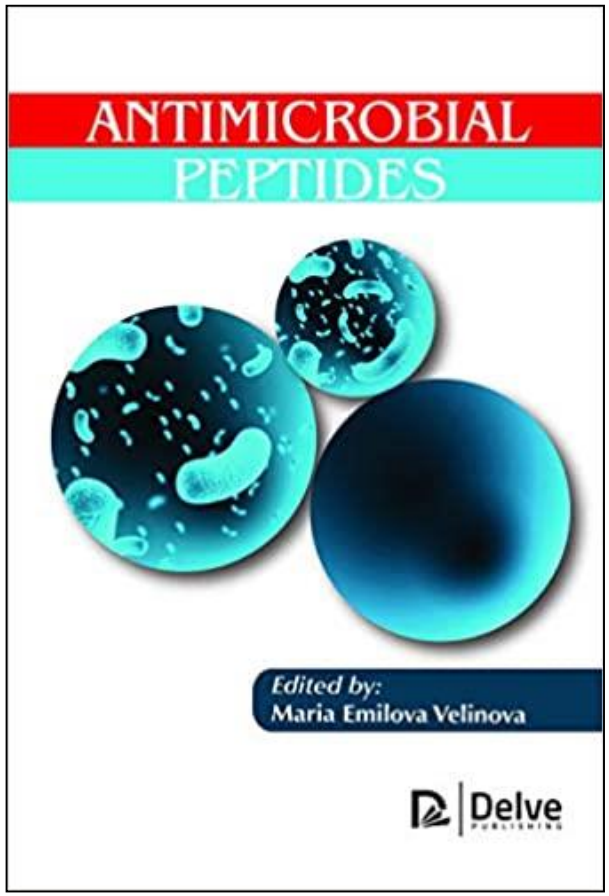
Antimicrobial Peptides

- **Antimicrobial Peptides**
- by Michael A. Zasloff (Author)
- Publisher: CRC
- 2008
- 256 pages



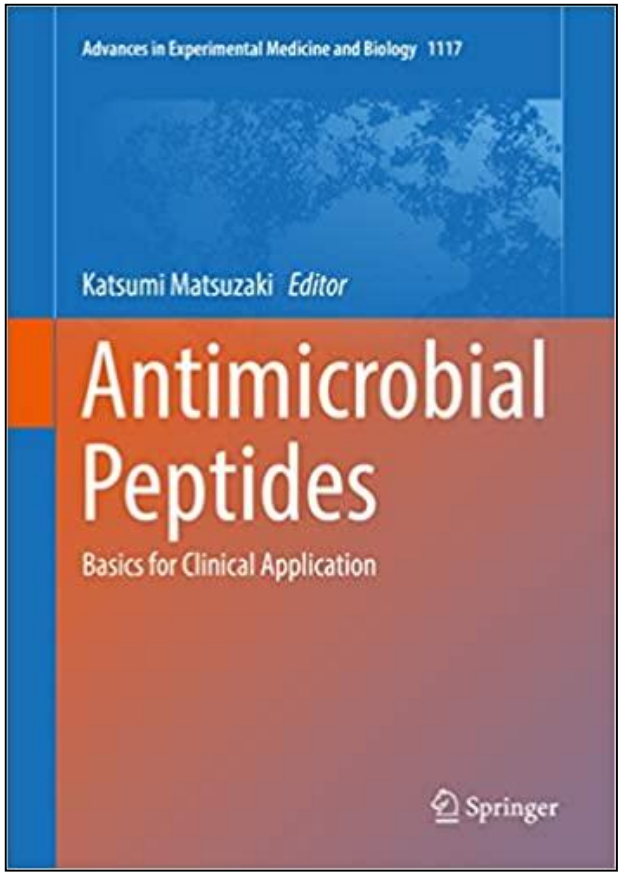
Antimicrobial Peptides

- **Antimicrobial Peptides**
- By Maria Emilova Velinova (Editor)
- Delve Publishing
- 2017
- 396 pages.



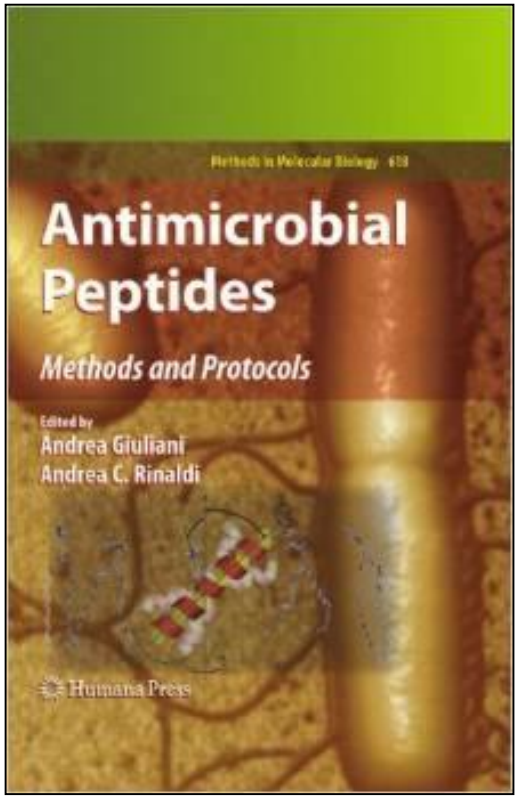
Antimicrobial Peptides: Basics for Clinical Application

- **Antimicrobial Peptides: Basics for Clinical Application.**
- Katsumi Matsuzaki (Editor)
- Publisher: Springer
- 2019
- 304 pages.



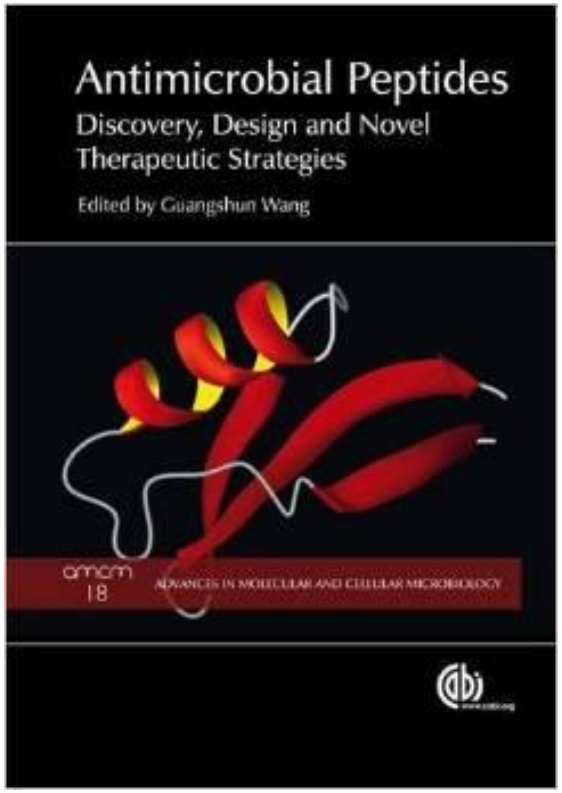
Antimicrobial Peptides: Methods and Protocols

- **Antimicrobial Peptides: Methods and Protocols**
- by Andrea Giuliani and Andrea C. Rinaldi
- Publisher: Humana Press
- 2010
- 378 pages.



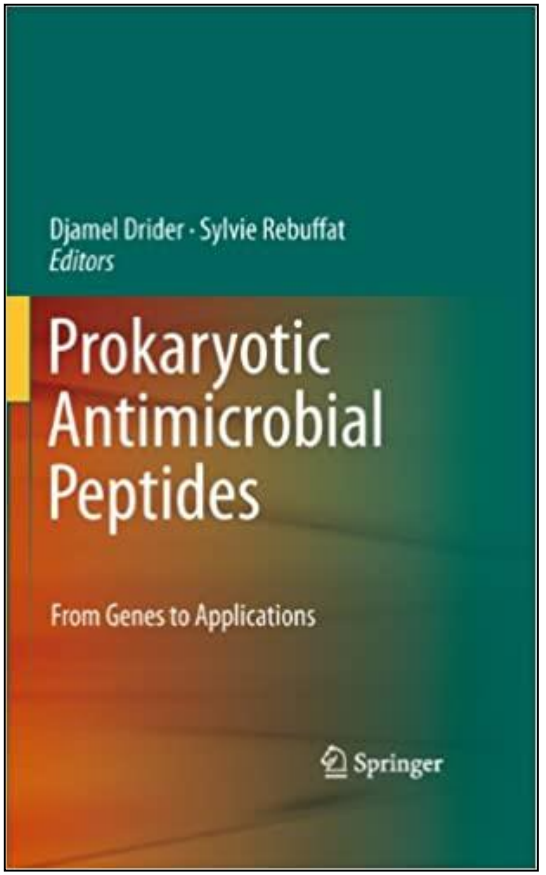
Antimicrobial Peptides: Discovery, Design and Novel Therapeutic Strategies

- **Antimicrobial Peptides: Discovery, Design and Novel Therapeutic**
- by Guangshun Wang (Editor)
- Publisher: CABI
- 2010
- 256 pages.



Prokaryotic Antimicrobial Peptides: From Genes to Applications

- **Prokaryotic Antimicrobial Peptides: From Genes to Applications**
- by Djamel Drider and Sylvie Rebuffat (Editors).
- Publisher: Springer
- 2011
- 465 pages.



Animal Antimicrobial Peptides
Review and articles

- Andreu, D. and L. Rivas.1998. Animal antimicrobial peptides: an overview. Biopolymers 47(6):415-33.
- Badosa,E., M. Planas, L. Feliu, L Montesinos, A. Bonaterra, and E. Montesinos.2022. Synthetic Peptides against Plant Pathogenic Bacteria. Microorganisms. 10(9): 1784.
- Cole, A.M. and T. Ganz. 2000. Human Antimicrobial Peptides: Analysis and Application. BioTechniques 29:822-831.
- Friberg, C. Haaber, J. K. *et al.* 2020. Human antimicrobial peptide, LL-37, induces non-inheritable reduced susceptibility to vancomycin in *Staphylococcus aureus*. Scientific Reports volume 10,

Animal Antimicrobial Peptides
Review and articles

- Kumar, R. Azmal, S.R. *et al.*, 2020. Peptides in Farm Animals: An Updated Review on Its Diversity, Function, Modes of Action and Therapeutic Prospects. *Vet. Sci.*, 7(4), 206.
- Li, J., Hu, S., Jian, W. *et al.* 2021. Plant antimicrobial peptides: structures, functions, and applications. *Botanical Studies* 62, 5.
- Rodrigues,G., L. Souza Santos and O. Luiz Franco1. 2022. Antimicrobial Peptides Controlling Resistant Bacteria in Animal Production. Front Microbiol. 13: 874153.
- Santos-Silva, CA and Zupin, L. *et al.* 2020. Plant Antimicrobial Peptides: State of the Art, In Silico Prediction and Perspectives in the Omics Era. Bioinformatics and Biology Insights.14,
- Wang, G. 2014. Human Antimicrobial Peptides and Proteins. Pharmaceuticals 7(5), 545-594.

Natural amino acids

The 20 proteinogenic natural amino acids

The structure of an amino acid

- An amino acid is a molecule containing two functional groups:

1. An amine group ($-NH_2$),
2. A carboxylic acid group (-COOH).

- There is an additional group called the side chain, designated with an R-group.
- A side-chain that is specific to each amino acid.
- Variation seen in naturally occurring amino acids arises from differences in this side chain.
- Only the R groups change.

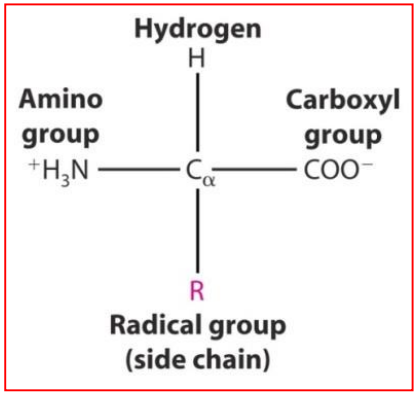
Natural amino acids

The 20 proteinogenic natural amino acids

The structure of an amino acid

- Proteins are long polymers made up of 20 different amino acid monomers.
- All 20 natural (proteinogenic) amino acids have the similar basic structure.
- The key elements of amino acid are:

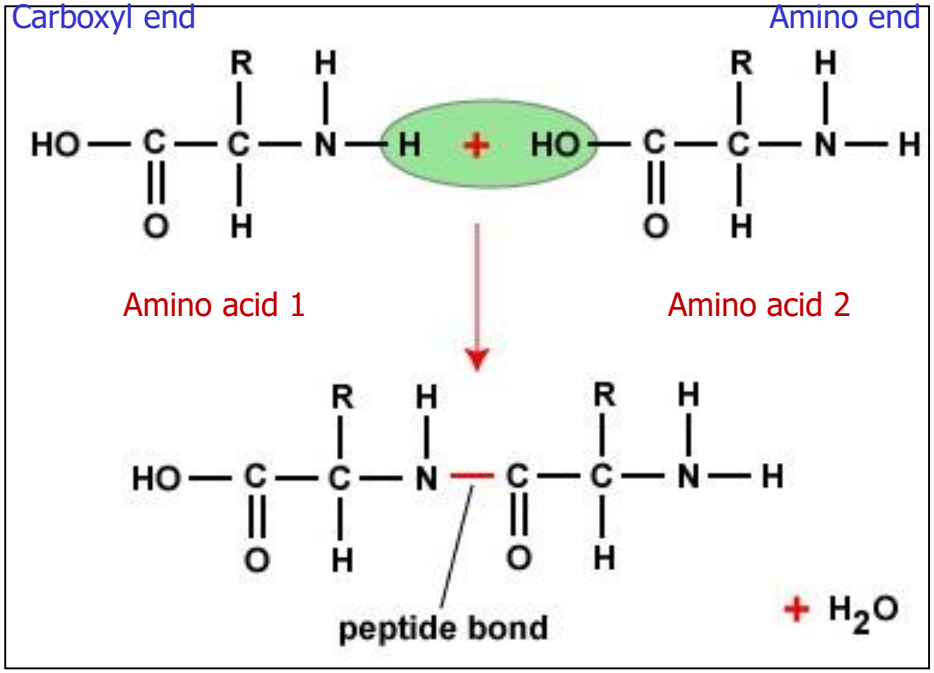
1. carbon,
2. hydrogen,
3. oxygen, and
4. nitrogen.



The α-carbon is where the different substituents attach to each different amino acid. R is a carbon containing side chain or branch. This carbon side chain may also contain sulfur, nitrogen or oxygen.

Peptide
Peptide bond

- A chain consisting of only two amino acid units(residues) is called a dipeptide.

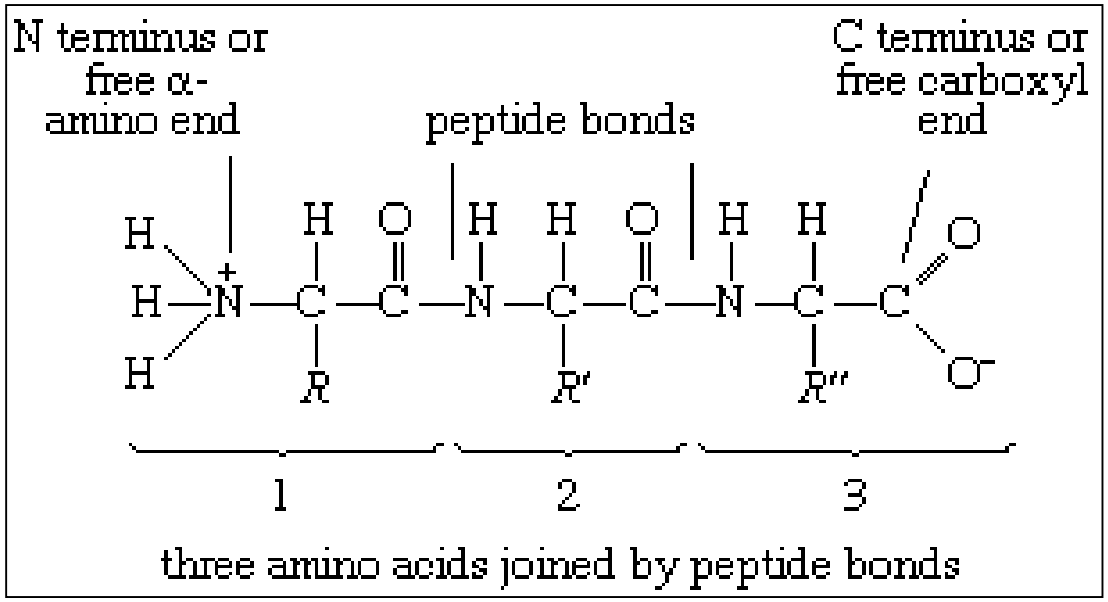


Each amino acid unit in a polypeptide is called a residue.

Protein or peptides

The joining of three amino acids yields the tripeptide

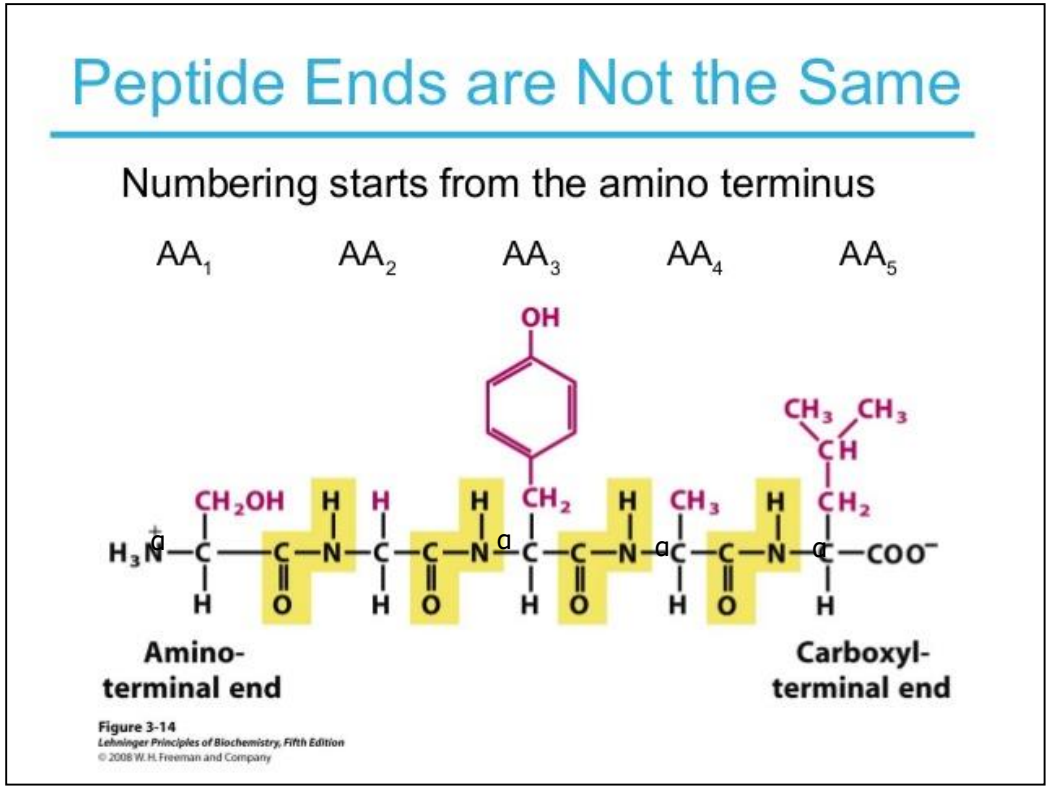
- A chain consisting of three amino acid units(residues) is called a tripeptide.
- R, R′ and R″ are side chains.



Haurowitz *et al.*,2020

Protein or peptides
Peptide bond

- A pentapeptide is a peptide comprised of five amino acids.



Protein or peptides

Classification of peptides and proteins

AMPs usually containing 12-100 amino acids

- **Peptides:**
- Peptides can be classified according to the number of AA residues.

1. An oligopeptide is comprised of 2 to 20 AA residues.
2. Those oligopeptides containing ≤ 10 AA residues are called small oligopeptides (or small peptides).
3. Those oligopeptides containing 10 to 20 AA residues are called large oligopeptides (or large peptides).
4. A peptide, which contains ≥ 21 AA residues and does not have a 3-dimensional structure, is termed a polypeptide.

- **Proteins:**

1. A protein consists of one or more high-molecular-weight polypeptides.

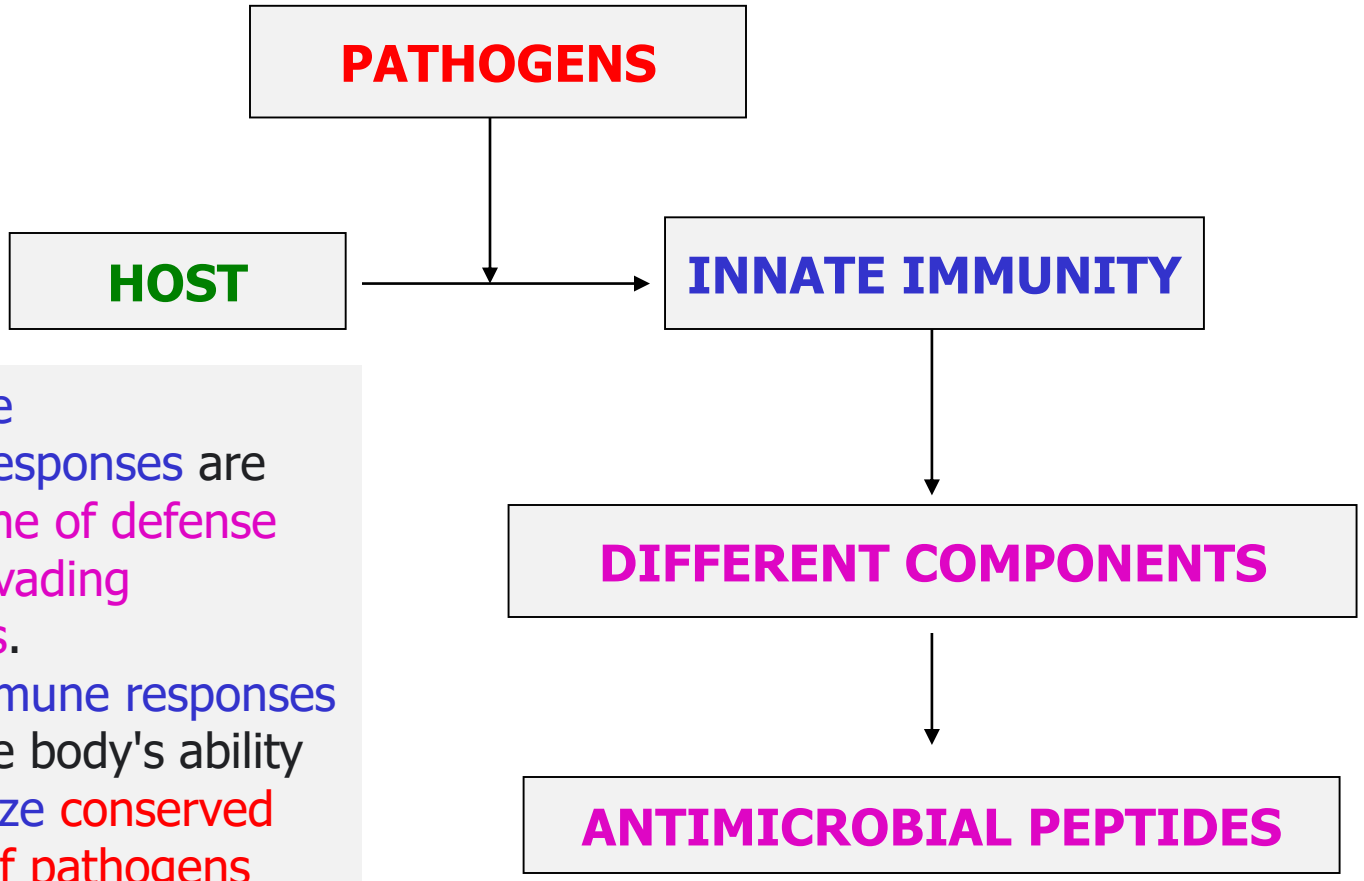
Protein or peptides

Classification of peptides and proteins

- Peptides are distinguished from proteins on the basis of size, they contain approximately 50 or fewer amino acids.
- Small molecules are low molecular weight (<900 daltons) organic compound.
- A one hundred residue (amino acid) protein weighs ~11,000 Da, or 11 kilodaltons (kD).

Dalton= A very small unit of mass, about the mass of a hydrogen atom(the atomic weight of hydrogen atom is about one dalton). Dalton was used to express the molecular weight of proteins. 1Da is about $1.660\ 54 \times 10^{-24}$ gram.

What are antimicrobial peptides (AMPs)



- The innate immune responses are the first line of defense against invading pathogens.
- Innate immune responses rely on the body's ability to recognize conserved features of pathogens that are not present in the uninfected host.

J. Mehla and R. A. Siddique

Protein or peptides
Antimicrobial peptides (AMPs)

- Antimicrobial peptides (AMPs), naturally encoded by genes and usually containing 12–100 amino acids, are the essential components of the innate immune system and can protect the host from fungi, viruses and various pathogenic bacteria.
- In general, AMPs are relatively small peptides (<10 kDa) with:

1. cationic nature (+vely charged)
2. amphipathic structure (having both hydrophilic and hydrophobic parts), and
3. have modes of action different from traditional antibiotics.

Liu *et al.*,2016;..

Antimicrobial peptides

Plant defense peptides

PRs vs. AMPs

- Antimicrobial peptides (AMPs) are non-specific proteins produced in most exposed tissues by induction or constitutively.
- Against invading pathogens such as fungi and bacteria as well as abiotic stress.
- Produced by almost all living organisms (including bacteria).

- Pathogenesis related proteins (PRs) are specific proteins generally induced.
- Some are constitutively expressed.
- Against mostly on fungal infection. Also other biotic (viruses, viroids, and bacteria) and abiotic factors.
- Produced mostly by plants.

Antimicrobial peptides

Plant host defense peptides

AMPs vs. PRs

- Antimicrobial peptides (AMPs) of plants are:

1. Small and low molecular weight peptides range in size from 2-9 kDa, generally between 12 and 50 amino acids (smaller than PRs).
2. Some stable wide pH range (3-12)
3. Thermostable between 0 and 80°C.
4. Resistance to chemical and proteolytic degradation.

- Most pathogenesis related proteins (PRs) in plants are:

1. Low-molecular proteins (6-43 kDa). E.g. in rice 17.6 kDa (168 amino acids);
2. Acid soluble (extractable and stable at low pH (<3);
3. Thermostable, and
4. Highly resistant to proteolysis (proteases).

11,000 Da, or 11 kilodaltons (kD)

Antimicrobial peptides

Plant ost defense peptides

AMPs vs. PRs

- In general, enzymatic mechanisms are not involved in the antimicrobial activities of AMPs.
- AMPs are positively charged compounds interact with membrane lipids of bacterial cell surface and cause cell death.

- Among the 17 PR protein families already described, at least 9 present enzymatic activity such
- glucanases (PR-2);
- osmotins and thaumatins (PR-5)
- protease inhibitors (PR-6);
- lysozymes (PR-8)
- peroxidase (PR-9)
- ribonucleases (PR10) and chitinases (PR-3, PR-4, PR-8, PR-11).

Antimicrobial peptides

Plant host defense peptides

AMPs vs. PRs

- Hypersensitive reaction (HR) is not due to AMPs.

1. Thionins,
2. plant defensins, and
3. nonspecific lipid transfer protein (nsLTPs) are a family of antimicrobial peptides (AMPs) which are included in the pathogenesis-related (PR) proteins.

- PRs are most common in hypersensitive reaction (HR).
- More recently, the PR-protein classification has been extended to include other inducible proteins, namely:

1. Thionins (PR-13),
2. Plant defensins (PR-12), and
3. Lipid transfer proteins (PR-14).

Antimicrobial peptides
Isolated from all organisms

- RAMPs are derived from a diverse range of species, from prokaryotes to humans.
- Synthesized at low metabolic cost.
- AMPs, either natural or synthetic can be developed as probiotic antibiotics against plant diseases.
- The use of plants as biofactories is presented as an alternative for the production of AMPs.

McClanahan,2009;..

Antimicrobial peptides
Antibiotics vs. probiotic antibiotics

- Unlikely antibiotics, which target specific cellular activities (e.g., synthesis of DNA, protein, or cell wall), AMPs are natural antibiotics target the lipopolysaccharide layer of cell membrane, which is ubiquitous in microorganisms.

Antimicrobial peptides
Multifunctional peptides

- AMPs are multifunctional peptides.
- Antimicrobial peptides comprise a host's natural defense against the daily exposure to millions of potential pathogens.
- These are having a wide spectrum of biological activities:

1. antiviral,
2. antiparasitic (protozoa parasites), and
3. antineoplastic activities (inhibit or halt the development of neoplastic cells (a tumor).

Antimicrobial/antibacterial peptides

The broad spectrum antimicrobial activities of AMPs

- Initially, AMPs were identified as endogenous antibiotics due to their potential to kill various pathogens by disrupting their membranes.
- They have broad spectrum antimicrobial activity and are able to kill:

1. Gram-positive and gram-negative bacteria,
2. Viruses,
3. Fungi, and
4. even transformed or cancerous cells.

Antimicrobial/antibacterial peptides
Common and specific AMP databases

- Some common databases: Common databases mainly include different kinds of AMPs, it does not include the sources and types of different kinds of AMPs.

1. The Collection of Antimicrobial Peptides (CAMP);
2. A database Linking Antimicrobial Peptides (LAMP);
3. The Antimicrobial Peptide Database (APD);
4. The Dragon Antimicrobial Peptide database (DAMPD);
5. The Data Repository of Antimicrobial Peptides (DRAMP).

Bajwa and Sharma,2021;..

Antimicrobial/antibacterial peptides
Common and specific AMP databases

- Some specific databases: To cater the need to accommodate more extensive subclasses of AMPs, various databases were established focusing on specific types, sources and characteristics of AMPs.

1. Defensins Knowledgebase (primarily focus on defensins family which are small cysteinerich cationic peptides, stabilized by 3-4 conserved cysteine disulfide bridges);
2. Antiviral peptide database AVPdb;
3. Antiparasitic peptide database ParaPep.

Bajwa and Sharma,2021;..

Antimicrobial/antibacterial peptides
Common AMP databases

- Detailed annotation present in APD, LAMP, CAMP, DAMPD, DRAMP are given below:

Annotation	APD	CAMP	LAMP	DAMPD	DRAMP
Name/Source	+	+	+	+	+
Sequence/Length	+	+	+	+	+
Physicochemical data	+	-	+	+	+
Structure	+	+	+	+	+
Antimicrobial activity	+	+	+	+	+
Hemolytic activity	-	+	+	-	-
Binding Target	+	-	-	-	+
Cross-linking	-	-	+	-	-
MIC with target organism	+	+	+	+	+
Post-translational modification	+	-	-	-	-

Bajwa and Sharma,2021

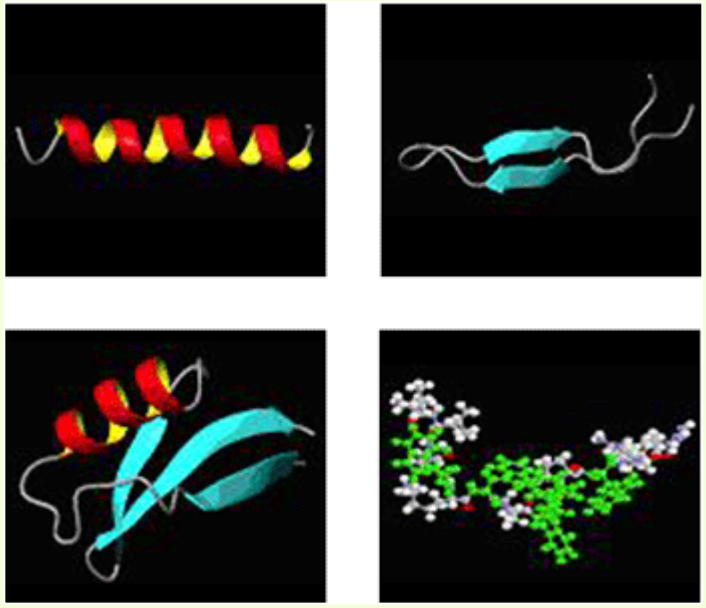
Antimicrobial/antibacterial peptides
Melittin, first reported AMP

- The first reported AMP, melittin, was isolated from bee venoms by Habermann *et al*., in 1952.
- Venom is a type of poison, especially one secreted by an animal.
- After that, a large number of natural AMPs have been reported, and these peptides were considered to be important components of their host defense system.
- More than 3000 antimicrobial peptides (AMPs) have been discovered, seven of which have been approved by the U.S. Food and Drug Administration (FDA).

Kang *et al*.,2019; Chen and Lu,2019

Antimicrobial/antibacterial peptides
AMP databases

- More than 3000 antimicrobial peptides were dicoverded with the following activity:
- Antibacterial peptides
- Antiviral peptides
- Antifungal peptides
- Antiparasitic peptides
- Anticancer/tumor peptides
- Antiprotistc peptides
- Insecticidal peptides
- Spermicidal peptides
- Anti_HIV-1 peptides
- AMPS with chemtactic activity.



The Antimicrobial Peptides Database,2013;..

Antimicrobial/antibacterial peptides
Antimicrobial-resistant (AMR) bacteria

- In human, infections caused by antimicrobial-resistant (AMR) bacteria have become a serious problem to global healthcare.
- It is low estimates that at least 700,000 people die from AMR infections each year.
- The emergence and worldwide spread of multiple-resistant "superbugs" (a harmful microorganism, typically a bacterium)cause an urgent need of novel antimicrobial medicine.
- A prospective weapon to fight against antimicrobial-resistant infections is antimicrobial peptides (AMPs).

Kang *et al.*,2019;..

Antimicrobial/antibacterial peptides

1. PhytAMP: A database of antimicrobial plant peptides

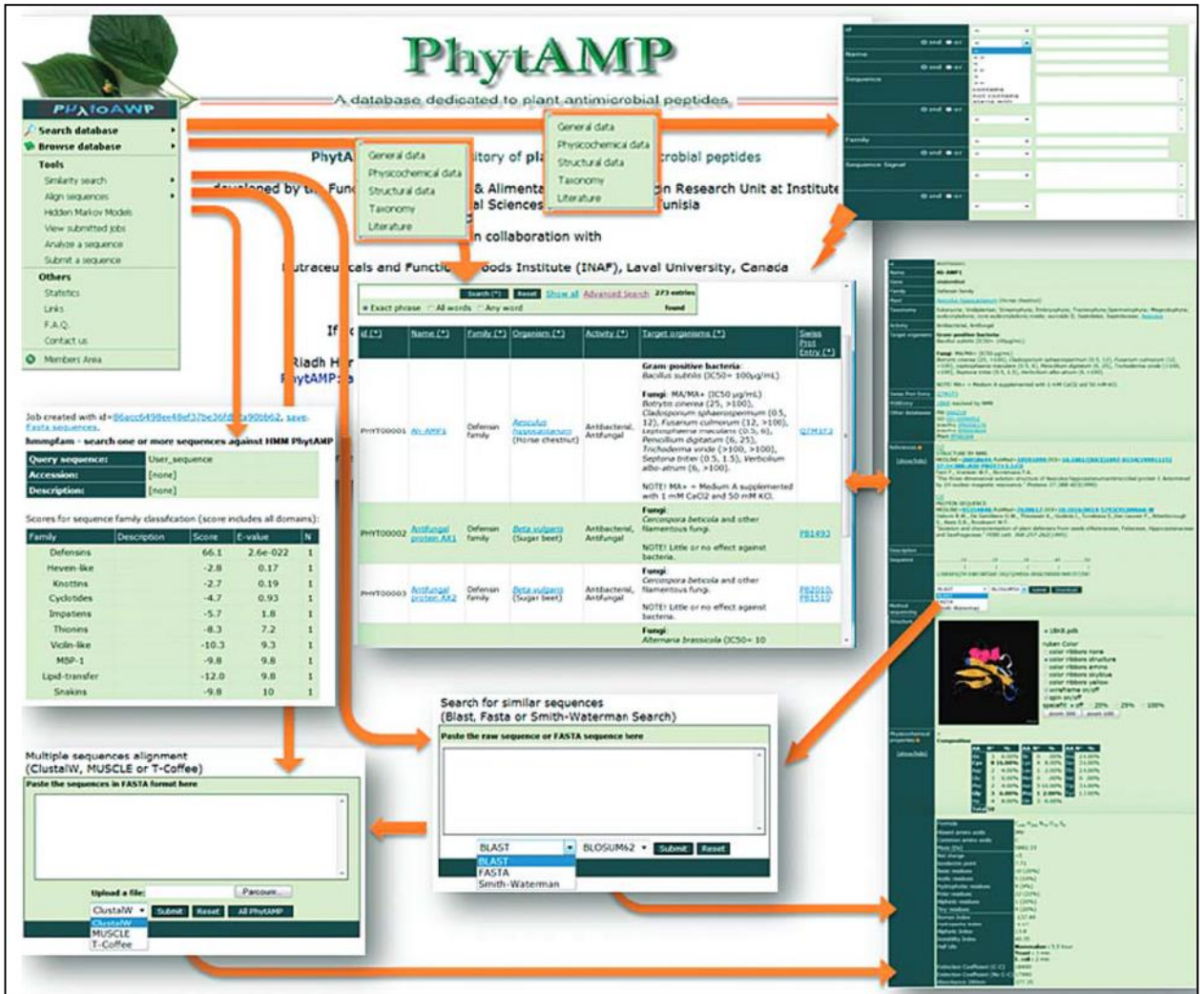
- PhytAMP is database of antimicrobial plant peptides.
- This database provides valuable information on antimicrobial plant peptides like taxonomic information, microbiological information and physiochemical information.
- This information is easy to access and allow:

1. rapid prediction of structure/function relationships which could be of beneficial use and may be exploited by the pharmaceuticals and agricultural sectors.
2. to study alternatives in response to increasing antibiotic resistance, or
3. For increasing plant resistance to pathogens by genetic engineering.

Hammam *et al.*,2009;..

Antimicrobial/anibacterial peptides

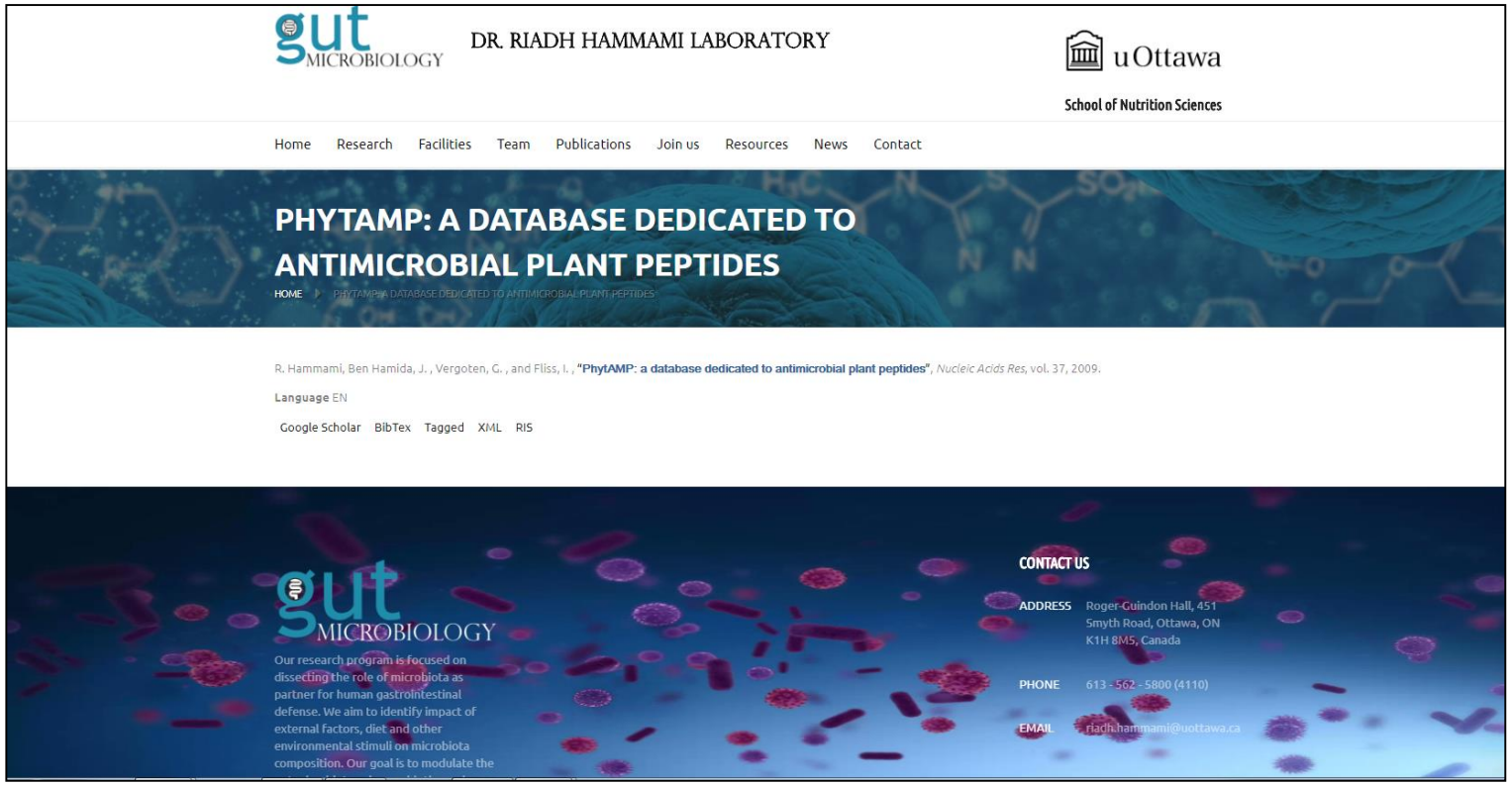
PhytAMP: a database dedicated to antimicrobial plant peptides



R. Hammami, Ben Hamida, J., Vergoten, G., and Fliss, I., "PhytAMP: a database dedicated to antimicrobial plant peptides", Nucleic Acids Res, vol. 37, 2009.

Antimicrobial/anibacterial peptides

PhytAMP: a database dedicated to antimicrobial plant peptides



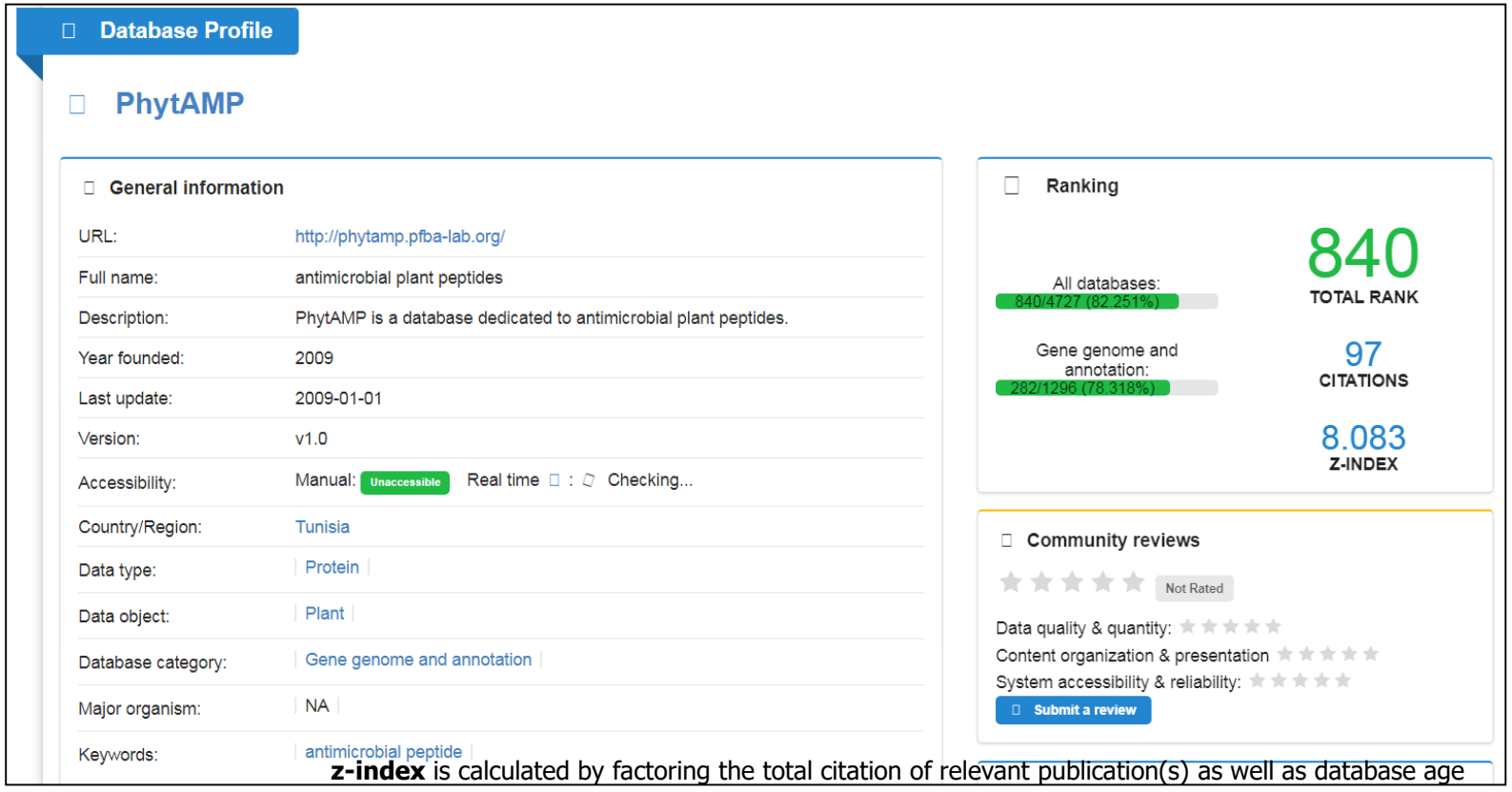
Antimicrobial/anibacterial peptides

PhytAMP: a database dedicated to antimicrobial plant peptides

Hammam *et al.*,2009

Antimicrobial/antibacterial peptides

PhytAMP: a database dedicated to antimicrobial plant peptides



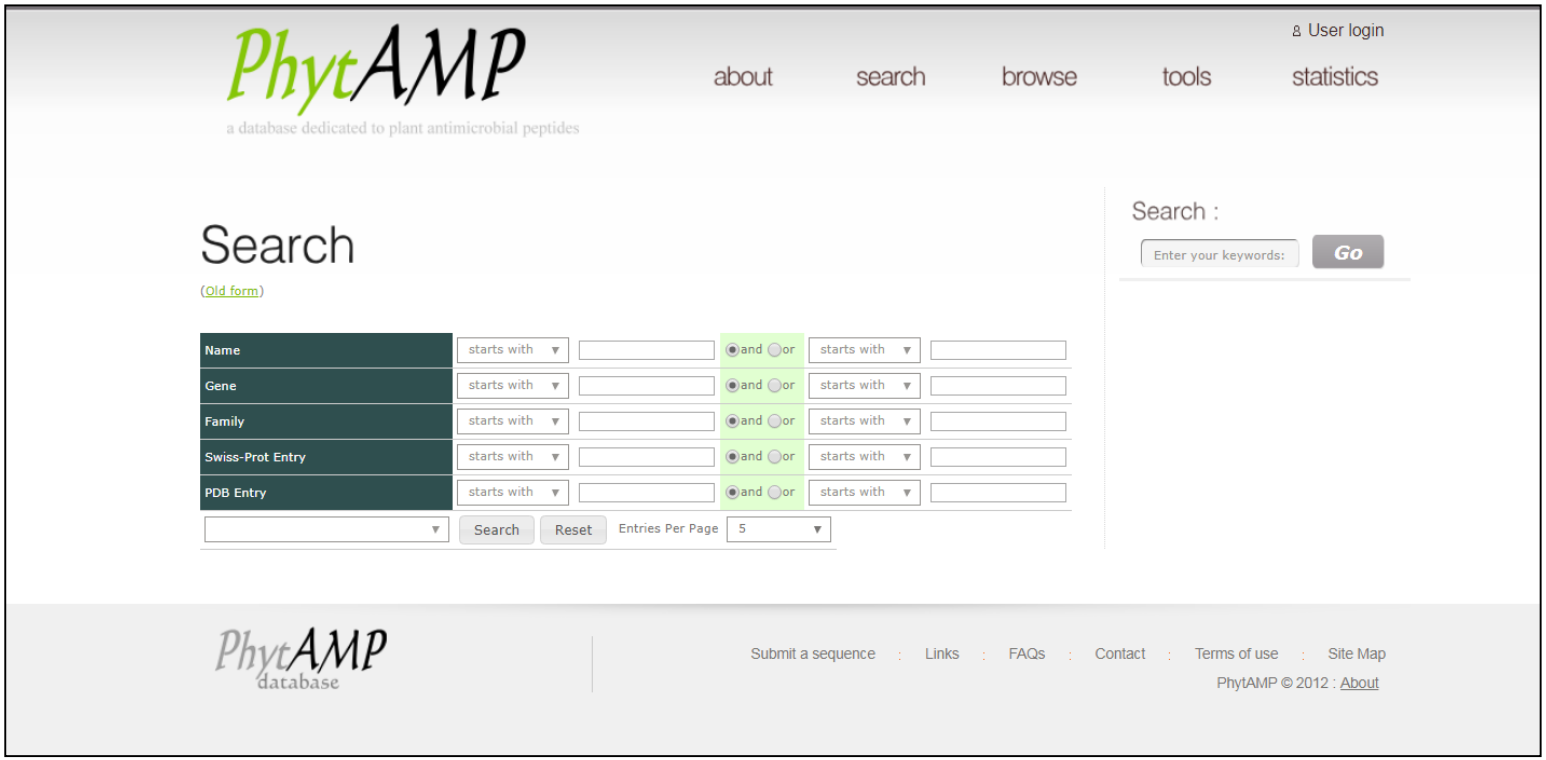
z-index is calculated by factoring the total citation of relevant publication(s) as well as database age

The National Genomics Data Center (NGDC), part of the China National Center for Bioinformation (CNCB), 2021

Antimicrobial/antibcterial peptides

PhytAMP: a database dedicated to antimicrobial plant peptides

Structural data

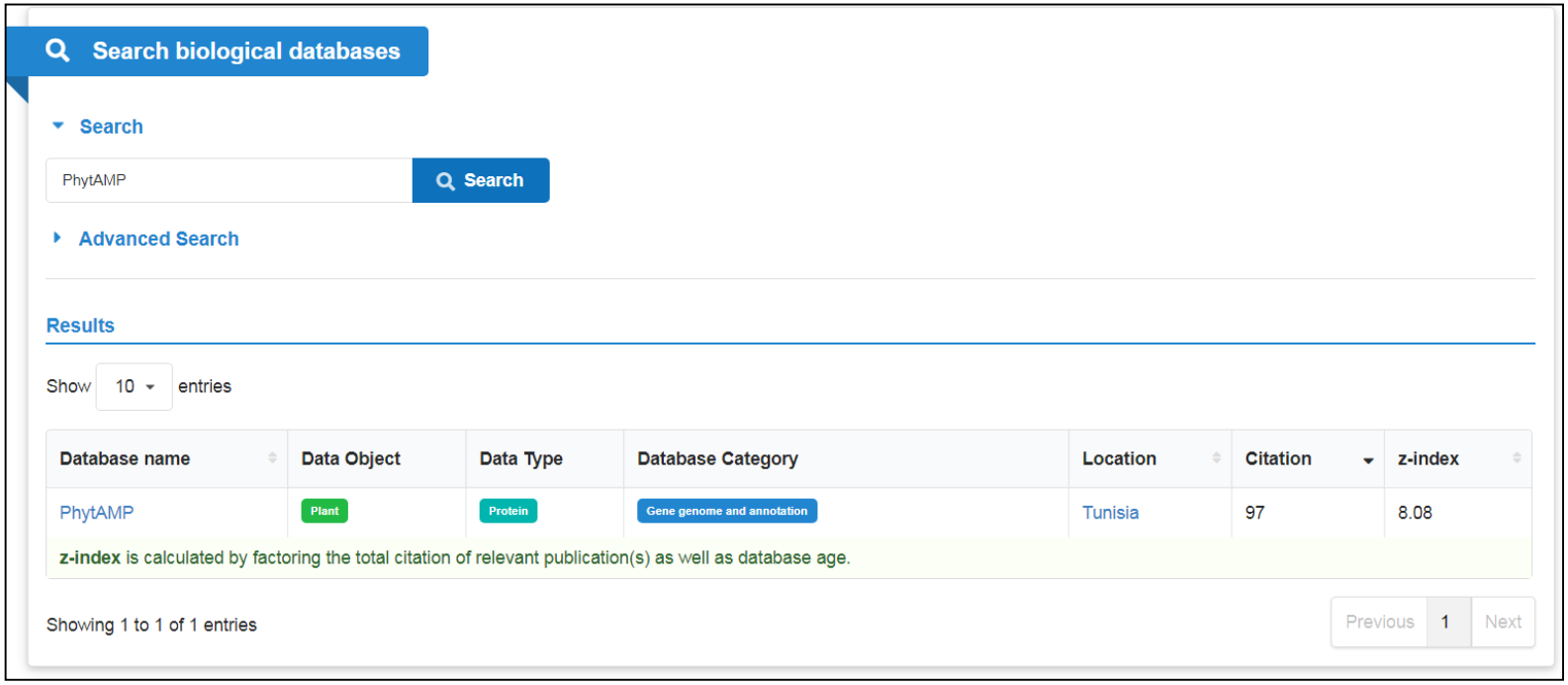


http://phytamp.hammamilab.org

Antimicrobial/antibacterial peptides

PhytAMP: a database dedicated to antimicrobial plant peptides

Structural data

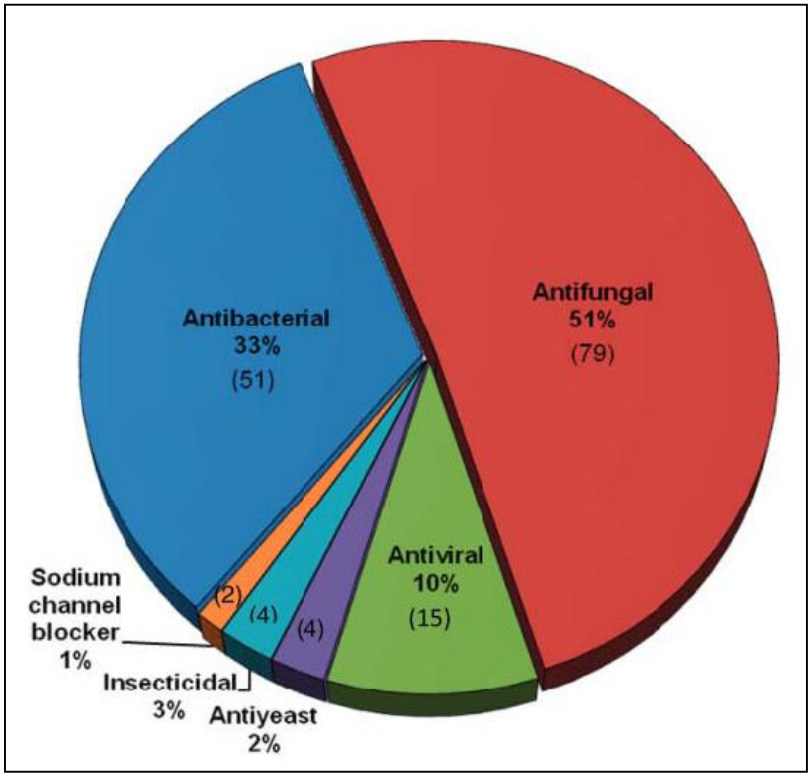


The National Genomics Data Center (NGDC), part of the China National Center for Bioinformation (CNCB), 2021

Sources of antibacterial peptides

Chart of reported activities for plant peptides compiled in the PhytAMP database

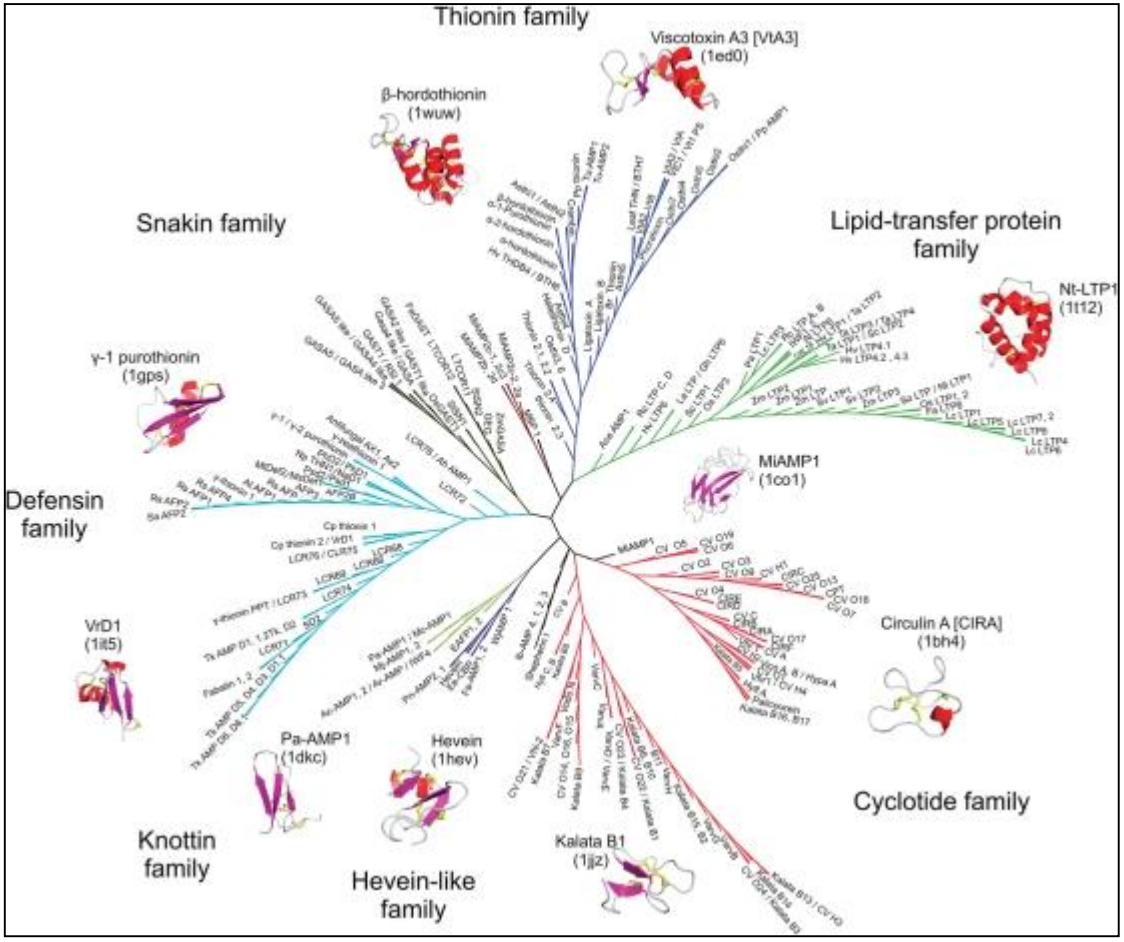
- The majority possesses:
  1. antifungal (51%),
  2. antibacterial (33%) and
  3. anti-viral (10%) activities.



Hammam *et al.*,2009

Sources of antibacterial peptides

Unrooted phylogenetic tree of plant AMPs compiled in the PhytAMP database



Hammam *et al.*,2009

Antimicrobial/antibacterial peptides
2. PlantPepDB: A manually curated(of online content) plant peptide database

- PlantPepDB is a manually curated database that consists of 3848 plant-derived peptides among which:

1. 2821 are experimentally validated at the protein level,
2. 458 have experimental evidence at the transcript level,
3. 530 are predicted and only 39 peptides are inferred (identified through) from homology.

Das *et al.*,2020

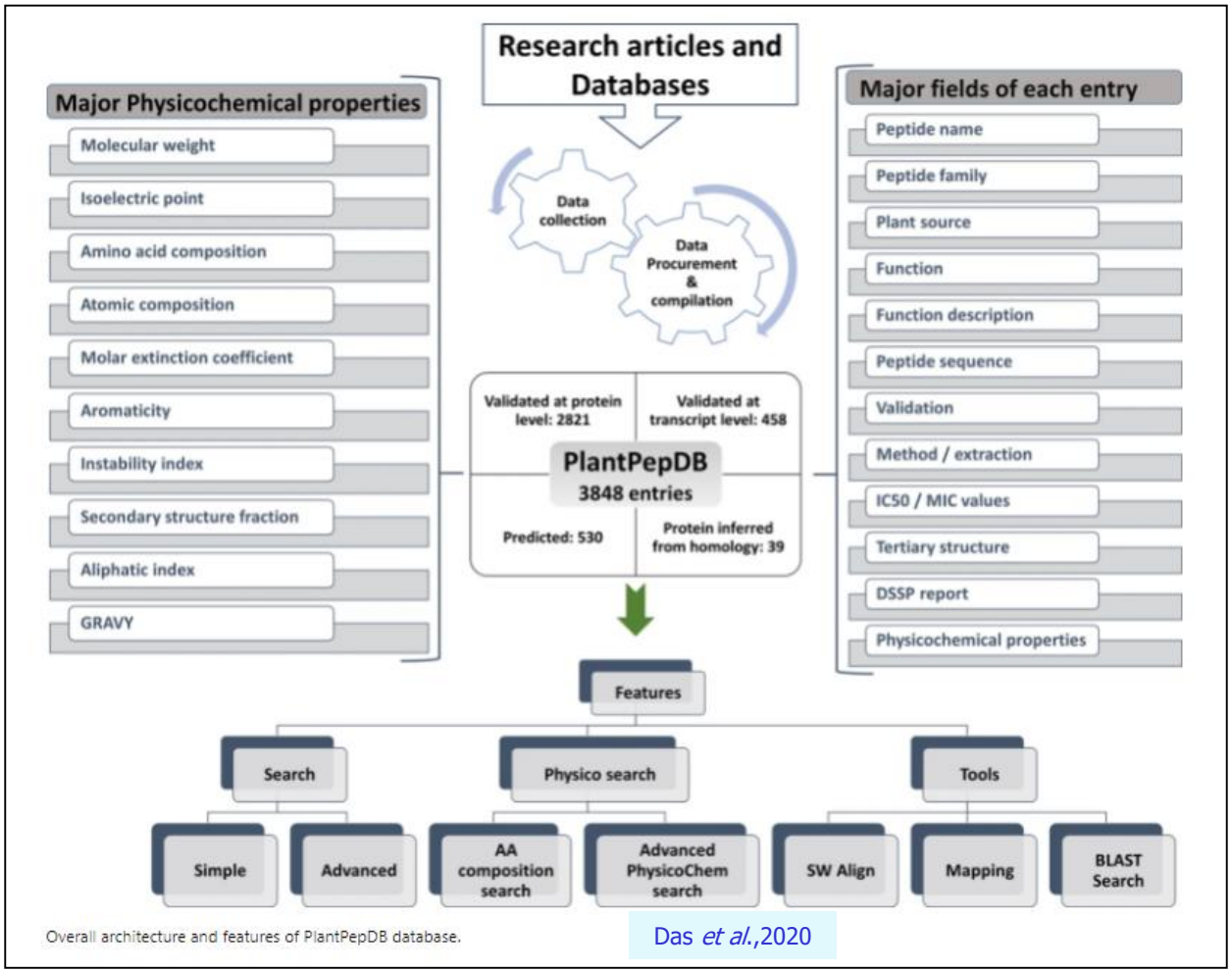
Antimicrobial/antibacterial peptides
PlantPepDB: A manually curated plant peptide database

- Overall, PlantPepDB is the first database comprising:
- detailed analysis and comprehensive information of phyto-peptides from a broad functional range which will be useful for peptide-based applied research.
- PlantPepDB is freely available at http://www.nipgr.ac.in/PlantPepDB/.
- PhytAMP is another plant peptide database, having only antimicrobial peptides.

Das *et al.*,2020

Antimicrobial/antibacterial peptides

PlantPepDB: A manually curated plant peptide database



Overall architecture and features of PlantPepDB database.

Das *et al.*,2020

Antimicrobial/antibacterial peptides

PlantPepDB: A manually curated plant peptide database

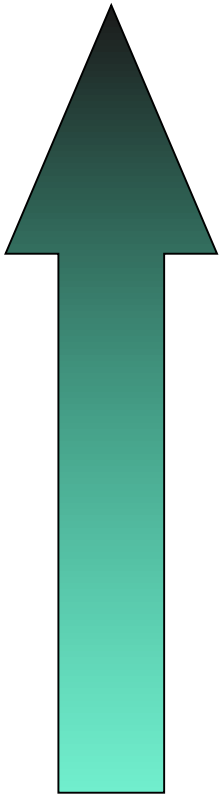
- List of functional and sub-functional category of peptides along with their response information incorporated in PlantPepDB.

Functional Category	Number of Peptides	Sub-functional Category	Response in Plants/Animal/Others
Inhibitory in nature	280	Protein translation inhibitor (2), Enzyme inhibitor (256), Protease inhibitor (13), Serine protease inhibitor (1), Tyrosinase and melanin inhibitor (3), Tyrosinase inhibitor (5)	Animal
Toxic	407	Toxin (37), Celiac toxic (201), Cytotoxic (169)	Animal
Immune system related	65	Immunomodulatory (35), Immunoregulator (10), Immunostimulating (1), Immunosuppressive (19)	Animal
Opioid	8	Opioid (6), Opioid agonist (1), Opioid antagonist (1)	Animal
Therapeutic	1465	Antiproliferative (9), Anticancer (156), Vasorelaxant (2), Antihypertensive (500), ACE-inhibitor (427), Hypotensive (5), Pore-forming (1), Antithrombotic (7), Antioxidant (227), Anti-inflammatory (13), Anti-amnestic (4), Anti-analgesic (1), Antinociceptive (3), Anxiolytic (2), Diuretic (2), Uterotonic (1), Anti-HIV (37), HIV-1-reverse-transcriptase inhibition (8), Antihyperglycemic (1), Antidiabetic (1), Hypoglycemic (1), DPP-IV inhibitor (3), Estrogen like activity (18), Phagocytosis stimulatory peptide (2), Bile acid binding inhibitor (1), Protein synthesis inhibitor (2), Cyclooxygenase inhibitor (8), HMG-CoA reductase inhibitor (3), Neurotensin inhibitor (1), Anti-allergen (11), Antimalarial (8)	Animal
Plant defense response	43	Alpha-amylase inhibitor (15), Defensive-proteinase inhibitor (3), Trypsin inhibitor (3), Gene expression activator (5), Gene expression stimulator (1), Antifeedant (7), Defense activator (3), Defense gene activator (6)	Plants
Microbe killing	2356	Antimicrobial (1393), Antiparasitic (15), Antiprotist (4), Antibacterial (323), Antiyeast (4), Antifungal (529), Antibiotic (2), Antiviral (83), Antibiofilm (3)	Others
Invertebrate killing	209	Anthelmintic (35), Anti-barnacle (1), Molluscicidal (6), Nematocide (56), Insecticidal (111)	Animal
Miscellaneous	231	Hemolytic (71), Hypocholesterolemic (2), Hypotriglyceridemic (3), Neuropeptide (92), Allergen (9), Enzymatic degradation (54)	Animal

Das *et al.*,2020

Antimicrobial/antibacterial peptides
Spectrum of biological activity of AMPs

High concentration



Low concentration

- **Tumor cell lysis**
- **Lysis of microbes**
- **Stimulation of keratinocyte growth(a growth factor)**
- **Inhibition of protein kinase C signal transduction**
- **Stimulation of cytokine (motility factor)**
- **Stimulation of adhesion molecule expression**

Wojciech Kamysz *et al.*,2003

Antimicrobial/antibacterial peptides
Classification of antimicrobial peptides

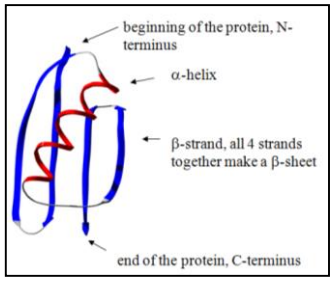
- There are numerous ways for classifying antimicrobial peptides:

1. Based on the biosynthetic machine: Natural peptides can be classified as gene coded and non-gene coded (i.e. multiple enzyme systems).
2. Based on biological source: Bacterial AMPs (bacteriocins), plant AMPs, animal AMPs.
3. Based on biological functions: Antibacterial, antiviral, antifungal, antiparasital, insecticidal, chemotactic, wound healing, growth promotion, etc.
4. Based on molecular properties;
5. Based on three-dimensional (3D) structure.

The Antimicrobial Peptides Database,2013; Wang,2015

Antimicrobial peptides

Four structural classes



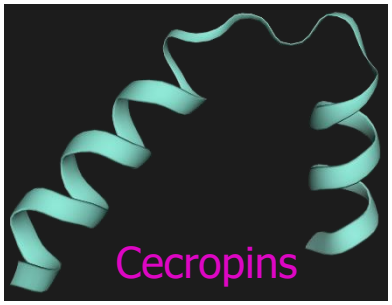
- AMPs are classified into four families: alpha, beta, alphabeta, and non-alphabeta based on the types of secondary structures.

1. **The alpha family** consists of AMPs with helical structures (e.g. magainins and LL-37).
2. **The beta family** is composed of AMPs with beta-strands (e.g. human alpha-defensins).
3. **The alphabeta family** comprises both helical and beta-strands in the 3D structure (e.g. beta-defensins),
4. **The non-alphabeta family** contains neither helical nor beta-strands (e.g. indolicidin).

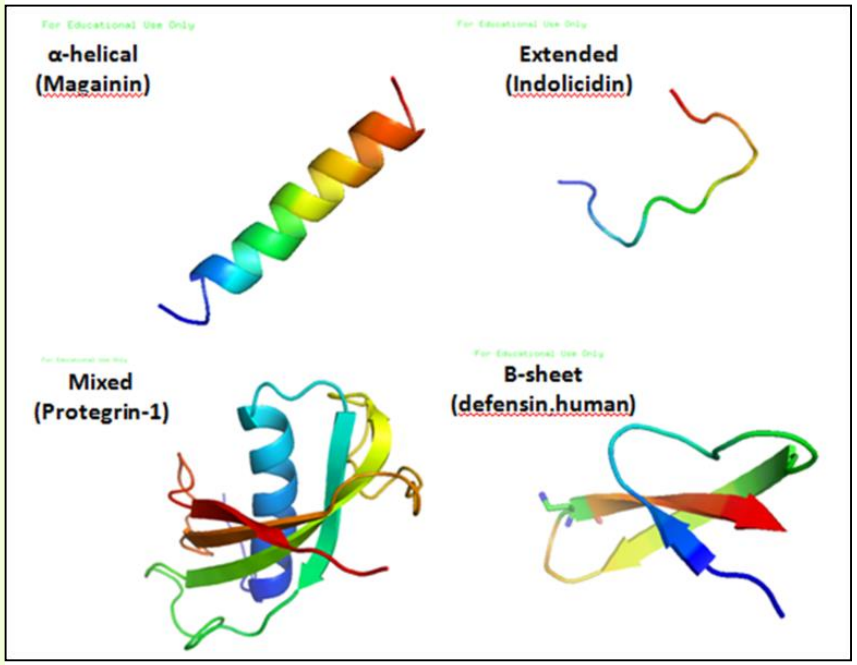
The Antimicrobial Peptides Database,2013

Antimicrobial peptides

Four structural classes



1. Magainin isolated from African clawed frog.
2. Defensins (isolated from plants and humans).Plant defensins have been isolated from seeds of various monocot and dicot species.
3. Cecropins, isolated from insects.
4. Indolicidin, isolated from bovine neutrophils (white blood cells).



The Antimicrobial Peptides Database,2013;Wikipedia,2015

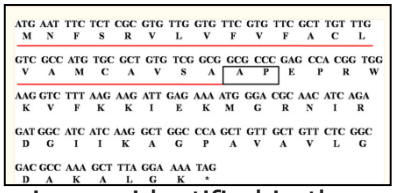
Antimicrobial peptides

Four structural classes

Cecropins, sequences andprice

Cecropia moth

- Cecropins were first isolated from the hemolymph of *Hyalophora cecropia*, whence the term cecropin was derived.
- Cecropins are small proteins anywhere from 31-37 amino acids long and are active against both gram-positive and gram-negative bacteria.
- Cecropins isolated from insects other than *Hyalophora cecropia* (Cecropia moth) have been given various names, such as bactericidin, lepidopterin, and sarcotoxin.
- All of these peptides are structurally related.



A novel cecropin was identified in the armyworm, and its gene and amino acid sequences

Price

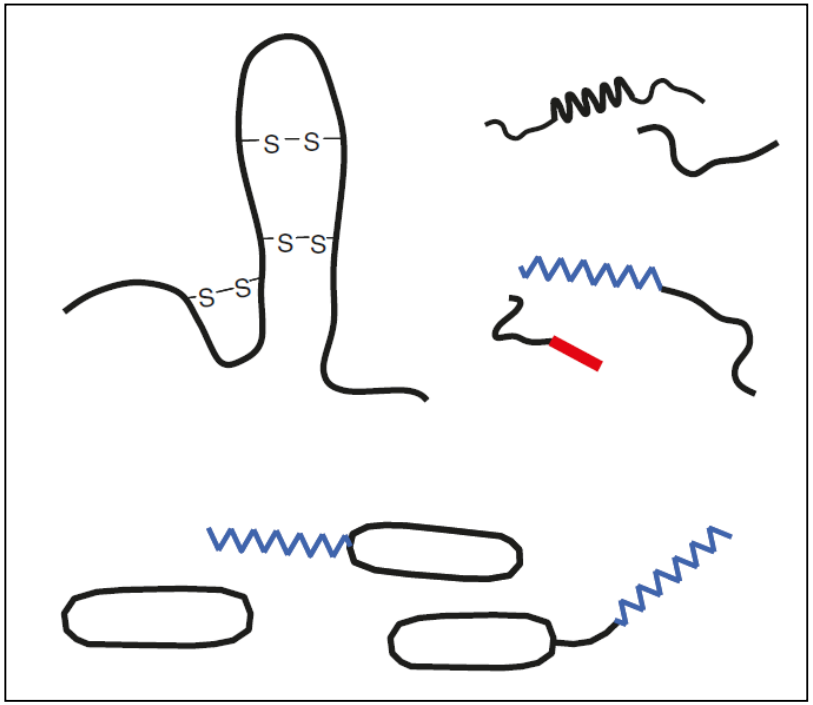
Product catalog	Size	Price € HT	Price $ USD
SB009-1MG	1 mg	115	134
SB009-5*1MG	5*1 mg	400	469

Wikipedia,2023;Lian *et al.*,2020; Smartox,2023

Antimicrobial peptides

Simplified structure of linear and cyclic antimicrobial peptides

- The peptidic moiety is represented in black adopting helical or extended conformation, or b-sheet structure with disulphide bonds (S).
- Fatty acyl substitutions in lipopeptides are shown in blue.
- Complex substitutions in pseudopeptides are represented in red.

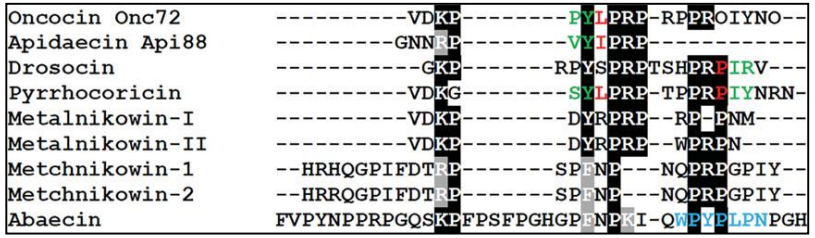


Montesinos,2007

Antimicrobial peptides

Sequence comparison of different proline-rich AMPs

- We compared the amino acid sequences of abaecin, the insect AMP with four other proline rich DnaK-binding AMPs (oncocin Onc72, apidaecin Api88, drosocin and pyrrhocoricin) in order to determine the functional sequence that interacts with DnaK.
- The alignment was generated with ClustalW and manually edited for the improved alignment of proline (P) residues.



Rahnamaeian *et al.*,2016

Sources of antimicrobial peptides
Classification of AMPs

1. Bacterial AMPs (bacteriocins),
2. Plant AMPs,
3. Animal AMPs.

- Animal AMPs are further classified into:
- Insect AMPs,
- Amphibian AMPs,
- Fish AMPs,
- Reptile AMPs, ...

Amphipathic molecule (of a molecule) having two different affinities, as a polar end that is attracted to water and a nonpolar end that is repelled by it. The amphipathic structure allows these peptides to be soluble in aqueous environments but also to interact with lipid membranes.

Sources of antimicrobial peptides
Classification of AMPs

- Hybrid peptides:
- Combining two known antimicrobial peptides (AMPs) into a hybrid peptide is one promising avenue in the design of agents with increased antibacterial activity.
- Broad-spectrum antimicrobial peptides (AMPs) kill bacteria indiscriminately, increasing the possibility of an ecological imbalance in the microbiota.
- To solve this problem, new types of AMPs, which kill pathogenic bacteria without breaking the micro-ecological balance of the body, were proposed.

Wade *et al.*,2019; Shang *et al.*,2020

Sources of antimicrobial peptides

Classification of AMPs

CLASS	EXAMPLE	STRUCTURE	ORIGIN
Anionic peptides	Dermicidin	Asp & Glu	Human
Cationic peptides	Cecropin	Helical	insects
Cathelicidin-type	LL37	Helical	Human
Cationic peptides with specific amino acids	PR 39	Pro (proline) & arg (arginine) rich	Pig
	Prophenin	Pro & Phe	Pig
	Indolicidin	Trp rich	cattle

Anionic- a negatively charged ion; Cathelicidins are small, cationic, antimicrobial peptides.

Sources of antimicrobial peptides

Classification of AMPs

CLASS	EXAMPLE	STRUCTURE	ORIGIN
Peptides that forms disulphide bridges	Brevinins	1-disulphide bridge	Amphibians
	Tachyplesin	2-disulphide bridges	Horse shoe crab
	Defensins	3-disulfide bridges	Plants and Human
	NK-lysin	3-disulfide bridges	Pig
	Drosomycin	More than 3-disulfide bridges	Fruit fly
Fragmented peptides	Lactoferricin	14-42 a.acids	Human

J. Mehla and R. A. Siddique

Antimicrobial peptides

Antimicrobial cyclic-peptides produced by microorganisms

Type	Compound	Composition*	Producer microorganism
Simple	Gramicidins	C10	*Bacillus brevis*
	Calophycin	C10	*Calothrix fusca*
	Laxaphycins	C11	*Anabaena laxa*
Tailed	Bacitracins	T5-C7	*Bacillus licheniformis*
Simple lipidic	Xanthostatin	R-C6	*Streptomyces spiroverticillatus*
	Echinocandins	R-C6	*Aspergillus* spp.
	Cryptocandins	R-C6	*Cryptosporiopsis quercina*
	Fusaricidins	R-C6	*Paenibacillus polymixa*
	Iturins	R-C7	*Bacillus* spp./ *Bacillus amyloliquefaciens*
	Aureobasidins	R-C8	*Aureobasidium pullulans*
	Syringomycins	R-C9	*Pseudomonas syringae/Pseudomonas viridiflava*
	Fengycins	R-C10	*Bacillus subtilis*
Tailed lipidic	Viscosins	R-T2-C7	*Pseudomonas fluorescens*
	Polymixins	R-T3-C7	*Paenibacillus polymixa*
	Agrastatins	R-T2-C8	*Bacillus subtilis*
	Amphisins	R-T2-C9	*Pseudomonas fluorescens*
	Putisolvins	R-T8-C4	*Pseudomonas putida*
	Tolaasins	R-T11-C4	*Pseudomonas tolaasi*
	Corpeptins	R-T17-C5	*Pseudomonas corrugata*
	Syringopeptins	R-T14-C8	*Pseudomonas syringae*
	Schizotrin A	R-T1-C12	*Schizotrix* sp.

Montesinos,2007

Antimicrobial peptides produced by microorganisms
Type: Non-lipidic

Producer microorganism	Composition*	Compound
Sepedonium sp.	Ac-P4-PheOH	Peptaibolin
Hypocrea murociana	Ac-P10-LeuOH	Hypomurocin
Trichoderma harzianum	Ac-P10-LeuOH	Harzianins
Sepedonium ampullosporium	Ac-P14-LeuOH	Ampullosporin
Emericellopsis microspora	Ac-P15-PheOH	Emericins
Clonostachys sp.	Ac-P15-C(6)OH	Clonostachin
Trichoderma virens	Ac-P17-LeuOH	Trichovirins
Trichoderma harzianum	Ac-P18-TrpOH	Trichorzianins
Apiocrea chrysosperma	Ac-P18-TrpOH	Chrysospermins
Trichoderma koningii	Ac-P19-PheOH	Trichokonin
Trichoderma polysporum	Ac-P19-PheOH	Polysporins
Trichoderma reesei	Ac-P19-PheOH	Paracelsin
Trichoderma viride	Ac-P19-PheOH	Alamethicin
Stilbella flaviceps	Ac-P19-ValOH	Stilboflavins

Montesinos,2007

Antimicrobial peptides produced by microorganisms

Type: lipidic

Producer microorganism	Composition*	Compound
Trichoderma viride	Dec-P5-LeuOH	Trichodecenin
Paecilomyces/Acremonium spp.	Hex-P8-MPD	Leucinostatins
Mycogone rosea	Oc-P8-AAE	Helioferins
Trichoderma polysporum	Dec-P9-AMAE	Trichopolyns
Tolypocladium geodes	Oc-P10-LeuOH	LP237
Trichoderma longibrachiatum	Oc-P10-LeuOH	Trichogin
Scleroderma texenense	FA-P20-ArgOH	Texenomycin
Trichoderma viride	Dec-P5-LeuOH	Trichodecenin

* Px, number of aminoacid residues; Ac, acetyl; Dec, decanyl; Hex, hexanyl; Oc, octanyl; FA, fatty acyl; MPD, N1-methyl-propane-1,2-diamine; AAE, 2-(2-aminopropyl)-aminoethanol; AMAE, 2-(2-aminopropyl)-N-methylamino-ethanol.

Montesinos,2007

Sources of antibacterial peptides
Plant antimicrobial peptides

- Plant antimicrobial peptides (AMPs) are a component of barrier defense system of plants.
- The repertoire of AMPs synthesized by plants is extremely large, with hundreds of different AMPs in some plant species.
- They have been isolated from roots, seeds, flowers, stems, and leaves of a wide variety of species.
- They have activities towards:

1. phytopathogens, as well as
2. against bacteria pathogenic to humans.

Folia Microbiogica,2013; García *et al.*,2012

Sources of antibacterial peptides
Plant antimicrobial peptides

- They are basic, amphypatic and cysteine-rich peptides with a stabilized structure by disulfide bonds.
- Plant AMPs are grouped into several families and share general features with other AMPs such as:

1. positive charge(-vely charged are few such as dermcidin from humans).
2. just in plants),
3. the presence of disulfide bonds (which stabilize the structure), and
4. the mechanism of action targeting outer membrane structures.

Antimicrobial peptides from plants have 3 or 4 disulfide bonds, such as thionin from barley and plant defensin from radish.

Sources of antibacterial peptides

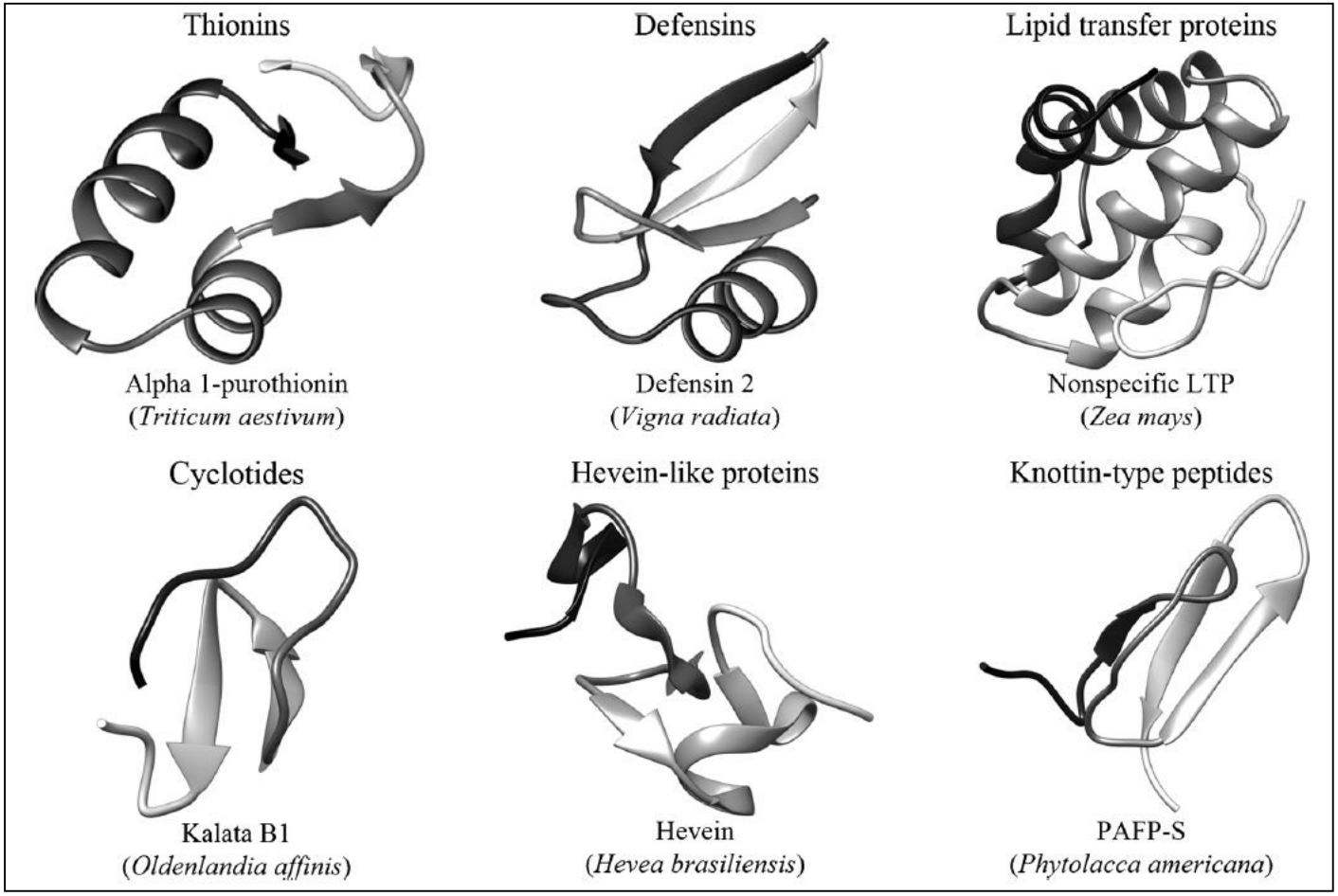
Plant antimicrobial peptides

- Based on amino acid sequence homology, these peptides were classified mostly as α-defensins, thionins, lipid transfer proteins, cyclotides, snakins and hevein-like.

1. Plant defensins the first plant defensins were isolated from wheat and barley.
2. Thionins (occurring ubiquitously in the plant kingdom).
3. cyclotides (small disulfide rich peptides isolated from plants),
4. glycine-rich proteins (isolated from plants such as wild tomato species),
5. snakins (a peptide from potato), and
6. hevein-type proteins, a lectin-like protein from rubber tree.

Sources of antibacterial peptides

Plant antimicrobial peptides



Nawrot *et al.*,2014

Sources of antibacterial peptides

Antibacterial agents from plants

Types of thionins

- Thionins are a family of small proteins found solely in higher plants.
- Thionins are toxic against bacteria, fungi, and yeast.
- Alpha- and beta- thionins are related to each other.
- Gamma-thionins have a similar structure but are an unrelated class of protein, now called plant defensins.

Nawrot *et al.*,2014;Wikipedia,2015

Antibacterial agents from plants

Screening for toxicity to transgenic plants and bacteria

Thionins

- Thionins are also plant antimicrobial proteins which are able to inhibit a broad range of pathogenic bacteria *in vitro*.
- Expression of alpha-thionin gene from barley in transgenic tobacco confers enhanced resistance to two pathovars of *P. syringae*.
- The drawback with most thionins, they can be toxic to animal and plant cells and thus may not be ideal for developing transgenic plants.

Antibacterial agents from plants, and insect and mammalian

Plants and insect and mammalian defensins

- Defensins are small cysteine-rich cationic proteins found widely in plants, mammals and insects.
- Plant defensins are structurally related to defensins found in other types of organism, including humans.
- They are active against bacteria, fungi and many viruses.
- Gamma-thionins also known as plant defensins. Its structure differs from that of the plant alpha- and beta-thionins, but is analogous to insect defensins.

In humans, two classes of defensins can be found: alpha-defensins and beta-defensins.

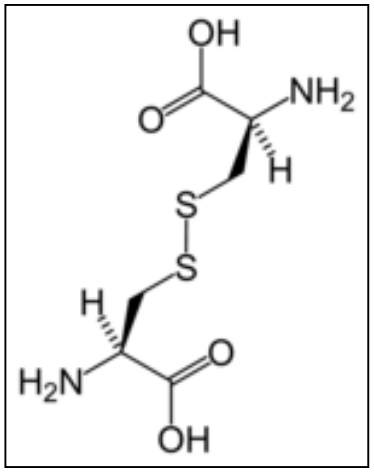
Antibacterial agents from plants, and insect and mammalian

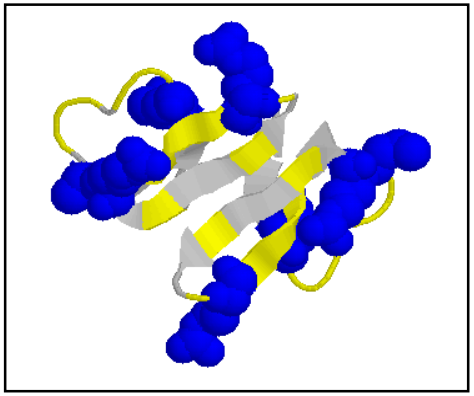
Plants and insect and mammalian defensins

- Insect and mammalian defensins have 3 disulfide bonds.
- Whereas plant defensins (PDFs) from radish (Rs-AFP1, 2, 3, 4), are small, cysteine-rich peptides consisting of 45-54 amino acids with 4 disulfide bonds.
- They are conserved in several plant species, including members of the Brassicaceae.
- Rice plants do not contain these peptides.

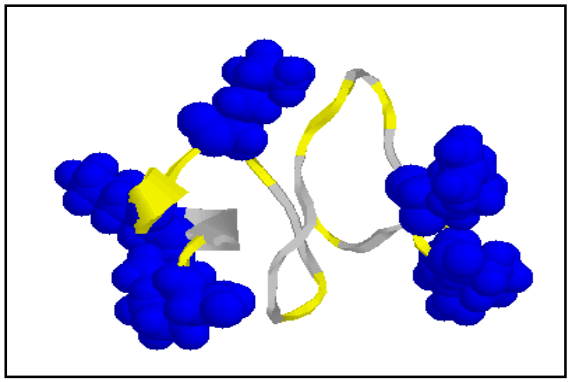
Antimicrobial/antibacterial peptides
Defensins

- Cystine is composed of two cystines linked by a disulfide bond (shown here in its neutral form).





Human



Plant

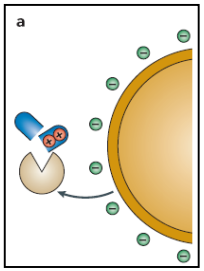
Antimicrobial/antibacterial peptides
Defensin-like peptides from plant species

- A high number of defensin-like peptides are present in various plant species.
- Defensin-like peptides are likely to be involved in both natural immunity and cell-to-cell communication.

Antimicrobial/antibacterial peptides
Natural peptides

- Are natural peptides that defend the host organism against bacterial infection.
- They typical contain both positively charged and hydrophobic residues.
- Cationic (+vely charged) peptides are the most widespread.
- However, cationic antimicrobial peptides(CAMPs) are very susceptible to proteolytic degradation by bacterial and host proteases.

Proteolysis of CAMPs



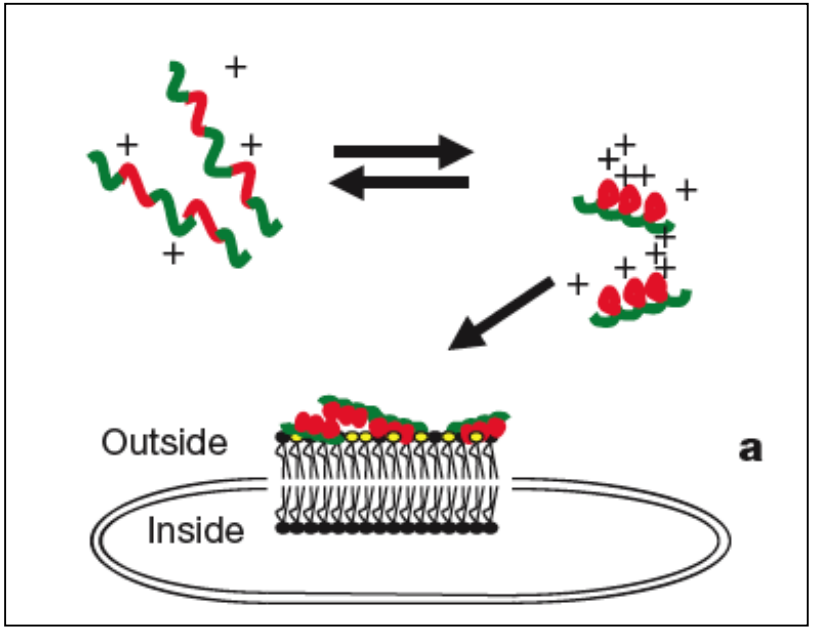
Antiifungal/antibacterial peptides
Natural peptides from different sources

Antimicrobial peptide	Species	Active against		
		Gram+ bacteria	Gram− bacteria	Fungi
Thionins	plants	++	++	++
Plant defensins	plants	(+)	(+)	+++
Knottin-type peptides	plants	++	(+)	++
Hevein-type peptides	plants	+	+	+
Cecropins	insects	(+)	+++	(+)
Drosocin	insects	(+)	+++	−
Metchnikowin	insects	+++	+++	+++
Insect defensins	insects	+++	(+)	−
Drosomycin	insects	−	−	+++
Clavanins	tunicates	++	++	++
Styelins	tunicates	+++	+++	(+)
Tachyplesin	shrimps	++	++	?
Penaeidins	shrimps	++	(+)	++
Lycotoxins	wolf spider	?	++	++

Mechanism of action

How do antibacterial peptides from plants act against bacteria

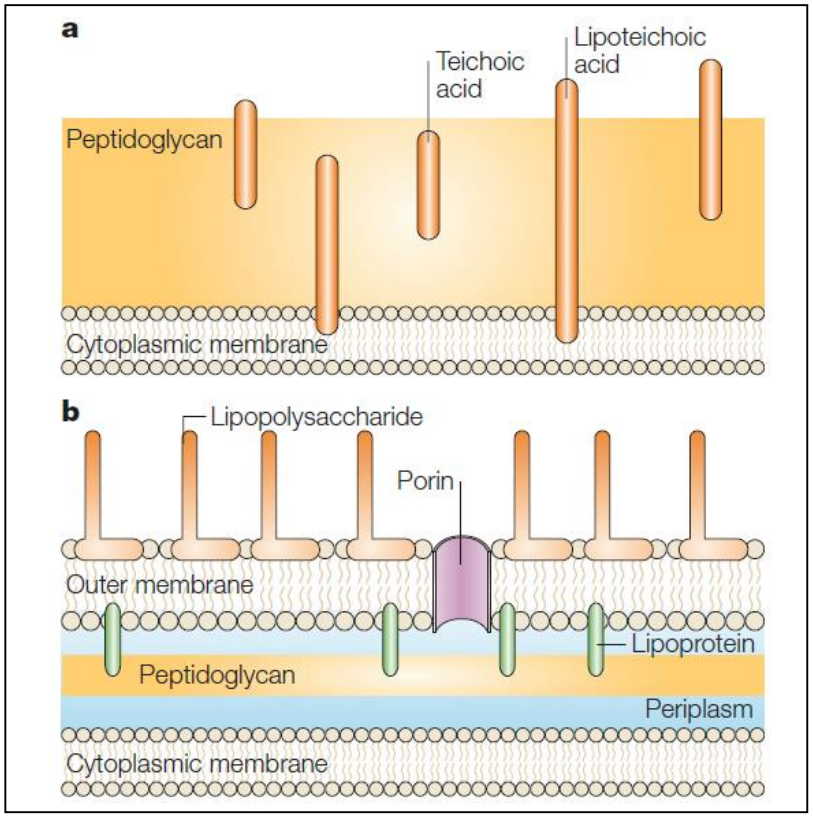
- The classical mechanism of action of cationic (+vely charged) AMPs, such as defensins, is the disruption of the anionic (-vely charged) bacterial membrane.



Cell wall composition

Differences between Gram-positive (a) and Gram-negative (b) cell walls

1. The cationic (+vely) peptides are attracted electrostatically to negatively charged molecules such as anionic phospholipids, lipopolysaccharides (LPS) (Gram-negative), and
2. Teichoic acid (Gram-positive), which are located asymmetrically in the membrane architecture.
3. The positively charged residues can also interact with membrane lipids through specific receptors at the surface of the cell.



Cabeen and Jacobs-Wagner,2005

Mechanism of action

How do antibacterial peptides act against bacteria

- AMPs can be broadly classified into two families:

1. **Cell surface-targeting peptides**, including both membrane-targeting and non-membrane targeting peptides, can be further classified based on specific targets such as

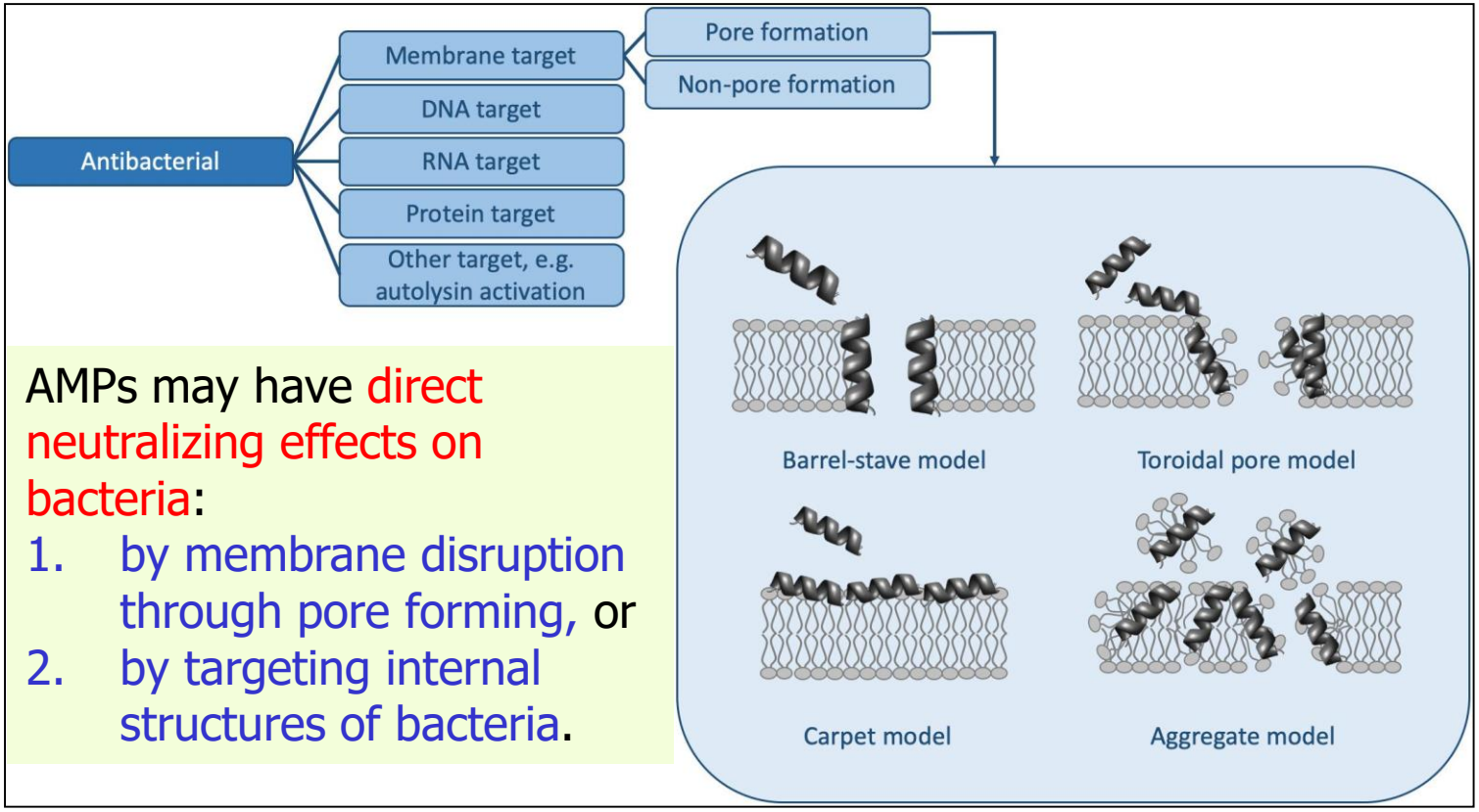
- ✓ cell wall/carbohydrates,
- ✓ lipids/membranes, and
- ✓ proteins/receptors.

2. **Intracellular targeting AMPs** can be further classified based on the specific target molecules (e.g. heat shock proteins, DNA, and RNA).

The Antimicrobial Peptides Database,2013

Mechanism of action

Four main models of membrane-pore formation by AMPs antimicrobial peptides



AMPs may have direct neutralizing effects on bacteria:

1. by membrane disruption through pore forming, or
2. by targeting internal structures of bacteria.

Martin *et al.*,2015; Carmona-Ribeiro and Araújo,2021

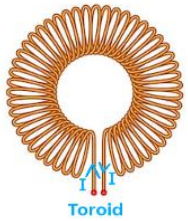
Mechanism of action

Four main models of membrane-pore formation by AMPs antimicrobial peptides

- There are four main models of membrane-pore formation, namely:

1. barrel-stave model,
2. toroidal-pore model,
3. carpet model, and
4. aggregate or "detergent–like" model model.

Stave

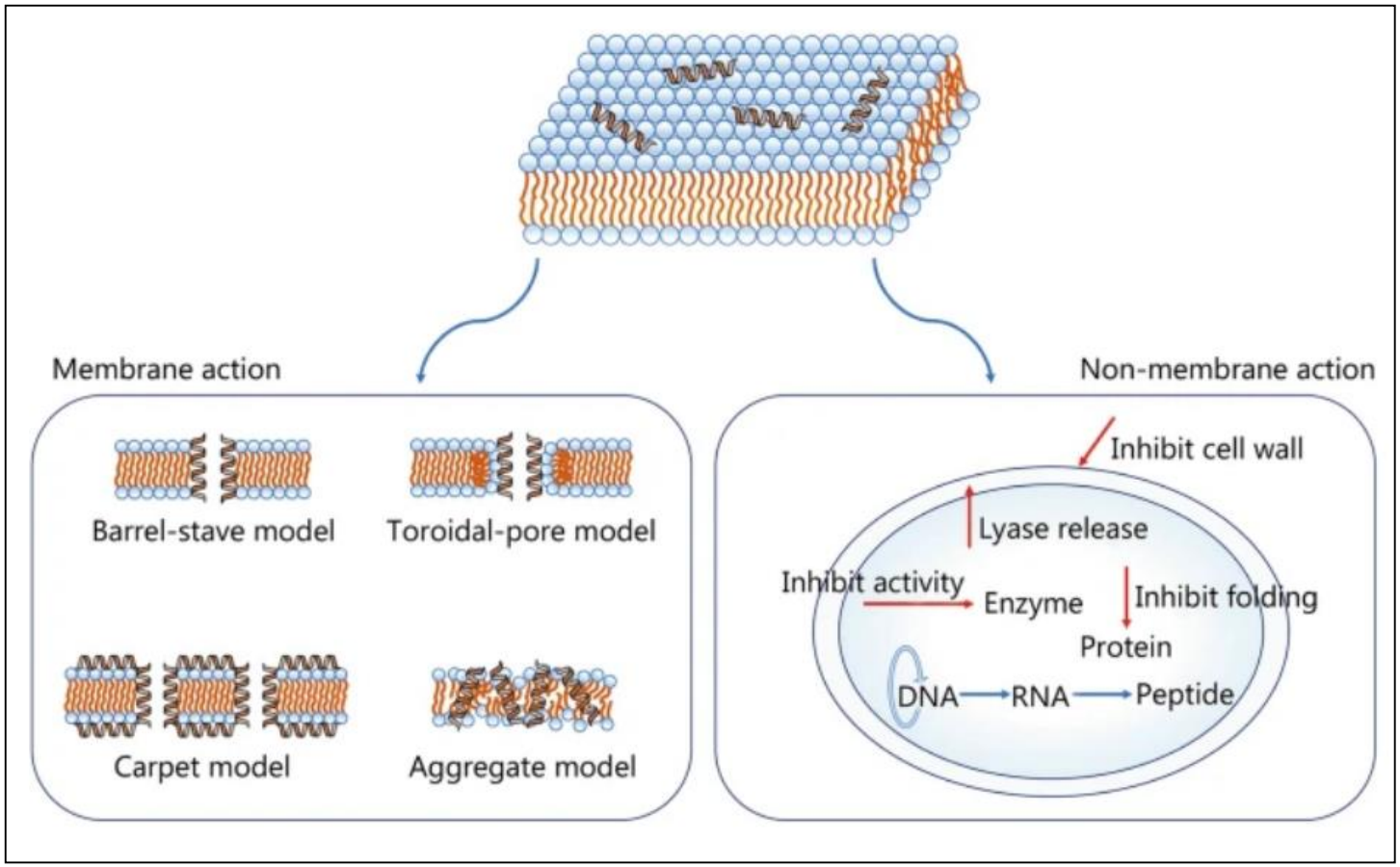


Toroid: Shape like a torus or toroid, a circle in three-dimensional space.
Stave: A narrow strip of wood forming part of the sides of a barrel.

Zhang *et al.*,2021

Mechanism of action

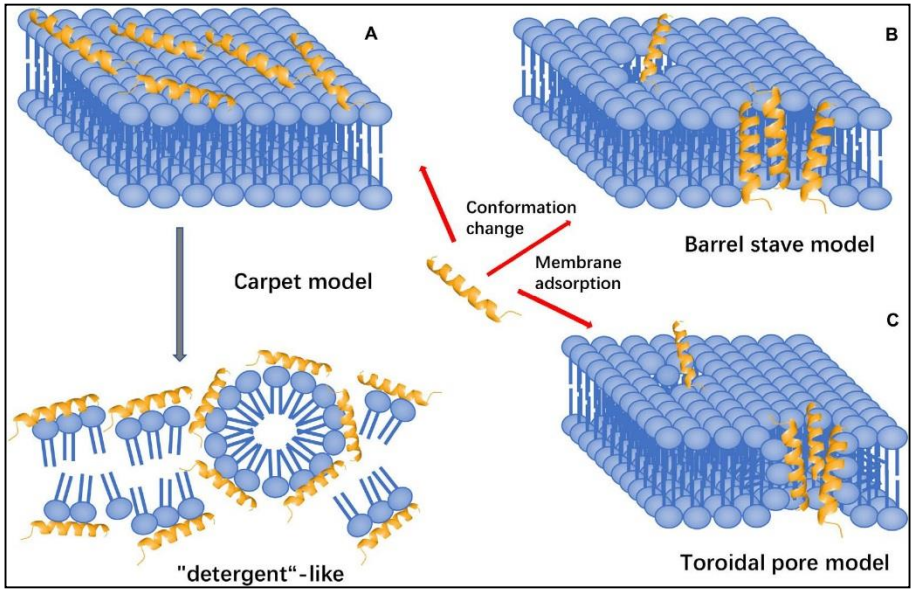
Four main models of membrane-pore formation by AMPs antimicrobial peptides

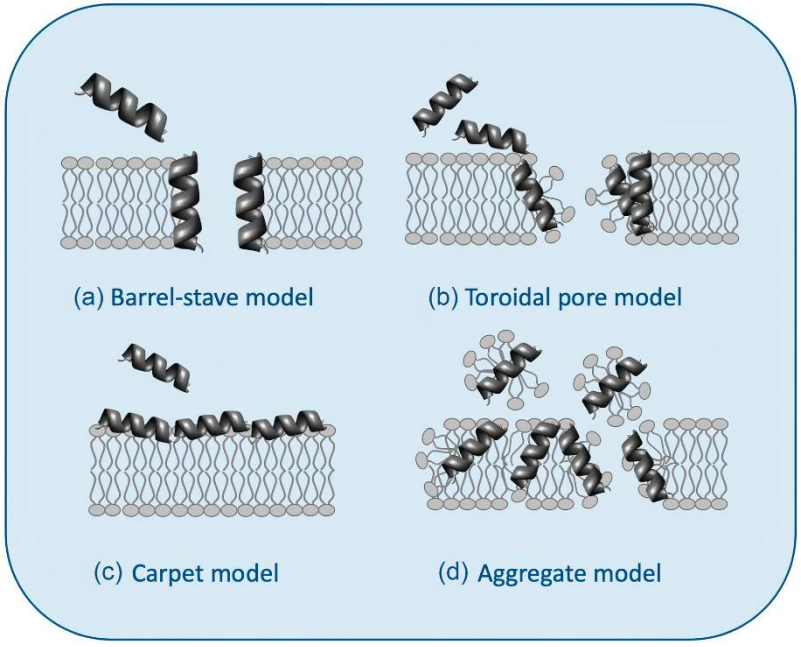


Zhang *et al.*,2021

Mechanism of action

Four main models of membrane-pore formation by AMPs antimicrobial peptides



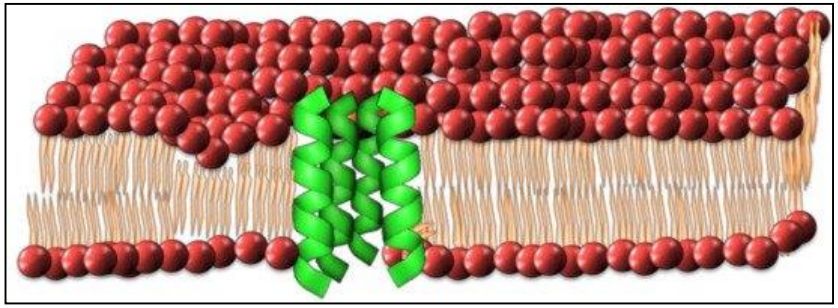


Huan et al.,2020; Carmona-Ribeiro and Araújo,2021

Mechanism of action

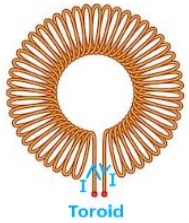
The first mechanism: barrel stave model

- Makes pores in the bilayers of bacterial membrane.
- The first is the "barrel stave model" whereby the antimicrobial peptides insert themselves into the membrane of the offending cell.
- The presence of one AMP attracts others, which quickly organize to form a pore.
- The cell's contents begin leaking out of the pore and the cell is destroyed (Reddy,2004).

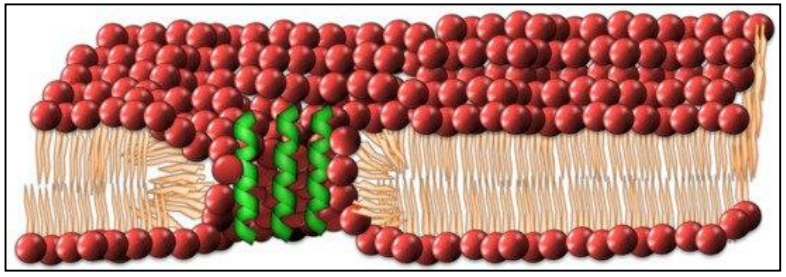


Mechanism of action

The second mechanism: toroidal pore or wormhole hypothesis



- Toroidal model **resembles** the Barrel-stave model, but AMPs are always in contact with phospholipid head groups of the membrane.
- The toroidal pore or wormhole hypothesis also postulates the formation of pores in a barrel-stave shape.

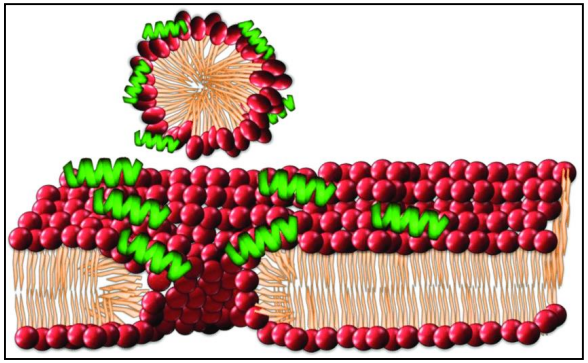


Pelegrini *et al.*,2011;..

Mechanism of action

The third mechanism: carpet model

- This involves AMPs carpeting the surface of the antagonizing organism, rather than inserting themselves into the cellular membrane.
- The carpet model, which suggests that peptides are absorbed parallelly in the bilayers and, after achieving a sufficient coverage, generate a detergent effect and destroy the membrane.

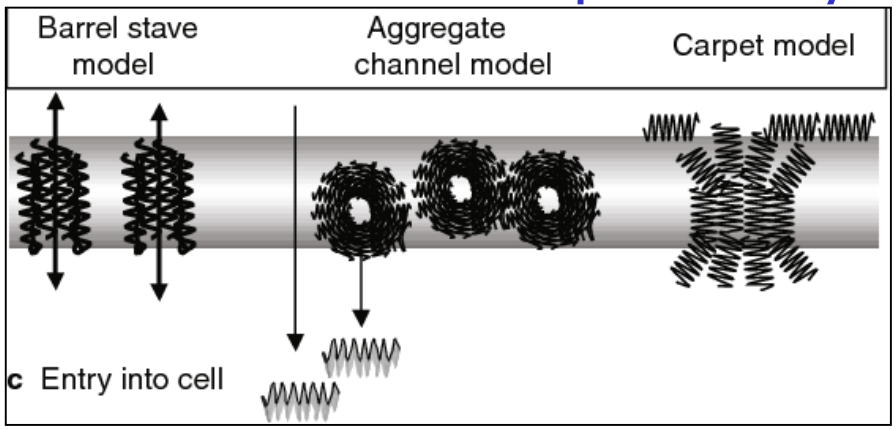


Amerikova *et al.*,2018; Alghalavini *et al.*,2019

Mechanism of action

The Forth mechanism: aggregate model

- This mechanism explains why AMPs not only target the cytoplasmic membrane, but may also cross the membrane into the cytoplasm to act on intracellular substances.
- Within the cell, AMPs aggregate in the cytoplasm and inhibit nucleic acid as well as protein synthesis.



Koczulla and Bals,2012; Spänig and Heider,2019; Zhang *et al.*,2021

Antimicrobial/antibacterial peptides

Antimicrobial pseudopeptides produced by microorganisms active against plant pathogens

Compound	Composition	Producer microorganism
Pantocines A and B	Alanine derivatives	*Pantoea agglomerans*
Polyoxins	Pyrimidinyl-dipeptide	*Streptomyces cacaoi*
Nikkomycins	Pyridinyl-dipeptide	*Streptomyces tendae*
Rhizocticin	Phosphono-oligopeptide	*Bacillus subtilis*
Bacilysin	Epoxycyclohexane-dipeptide	*Bacillus subtilis*
Blasticidin	Nucleopeptide	*Streptomyces griseochromogenes*
Mildiomycin	Nucleopeptide	*Streptoverticillium rimofaciens*

Montesinos,2007

Antimicrobial activity of the peptides

Several methods have been used to determine the mechanisms of antimicrobial peptide activity

Methods	Applications
Atomic-force microscopy (AFM) or scanning-force microscopy (SFM)	to visualize the effects of antimicrobial peptides on microbial cells
Atomic emission spectroscopy	to detect loss of intracellular potassium (an indication that bacterial membrane integrity has been compromised)
Fluorescent dyes	to measure ability of antimicrobial peptides to permeabilize membrane vesicles
Ion channel formation	to assess the formation and stability of an antimicrobial-peptide-induced pore
Circular dichroism and orientated circular dichroism	to measure the orientation and secondary structure of an antimicrobial peptide bound to a lipid bilayer
Dual Polarization Interferometry	to measure the different mechanisms of antimicrobial peptides
Solid-state NMR spectroscopy	to measure the secondary structure, orientation and penetration of antimicrobial peptides into lipid bilayers in the biologically relevant LIQUID-CRYSTALLINE STATE
Neutron and X-ray diffraction	to measure the diffraction patterns of peptide-induced pores within membranes in oriented multilayers or liquids

Wikepedia,2015

Antimicrobial activity of the peptides
Atomic-force microscopy (AFM) or scanning-force microscopy (SFM)

- AFM has been increasingly applied to investigate the morphology and ultrastructure of cell surface of:

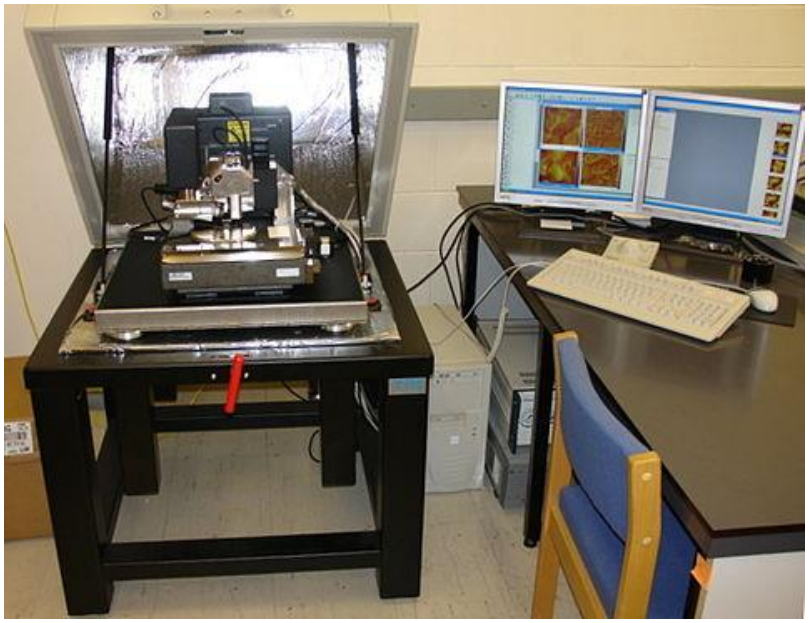
1. Gram-negative bacteria (e.g. *Escherichia coli*, *K. pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*), and
2. Gram-positive bacteria (e.g. *Bacillus cereus*, *Bacillus circulans*, *Micrococcus luteus*, *Staphylococcus aureus* and *Streptococcus pyogenes*), as well as
3. The biological effects of various compounds like antibiotics and antimicrobial peptides on bacterial cells.

- When a bacterium was exposed to antimicrobial peptides, alterations in the bacterial cell surface was imaged by AFM.
- These include topography as well as nanomechanical properties in comparison with control bacteria.

Palusinska-Szysz, *et al.*,2015

Antimicrobial activity of the peptides
Atomic-force microscopy (AFM) or scanning-force microscopy (SFM)

- Atomic force microscopy (AFM) has played a crucial role in nano-scale science and technology.
- An atomic-force microscope on the left with controlling computer on the right.

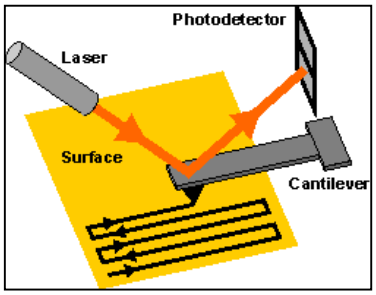


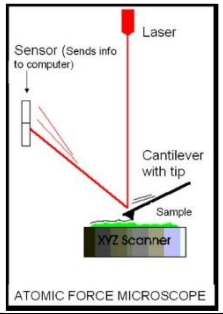
Another major application of AFM (besides imaging) is force spectroscopy, the direct measurement of tip-sample interaction forces as a function of the gap between the tip and sample. Force spectroscopy measures the mechanical properties.

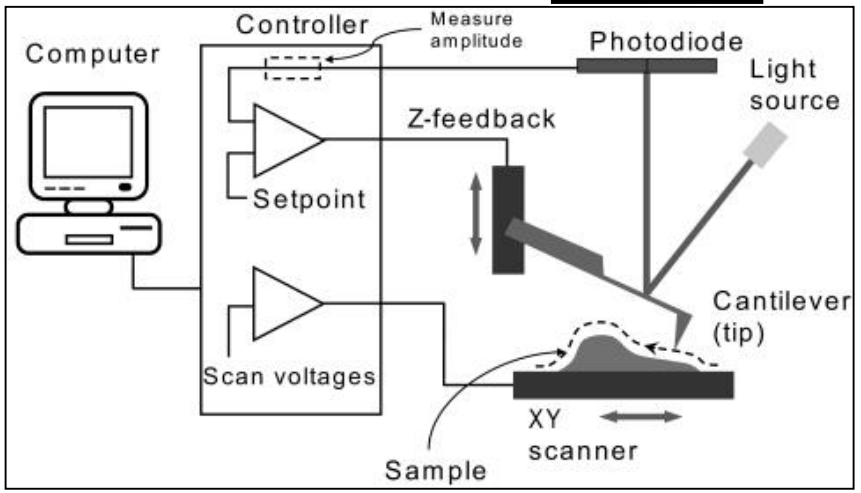
Wikipedia,2017;..

Antimicrobial activity of the peptides
Atomic-force microscopy (AFM) or scanning-force microscopy (SFM)

- Schematic of the operation of the AFM.
- To obtain an image, a cantilever is scanned over the sample surface.
- A laser beam is deflected off the back of the cantilever, and changes in deflection are monitored with a photodiode detector.







cantilever is a rigid structural element, such as a beam or a plate, anchored at only one end to a (usually vertical) support from which it is protruding.

Last *et al.*,2010

Antimicrobial activity of the peptides

Atomic-force microscopy (AFM) or scanning-force microscopy (SFM)


Meca discs

- Forty microliters of the bacterium *Legionella pneumophila* suspension (OD=0.2) was incubated without (control) or in the presence of antibacterial peptide, apoE (final concentration 0.4 mg mL^{-1})at 37°C for 1h.
- After centrifugation (8000 g, 10 min, 4°C), the bacteria were prepared on afm mica discs for imaging as described previously (Zdybicka-Barabas *et al.*,2011).
- Note: The highest quality mica (V-1 or V-2) is for AFM applications and Medium Quality (V-4 to V-6) discs for replication and thin film deposition.
- The bacterial cell surface was imaged using a NanoScopeV AFM(Veeco)(Analytical Laboratory, Faculty of Chemistry, UMCS, Lublin, Poland) in the 'Peak Force QNM' operation mode using a silicon tip with a spring constant of 20 N m^{-1} (NSG30, NT-MDT, Russia).

Palusinska-Szysz *et al.*,2015

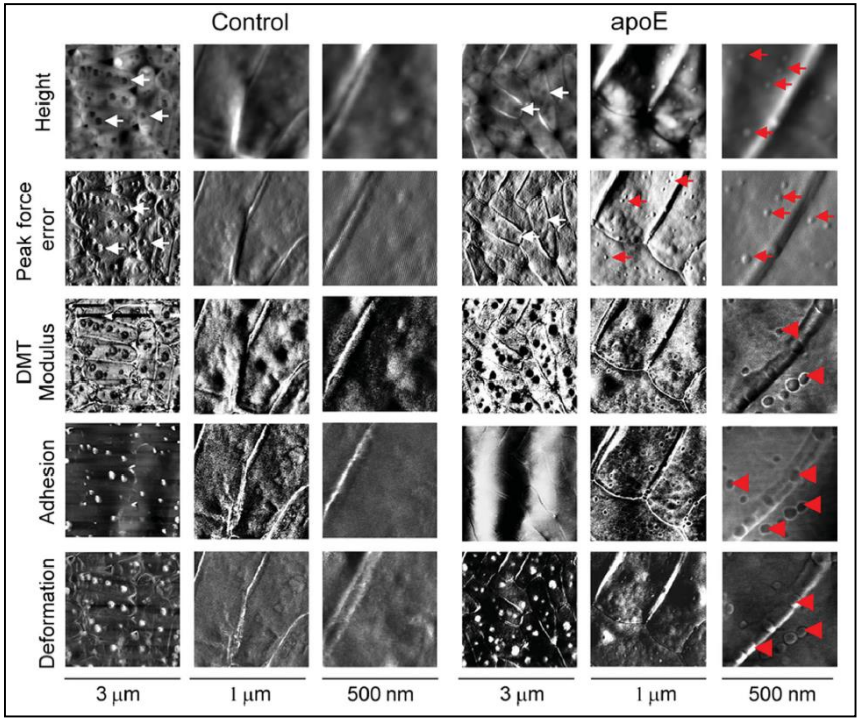
Antimicrobial activity of the peptides
Atomic-force microscopy (AFM) or scanning-force microscopy (SFM)

- Three fields were imaged on each mica disc.
- The topography of the examined samples was presented as the height and peak force error images. The 1) DMT (Derjaguin, Muller and Toporov) modulus, 2) adhesion and 3) deformation maps reflected bacterial cell surface stiffness, adhesion forces between the cell surface and a tip and penetration of the tip into the cell surface, respectively.
- The values of average root mean square (RMS) roughness, DMT modulus, adhesion forces and the deformation rate of the cell surface were calculated from measurements of 60 fields (120 × 120 nm) in 1 × 1 μm images of the bacterial cell surface.
- The data were analysed with nanoscope analysis ver. 1.40 software (Veeco).
- The section profiles of the cells were generated using wsxm 5.0 software (Nanotec, Spain; Horcas *et al.*,2007).

Palusinska-Szysz *et al.*,2015

Antimicrobial activity of the peptides
Atomic-force microscopy (AFM) or scanning-force microscopy (SFM)

- AFM imaging of *Legionella pneumophila* cells treated with human antipeptide apoE.
- The bacteria were incubated without (control) or in the presence of apoE (0.4 mg mL^{-1}) and imaged by AFM.
- The height, peak force error, adhesion, elasticity (DMT modulus) and deformation images are presented.
- The brighter and darker areas of the images correspond to the higher and lower values of the parameters, respectively.
- The round structures reflecting the vacuoles and granule-like protuberances are marked by white and red arrows, respectively.
- In the DMT modulus, adhesion and deformation maps of the apoE-treated bacteria the red arrowheads indicate separate areas of distinct properties in comparison with the rest of the surface.



Palusinska-Szysz *et al.*,2015

Antimicrobial activity of the peptides
Force spectroscopy

- Another major application of AFM (besides imaging) is force spectroscopy.
- Force spectroscopy is a set of techniques for the study of the interactions and the binding forces between individual molecules.
- These methods can be used to measure the mechanical properties of single polymer molecules or proteins, or individual chemical bonds.

Antimicrobial activity of the peptides
Method for growth inhibition

- In general, try to dissolve the peptide in sterile distilled water or sterile dilute acetic acid (0.1%) solution to give a stock solution at a higher concentration than required for the assay.
- Below the names, properties and amount of water needed to prepare 1mM of the solutions were measured.
- For best preservation, store them under refrigeration at 4°C or colder, away from bright light. PI, protease inhibitor (inhibitor of pathogen's protease).

No	Organism	Name	Sequence	pI	MW (Da)	delivered (mg)	µL sterile water to be added to the tube to have 1mM stock
BR001	Hyalophora cecropia	Cecropin A	kwklfkkiekvgqnirdgiikagpavavvgqatqiak*-NH2	10,75	4005	1	250
BR002	Sarcophaga peregrina	Sarcotoxin IA	gwlkkigkkiervgqhtrdatigqlqiagqaanvaatar*-NH2	11,74	4157	1	240
BR004	Ceratitis capitata	Ceratotoxin	sigsafkkalpvakkigkaalpiakaalp	10,70	2861	1	345
BR005	Stomoxys calcitrans	Scal-stomoxyn	rgfrkhfnklvkkvkhtisetahvakdtaviagsgaavvaat*-NH2	11,26	4416	1	225
BR006	Pseudacanthotermes spiniger	Spinigerin	hvdkkvadkvlllkqlrimrlltrl	11,07	3001	1	335
BR007	Apis mellifera	Apidaecin Ia	gnnrpvyipqprpphpri	11,71	2108	1	475
BR009	Myrmecia gulosa	Formaecin-1	grpnpvnnkptphprl	12,01	1794	1	555
BR016	D. melanogaster	Metchnikowin-1	hrhqgpifdtrpspfnpnqprpqpiy	10,74	3026	1.1	365
BR017	D. melanogaster	Metchnikowin-2	hrrqgpifdtrpspfnpnqprpgpiy	11,54	3045	1.26	415
BR033	Lucilia sericata	Lser-Cecropin1	GWLKKIGKKIERVGQHTRDATIQTIGVAQQAANVAATLKG	10,56	4256	1	235
BR036	Lucilia sericata	Lser-Cecropin3	GWLKKIGKKIERVGQHTRDATIQVLGVAQQAANVAATARG	11,07	4242	1	235
BR039	Lucilia sericata	Lser-PRP2	EWRPHGSIGGSGLRPGRPQTLPPQRPRRPDFNGPRHRF	12,22	4371	1	230
BR040	Lucilia sericata	Lser-PRP3	SPFVDRPRRPIQHNGPKPRIITNPPFNPNARPAW	12,18	3945	1	255
BR044	Lucilia sericata	Lser-Stomoxyn	GFRKRFNKLVKKVKHTIKETANVSKDVAIVAGSGVAVGAAMG	10,73	4384	1	230
BR080	Sarcophaga peregrina	Sapecin	atcdllsgtginhsacaahcllrgnrggycngkavcvcrn	8.69	4081	1.15	280
BR081	Aeschna cyanea	Defensin	gfgcpldqmqchrhcqtitgrsggycsgplkltctcyr	8.68	4180	1.15	275
BR083	Heliothis virescens	Heliomicin	dkligscvwgavnytsdcngeckrrgykgghcgsfanvncwcet	7.77	4790	2.14	445
BR097	Galleria mellonella	GmelCecropinA	KWKIFKKIEKAGRNIRDGIIKAGPAVSVVGEAATIYKTG*-NH2	10,21	4215	1	235
Br098	Galleria mellonella	GmelCecropinB	KWKFFKKIERVGQNIRDGIIKAGPAVQVVGQAATIYKGK*-NH2	10,46	4344	1	215
BR099	Galleria mellonella	GmelCecropinC	RWKVFKKIERMGQHIRDGIIKAGPAVAVVGQASTIISG*-NH2	11,07	4119	1	240
BR100	Galleria mellonella	GmelCecropinD	ENFFKEIERAGQRIRDAIISAAPAVETLAQAQKIIKGGD*-NH2	6,43	4256	1	235

Mohammad Rahnamaeian; A Guide to Handling and Storing Peptides, MIMOTOPES

Antimicrobial activity of the peptides
Method for growth inhibition

- The antimicrobial activity of the peptides was examined in sterile 96-well plates (Nunc F96 microtitre plates) in a final volume of 100 µl as follows:
- Aliquots (50 µl) of a suspension containing bacteria at a concentration of 1×10^6 colony-forming units (CFU)/ml in Lurie-Bertani culture(LB) medium were added to 50 µl of water containing the peptide in serial 2-fold dilutions.
- Inhibition of growth was determined by measuring the attenuance (absorbance) at 492 nm with a Microplate Autoreader after an incubation time of 18±20 h at 37°C.
- Antimicrobial activities were expressed as the minimal inhibitory concentration (MIC), the concentration at which 100% inhibition of growth was observed after 18±20 h of incubation.
- The bacteria used were: *Escherichia coli* D21, a Gram-negative bacteria, and *Bacillus megaterium* Bm11, a Gram positive bacteria.

Oren *et al.*,1999

Antimicrobial/antibacterial peptides

Antimicrobial activity against three plant-pathogenic bacteria and cytotoxicity of selected peptides

- Assessment of toxicity (hemolytic activity) of the selected AMPs against *E. amylovora*, *Pseudomonas syringae* and *X. vesicatoria*.

Peptide	MIC (μM)			ED_{50} (μM)			HD_{50} (μM)
	E. amylovora	*P. syringae*	*X. vesicatoria*	*E. amylovora*	*P. syringae*	*X. vesicatoria*	
Pep3	7–10	7–10	7–10	5.5	5.5	3.6	104
BP08	10–12	7–10	2–5	9.0	4.3	2.0	17
BP09	12–15	12–15	<2	11.2	6.9	ND[a]	10
BP10	15–20	15–20	<2	15.1	9.5	ND	11
BP11	7–10	5–7	2–5	5.0	3.8	2.5	30
BP12	50–100	50–100	25–50	60.0	56.8	14.7	6
BP15	5–7	2–5	12–15	4.3	1.6	7.3	334
BP18	5–7	5–7	<2	3.0	2.5	ND	26
BP19	5–7	5–7	<2	1.3	1.9	ND	32
BP20	2–5	2–5	2–5	3.2	2.7	2.3	42
BP33	5–7	5–7	10–12	4.3	3.2	4.1	190
BP76	2–5	2–5	2–5	2.5	2.1	1.9	203
Cecropin A[b]	<1	<1	<1	<0.3[c]	<0.3	<0.3	ND

[a] ND, not determined.
[b] Cecropin A was included for comparison purposes.
[c] Estimated visually from graphs; lowest concentration tested.

Fifty percent hemolysis (HD50) values; oral 50% effective doses (ED50).

Ferre *et al.*,2006

Antimicrobial/antibacterial peptides

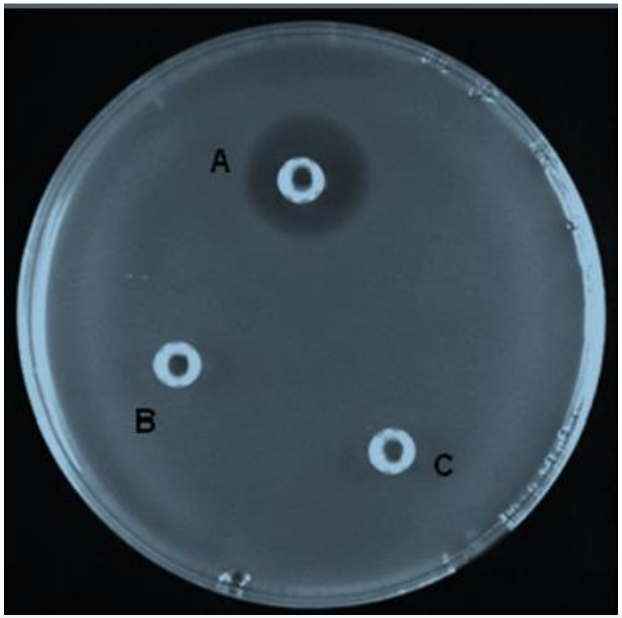
Screening for toxicity to antimicrobial activity against plant-pathogenic bacteria

- Many of the above toxic peptides may be useful for the control of bacterial pathogens in plants and they should be screened for activity in laboratory assays to determine if they have potential for use in transgenic plants.
- In addition, more efficient synthetic compounds designed by combining different protein domains responsible for toxicity to bacteria could also be tested.
- Ideally, ecological risks and human health hazards could also be evaluated in preliminary experiments.

Antimicrobial/antibacterial peptides

AMP produced by the plant pathogen *C. michiganensis* subsp. *michiganensis* against *Cms*

- It has previously been shown that the tomato pathogen *Clavibacter michiganensis* subsp. *michiganensis* secretes a 14-kDa protein, *C. michiganensis* subsp. *michiganensis* AMP-I (*Cmm*AMP-I), that inhibits growth of *Clavibacter michiganensis* subsp. *sepedonicus*, the causal agent of bacterial ring rot of potato.



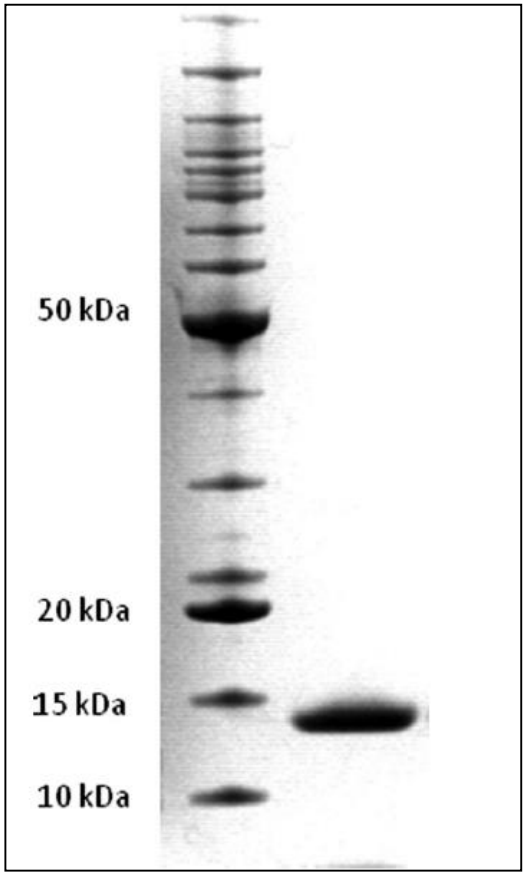
Inhibition of *C. michiganensis* subsp. *sepedonicus* by recombinant *Cmm*AMP-I. An NBY plate with a confluent layer of bacterial *C. michiganensis* subsp. *sepedonicus* cells, except for a halo around the well labeled "A", is shown. The following samples were added to the wells: A, 25 l of 5 M*Cmm*AMP-I in TN buffer; B and C, 25 l TN buffer.

Liu *et al.*,2013

Antimicrobial/antibacterial peptides

AMP produced by the plant pathogen *C. michiganensis* subsp. *michiganensis* against *Cms*

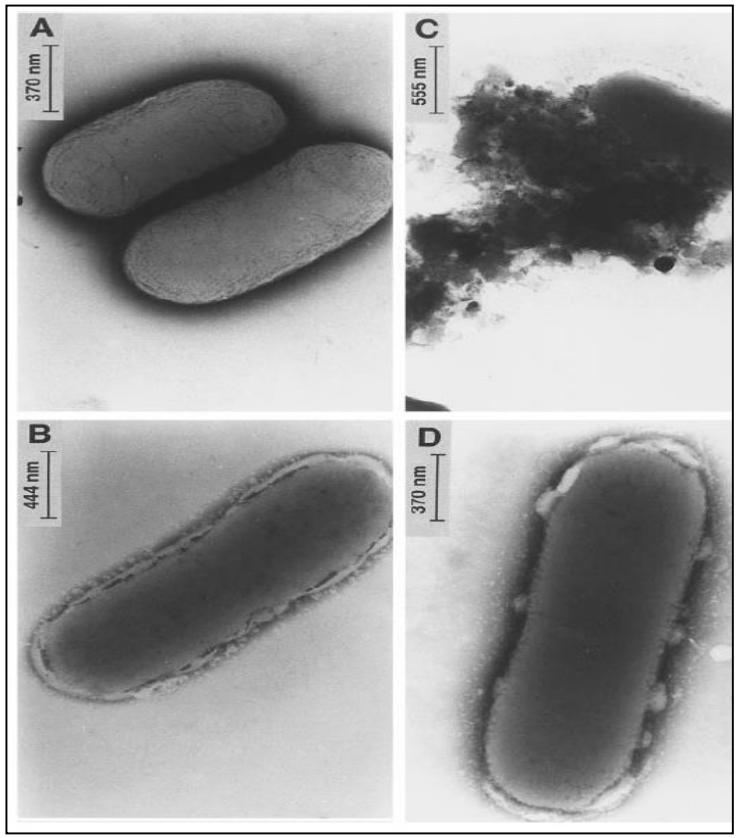
- SDS-PAGE analysis of purified recombinant *Cmm*AMP-I.
- Right lane, purified *Cmm*AMP-I;
- left lane, BenchMark protein ladder (Life Technologies)with 15 proteins ranging from 10 to 220 kDa.



Liu *et al.*,2013

Electron micrographs of negatively stained *E. coli* untreated and treated with antimicrobial peptides LL-37 and cecropin B

A. Control;
B. After treatment of the bacteria with LL-37 at a concentration lower than the MIC (7±5 lM);
C. After treatment of the bacteria with LL-37 at the MIC concentration (12±5 lM);
D. After treatment of the bacteria with insect peptide cecropin B at a low concentration.



The first active cationic peptide identified was LL-37, a 37 amino acid long peptide with broad antimicrobial activity.

Antimicrobial/antibacterial peptides

Synthetic antimicrobial peptides active against plant pathogens

Compound	Size	Sequence	Source
PEP6	6	FRLKFH	Synthetic
PAF26	6	Acetyl-RKKWFW-NH_2	Synthetic
BPC194	10	c(KKLKKFKKLQ)	Synthetic
PEP3	11	WKLFKKILKVL-NH_2	Cecropin–melittin hybrid
PEP11	11	WKLFKKILKVL	Cecropin–melittin hybrid
BP76	11	KKLFKKILKFL-NH_2	Cecropin–melittin hybrid
CAMEL	15	KWKLFKKIGAVLKVL-NH_2	Cecropin–melittin hybrid
Iseganan	17	RGGLCYCRGRFCVCVGR-NH_2	Protegrin
D4E1	17	FKLRAKIKVRLRAKIKL	Cecropin
TPY	17	KWVFRVNYRGIKYRRQR	Tachyplesin
ESF12	18	MASRAAGLAARLARLALR	Magainin
ESF1	20	MASRAAGLAARLARLALRAL	Magainin
Pexiganan	22	GIGKFLKKAKKFGKAFVKILKK-NH_2	Magainin
MSI-99	23	GIGKFLKSAKKFGKAFVKILNS	Magainin
MB-39	39	HQPKWKVFKKIEVVGRNIRNGI VKAGPAIAVLGEAKALG	Cecropin
Pen4-1	46	HSSGYTRPLRKPSRPIFIRPIGCDVCYGI PSSTARLCCFRYGDCHL-NH_2	Penaedin
D32R	47	KSCCRNTWARNCYNVCRLPGTISREI CAKKCRCKIISGTTCPSDYPK	Thionin

Montesinos,2007

Antimicrobial/antibacterial peptides

Antibacterial activity of the small-molecule compounds isolated from marine bacterium *Pseudoalteromonas flavipulchra* JG1 against the test organisms indicated

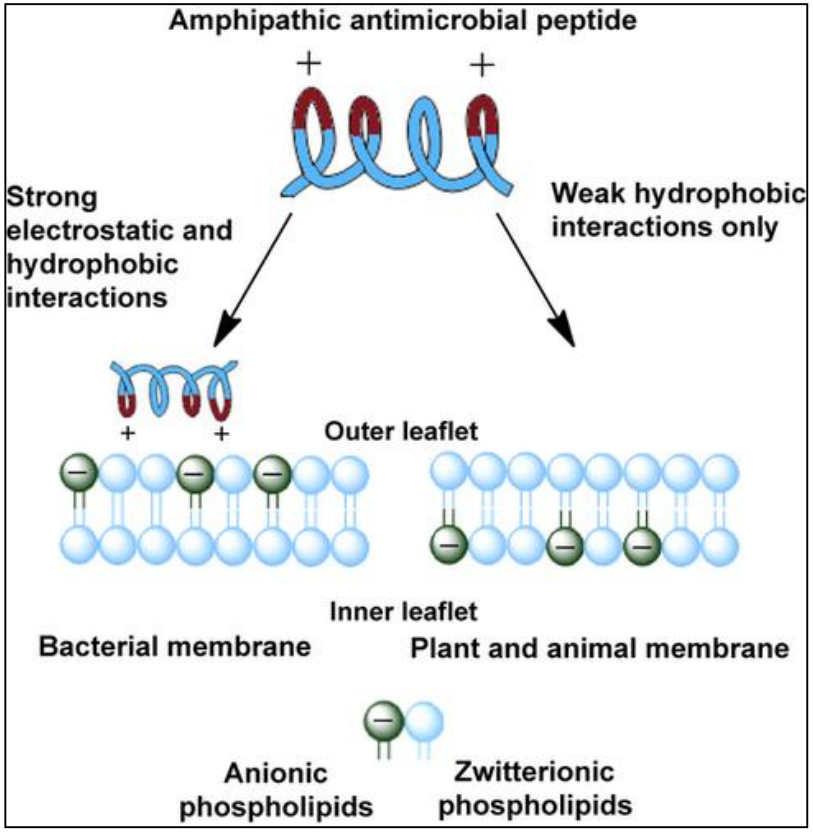
Compound	*V. anguillarum*	*V. harveyi* VIB 286	*Ph. damselae* subsp. *damselae*	*A. hydrophila*	*S. aureus*	*B. subtilis*
1. *p*-Hydroxybenzoic acid	+	+	+	+	−	+
2. *trans*-Cinnamic acid	+	+	+	+	−	−
3. 6-Bromoindolyl-3-acetic acid	+	+	+	+	+	+
4. *N*-Hydroxy-benzoisoxazolone	+	+	−	+	+	−
5. 2′-Deoxyadenosine	+	−	−	−	−	−

+, Antibacterial activity observed by TLC bioautography overlay assay; 2, no inhibition zone detected.

Yu *et al.*,2012

Antimicrobial/antibacterial peptides
Bacterial canker of kiwifruit
Pseudomonas syringae pv. *actinidiae*

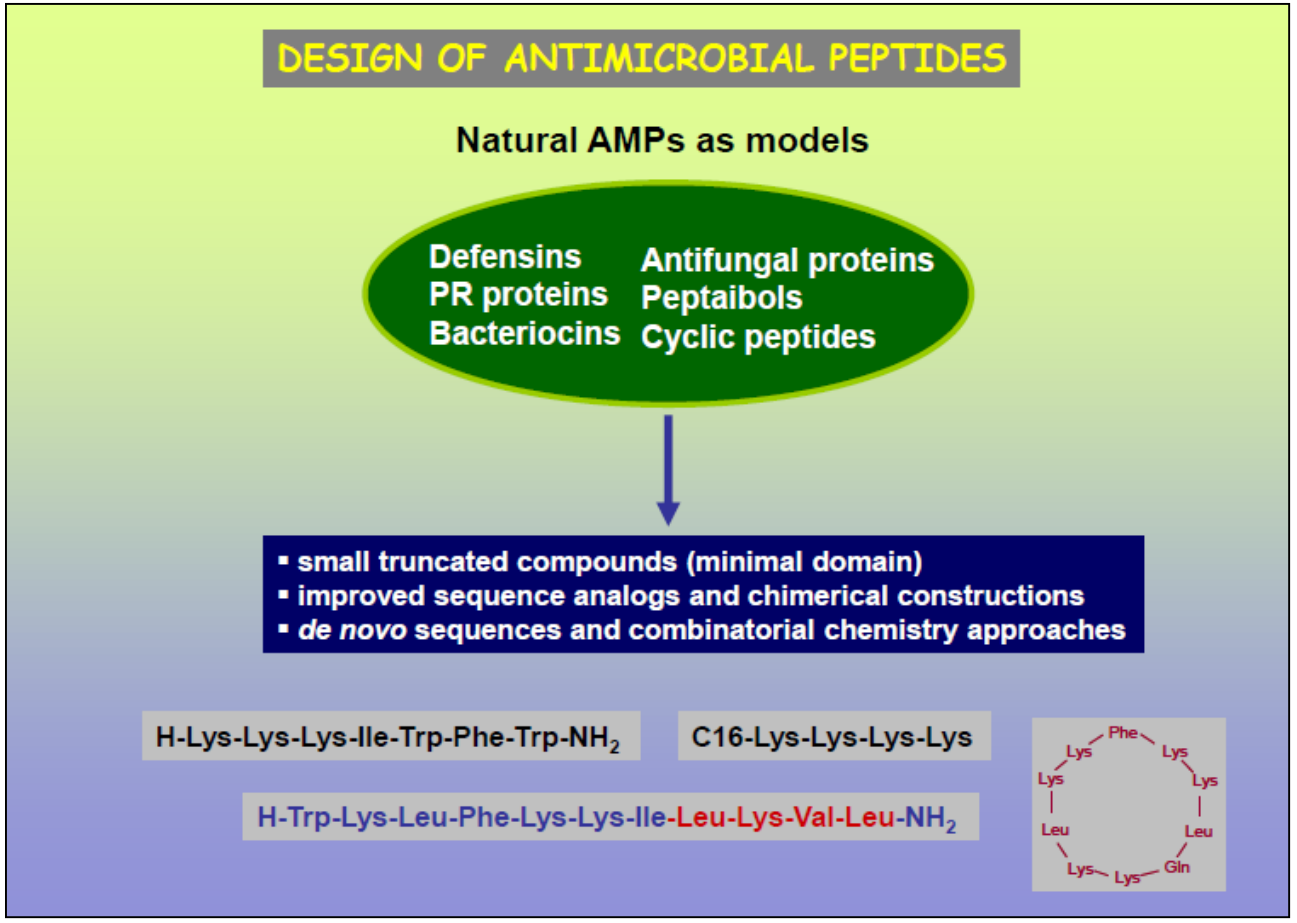
- A theoretical amphipathic α-helical antimicrobial peptide showing selectivity for the bacterial plasma membrane over those of plants and animals.
- Dark grey (red on-line) = cationic (+vely charged) and polar residues,
- light grey (blue on-line) = hydrophobic residues.



Cameron and Sarojini,2013

Antimicrobial/antibacterial peptides
Design of antimicrobial peptides

The bacteriocins (peptide antibiotics from bacteria) are proteinaceous substances usually have narrow spectrum. Whereas, the antibiotics (secondary metabolites; usually have broader spectrum.



Truncated: shorten
Chimerical: fantastically
Analogs: high chemical similarity
Peptaibols: a class of linear peptides of fungal origin with 7–20 residues.

Moragrega *et al.*,

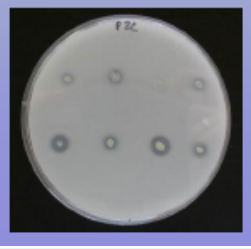
Antimicrobial/antibacterial peptides

Antibacterial agents from microorganisms (bacteria)

Biological control agents of plant diseases in which there are strong evidences that the mechanism of action involves antimicrobial peptides

Biocontrol microorganism	Target pathogen	Disease controlled	Antimicrobial peptide	Reference
B. subtilis FZB42	*F. oxysporum*	Plant growth promotion	bac, fen, itu	Koumoutsi et al. 2004
B. subtilis ATCC6633	*Phytium*	tomato	myc	Leclrerc et al. 2005
B. subtilis 6051	*Ps. syringae*	*Arabidopsis*	sur	Bais et al. 2004
B. subtilis GA1	*Botrytis*	postharvest rot apple	fen	Touré et al. 2004
Pantoea agglomerans Eh318	*E. amylovora*	fire blight	pan	Wright and Beer 1996
Pseudomonas fluorescens SS101	*Phytium, Phytophthora*	root rot	mas	De Souza et al. 2003
P. fluorescens Pf5	*P. syringae*	–	bat	Parret et al. 2005
Bacillus subtilis QST713	*Erwinia amylovora*	Fire blight, fungal dis. veg.	bac, fen, sur, itu	Joshi & McSpaden 2005
B. subtilis GB03	Fus, Asp, Rhi, Alt	root rot	bac, fen, sur	Joshi & McSpaden 2005
B. subtilis MBI600	Fus, Rhi, Asp, Bot	vegetables	bac, fen, sur	Joshi & McSpaden 2005
B. subtilis M4	*Phytium, Botrytis*	bean	fen	Ongena et al. 2005
P. fluorescens DR54	*Phythium, Rhizopus*	damping-off	vis	Nielsen and Sorensen 2003
Trichoderma harzianum ATCC36042 (Rifai strain)	*Botrytis*	–	tric	Schirmbock et al. 1994

bac, bacillomycin; fen, fengycin; itu, iturin; myc, mycosubtilin ; sur, surfactin; pan, patocine; mas, massetolyde; vis, viscosin amide; tric, trichorzianins; bat, bacteriocin



Moragrega *et al.*,

Antimicrobial/antibacterial peptides
Synthetic AMPs

Synthetic antimicrobial peptides active against plant pathogens

Sequence	Name	Size	Target
C16-KLLK	–	4	wide
FRLHF	PPD1	5	fungi
FRLKFH	66-10	6	fungi
Ac-RKKWFW-NH_2	PAF26	6	fungi
Ac-RRWQWR-NH_2	LfcinB $_{20\ 25}$	6	fungi
FRLKFHF	77-3	7	fungi
c(KKLKKFKKLQ)	BPC194	10	bacteria
WKLFKKILKVL-NH_2	PEP3	11	fungi
KKLFKKILKYL-NH_2	BP100	11	bacteria
KWKLFKKIGAVLKVL-NH_2	CAMEL	15	bacteria
RGGLCYCRGRFCVCVGR-NH_2	Iseganan	17	bacteria
FKLRAKIKVRLRAKIKL	D4E1	17	wide
KWVFRVNYRGIKYRRQR	TPY	17	fungi
MASRAAGLAARLARLALR	ESF12	18	fungi
KWKLFKKIPKFLHLAKKF	P18	18	wide
MASRHMFLPLIGRVLSGIL	MsrA3	19	wide
ARHGSCNYVFPAHKCICYF	MBG01	19	fungi
MASRAAGLAARLARLALRAL	ESF1	20	fungi
GIGKFLKKAKKFGKAFVKILKK-NH_2	Pexiganan	22	bacteria
GIGKFLKSAKKFGKAFVKILNS	MSI-99	23	wide

Moragrega *et al.*,

Antimicrobial/antibacterial peptides
Synthetic AMPs

Synthetic antimicrobial peptides designed (patented) by University of Girona research groups

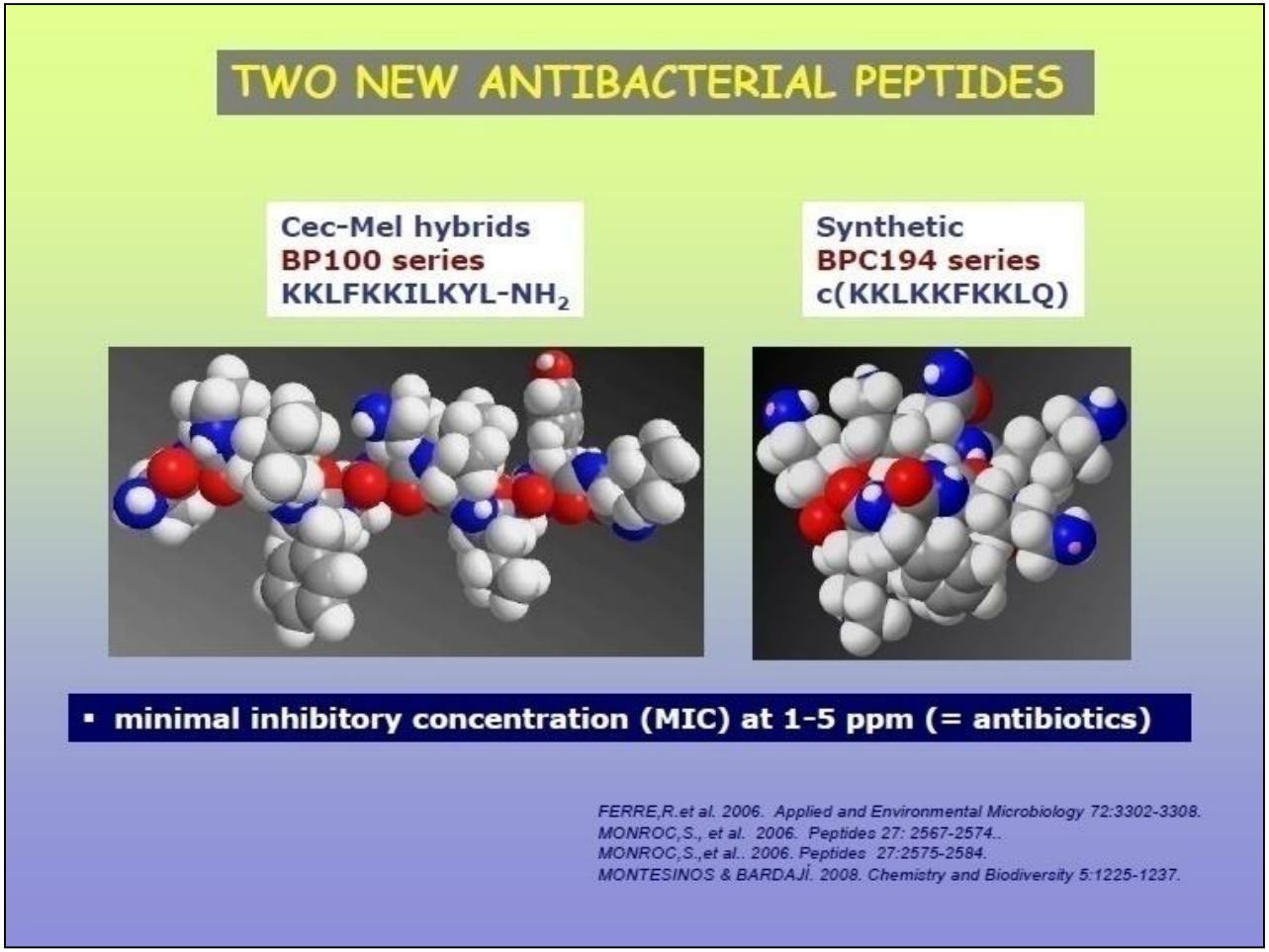
LIPPSO Laboratori d'Innovació en Processos i Productes de Síntesi Orgànica

CIDSAV Centre d'Innovació i Desenvolupament en Sanitat Vegetal

Sequence	Name	Size	Target
C (KKLKKFKKLQ)	BPC194	10	bacteria
KKLFKKILKY L-NH	BP100	11	bacteria

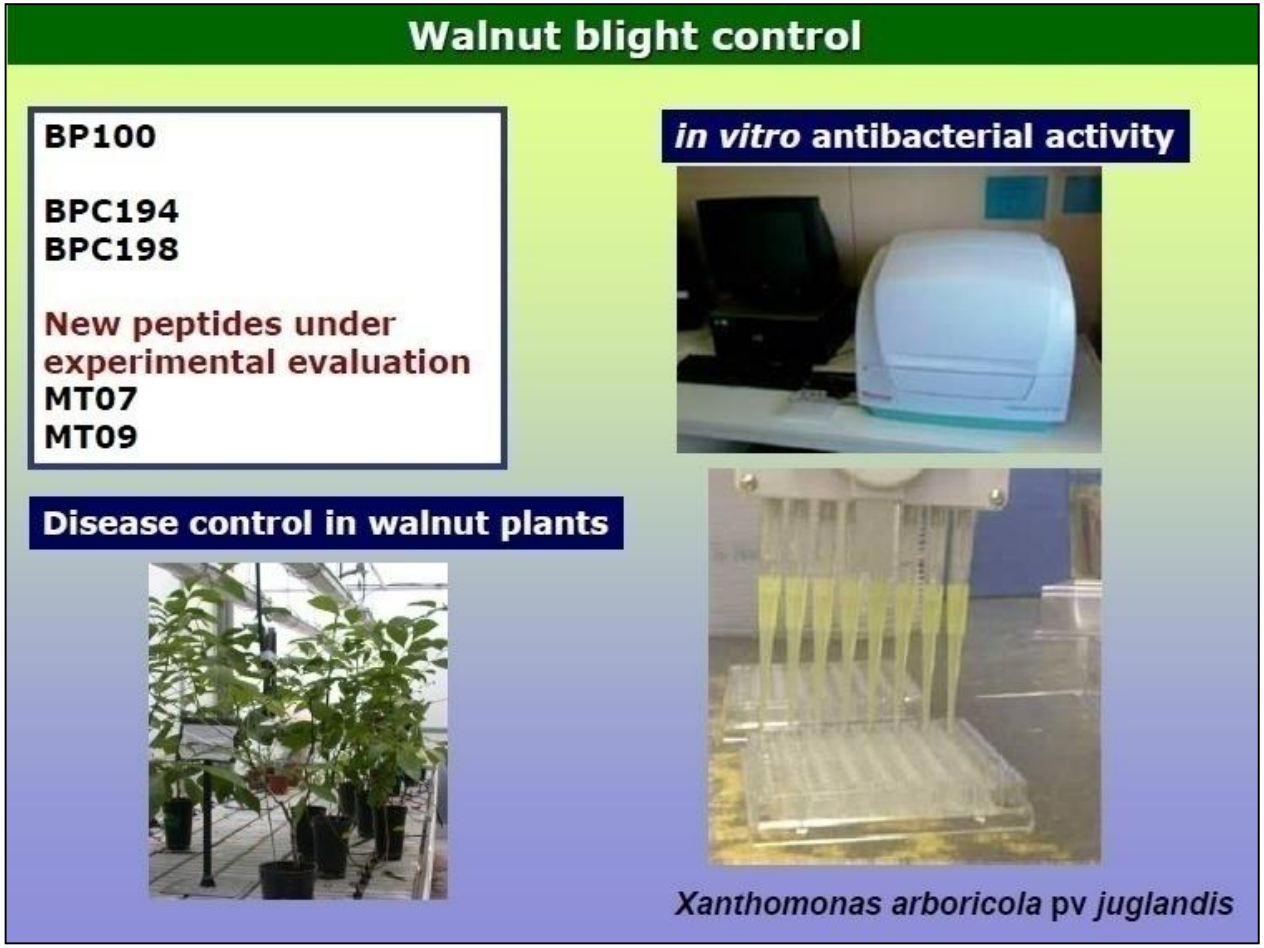
Moragrega *et al.*,

Antimicrobial/antibacterial peptides
Synthetic AMPs



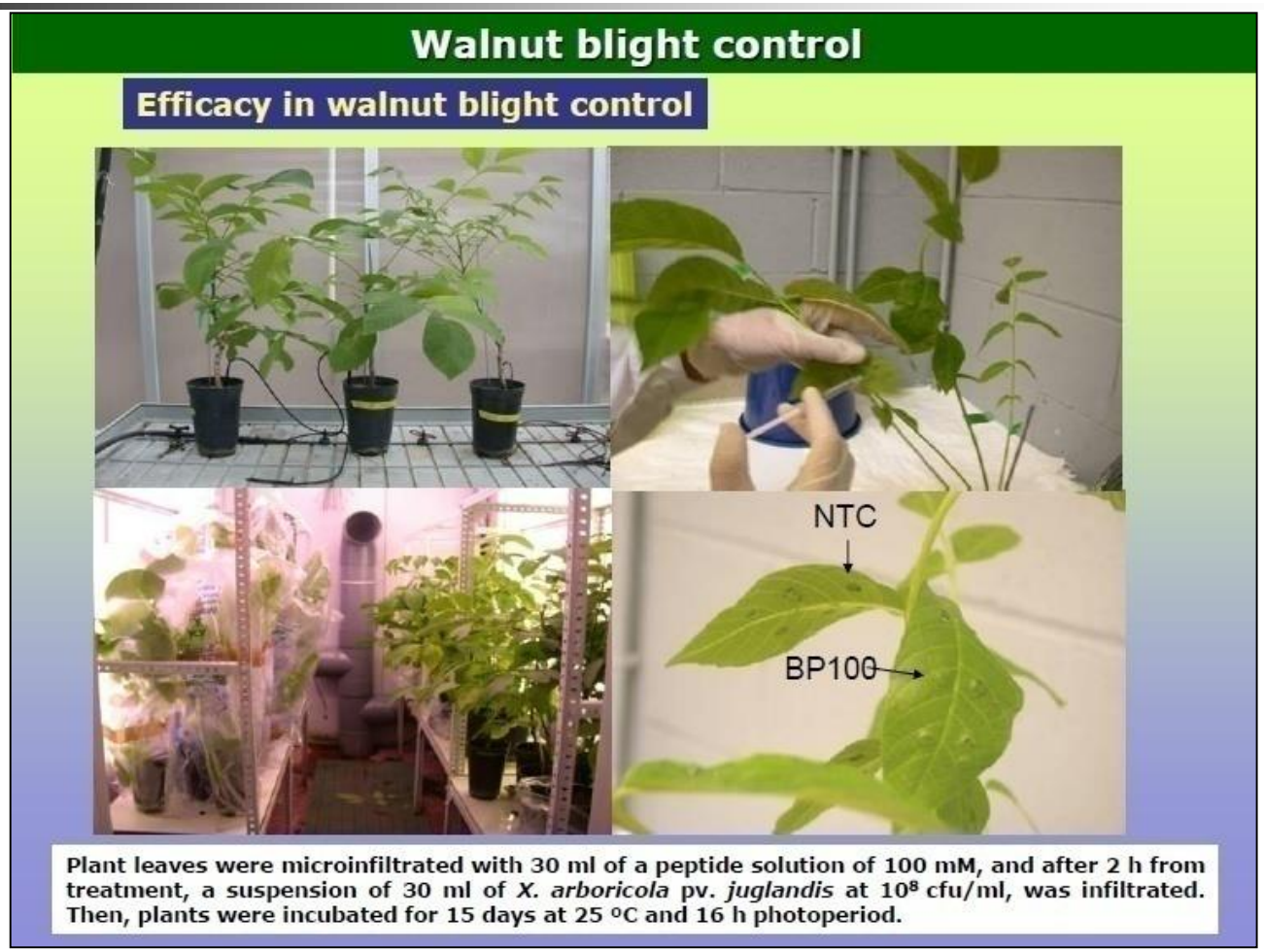
Moragrega *et al.*,

Antimicrobial peptides or proteins
Synthetic AMPs
Walnut blight control



Moragrega *et al.*,

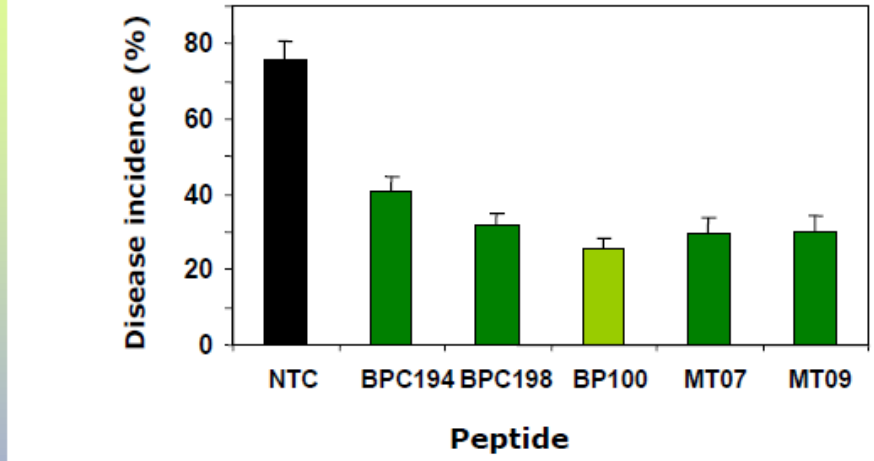
Antimicrobial/antibacterial peptides
Synthetic AMPs
Walnut blight control



Moragrega *et al.*,

Antimicrobial/antibacterial peptides
Synthetic AMPs
Walnut blight control

Efficacy of AMPs in walnut blight control

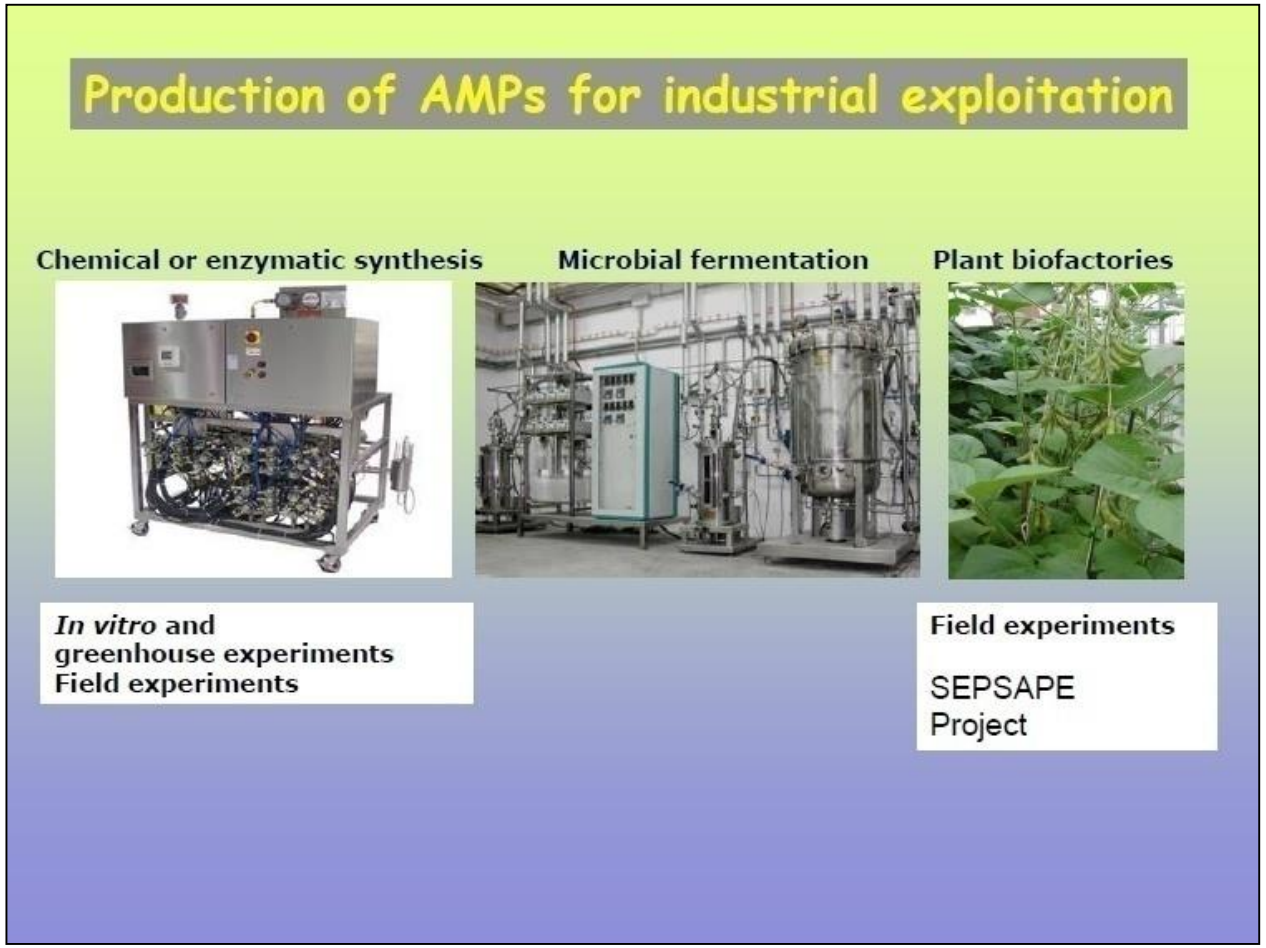


Peptides significantly reduced *Xaj* infection in walnut plants

BP100	**good in walnut blight control**
MT07 and MT09	**reduction of disease incidence similar to BPC series**

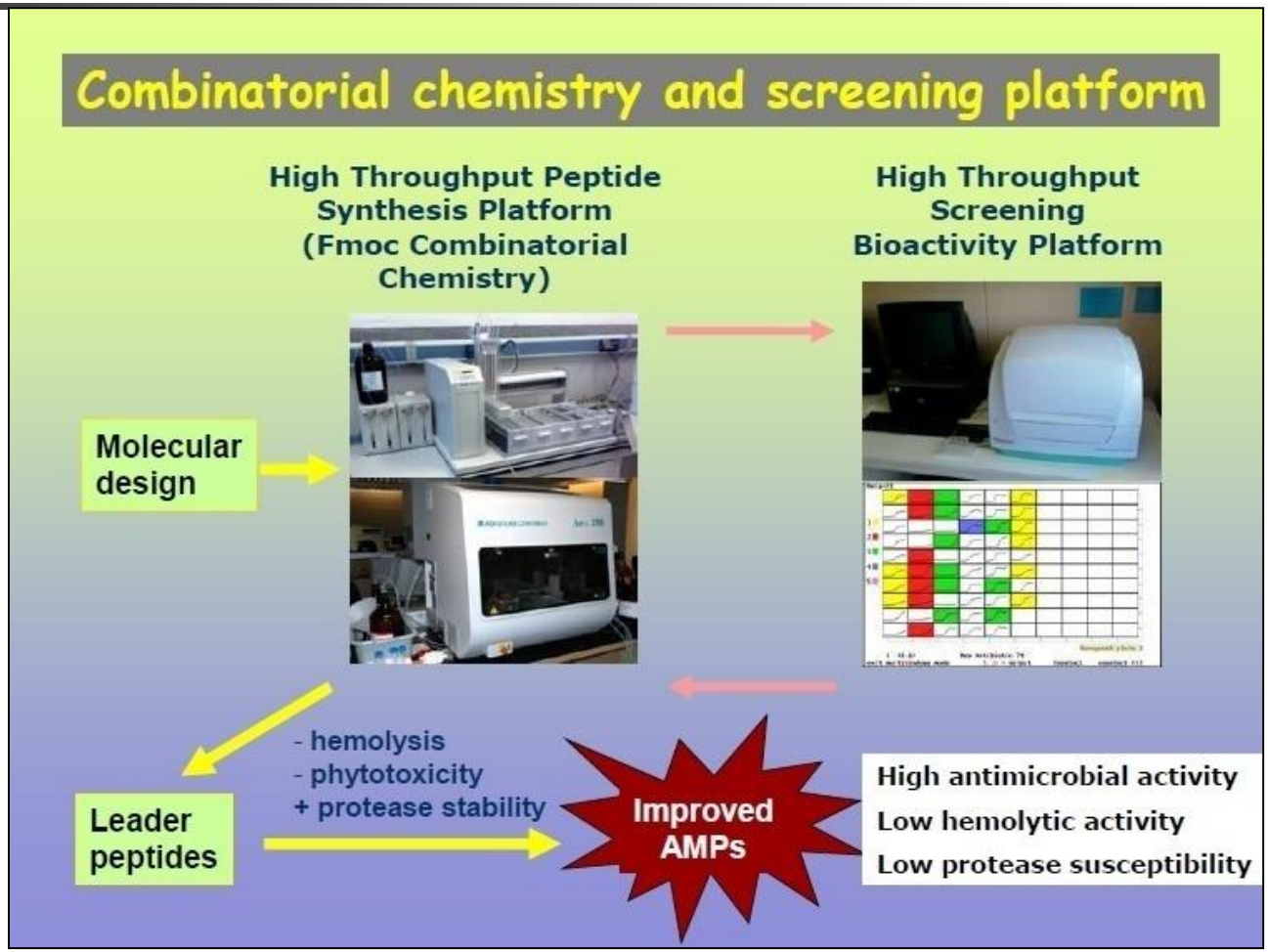
Moragrega *et al.*,

Antimicrobial/antibacterial peptides
Synthetic AMPs



Moragrega *et al.*,

Antimicrobial/antibacterial peptides
Synthetic AMPs



Moragrega *et al.*,

Antimicrobial peptides

New drugs for bad bugs

Drug-resistant bacteria

- Antibiotic-resistant bacteria are emerging as critical public health threats, with recent accounts of bacterial strains resistant to all approved antibiotics.
- Antimicrobial peptides (AMPs) are naturally occurring molecules with the potential to serve as the basis for a new class of anti-infectives targeting these difficult-to-treat bacteria.
- Antimicrobial drugs either:
- kill microbes (microbiocidal), or
- prevent the growth of microbes (microbiostatic).

Antimicrobial/antibacterial peptides
Transgenic approaches

- A resulting new generation of antimicrobial peptides (AMPs) with:

1. higher specific activity, and
2. wider microbe-range of action could be constructed, and hopefully endogenously expressed in genetically-modified organisms.

- Many authors have reported the enhancement of disease resistance by transgenic approaches, as demonstrated in tobacco, potato, and rice.
- It is also possible to utilize antimicrobial peptides for therapeutic and herbicidal uses.

Antimicrobial/antibacterial peptides

Synthetic antimicrobial peptides active against plant pathogens

Compound	Size	Sequence	Source
PEP6	6	FRLKFH	Synthetic
PAF26	6	Acetyl-RKKWFW-NH_2	Synthetic
BPC194	10	c(KKLKKFKKLQ)	Synthetic
PEP3	11	WKLFKKILKVL-NH_2	Cecropin–melittin hybrid
PEP11	11	WKLFKKILKVL	Cecropin–melittin hybrid
BP76	11	KKLFKKILKFL-NH_2	Cecropin–melittin hybrid
CAMEL	15	KWKLFKKIGAVLKVL-NH_2	Cecropin–melittin hybrid
Iseganan	17	RGGLCYCRGRFCVCVGR-NH_2	Protegrin
D4E1	17	FKLRAKIKVRLRAKIKL	Cecropin
TPY	17	KWVFRVNYRGIKYRRQR	Tachyplesin
ESF12	18	MASRAAGLAARLARLALR	Magainin
ESF1	20	MASRAAGLAARLARLALRAL	Magainin
Pexiganan	22	GIGKFLKKAKKFGKAFVKILKK-NH_2	Magainin
MSI-99	23	GIGKFLKSAKKFGKAFVKILNS	Magainin from higher animals and mammals
MB-39	39	HQPKWKVFKKIEVVGRNIRNGI VKAGPAIAVLGEAKALG	Cecropin from insects
Pen4-1	46	HSSGYTRPLRKPSRPIFIRPIGCDVCYGI PSSTARLCCFRYGDCHL-NH_2	Penaedin
D32R	47	KSCCRNTWARNCYNVCRLPGTISREI CAKKCRCKIISGTTCPSDYPK	Thionin from plants

Montesinos,2007

Antimicrobial peptides expressed in transgenic plants that confer partial resistance to pathogens

Origin	AMP	Source	Plant transformed
Animal	Cecropin A, B	Moth haemolynph	Rice
	Tachyplesin	Crab haemolynph	Potato
	Heliomicin/drosomycin	Insect defensin	Tobacco
	Sarcotoxin IA	Fruit fly haemolynph	Tobacco
	Mussel defensin	Mussel	Tobacco
	Magainin	Frog skin	Tobacco
	Esculentin-1	Frog skin	Tobacco
Plant	Rs-AFP2	Radish defensin	Tobacco/tomato
	Alf-AFP	Alfalfa defensin	Potato
	Spi1	Spruce defensin	Tobacco
	DRR230-a	Pea defensin	Canola/tobacco
	BSD1	Cabbage defensin	Tobacco
	WT1	Wasabi defensin	Rice
	Dm-AMP1	Dahlia defensin	Eggplant
	Mj-AMP1	Jalapa defensin	Tomato
	Pn-AMP	Hevein	Tobacco
	Hordothionin	Barley	Tobacco
	Alpha thionin	Barley	Tobacco
Fungal	AFP	Fungal defensin	Rice
Synthetic	SB-37	Cecropin analogue	Potato, apple
	Shiva-1	Cecropin analogue	*Anthurium, Paulownia*
	SB37, Shiva-1	Cecropin analogues	Tobacco
	MB-39	Cecropin analogue	Apple
	MsrA1	Cecropin–melittin hybrid	Potato
	MSI-99	Magainin analogue	Grapevine/banana
	Myp30	Magainin analogue	Tobacco
	Rev4	Indolicidin analogue	Tobacco/arabidopsis
	D4E1	Synthetic	Tobacco/cotton/poplar

Montesinos,2007

Volatile Organic Compounds

The volatome

Natural volatile compounds

1. Plant volatile organic compounds
2. Microbial (fungi and bacteria) volatile organic compounds

See also Bacterial disease management file-Part2.

Volatile Organic Compounds (VOCs)
Novel technologies for employing VOCs in smart agriculture practices

- This perspective article explores the potential of natural Volatile Organic Compounds (VOCs) emitted by plants as an eco-sustainable strategy to implement future smart agricultural practices and enhance plant protection and productivity.

Brilli *et al.*,2019

Volatile Organic Compounds
VOCs

- Volatile organic compounds (VOCs) are defined as any organic compound with vapor pressures high enough under normal conditions to be vaporized into the atmosphere.
- In general, VOCs have:
- Low boiling points (below 200°C)
- high vapor pressures at room temperature.
- Low-to-medium water solubility
- Organic compounds
- Low molecular weights
- low molecular weights.

Spinelli *et al.*,2011;..

List of Common Volatile Organic Compounds(VOCs)

- These organic chemicals are substances made up of carbon and other elements, and they encompass nearly all carbon compounds with the exception of carbon dioxide and carbon monoxide.
- Acetone
- Acetic Acid
- Butanal
- Carbon Disulfide
- Ethanol
- Alcohol
- Formaldehyde
- Methylene Chloride.

Volatile Organic Compounds
VOCs

1. **Plant volatile organic compounds;**
2. **Microbial volatile organic compounds (MVOCs):**

- ✓ **Fungal, and**
- ✓ **Bacterial volatile organic compounds(BVOCs)**

Spinelli *et al.*,2011;..

Volatile Organic Compounds (VOCs)
Plant volatile organic compounds

- Plants can produce a high diversity of volatile organic compounds (VOC).
- The emission of these secondary metabolites can be strongly increased as a result of certain biotic or abiotic stresses.
- Several VOC are emitted as a natural defense mechanism (NDM) against the attack of:

1. Arthropods, and
2. Pathogens.

Villamar-Torres *et al.*,2018

Volatile Organic Compounds (VOCs)

1. Plant volatile organic compounds

- These are natural compounds referred as secondary metabolites.
- More than 100,000 chemical products are known to be produced by plants,
- At least 1,700 of these are known to be volatiles.
- The agronomic potential of volatile organic compounds (VOCs) emitted from leaves, as a natural and eco-friendly solution to defend plants from:

1. Stresses, and
2. to enhance crop production.

Brilli *et al.*,2019

Volatile Organic Compounds (VOCs)
Plant volatile organic compounds

- VOCs have been extensively demonstrated to prime defenses against:

1. herbivorous insects,
2. Pathogens, and
3. environmental stresses.

Brilli *et al.*,2019

Volatile Organic Compounds (VOCs)
Plant volatile organic compounds

- According to their biosynthetic origin and chemical structure, plant volatiles can be grouped into:

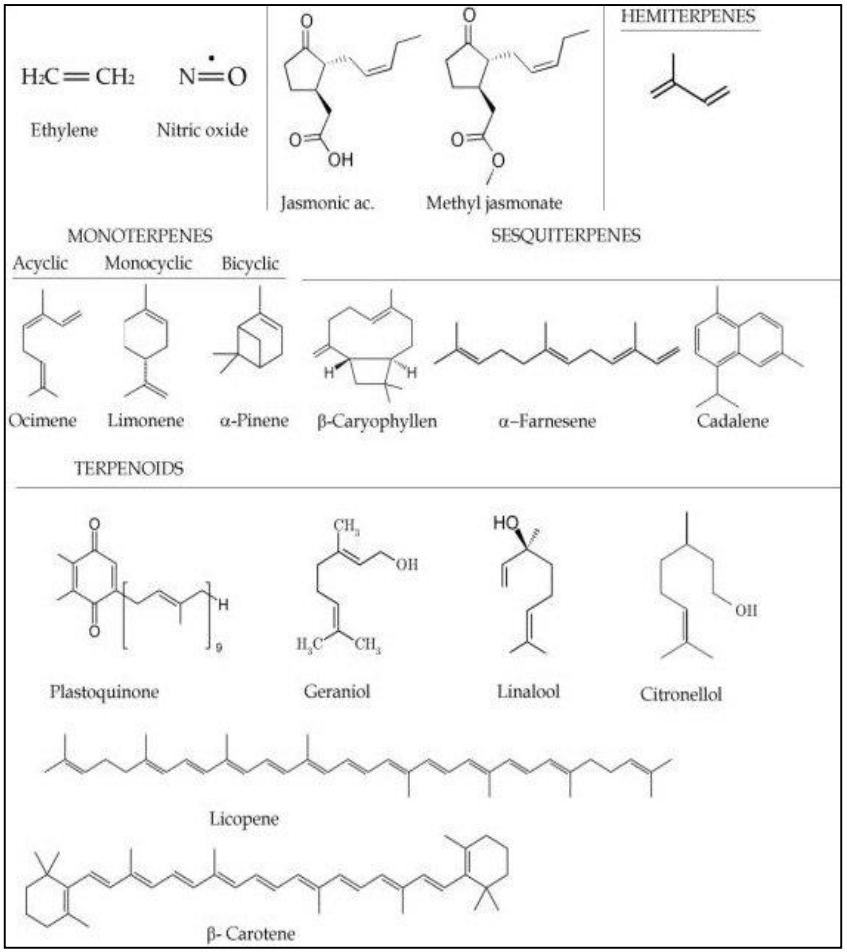
1. Isoprenoids or terpenoids, but also oxygenated VOCs (OVOCs), such as methanol (CH_4O), acetone (C_3H_6O), acetaldehyde (C_2H_4O), methyl-ethyl-ketone (MEK, C_4H_8O) and methyl-vinyl-ketone (MVK, C_4H_6O).
2. In few cases, sulfur compounds (e.g. in Brassicales) and furanocoumarins and their derivatives (e.g. in Apiales, Asterales, Fabales, Rosales) are also found.

Vivaldo *et al.*,2017

Volatile Organic Compounds (VOCs)
Plant volatile organic compounds

- Monoterpenes, such as eucalyptol, linalool, camphor, α-pinene, β-pinene, α-terpineol, borneol and many others, are the principal components of plant volatile oils.
- These volatile essential oils(EOs) are involved in:

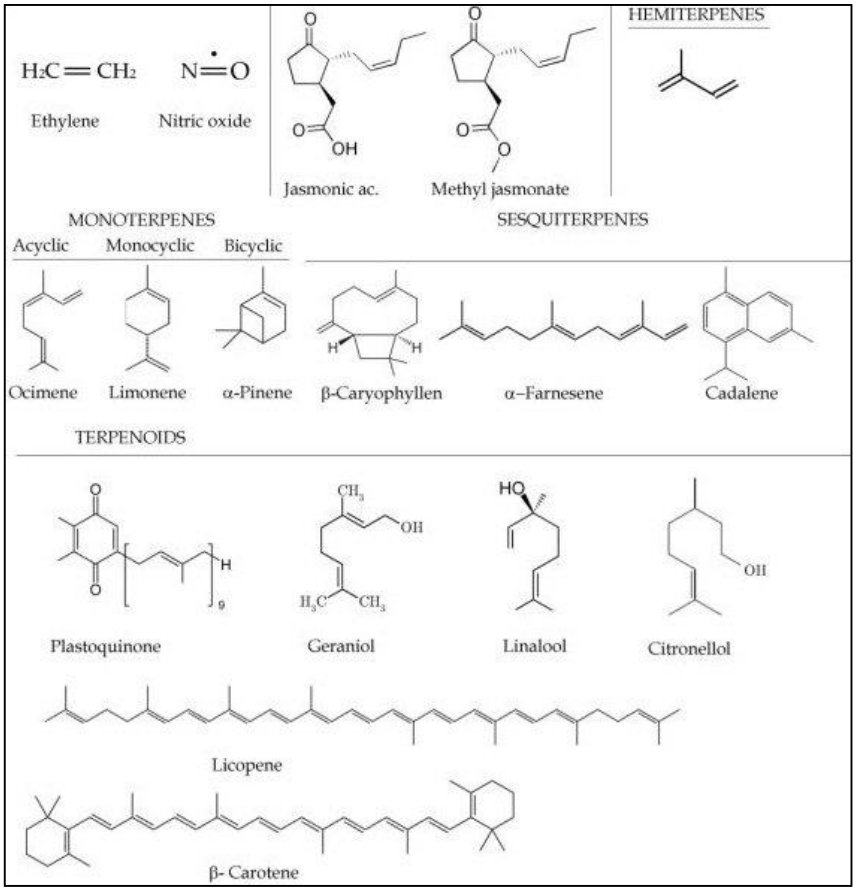
1. antimicrobial; and
2. antioxidant activity.



Spinelli *et al.*,2011

Volatile Organic Compounds (VOCs)
Plant volatile organic compounds

- Chemical diversity of the different VOCs, and related compounds, present in the plant.
- The low molecular weight compounds (i.e. NO, ET, JA, ISOPRENE) usually act as stress signals.
- Isoprene, NO and the majority of the other compounds may also directly act as antioxidants.



Spinelli *et al.*,2011

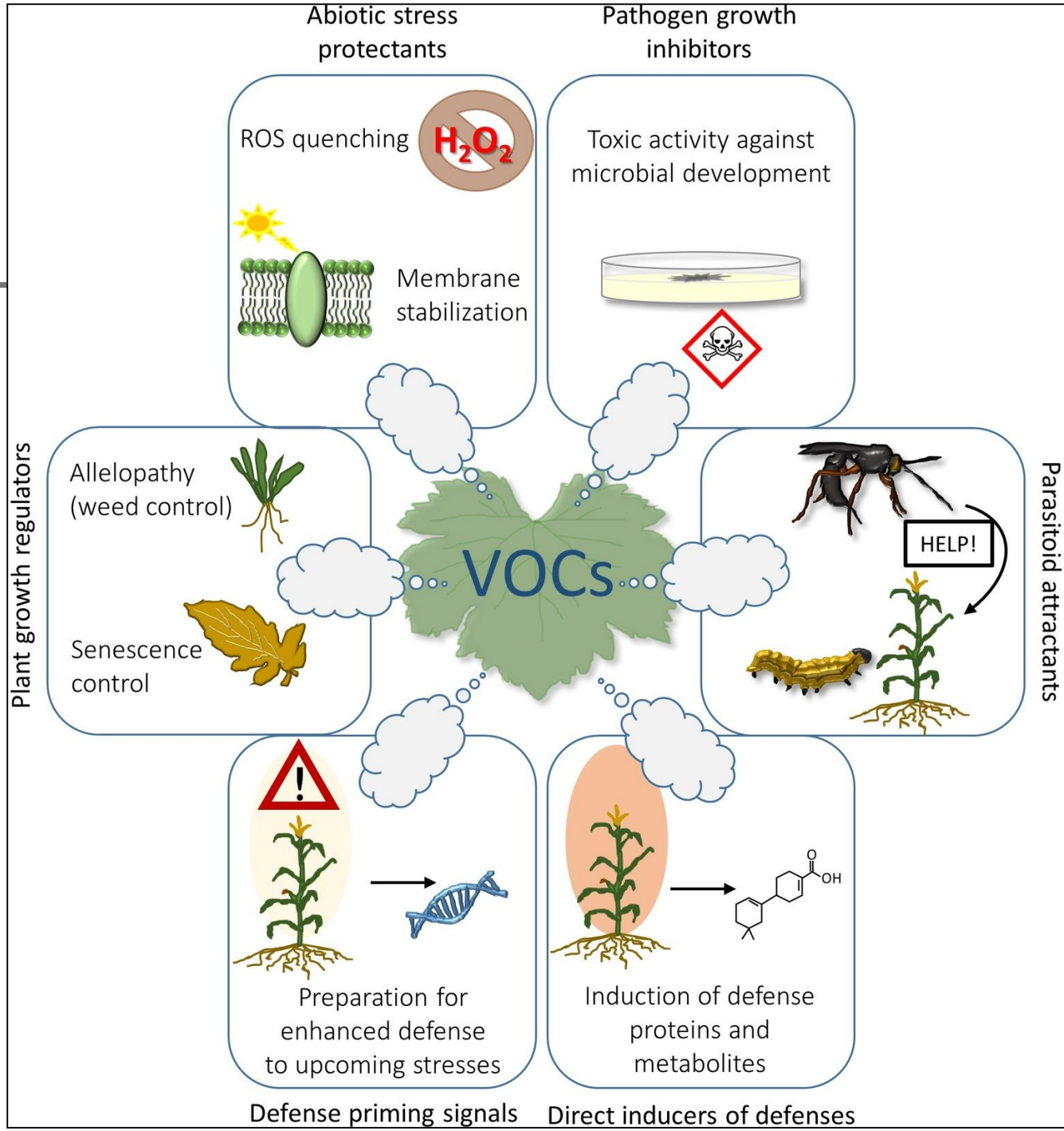
Volatile Organic Compounds (VOCs)
Plant volatile organic compounds

- Plant VOCs are involved in a range of ecological functions, including:

1. plant's defense mechanisms for an enhanced resistance/tolerance to the upcoming stress,
2. quench reactive oxygen species (ROS),
3. have potent antimicrobial as well as allelopathic effects, and
4. might be important in regulating plant growth, development, and
5. senescence through interactions with plant hormones.

Brilli *et al.*,2019

Emission of VOCs can be induced at any time from leaves of all plant species following abiotic or biotic stresses.

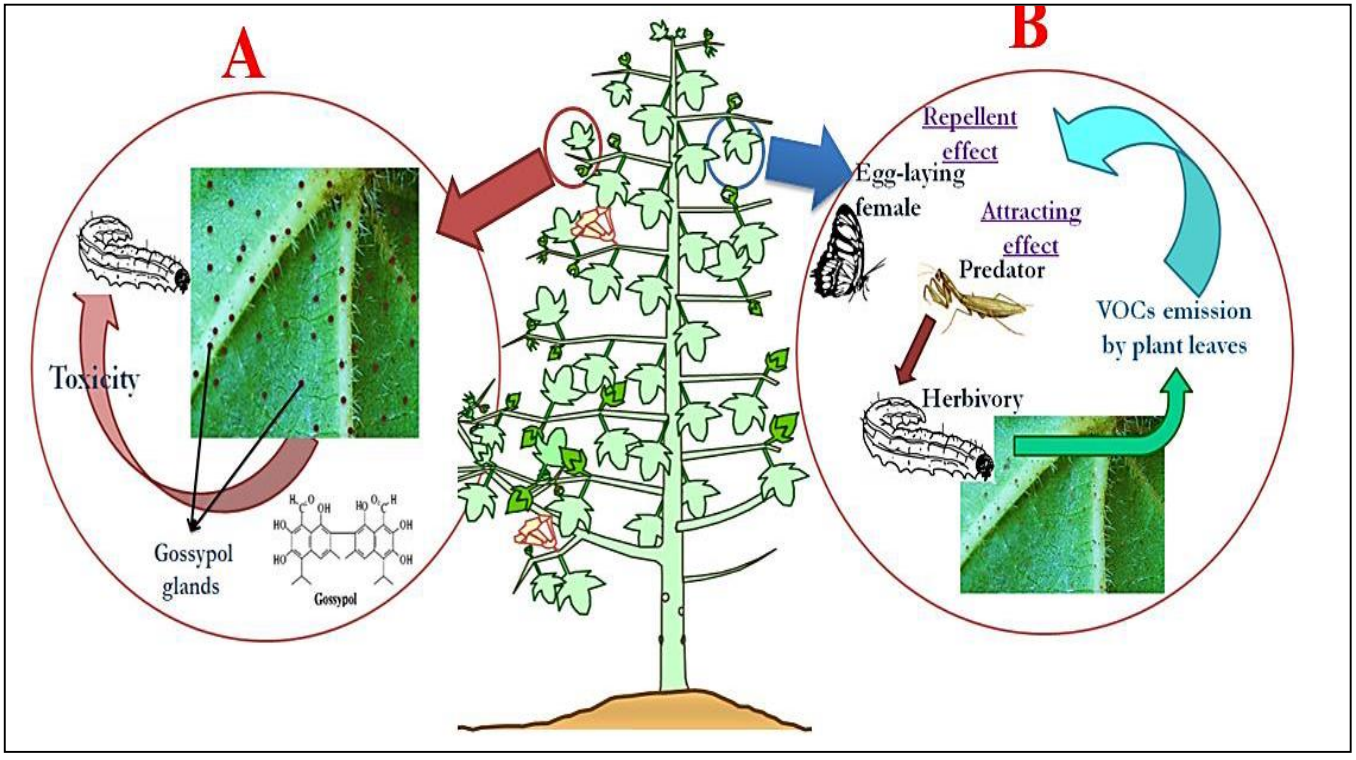


Brilli *et al.*,2019

Volatile Organic Compounds (VOCs)

Plant volatile organic compounds

Against insect pests

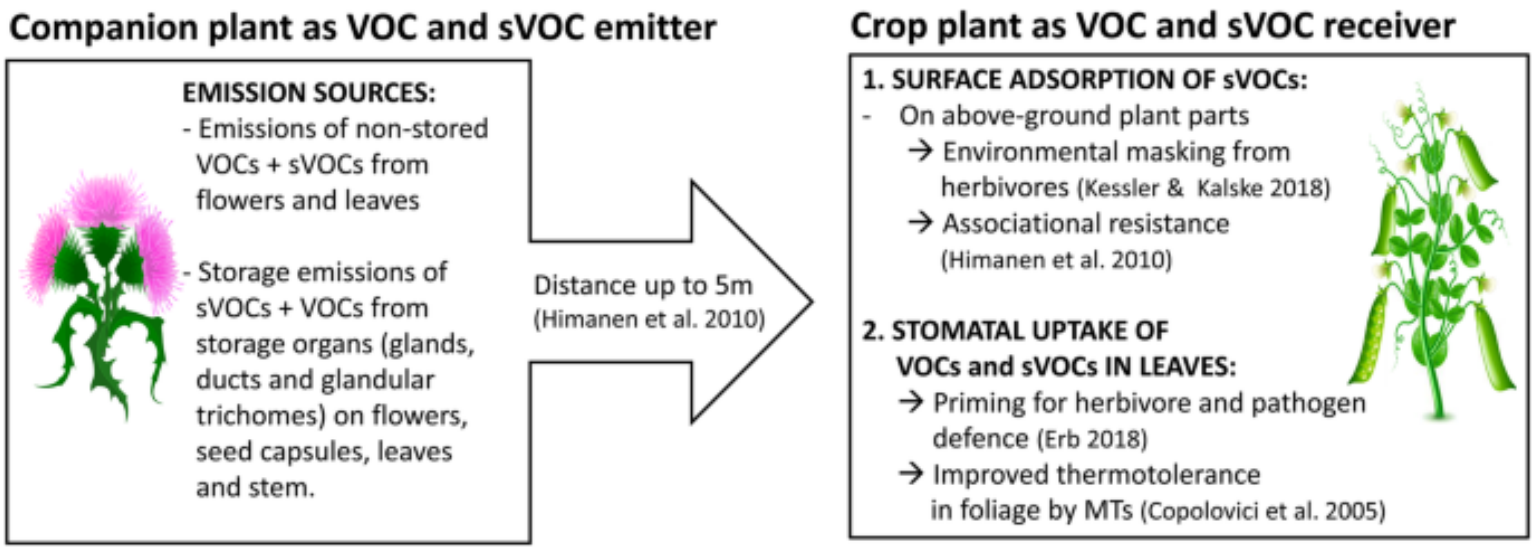


A) Direct mechanism of natural defense in the cotton plant: gossypol glands containing highly toxic terpenoids affecting the physiology of the herbivorous arthropods and causing mortality or retarded growth, B) Indirect mechanism of natural defense by means of volatile organic compounds (VOC) in the cotton plant.

Villamar-Torres *et al.*,2018

Volatile Organic Compounds (VOCs)

Foliar behaviour of biogenic semi-volatiles: potential applications in sustainable pest management



A schematic illustration of potential VOCs and semi-volatiles (sVOCs) functions in companion/secondary plant–crop plant interactions related to surface adsorption and stomatal uptake of companion plant emissions by crop plant and potential consequences for herbivore tolerance of crop.

Mofikoya *et al.*,2019

Volatile Organic Compounds (VOCs)
Plant volatile organic compounds
Against plant pathogens

- Defense priming against pathogens has also been considered as a sort of green vaccination.
- Green leaf volatiles (GLVs) such as Z-3-hexenyl acetate, ubiquitously and rapidly released after mechanical damage of leaf tissues have been reported to prime resistance of wheat plants to the fungal pathogen *Fusarium graminearum*.

Brilli *et al.*,2019

Volatile Organic Compounds (VOCs)
Plant volatile organic compounds
Against plant pathogens

- A number of experimental trials have shown the capacity of various VOCs produced by leaves to inhibit germination and growth of plant pathogens, yet the mechanisms of action remain unknown.
- Citral, carvacrol, and trans-2-hexenal were reported to be effective in hampering *in vitro* growth and germination of *Monilinia laxa*, the agent of brown rot of stone fruit.

Brilli *et al.*,2019

Volatile Organic Compounds (VOCs)
Plant volatile organic compounds
Against plant pathogens

- A screening on the efficacy of 22 different VOCs, known to be emitted from leaves, against the fungal pathogens *Colletotrichum lindemuthianum*, *Fusarium oxysporum*, and *B. cinerea*.
- These fungi were grown in Petri dishes in which the headspace had been enriched, each time, with a single VOC.
- Results showed that exposure to nonanal, (+)-carvone, citral, *trans*-2-decenal, *L*-linalool, nerolidol, or eugenol significantly inhibited the growth of all these three fungal species, with eugenol demonstrating the strongest activity.

Brilli *et al.*,2019

Volatile Organic Compounds (VOCs)
Plant volatile organic compounds
Against plant pathogens

- Other VOCs such as cuminaldehyde and *p*-cymene have been also demonstrated to possess antifungal activity against:

1. *B. cinerea*
2. *F. oxysporum*
3. *Verticillium dahliae*, and
4. *Alternaria mali*.

Brilli *et al.*,2019

Volatile Organic Compounds (VOCs)
Plant volatile organic compounds
Against bacterial pathogen

- Even methanol, ubiquitously emitted from plant leaves during cell division and cell wall expansion, seems to act as a priming stimulus when released from damaged tobacco leaves by enhancing resistance to the pathogenic bacterium *Ralstonia solanacearum*.

Brilli *et al.*,2019

Volatile Organic Compounds (VOCs)

2. Microbial volatile organic compounds

A. Fungal and bacterial volatile organic compounds

- One group of secondary metabolites produced by soil and plant-associated microorganisms, but largely unexplored to date, are the volatile organic compounds (VOCs).
- VOCs are typically small, odorous compounds (<C15) with low molecular mass (<300 Da), high vapor pressure, low boiling point, and a lipophilic moiety.
- The production of mVOCs in soil is influenced by various factors including the growth stage of the microbes, nutrient availability, temperature, oxygen availability, pH, and soil moisture content.

Schulz-Bohm *et al.*,2017

Volatile Organic Compounds (VOCs)

Microbial volatile organic compounds (MVOCs)

Fungal and bacterial volatile organic compounds

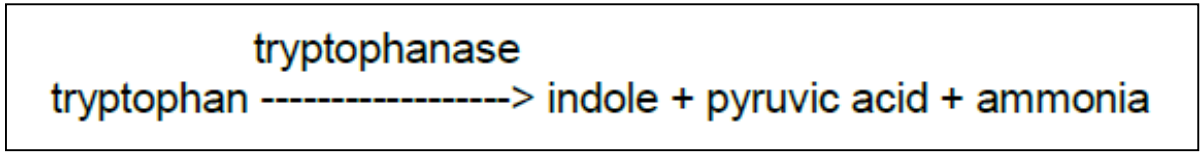
- Microbial volatile organic compounds (MVOCs) were often considered to be by-products of primary metabolism, but recent findings revealed that many mVOCs demonstrate biological activity.
- These findings clearly disagree with the opinion that mVOCs are just waste products.
- Bacterial volatile compounds (BVCs) are not waste or by-products of primary metabolism but rather have critical roles in the biology and ecological competence of bacteria.
- BVCs are exploited as a source of:

1. Nutrients, and
2. information in plant-bacteria interactions.

Audrain *et al.*,2015; Schulz-Bohm *et al.*,2017

Volatile Organic Compounds (VOCs)
Bacterial volatile compounds(BVC)

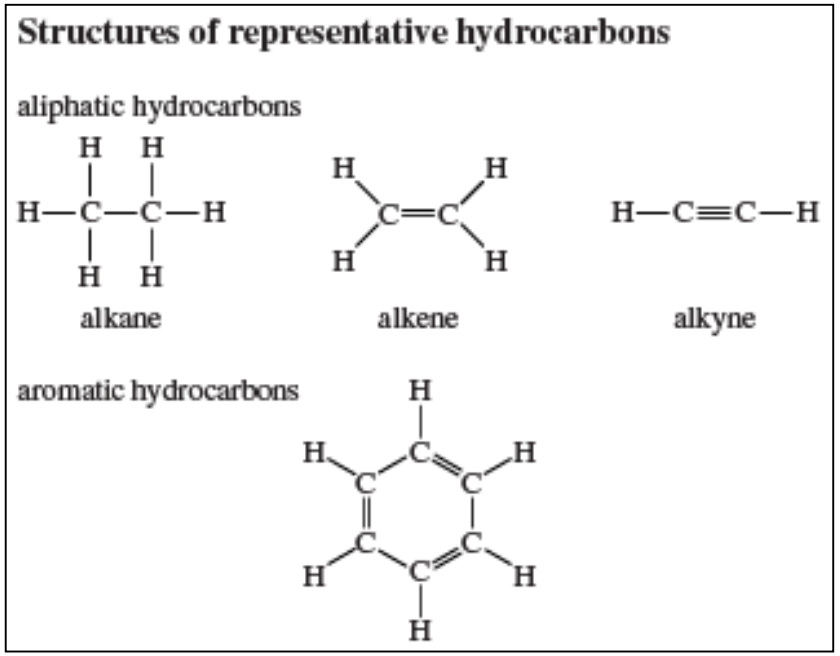
- VOCs are thought to evolve as products or by-products of metabolic pathways; for example,
- The generation of hydrocarbons, aliphatic alcohols and ketones from fatty acid biosynthesis,
- Indole evolves from the breakdown of the amino acid tryptophan.



Tait *et al.*,2013;..

Volatile Organic Compounds (VOCs)
Bacterial volatile compounds(BVC)

- In organic chemistry, hydrocarbons are divided into two classes:
1. aromatic compounds and
2. aliphatic compounds also known as non-aromatic hydrocarbons such as alcohol (ethanol) and isopropyl alcohol.
- Hydrocarbons are naturally-occurring compounds and form the basis of crude oil, natural gas, coal, and other important energy sources.



Hydrocarbon is an organic chemical compound composed exclusively of hydrogen and carbon atoms.

Volatile Organic Compounds (VOCs)

Microbial volatile organic compounds (MVOCs)

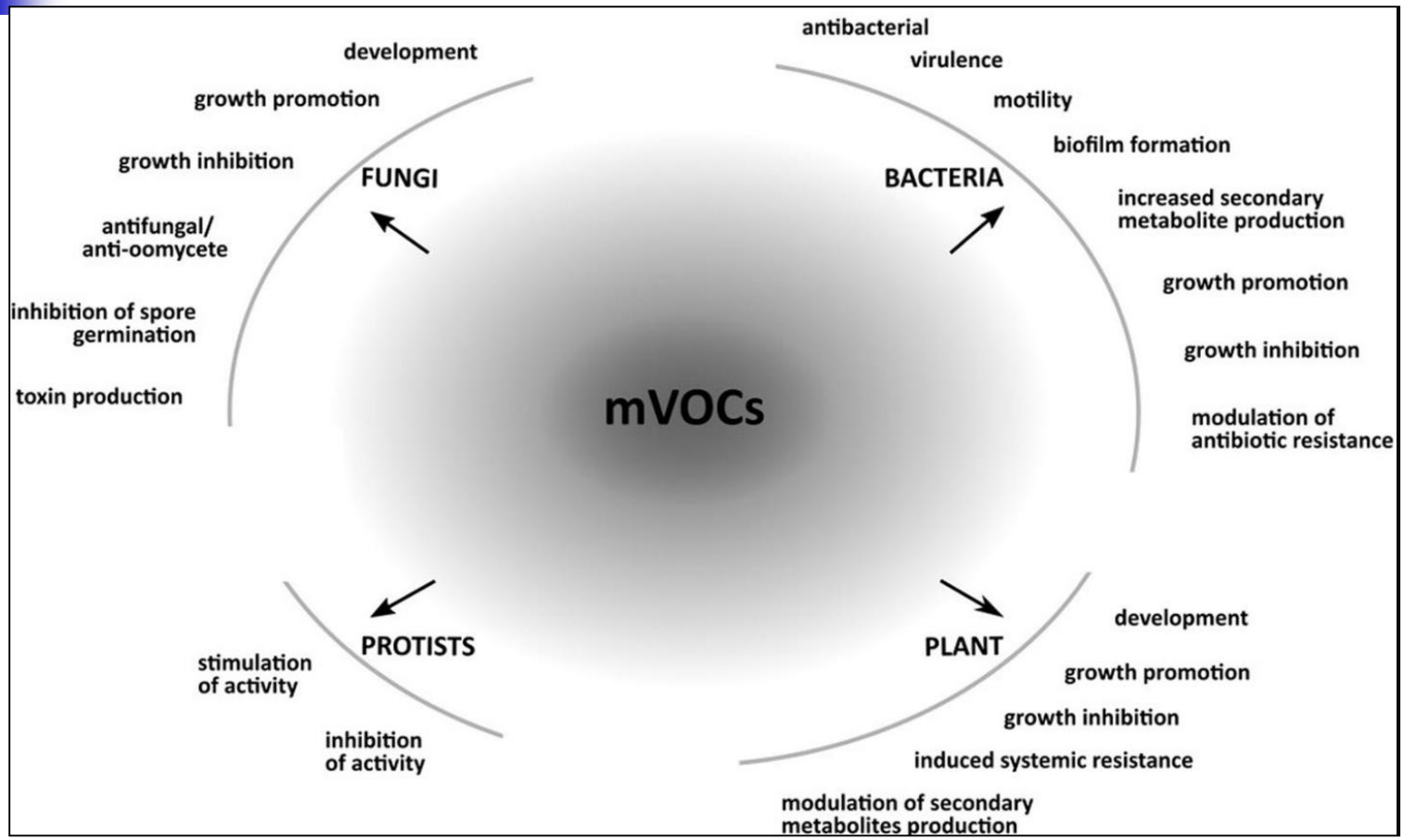
Plant-microbe interactions

- There are many types of microbial interactions occurring belowground such as:

1. Bacteria-bacteria,
2. Fungi-fungi,
3. Fungi-bacteria,
4. Bacteria-protists,
5. Fungi-plant,
6. Bacteria-plant, and
7. Bacteria-fungi-plant interactions.

Audrain *et al.*,2015; Schulz-Bohm *et al.*,2017

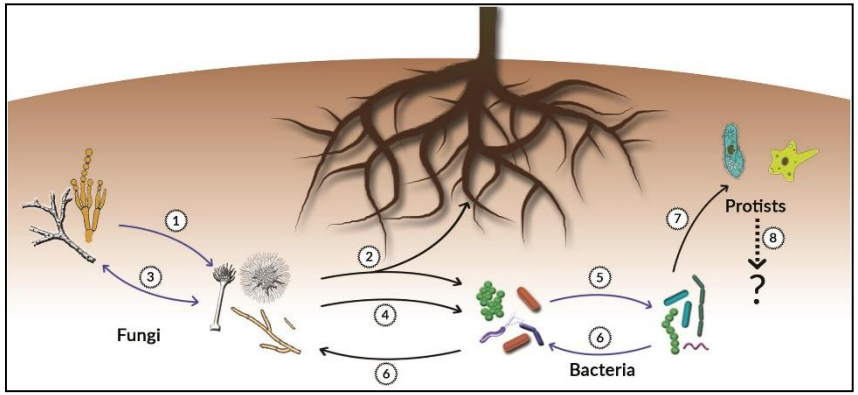
Volatile Organic Compounds (VOCs)
Microbial volatile organic compounds (MVOCs)
Plant-microbe interactions



Schulz-Bohm *et al.*,2017

Volatile Organic Compounds (VOCs)
Microbial volatile organic compounds (MVOCs)
Plant-microbe interactions

- Terpenes-mediated belowground interactions.
- An examples of interactions between different organisms in the rhizosphere that are mediated by microbial terpenes.
- Blue arrows indicate intra-kingdom interactions while black arrows indicate inter-kingdom interactions.
- See the next table for corresponding numbers.



Schulz-Bohm *et al.*,2017

Volatile Organic Compounds (VOCs)
Microbial volatile organic compounds (MVOCs)
Plant-microbe interactions

- Examples of terpenes involved in belowground microbial interactions.

Origin	Nr	Compound	Biological activity
Fungal	1	α –Humulene	Antimicrobial (antifungal)
	2	β -Caryophyllene	Antimicrobial (antibacterial)
			Plant growth promotion
	3	Farnesol	Infochemical
	4	β-Phellandrene	Affects motility
Bacterial	5	Albaflavenone	Antimicrobial (antibacterial)
	6	β-Pinene	Antimicrobial (antifungal, antibacterial)
	7	Volatile terpenes from *Collimonas*	Stimulation of protists activity
Protist	8	(E,E)- α-farnesene β-barbatene	Unknown

Schulz-Bohm *et al.*,2017

Volatile organic compounds(VOCs)

Microbial volatile organic compounds (MVOCs)

Identification of MVOCs

- Identification of compounds present in a volatile sample can be realized by comparing mass spectra with spectra from different databases like:

1. the Wiley, or
2. NIST(National Institute of Standards and Technology) libraries.
3. A database of microbial volatiles, called mVOC, is now available online at: (http://bioinformatics.charite.de/mvoc).

Audrain *et al.*,2015

Microbial volatile organic compounds (MVOCs)
Identification of MVOCs
mVOC 2.0: a database of microbial volatiles

- Metabolic capabilities of microorganisms include the production of secondary metabolites (e.g. antibiotics).
- The analysis of microbial volatile organic compounds (mVOCs) is an emerging research field with huge impact on medical, agricultural and biotechnical applied and basic science.
- The mVOC database (v1) has grown with microbiome research and integrated species information with data on emitted volatiles.
- Here, we present the mVOC 2.0 database with about 2000 compounds from almost 1000 species and new features to work with the database.

Lemfack *et al.*,2017

Microbial volatile organic compounds (MVOCs)
Identification of MVOCs
mVOC 2.0: a database of microbial volatiles

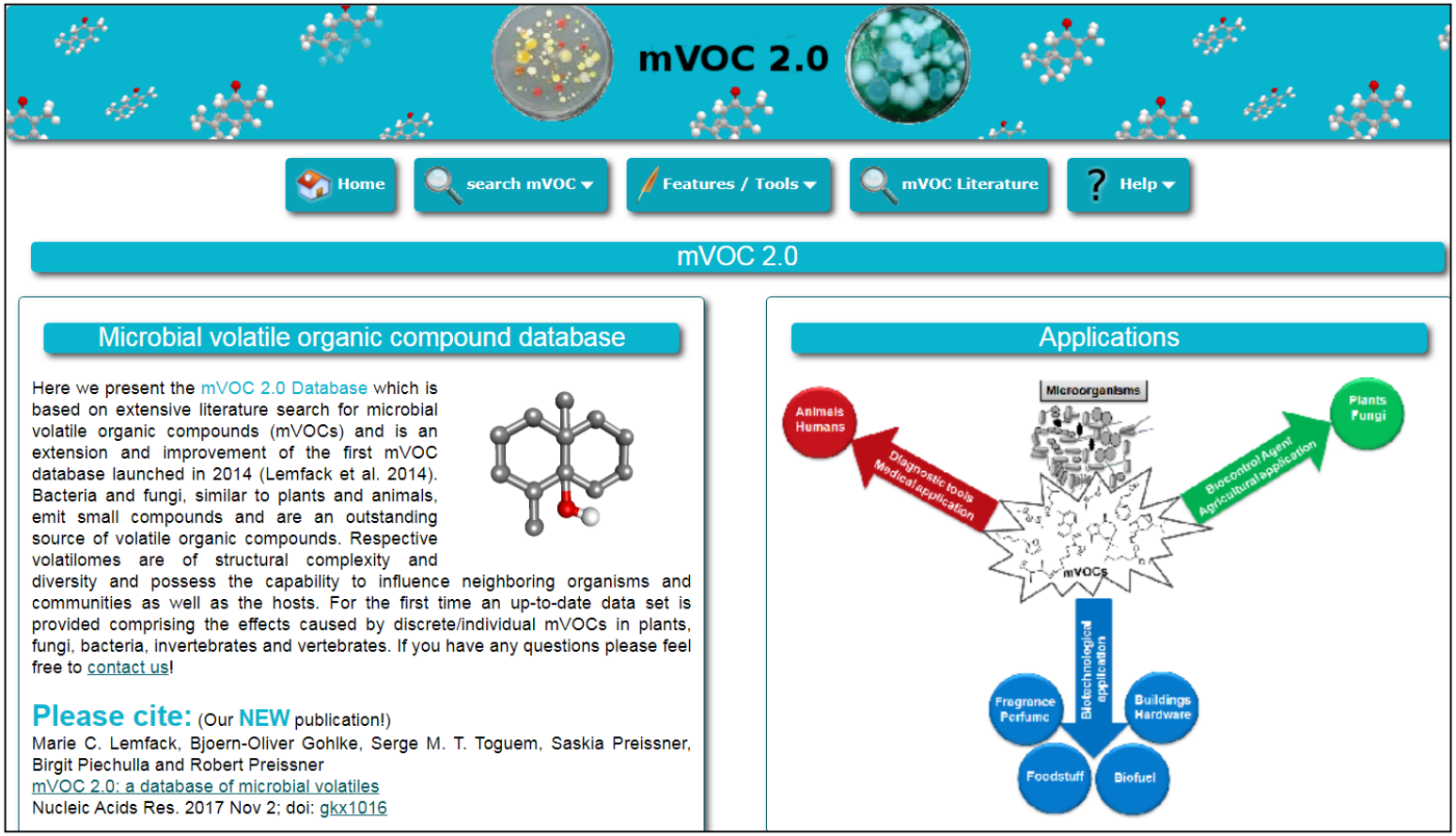
- The extended collection of compounds was augmented with data regarding mVOC-mediated effects on plants, fungi, bacteria and (in-)vertebrates.
- The mVOC database 2.0 now features a mass spectrum finder, which allows a quick mass spectrum comparison for compound identification and the generation of species-specific VOC signatures.
- Automatic updates, useful links and search for mVOC literature are also included.
- The mVOC database aggregates and refines available information regarding microbial volatiles, with the ultimate aim to provide a comprehensive and informative platform for scientists working in this research field.

Lemfack *et al.*,2017

Volatile organic compounds(VOCs)

Microbial volatile organic compounds (MVOCs)

Identification of MVOCs

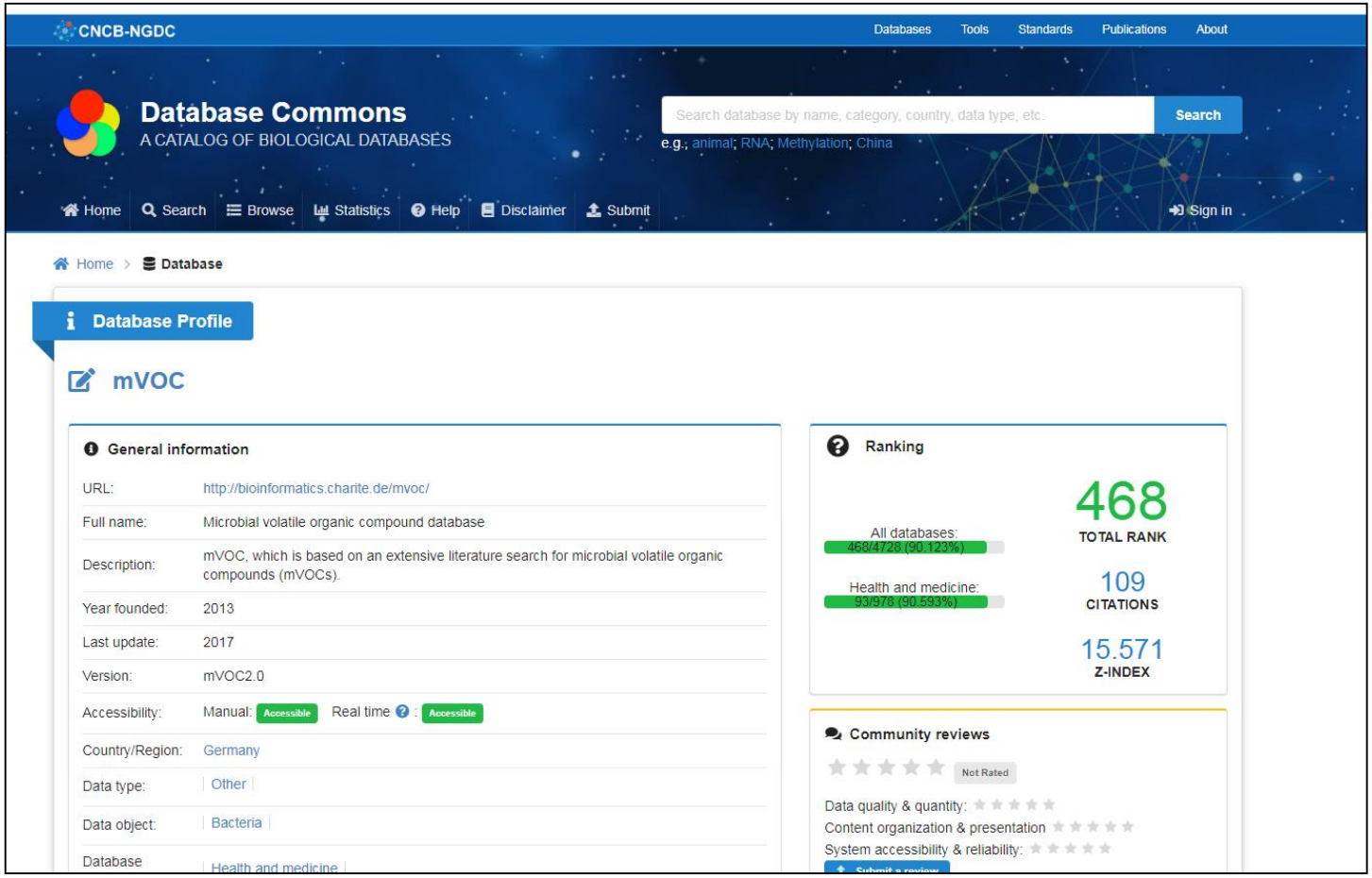


https://bioinformatics.charite.de/mvoc/

Volatile organic compounds(VOCs)

Microbial volatile organic compounds (MVOCs)

Identification of MVOCs



https://bioinformatics.charite.de/mvoc/

Bacterial volatile compounds(BVC)

Analysis of bacterial volatile compounds(BVC)

1. Standard approach to analyze BVC profiles relies on gas chromatography coupled with mass spectrometry (GC-MS),
2. Selected-ion flow-tube mass spectrometry (SIFT-MS),
3. Ion-mobility spectrometer (IMS) and
4. electronic noses (eNoses) are therefore often preferred for real-time analysis of volatiles.

- Indeed, SIFT-MS and IMS are compatible with *in-situ* real-time measurement of BVC, whereas eNoses rely on pattern recognition.

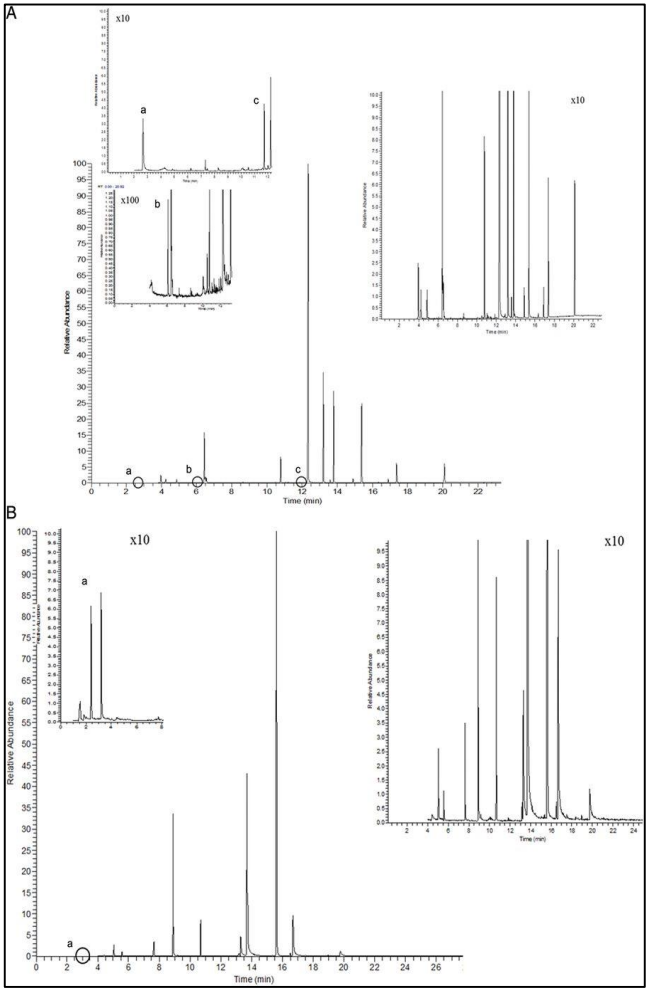
Audrain *et al.*,2015

Bacterial volatile compounds(BVC)

Chromatographic separation of bacterial volatile compounds(BVC)

- The identification of VOCs was achieved by using:

1. The National Institute of Standards and Technology (NIST) reference library and
2. The comparison of the retention times (t_R) and mass spectra of authentic standards.



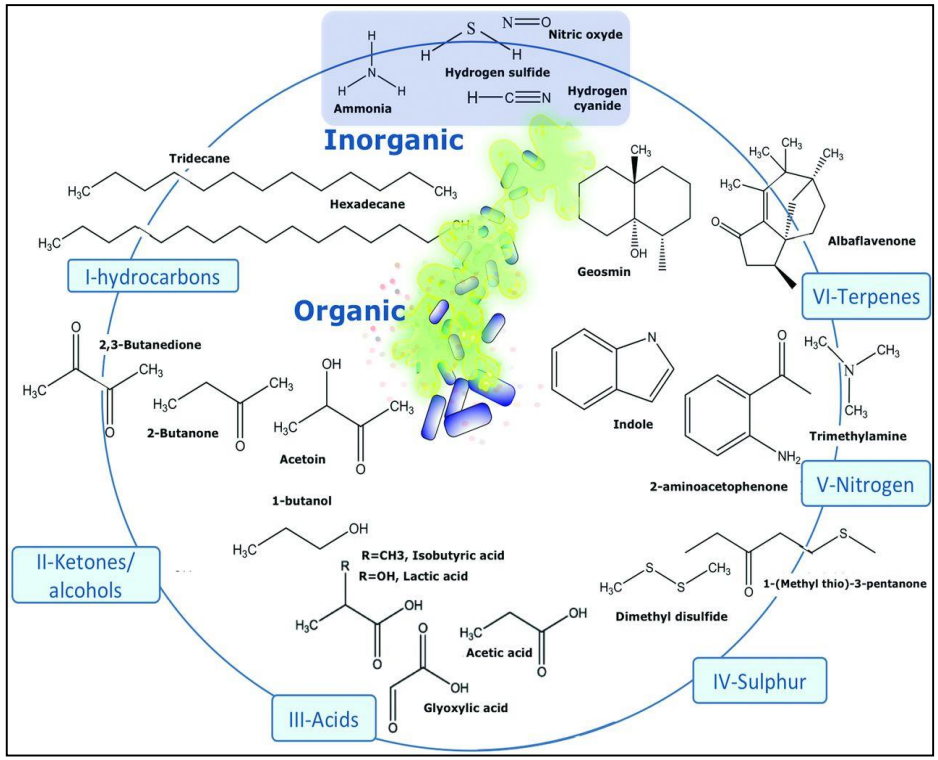
Tait *et al.*,2013

Bacterial volatile compounds(BVC)

Chemical classes of volatile compounds released by bacteria

- The structure of biologically active organic volatile compounds are regrouped in six chemical classes, including :

I. Hydrocarbons,
II. ketones/alcohols,
III. Acids(short-chain fatty acids),
IV. Sulfur compounds,
V. Nitrogen-containing compounds, and
VI. Terpenes.



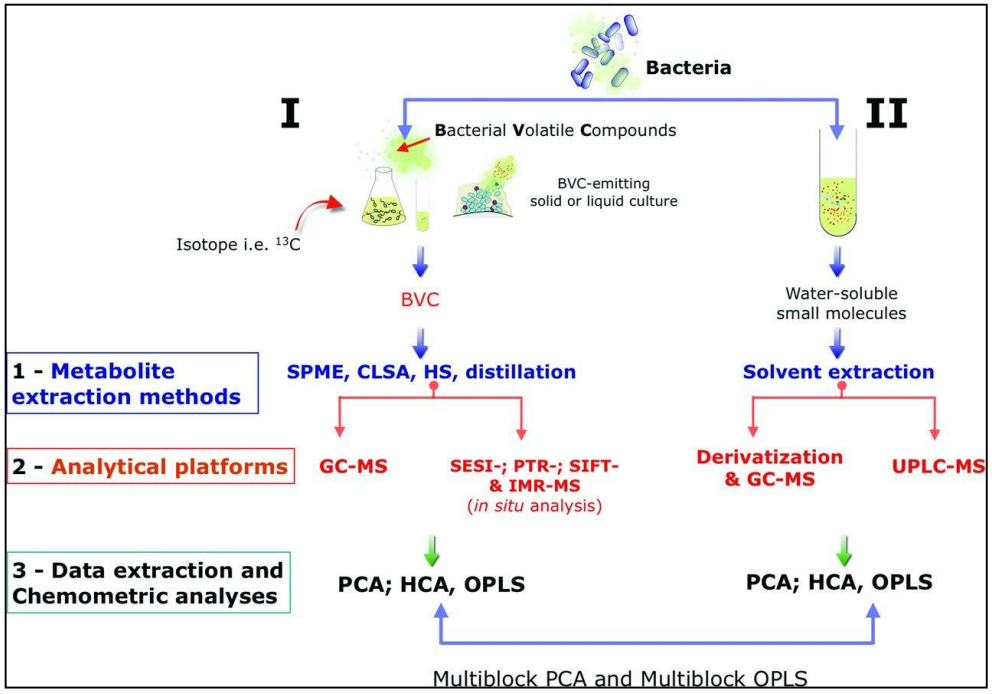
The simplest ketone($R_2C=O$) is acetone with the formula $CH_3C(O)CH_3$.

Audrain *et al.*,2015

Bacterial volatile compounds(BVC)

A workflow showing key steps for the analysis of BVC

- Volatile profiles of BCCs obtained by three extraction methods and gas chromatography–mass spectrometry (GC–MS) analysis.
- Simultaneous distillation extraction (SDE) and closed-loop stripping analysis (CLSA) and head space-solid phase micro extraction (HS-SPME).



Audrain *et al.*,2015;..

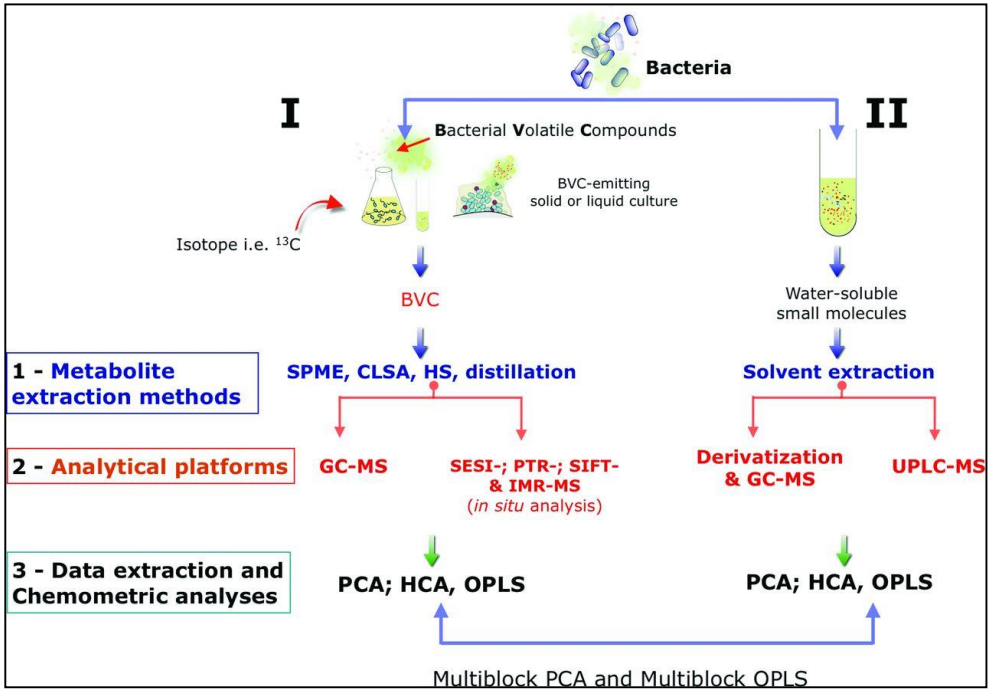
Bacterial volatile compounds(BVC)

A workflow showing key steps for the analysis of BVC

- Bacteria were inoculated into a liquid or solid culture medium, (i.e. a broth or agar).
- A workflow showing key steps for

I. the analysis of BVC, and

II. water soluble primary metabolites serving as volatile precursors.



Volatile and non-volatile metabolite data are extracted and analyzed using chemometric analyses including PCA, hierarchical cluster analysis (HCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA); for combining metabolites, data derived from the two different platforms I and II, multiblock (PCA) and multiblock (OPLS) should to be used.

Audrain *et al.*,2015

Bacterial volatile compounds(BVC)
Rapid and reliable bacterial identification

- **BVC can also be used for rapid bacterial identification:**
- Recent advances in methods to detect and analyze bacterial-specific pattern of emission suggest that rapid and reliable bacterial identification through BVC could be used as potential diagnostic tool in some clinical situations.
- Several studies reported that direct mass spectrometric methods such as SIFT-MS, IMS or SESI-MS allow *in vitro* detection of bacterial growth and differentiation of pathogenic bacteria after 5, 8 or 24 h of growth in synthetic media.

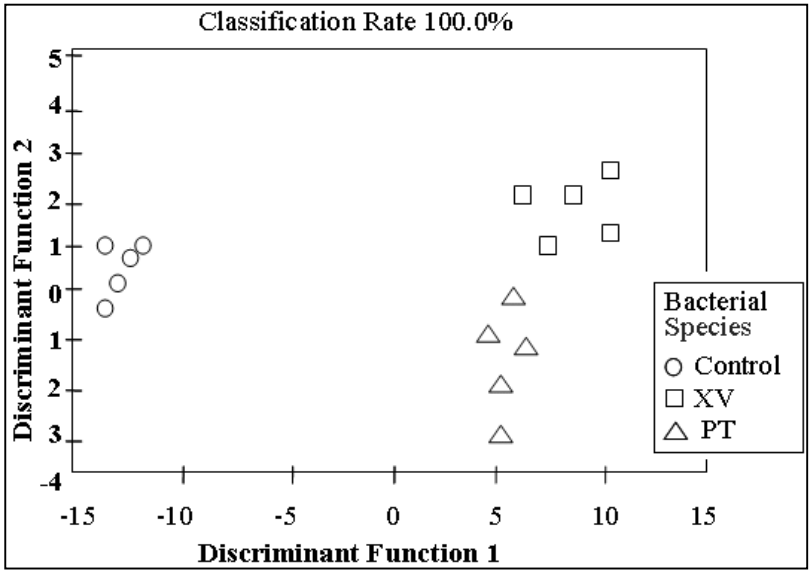
Audrain *et al.*,2015

Bacterial volatile compounds(BVC)

Electronic Nose(EN or e-nose)

The correct classification rate for two bacterial species at a time

- Discriminant function analysis of two bacterial species based on electronic nose readings.
- Abbreviations used:
- Control = bacteria free,
- XV = *Xanthomonas campetris* pv. *vesicatoria*;
- PT = *Pseudomonas syringae* pv. *tomato*.



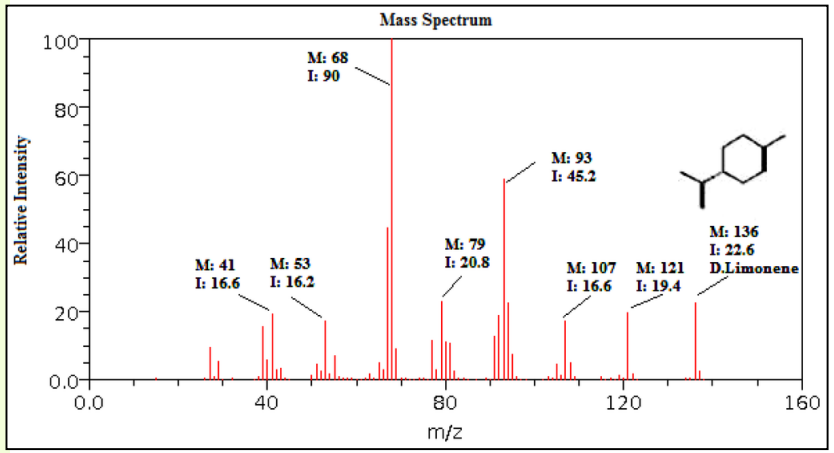
The correct classification rate for two bacterial species at a time (i.e., *Xanthomonas campestris* pv. *vesicatoria* versus *Pseudomonas syringae* pv. *tomato*) and control samples was 100%.

Momol *et al.*,2004

Bacterial volatile compounds(BVC)

Mass spectrum of the main volatile compound produced by *Burkholderia gladioli* pv. *agaricicola*

- GC-MS analysis of VOCs produced by *Burkholderia gladioli* pv. *agaricicola* strain ICMP 11096 indicated the presence of 1-methyl-4-(1-methylethenyl)-cyclohexene, which was detected at retention time 11.61 min and has a molecular weight of 136.
- This isolated main volatile compound is a liquid hydrocarbon that can be classified as cyclic terpene (an isomer of limonene).



This compound(terpene) could be responsible for the antifungal activity of Bga strain ICMP 11096 against all studied phytopathogenic fungi.

Elshafie *et al.*,2012

Role of bacterial volatile compounds(BVC) in bacterial biology

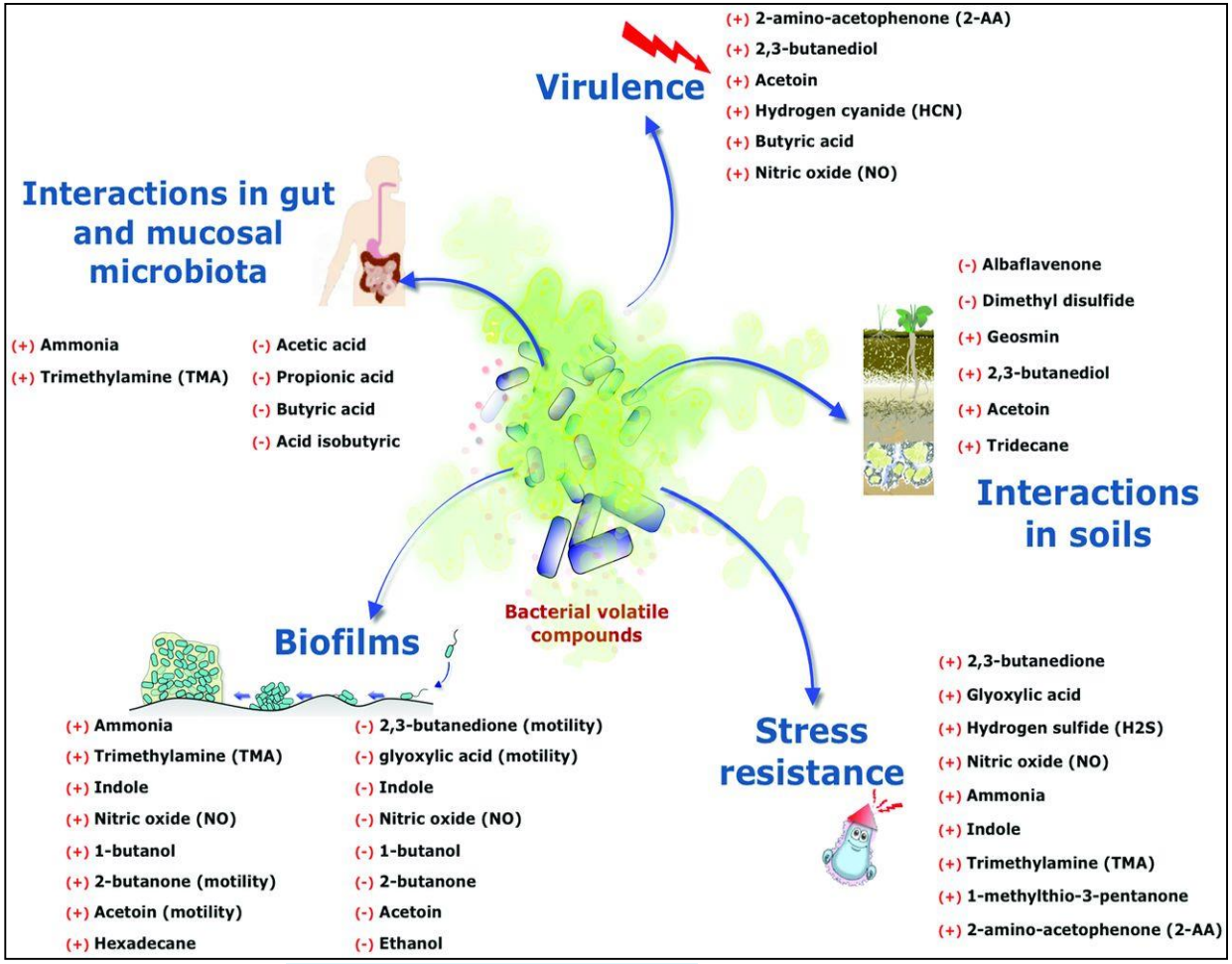
Impact of BVC on bacterial growth and stress resistance

Phenotypic consequences of exposure to BVC in various environments.

Volatile compounds released from bacteria are listed according to their positive and/or negative influence on different bacterial phenotypes in various environments.

+, stimulation;

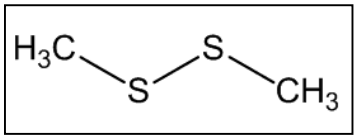
–, inhibition.



Audrain *et al.*,2015

Role of bacterial volatile compounds(BVC) in bacterial biology

Impact of BVC on bacterial growth and stress resistance



- **Dimethyl disulfide (DMDS):**
- DMDS is an organic chemical compound with the molecular formula CH_3SSCH_3 which is the simplest disulfide.
- Several groups investigated the impact of BVC produced by soil-associated bacteria on fungal and bacterial differentiation and growth.
- Emission of dimethyl disulfide from two rhizospheric bacteria, *P. fluorescens* and *Serratia plymuthica*, shows bacteriostatic effects against two plant bacterial pathogens *Agrobacterium tumefaciens* and *Agrobacterium vitis*.

Audrain *et al.*,2015

Role of bacterial volatile compounds(BVC) in bacterial biology

Impact of BVC on bacterial growth and stress resistance

- **Boosting antibiotic and stress resistance:**
- BVC were also reported to modulate the bacterial response to different stresses, including exposure to antibiotics (next Fig.).
- Volatile ammonia released from a bacterial population of high density increases at-a-distance resistance to tetracycline and ampicillin, and decreases resistance to aminoglycosides, in several Gram-negative and Gram-positive bacteria.

Audrain *et al.*,2015

Role of bacterial volatile compounds(BVC) in bacterial biology
Boosting antibiotic and stress resistance

- **H_2S: a universal defense against antibiotics in bacteria:**
- The production of endogenous hydrogen sulfide (H_2S) has been shown to confer antibiotic tolerance in all bacteria studied to date.
- This gas confers multidrug resistance upon different pathogens (*Bacillus anthracis*, *P. aeruginosa*, *Staphylococcus aureus* and *E. coli*) under aerobic conditions.

Audrain *et al.*,2015, Yong Ng *et al.*,2020

Role of bacterial volatile compounds(BVC) in bacterial biology
Promoting bacterial virulence

- **Bacterial virulence:**
- BVC can also play a critical role in completion of bacterial pathogenesis by affecting bacterial virulence.
- Several reports describe the effect of VOCs in bacterial virulence.
- For instance, 2,3 butanediol and acetoin are required for full virulence in *Pectobacterium carotovorum*.
- The same compounds can increase the production of virulence factors in *Pseudomonas aeruginosa*.

Audrain *et al.*,2015

Role of bacterial volatile compounds(BVC) in bacterial biology
Biofilm formation

- **Role of BVC in bacterial biofilm formation:**
- Recent studies demonstrated the influence of volatile compounds on different stages of the development of bacterial biofilms, from bacterial motility to biofilm dispersal.
- For instance, volatile ammonia induced biofilm formation in *Bacillus licheniformis*, *B. subtilis* and *S. aureus*.

Audrain *et al.*,2015

Role of bacterial volatile compounds(BVC) in bacterial biology
Biological control

- **BVC can also be used for plant disease control:**
- For instance, direct application of volatile 2,3-butanedione $(CH_3CO)_2$ reduced soft-rot symptoms of various vegetables by modulating QS-mediated virulence of the plant pathogen *P. carotovorum* subsp. *carotovorum*.
- Hence, besides promising biomarker applications in clinic, BVC could also be used for plant disease control, growth promotion or abiotic stress resistance.

Audrain *et al.*,2015

Role of bacterial volatile compounds(BVC) in bacterial biology
Biological control

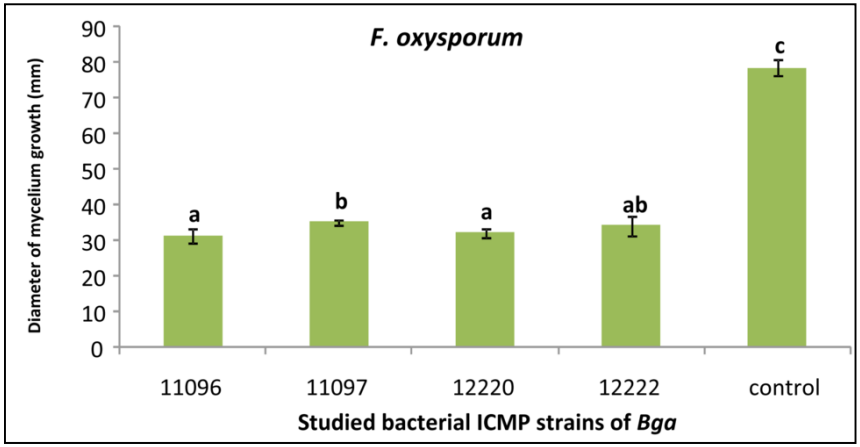
- Strains of *Burkholderia gladioli* pv. *agaricicola* (Bga) produced VOCs which reduced the mycelium growth of *F. oxysporum*.
- After four days of incubation, the fungal growth appeared to be almost stopped.
- Strains Bga ICMP11096, ICMP11097 and ICMP 12220 showed the highest significant reduction of fungal growth compared to strain Bga ICMP 12322.
- In contrast, Kai *et al.*,2007 found that volatiles of *Pseudomonas* spp., *Serratia* spp., *Stenotrophomonas* spp. drastically inhibited the growth of *R. solani*.

Elshafie *et al.*,2012

Role of bacterial volatile compounds(BVC) in bacterial biology

Biological control

- Antifungal activity of volatile organic compounds of *Burkholderia gladioli* pv. *agaricicola* (Bga) strains vs. *F. oxysporum* (5 days growth).
- Bars with different letters indicate mean values significantly different at $p < 0.05$ according to Duncan test.
- Data are expressed as mean of three replicates ± SD.



Elshafie *et al.*,2012

Role of bacterial volatile compounds(BVC) in bacterial biology
Biological control

- Candidate attractants among the VOCs emitted by bacteria and their attracting ability toward *Caenorhabditis elegans*, a free-living terrestrial nematode that feeds on bacteria in its environment.

Unique candidate attractants in *Bacillus nematocida*

VOC (no.)	% Relative content (SD)	AC_{50} (SD)
Benzaldehyde (1)	16.7 (0.9)	46.7 ppm (15.3)
Chloromethyl 4-Chloroheptanoate (3)	10.7 (0.7)	—
2-Pentanone (2)	4.1 (0.4)	n.d.
2-Heptanone (6)	3.4 (0.3)	123.3 ppm (25.2)
2-Heptanone, 6-methyl- (7)	2.1 (0.4)	—
1-Hexanol, 2-ethyl- (4)	0.7 (0.2)	—
Acetophenone (5)	0.6 (0.2)	93.3 ppm (11.5)
2-Tetradecanone (9)	0.5 (0.1)	—
2-Nonanone (8)	0.4 (0.2)	n.d.
Benzyl benzoate (11)	0.3 (0.1)	25 ppm (5)
1,3,5-Cycloheptatriene (10)	0.2 (0.1)	—

Shared candidate attractants

VOC (No.)	% Relative content (SD) in *B. nematocida*	% Relative content (SD) in *E. coli*	AC_{50} (SD)
Indole (14)	5.5 (0.6)	2.0 (0.3)	1 mM (0.5)
Naphthalene (17)	2.3 (0.3)	1.8 (0.2)	1.5 mM (0.5)
2-Butanone (12)	1.3 (0.3)	0.9 (0.4)	—
Pyrazine, 2,6-dimethyl- (15)	0.9 (0.3)	0.2 (0.1)	—
2,5-Dimethy-lanisole (16)	0.4 (0.1)	0.1 (0.02)	323.3 ppm (20.5)
Acetone (13)	0.1 (0.03)	0.2 (0.03)	n.d.

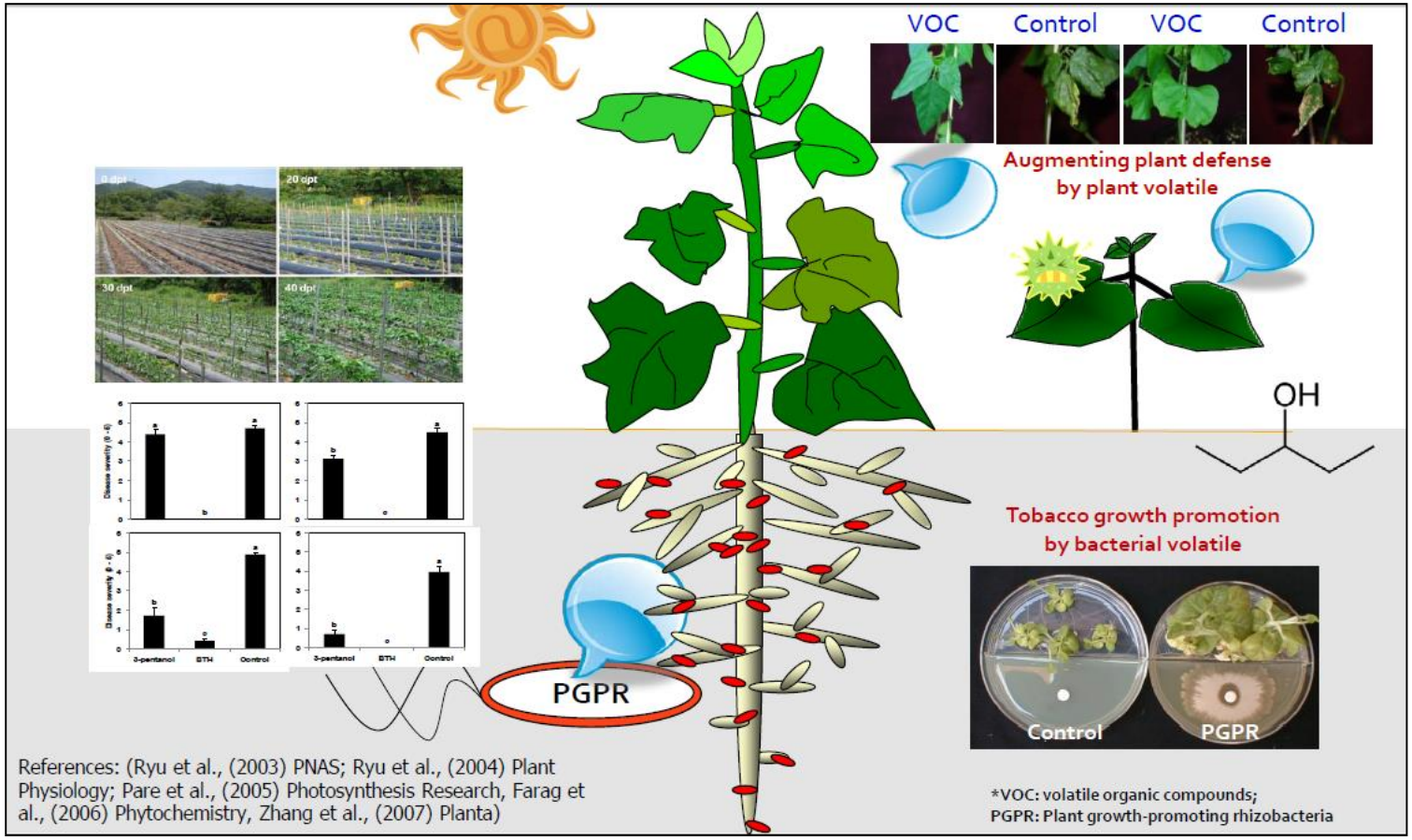
AC_{50} [the concentration of the pure tested compound at which the nematode-attracting abilities (AAs) reached 50% within 30 min].

Niu *et al.*,2010

Role of bacterial volatile compounds (BVC) in bacterial biology

ISR-induced host resistance

Volatile-mediated induced resistance

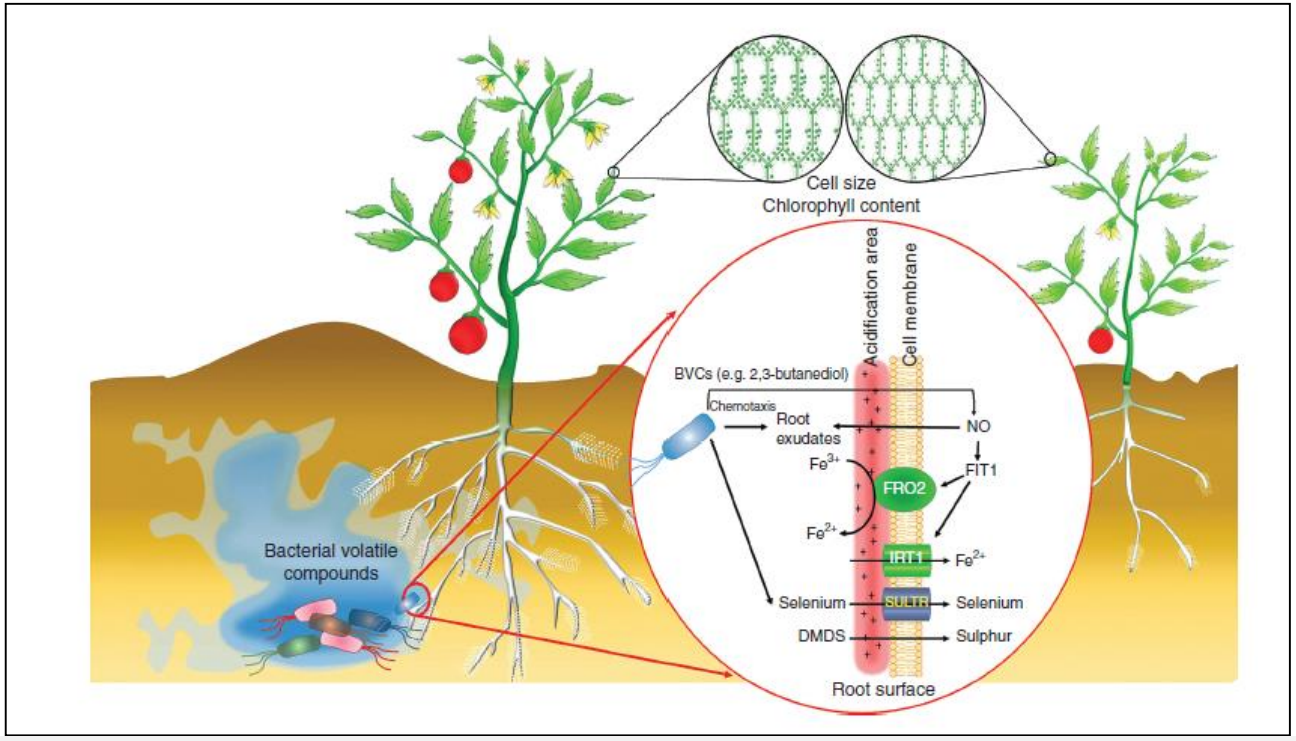


Choong-Min Ryu, Geun-Cheol Song

Role of bacterial volatile compounds (BVC) in bacterial biology

Plant growth promotion

BVCs promote above-ground plant growth by stimulating photosynthesis and sugar accumulation and by modulating phytohormone signalling



Bacterial volatiles improve plant growth and yield, leaf size, flower and fruit production, root proliferation, root hair formation, cell size, and chlorophyll content. Bacterial volatiles can help plants take up sulphur, selenium and iron. In the case of iron, volatiles enhance proton release to the rhizosphere and increase the expression of *FRO2* and *IRT1*, which are involved in the reduction and transport of iron, respectively. These genes are regulated by FIT1, expression of which is induced by nitric oxide (NO). Bacteria volatiles enhance NO accumulation in plants. Volatiles also increase selenium uptake by upregulating sulphate transporter genes (SULTRs). DMDS, dimethyl disulphide.

Sharifi and Ryu,2018

Role of bacterial volatile compounds (BVC) in bacterial biology

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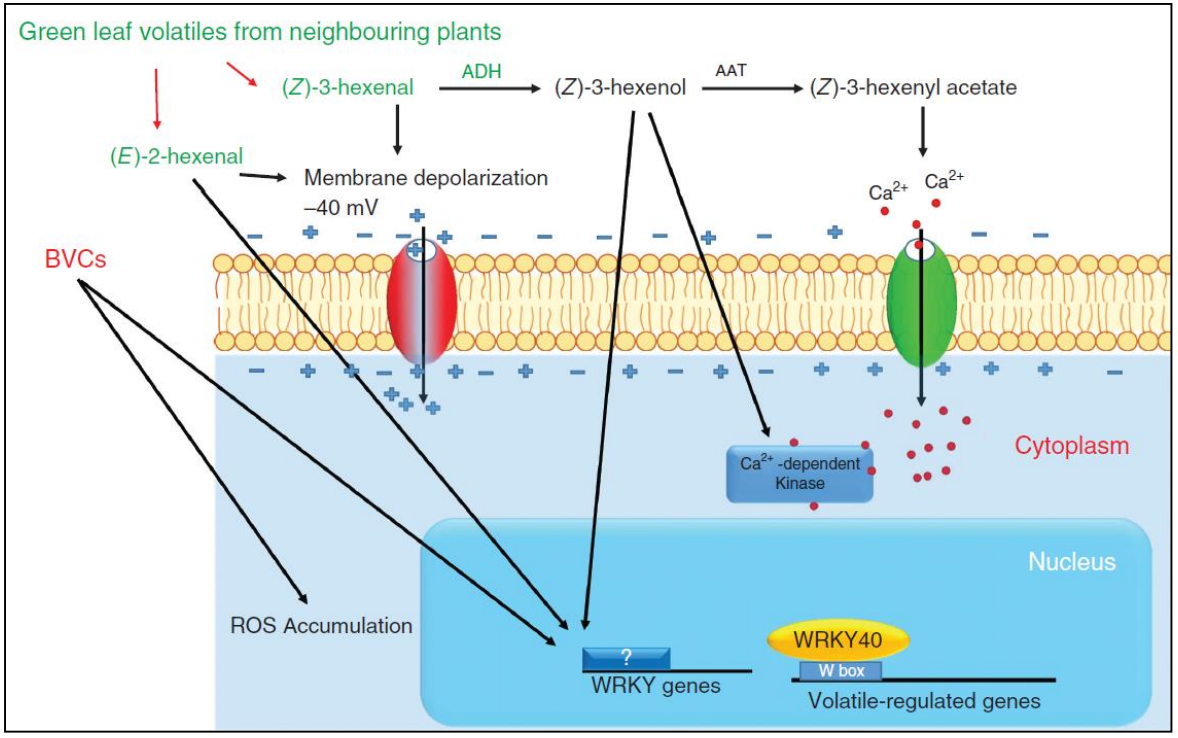

Volatile perception and signalling in plants. Herbivore-wounded plants release volatiles such as (*z*)-3-hexenal and (*E*)-2-hexenal, which deter herbivores from attacking the wounded leaves and inform neighbouring plants of the attack. These compounds elicit changes in plasma membrane potential depolarization and activate several regulatory proteins such as WRKY transcription factors. These volatiles are also converted to more active, highly volatile compounds such as (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate, which induce calcium influx and the expression of several regulatory genes, such as calcium-dependent kinase and WRKY genes. Bacteria volatiles induce the expression of *WRKY18* and ROS accumulation in plants.

Sharifi and Ryu,2018