ADVANCED SOLUTIONS TO REDUCE PESTICIDE USE ON GRAPE

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Grape protection: precise and careful protection management

- Main pests and pathogens:
  - **Downy mildew (*Plasmopara viticola*)**
  - **Powdery Mildew (*Erysiphe necator*)**
  - **Grey mold (*Botrytis cinerea*)**
  - **Esca disease (*Phaeomoniella chlamydospora, Phaeoacremonium aleophilum*)**
  - Crown gall (*Agrobacterium vitis*)
  - **Grapevine moth *(Lobesia botrana)***
  - **Vector of Flavescence Dorée (*Scaphoideus titanus*)**
  - Leafhoppers (*Empoasca vitis, E. decipiens*)
  - **Mealy bugs *(Planococcus ficus)***
  - Longidorid nematodes (*Xiphinema index*)

EU legislation on pesticides

- REGULATION (EC) No **1107**/2009 concerning the **placing of plant protection products on the market** and repealing Council Directives 79/117/EEC and 91/414/EEC
- DIRECTIVE 2009/**128**/EC establishing a framework for Community action to achieve the **sustainable use of pesticides**
- REGULATION (EC) No **396**/2005 on **maximum residue levels of pesticides** in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC

REGULATION (EC) No 1107/2009

- *This Regulation shall apply to substances, including micro-organisms having general or specific action against harmful organisms or on plants, parts of plants or plant products, referred to as 'active substances*'.
- **Biopesticides (microbial biocontrol agents, botanicals) should be registered**
- **Active substance** (EU approval) and **plant protection products** (Member state approval, mutual recognition in the 3 zones)
- **High cost** for registration, difficult (and often useless) to study all **metabolites**, **difficult to protect IP** for "natural active substances", **long process**

Basic substances

- is not a substance of concern
- does not have an inherent capacity to cause endocrine disrupting, neurotoxic or immunotoxic effects
- **is not predominantly used for plant protection purposes** but nevertheless is useful in plant protection either directly or in a product consisting of the substance and a simple diluent
- **is not placed on the market as a plant protection product**

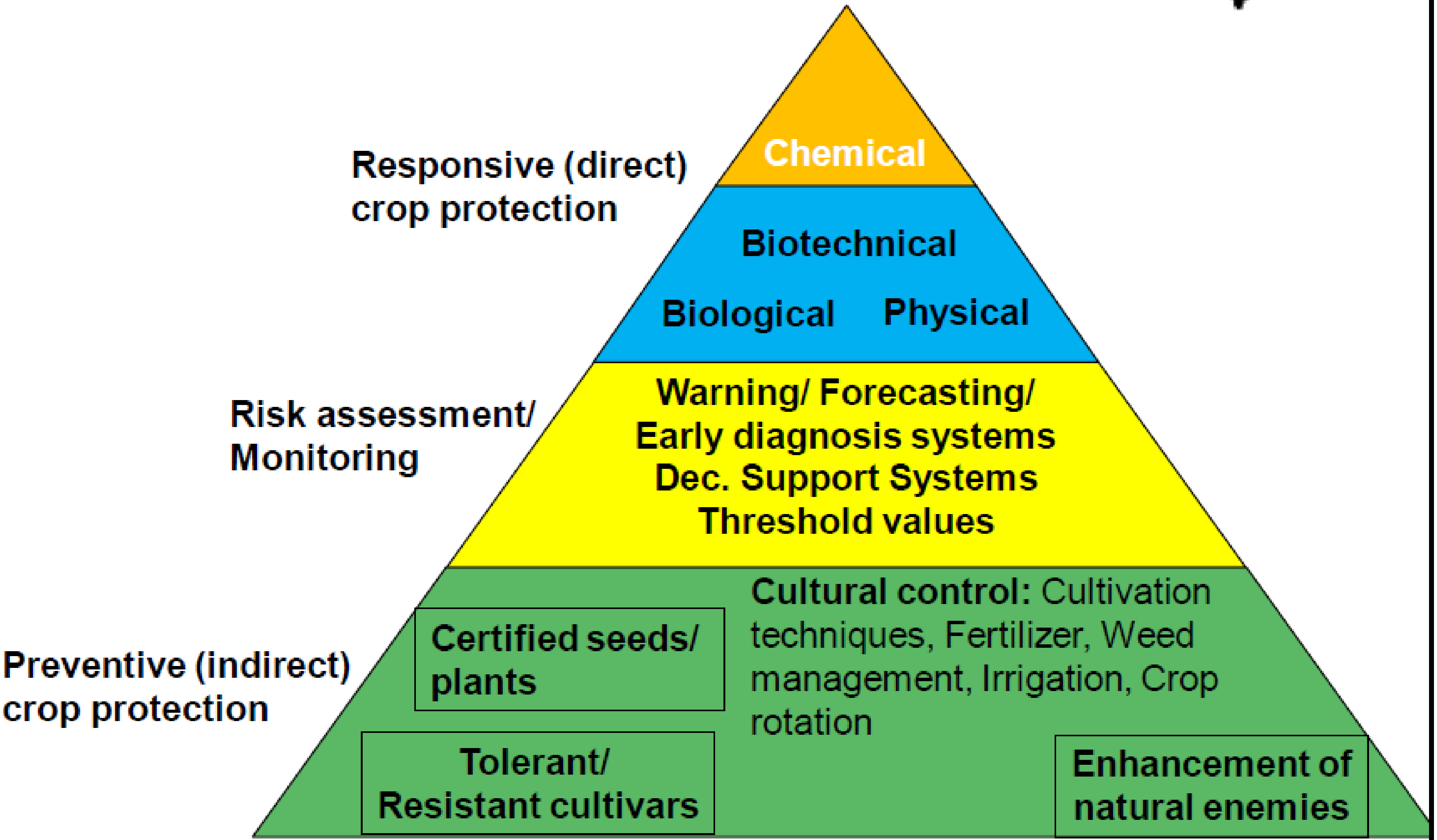
Example: milk, molasses

Microbial pesticides and medicinal plants: no basic substances!

For details: Working document SANCO/10363/2012 rev.9 (2014)

Integrated Pest Management – 128/2009

- Not only pesticides, but **other options:**
  - crop rotation (not grape after grape)
  - adequate cultivation techniques (e.g. pruning)
  - resistant/tolerant cultivars (several breeding programs on grape) and standard/certified planting material (virus free)
  - balanced fertilisation, and irrigation/drainage practices
  - preventing the spreading of harmful organisms by hygiene measures
  - protection and enhancement of important beneficial organisms
- …
- Sustainable **biological, physical and other non-chemical methods** must be preferred to chemical methods if they provide satisfactory pest control
- …



Meissle et al. 2011.Pest Manag. Science,67

Are growers ready for the alternatives to chemicals?

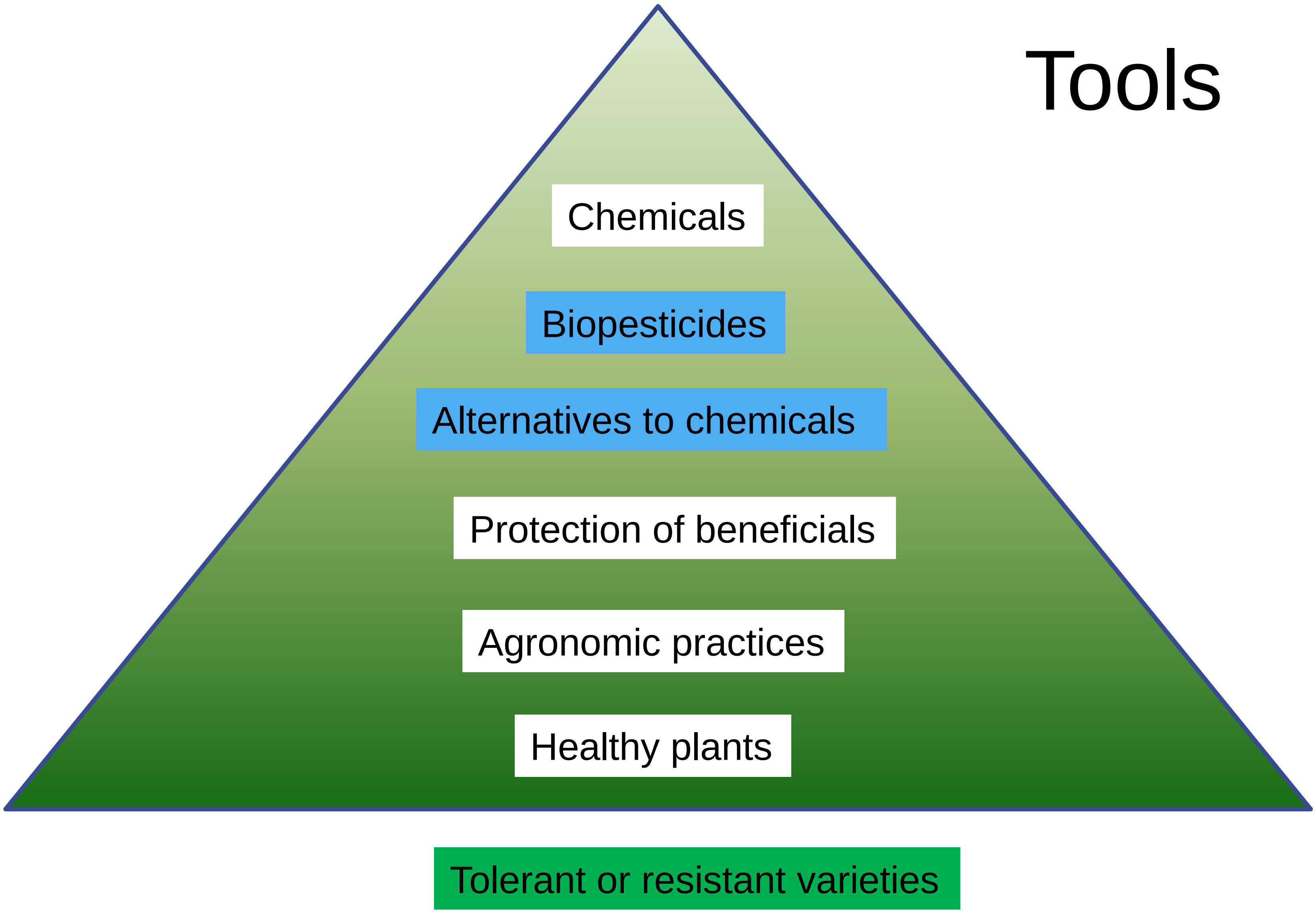
- **Precise timing**: is it feasible?
  - Treatments in a short time: dimension of the farm, availability of workers, weather, etc.
  - Knowledge: to determine the right timing based on right conditions
- **Costs**: are they acceptable?
  - Pressure on chemical residue reduction (food retailers) and pesticide reduction (consumers directive 128), space for competition with chemicals (before harvest, integration in IPM)
- **Efficacy**: is it sufficient and consistent?
  - Need for improved active ingredients and formulation, roboust systems
- **Integration in IPM**: do we have enough knowledge?
  - Change of mentality, strategic use of alternatives

Building and IPM program

- Knowledge of the crop, pest/pathogens and environment
- Assessment of the present situation (indicators)
- Identification of key actions to take (i.e. substitution of a specific chemical with and alternative, reduction in spray volume, dosage, number of treatments, etc.)
- Taking preventative measures to prevent pest/pathogen buildup

- Monitoring
- Assessing the pest situation
- Determining the best action to take (tools)

Tools


Chemicals
Biopesticides
Alternatives to chemicals
Protection of beneficials
Agronomic practices
Healthy plants
Tolerant or resistant varieties

Resistant/tolerant cultivars

- several breeding programs on grapevine and standard/certified planting material (virus free) available in EU

Advantages:

No need for treatment against the pest, simple to use

Constrains:

The others pests/pathogens should still be controlled

Low durability of certain resistance genes (pathogen overcome resistance) – gene pyramidization

No resistance known against grape insects (only powdery and downy mildew, tolerance to grey mold)

Long and expensive breeding programs for perennials

Need to learn the technology of making the wine with a new CV

Terroir, image of a wine (Barolo, Bordeaux, Merlot…)

New consumers, different market, low price, …

Crop rotation

- Crop rotation is mandatory for arable crops, vegetables and mixed farming systems
- Not a solution for grape, alternation almost impossible, too (appellation d'origine contrôlée, DOC/IGT)
- Advantages:

Theoretically easy to apply

Constrains:

Useless for grape (except for nematodes), soilborne pathogens of grape are polyphagous

Almost impossible on high price level wines and regional wine

More equipment and machines

Shape and slope of vineyards

Preventing the spreading of harmful organisms by hygiene measures

- Plant material record/certificate of quality, health, variety/clone name, nursery name must be kept available
- Situation on grape is in average satisfactory (open questions on Esca and Eutypa, new viruses, *Xylella fastidiosa* and quarantine and possible invasive alien species)
- All propagation material must be inspected by the grower to be free of pests and diseases (i.e. Agrobacterium)
- Crop residues should be remove from crop if they contain inoculum/pests or can be substrates for growth of pest and pathogens (composted, or in some case burned)
- Particularly important for Root rot diseases (Armillaria, Roesleria)

Adequate cultivation techniques (e.g. pruning in right time)

- Timing (pruning, weed control, etc.) can help to secure healthy crop development, to limit the negative impact of weeds, pathogens and pests and to minimise nutrient losses
- Trellis and training systems: normally optimised per each variety and environment (tradition)
- Summer pruning: highly relevant, compromise between photosynthesis and sugar accumulation (canopy) and air circulation (prevention of disease)

Protection and enhancement of important beneficial organisms

- Prefer highly selective pesticides (highly relevant on grape: predatory mites and insects)
- Avoid pesticides with broad spectrum of activity (although some advantages with pesticide with effect on more than one disease: downy + powdery, downy + black rot, powdery + aspergillus, etc.)
- Right timing of application (DSS)
- Ecological infrastructures (plants, bushes, flowering stripes, etc. to give shelter to beneficial organisms)
- Biodiversity
- See IOBC-wprs book on ecological infrastructures

Balanced fertilisation and irrigation/drainage practices

- These practices are normally applied on grape, primarily to enhance quality of berries which is then reflected into wine
- The use of nitrogen needs particular care, excess of N has impact on growth and some diseases, but also on the quality of the wine
- The trend is for a reduction of OM in vineyards across EU, thus organic fertilizers should be preferred
- Irrigation is normally not of concern
- Good drainage is necessary on grape (physiology of the plant)

Optimization: Decision support systems

- Several softwares, web-based solutions, Apps
- Optimization of dosage, volumes, identification of thresholds (population, risk of infection, etc.)

Advantages:

Optimization of treatments, reduction of quantities of pesticides and impact

Constrains:

Organization (treatments should be applied in a short time)

IT skills and validation

Acceptance of risk

More in the presentation of Caffi T. (CNR-UNICATT)

Innovative tools for managing major diseases

Biological control

- Insect and mite biocontrol (predators and/or parasites)
- Microbial biocontrol agents = biopesticides (the active ingredient is a microorganism or part of it)

Advantage:

- No or minimized impact, safe for humans and environment

Constrains

- Inundative biocontrol with insects: expensive in open areas
- Knowledge
- Exact timing, right condition (pest/disease pressure, environment)
- Inconsistent efficacy

Erysiphe necator: powdery mildew

- Prevalent in **Mediterranean climates**
- **Reduction of production and quality**, cracks on berries (infections of *B. cinerea*)
- Sulphur is still the **most used active ingredient**
- **High quantities**, **side effects** on beneficials, bad smell in wine



Botrytis cinerea: Grey mold or Botrytis bunch rot

- Crop losses and **reductions in wine quality**
- Worldwide distribution
- Rainy and wet seasons, especially with **frequent rainfall during fruit ripening**
- Fermentation problems and reduction in wine quality
- Often associated with sour rot and now with *D. suzukii*



Downy mildew: *Plasmopara viticola*

- The most important diseases **in warm and wet climates**
- Zoospores swim in water to reach stomata, once they entered, only curative products can partially stop the disease (**short time**)
- Difficult to address with microbial biocontrol agents (presence and activity during rain)
- Some resistant/tolerant varieties



Esca disease: complex

- *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora* cause a **vascular disorder associated** with Esca and Petri disease
- **Penetration through pruning wounds**
- **Chemicals are not sufficiently persistent** to protect wounds all over the season

BIOCONTROL AGENTS AGAINST GRAPE DISEASES AND BIO-FUNGICIDES

State of the art

Case studies

Future challenges

Subtance	Status under Reg. (EC) No 1107/2009	Date of approval	Expiration of approval	Authorised	Authorisation in progress
Ampelomyces quisqualis strain AQ10	Approved	01/04/2005	31/03/2015	BG, CY, DE, EL, ES, FR, IT, SI, UK	NL
Aureobasidium pullulans	PENDING			AT, BE, FR, HU	ES, IT, SI, SK
Bacillus amyloliquefaciens subsp. plantarum D747	Approved			ES, IT	
Bacillus pumilus QST 2808	PENDING				ES
Bacillus subtilis str. QST 713	Approved	01/02/2007	31/01/2017	DE, ES, FR, IE, IT, SI, UK	EL
Candida oleophila strain O	PENDING			FR, UK	ES
Coniothyrium minitans	Approved	01/01/2004	31/12/2013	AT, BE, CZ, DE, DK, EL, ES, FR, HU, IE, IT, LU, NL, PL, SE, SK, UK	PT
Gliocladium catenulatum strain J1446	Approved	01/04/2005	31/03/2015	BE, DK, EE, FI, SE, UK	AT
Paecilomyces fumosoroseus Apopka strain 97	Approved	01/07/2001	31/12/2015	BE, FI, LU, NL, SE	AT
Phlebiopsis gigantea (several strains)	Approved	01/05/2009	30/04/2019	DK, EE, FI, LV, SE, UK	
Pseudomonas chlororaphis strain MA342	Approved	01/10/2004	30/09/2014	AT, BE, DE, DK, ES, FI, FR, IT, LT, LU, NL, SE, UK	PT
Pseudozyma flocculosa	PENDING				ES
Pythium oligandrum M1	Approved	01/05/2009	30/04/2019	CZ, HU, PL, SK	AT
Streptomyces K61 (formerly S. griseoviridis)	Approved	01/05/2009	30/04/2019	BE, CY, EE, EL, ES, FI, HU, IT, LT, NL, SE	
Streptomyces lydicus WYEC 108	PENDING				ES
Trichoderma asperellum (formerly T. harzianum) strains ICC012, T25 and TV1	Approved	01/05/2009	30/04/2019	ES, FR, IT, SI	
Trichoderma asperellum (strain T34)	PENDING				ES
Trichoderma atroviride (formerly T. harzianum) strains IMI 206040 and T11	Approved	01/05/2009	30/04/2019	ES, IT, SE	
Trichoderma atroviride strain I-1237	PENDING			FR	ES
Trichoderma gamsii (formerly T. viride) strain ICC080	Approved	01/05/2009	30/04/2019	ES, SI	
Trichoderma polysporum strain IMI 206039	Approved	01/05/2009	30/04/2019	ES, SE	
Verticillium albo-atrum (formerly Verticillium dahliae) strain WCS850	Approved	01/05/2009	30/04/2019	NL	
Zucchini yellow mosaic virus (ZYMV mild strain)	Not Approved				
Zucchini Yellow Mosaik Virus, weak strain	PENDING			FR	ES

Bacillus subtilis, B. amyloliquefaciens

- Active against several disease, e.g. Botrytis bunch rot, powdery mildew, sour rot
- Several advantages over gram-negative bacteria:
  - Production of endospores that are tolerant of heat and desiccation
  - Production of secondary metabolites with broad-spectrum activities
  - Induction of resistance
- *Bacillus* spp. offers advantages to commercial formulation because of spore production, long shelf-life
- **Advantage vs. chemicals**: no residues, no interference with fermentation, secondary activity against sour rot (can be applied before harvest)
- **Disadvantage:** inconsistent efficacy, only preventive

Ampelomyces quisqualis

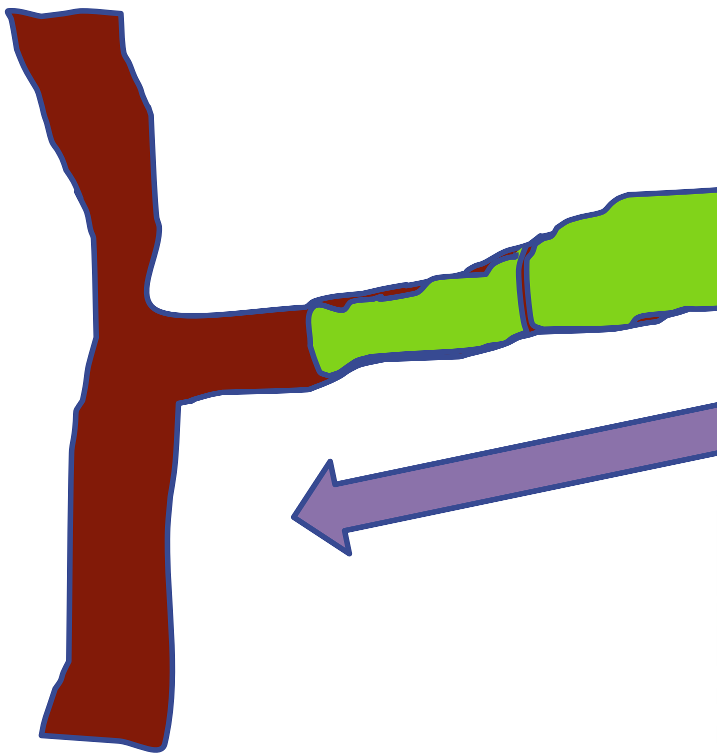
- Oldest commercial biofungicide
- Mycoparasite (needs the presence of the pathogen)
- Best conditions for application: low-moderate level of the disease, second part of the season, high relative humidity
- **Advantage:** no residues, no interference with fermentation, substitute of sulphur (no bad smells), reduction of overwintering inoculum
- **Disadvantage**: very susceptible to adverse environmental conditions, reduction of the disease (hyperparasite), slow effect, to reduce overwintering inoculum the right stage of ripening of chasmothecia should be identified

Trichoderma spp.

- The mechanism of action varies according the strain
- It may involve:
  - competition for nutrients
  - plant root colonization and/or rhizosphere modification (competition for space)
  - biofertilization and growth promotion (phytohormones, ACC-deaminase, organic acids, etc.)
  - stimulation of plant resistance and defence mechanisms (ISR)
  - mycoparasitism (coiling of parasite hyphae around the host and the formation of specialized appressorium-like structures)
  - formation and synergistic action of hydrolytic enzymes (i.e. endochitinases, hexosaminidases, and aspartyl proteases) and toxins/antibiotics (peptaibiotics)

Wood diseases: Trichodermas quickly colonize wounds

- After spraying, they colonizes the wound and penetrates into the dead wood preventing the infection of the pathogens



Treated

Untreated

Yeasts (*Aureobasidium pullulans*)

- Grape berry cuticles **host several non-Saccharomyces yeast** that can interact with other microorganisms such as filamentous fungi and bacteria
- **A part of the yeast** species associated with grapes (less than 5% of the population) have a broad spectrum of **antagonistic activity** against fungal pathogens
- Main mechanisms of action: **competition for space** (rapid colonization of wounds) **and nutrients** (nitrogen and/sugar competition in wounds may be a key mechanism)
- **Advantage:** no residues, easy registration process
- **Disadvantage:** partially effective if fruit sap leaking

B. cinerea: several experimental antagonists

Organism	Product (manufacturer)	Laboratory/Field Assessments	Reference
Filamentous fungi and oomycetes			
Gliocladium spp.		Grape berry assays	Machowicz-Stefaniak (1998)
Epicoccum nigrum		Necrotic grape leaf discs	Stewart *et al.* (1998)
		Grape rachii	Fowler *et al.* (1999)
Pythium radiosum		*In vitro* grape vine plantlets	Paul (1999a)
P. periplocum		*In vitro* grape vine plantlets	Paul (1999b)
Trichoderma atroviride (LC52)	Sentinel (Agrimm Technologies, NZ)	Grapevine field studies	www.agrimm.co.nz
T. harzianum (T39)	Trichodex (Makhteshim-Agan, Israel)	Multi-country grapevine field studies	O'Neill *et al.* (1996); Elad (2001); Elad & Stewart (2004)
T. harzianum (P1)		Grapevine field studies	Latorre *et al.* (1997)
T. viride (Td50)	Trichopulvin 25WP (Research Institute of Plant Protection, Romania)	Grapevine field studies	Sesan *et al.* (1999)
Ulocladium atrum (U385)		Multi-country grapevine field studies	Lennartz *et al.* (1998); Schoene & Kohl (1999); Roudet & Dubos (2001); Schoene *et al.* (2000); Metz *et al.* (2002)
Ulocladium oudemansii (HRU3)	Botry-Zen (Botry-Zen Ltd, NZ)	Grapevine field studies	Reglinski & Kingston (2001); Elmer *et al.* (2003); Reglinski *et al.* (2005); Elmer *et al.* (2005)
Ulocladium spp.		Grape rachii	Fowler *et al.* (1999)

Elmer and Reglinski, 2006

Yeasts and yeast-like fungi			
Acremonium cephalosporium (B11)		Grape berry assays	Zahavi *et al.* (2000)
Aureobasidium pullulans		Grape berry assays and field studies	Lima *et al.* (1996, 1997, 1999); Schena *et al.* (1999); Castoria *et al.* (2001)
Candida guilliermondii		Grape berry assays Grapevine field studies	McLaughlin *et al.* (1992) Zahavi *et al.* (2000)
C. saitoana	Bio-Coat and Biocure	Grapevine field studies	Schena *et al.* (2004)
Cryptococcus laurentii LS-28		Grape berry assays	Lima *et al.* (1998, 1999)
Metschnikowia spp. LS15		Grape berry assays and grapevine field studies	Schena *et al.* (2000)
Metschnikowia fructicola	Shemer (AgroGreen, Minrav Group, Israel)	Grapevine field studies	Kurtzmann & Droby (2001); Keren-zur *et al.* (2002)
Metschnikowia pulcherrima 320		Grape berry assays	Nigro *et al.* (1999)
Pichia membranifaciens (FY 101)		*In vitro* grape vine plantlets	Masih *et al.* (2000, 2001)
Rhodotorula glutinis (LS-11)		Grape berry assays	Lima *et al.* (1998, 1999)
Saccharomyces chevalieri	Saccharopulvin 25 PU (RIPP) Bucharest, Romania	Grapevine field studies	Sesan *et al.* (1999)
Trichosporon pullulans (RB9)		Grape berry assays Grapevine field studies	Schmidt *et al.* (1996); Holz & Volkmann (2002)
Bacteria			
Bacillus spp.		Grape berry assays *In vitro* grape vine plantlets	Ferreira (1990) Paul *et al.* (1998)
Bacillus subtilis (QST-713)	Serenade (Agra Quest, USA)	Grapevine field studies	Esterio *et al.* (2000); Schilder *et al.* (2002)
Bacillus circulans		*In vitro* grape vine plantlets	Paul *et al.* (1997)
Brevibacillus brevis		Grape berry assays Grapevine field studies	Ellis (1996); Seddon *et al.* (2000) Schmitt *et al.* (2002)
Pseudomonas fluorescens		Grape berry assays	Krol (1998)
Serratia liquefaciens		Necrotic grape leaf disc assays	Whiteman & Stewart (1998)

Elmer and Reglinski, 2006

Challenges: what is missing to bring biopesticides on the market?

- **Registration process** (long, expensive)
- **Investment** (low RoI, small market)
- **Cost of production** (ease of growing the organism, dilution factor needed for preparing the culture suspensions for a commercial spray)
- **Efficacy** (level, consistency)
- **New strains** (most of the registered strains: isolated 30 years ago)
- Development of a **stable, formulated product** retaining biocontrol activity
- **Storage conditions (logistics) and shelf-life**

Phosphonates

- Any compound containing a carbon phosphorus bond
- Examples: potassium phosphite, fosetyl-Al, etc. as fungicides
- Phosphite ions are taken up by plants, but not used in phosphorous metabolism; they have fungicidal properties
- Used as fertilisers with fungicide side effect
- Recently registered as PPP according REG. 1107/2009
- Direct effect mainly against oomycetes
- Hp: induced resistance (to be further proven)
- Low toxicity (mammals), low skin and eye irritation, no genotoxicity, no environmental side effects
- Residues in the plant (and in wine)!!

Plant extracts - botanicals

- No products registered as fungicides
- Several experimental products (Rheum extracts, extracts form Labiatae family, etc.)
- Pyrethrum, azadiractine, etc., vegetable oils as insecticides
- Cost/efficacy
- Clear guidelines for registration of plants extracts are needed (some progress with the ‘technical grade’)
- Large potential market for botanicals on grape (downy and powdery mildew and grey mold)

INNOVATIVE NON-CHEMICAL TOOLS AGAINST GRAPE INSECTS

State of the art

Future challenges

Pheromone mating disruption

- Reality in several grape growing areas
- *Lobesia botrana* and/or *Eupoecilia ambiguella*
- High technical and economic feasibility
- Area-wide approach (hectares and not single vineyards)
- Organization
- Monitoring of populations (some treatments often needed in the first year of application)
- Outbreak of secondary pests (mealy bugs, trips, other lepidoptera, etc.)

Bioinsecticides and biocontrol agents

- Botanicals, plant extracts
- Microbial bioinsecticides
- Biocontrol agents as predatory and hyperparasite insects and mites

More in the presentation of Duso C. (CNR-UNIPD)

Alternatives to conventional insecticides to cotrol berry moth and mealybugs

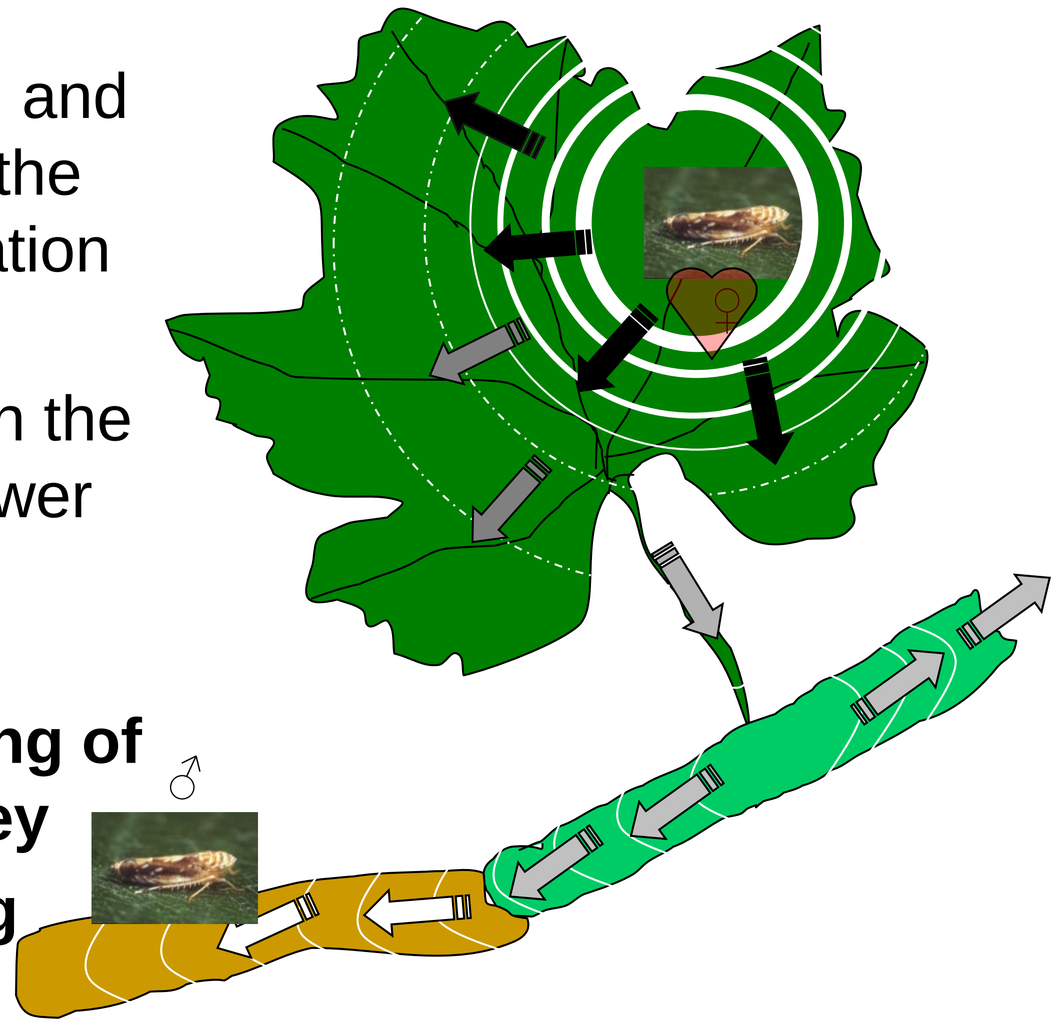
Vibrational mating disruption

- Several insects communicate by odors (pheromones)
- Pheromone mating disruption: commercially available
- **Others by vibrations: *Scaphoideus titanus* leafhopper**

Vibrations let male and female communicate on the plant

- The spectral (Hz, db) and temporal features of the signal allow identification and location
- Signals travel through the substrate and the power vanishes along the distance
- **Alteration or masking of the signals is the key**

to prevent the mating



Mazzoni et al., 2009. Bull. Entomol. Res. 99: 401-413

A LIGHT AT THE END OF THE TUNNEL, BUT STILL A LONG WAY TO GO…

Thank you for your attention!