Science For A Better Life

Micro-organisms as plant protection agents

Peter Lüth

Agenda/
Content

- Introduction of the Speaker
- Biocontrol based on Viruses and Bacteria
- Biocontrol based on Fungi
- The Technology
- Strain Selection
  - The Efficacy
  - The Applicability
  - The Economics
- Product Example
- Product Perception
- Where and how to apply the products?

Introduction

The Speaker

- Study at the University of Rostock
- PhD about ectoparasitic nematodes on cereals
- Scientist at a plant breeding institute in the former GDR
- 1992 foundation of Prophyta Biologischer Pflanzenschutz GmbH
- Development of the technological bases for the production of fungal BCA's
- International registration of the biological fungicide Contans WG
- International registration of the product BioAct
- Now working with Bayer CropScience, responsible for the development of new fungal BCA's

Introduction

The company

- Founded 1992
- Develops, produces and distributes biological plant protection agents
- 32 employees
- 2013 acquired by Bayer CropScience → market access

Biocontrol based on Microorganisms

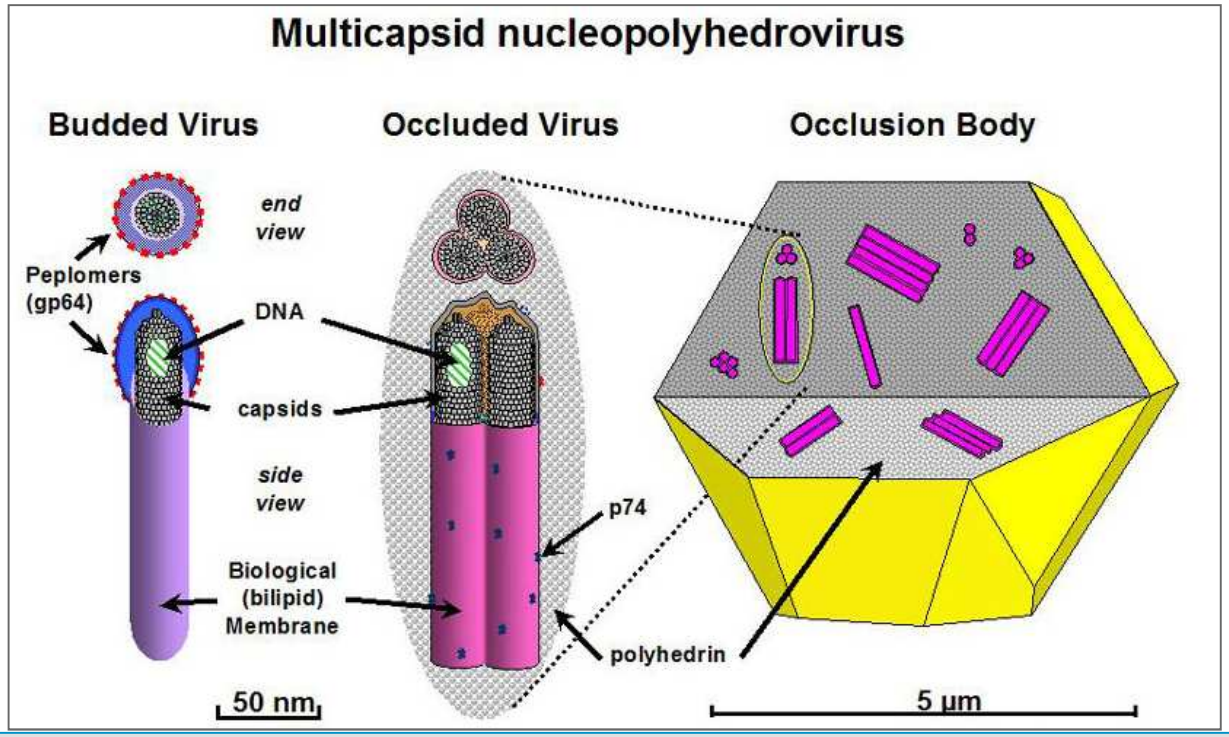
Overvew

Biocontrol based on Viruses

Insecticides

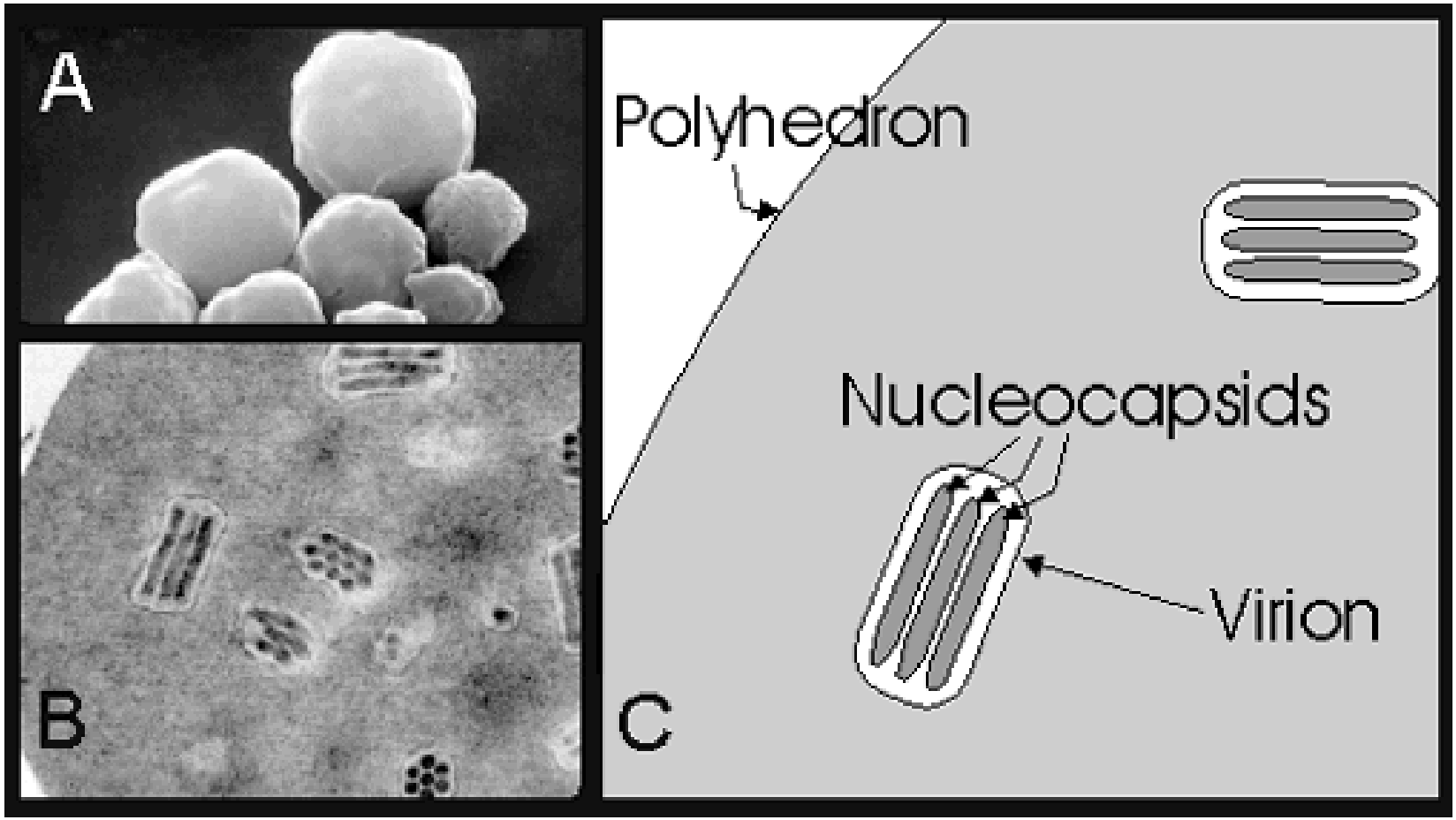
Baculoviruses

- First found in the 16th century on silk caterpilars
- 1949 first biological insecticide



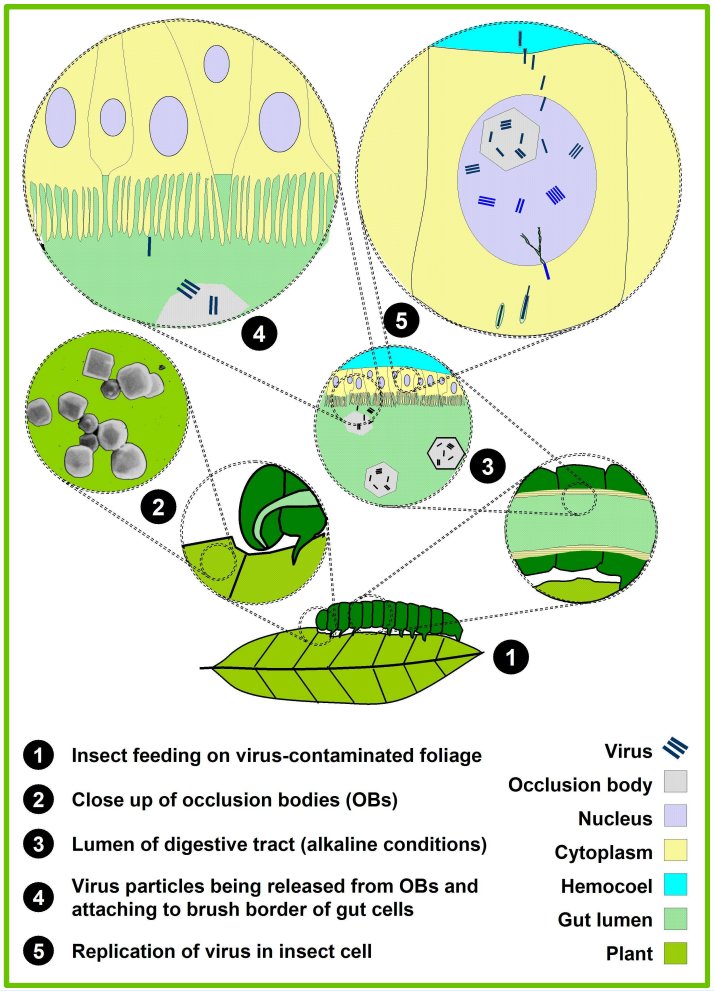
Biocontrol based on Viruses

Insecticides



Biocontrol based on Viruses

Insecticides



Biocontrol based on Viruses

Insecticides

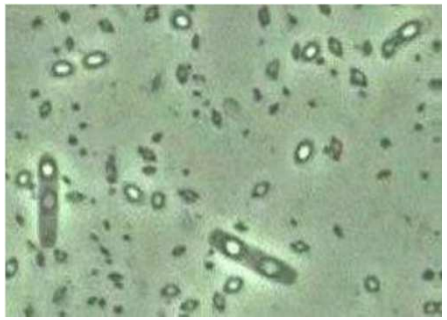
Baculovirus products

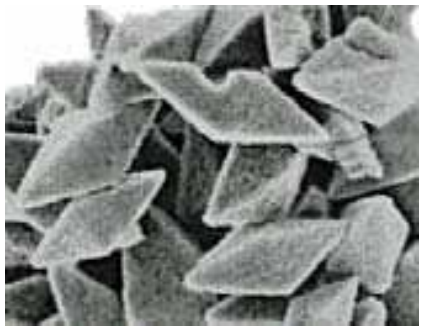
- Spod-X® (Certis) *Spodoptera exigua* NPV
- GemStar® (Certis) *Helicoverpa zea* NPV
- Elcar® (Syngenta) *Helicoverpa zea* NPV
- Cyd-X® (Certis) *Cydia pomonella* GV
- Madex® (Andermatt Biocontrol) *Cydia pomonella* GV
- Granupom® (Neudorf) *Cydia pomonella* GV
- Carpovirusin (NPP) *Cydia pomonella* GV
- Mamestrin® (NPP) *Mamestra brassicae*
- Capex (Andermatt Biocontrol) *Adoxophyes orana*
- VPN® (Agricola del Sol) *Anticarsia gemmatalis* NPV
- Gusano® (Certis) *Autographa californica* NPV
- Spodopterin® (Ajay Bio-Tech Ltd.) *Spodoptera littoralis* NPV

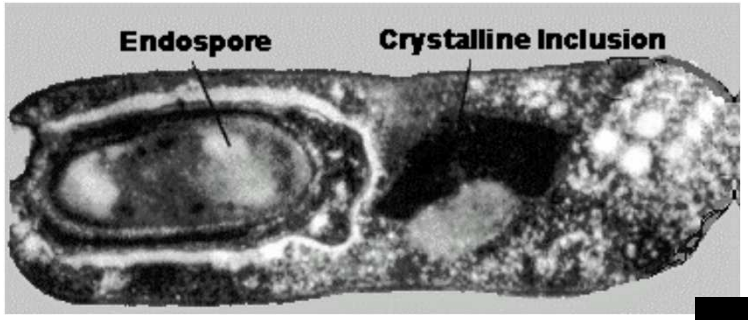
Biocontrol based on Bacteria

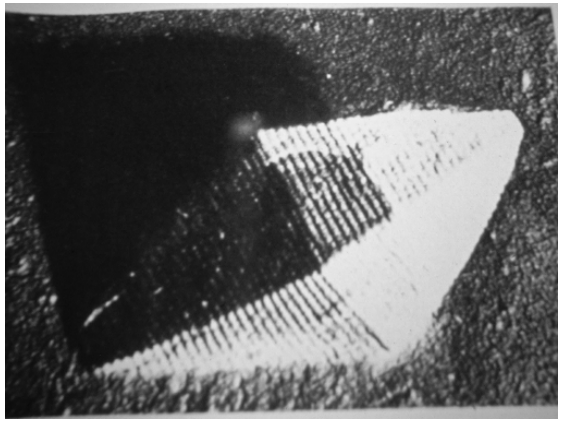
Insecticides

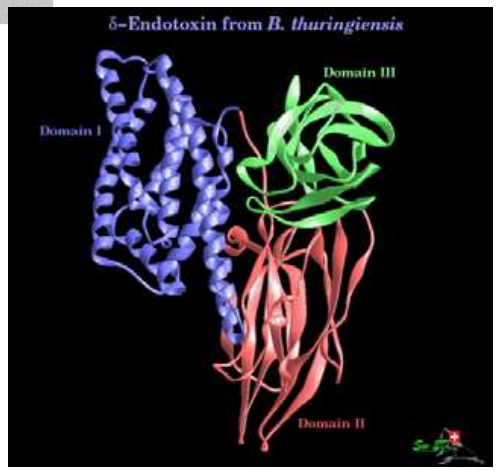
Bt products











In the alkaline environment of the susceptible insect's digestive system, the crystals are dissolved and converted into toxic protein molecules that destroy the walls of the insect's stomach. The insect usually stops feeding within hours and dies within days.

Biocontrol based on Bacteria

Insecticides

Bt products

- Bacillus thuringiensis serovar israelensis (controls the larvae stages of certain pests belonging to the Diptera)
  - Teknar (Certis)
  - Vectobac (Valent)
  - Biomükk (Biofa)
  - Neudomück (Neudorff)

- Bacillus thuringiensis serovar kurstaki or aizawea (controls the larvae stages of certain pests belonging to the Lepidoptera)
  - Dipel
  - Thuricide
  - Caterpillar
  - Attack
  - XenTari

Biocontrol based on Bacteria

Insecticides

- Bacillus thuringiensis serovar tenebrionis (controls the larvae stages of certain pests belonging to the Coleoptera)
  - Novodor
  - Jackpot
  - Perizin

Biocontrol based on Bacteria

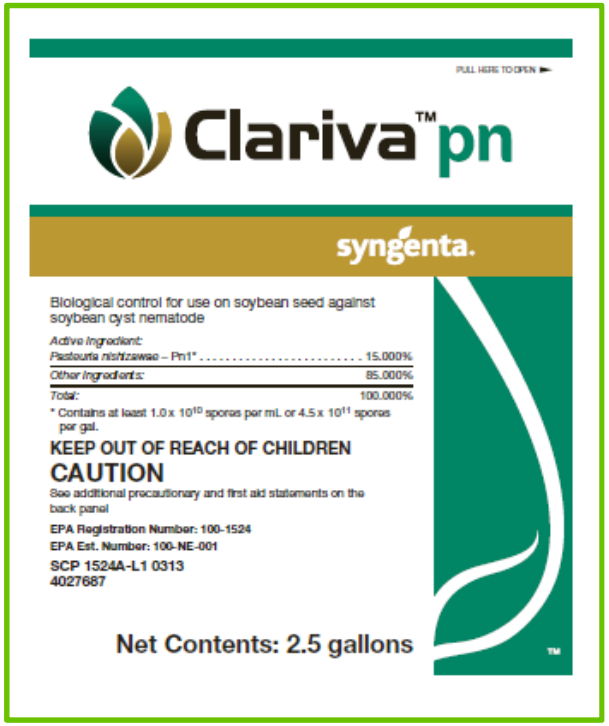
Fungicides

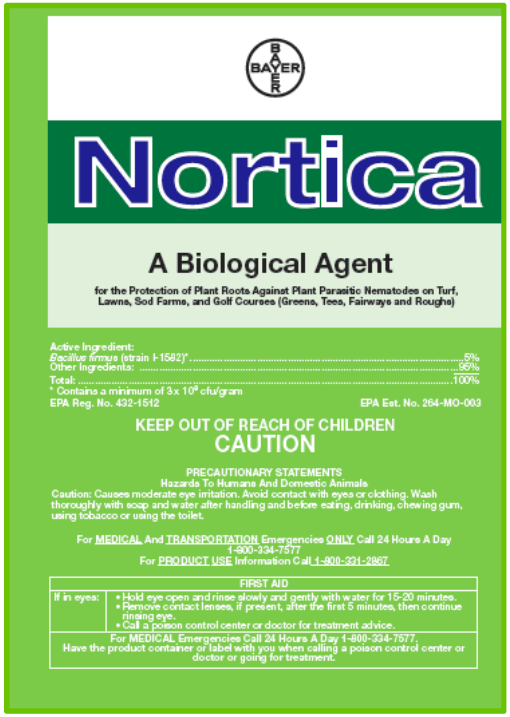
SERENADE	SONATA	Taegro, Rhizoctrol	EcoShot, Double Nickel, AmyloX, BacStar	Integral, HiStick, Subtilex, Clarity	Companion, Kodiak
B. subtilis QST 713	B. pumilus QST 2808	B. amyloliquifaciens FZB24	B. amyloliquifaciens D747	B. subtilis MBI600	B. subtilis GB03

Biocontrol based on Bacteria

Nematicide

- Pasteuria nishizawae (Econem, Clariva)
- Bacillus firmus (VOTiVO, Flocter, Nortica)





Biocontrol based on Fungi

Herbicides

- DeVine (Phytophthora palmivora)
  - Controls stranglervine (Morrenia odorata)
- Collego (Colletotrichum gloeosporioides f. sp. aeschynomene)
  - Controls northern jointvetch (Aeschynomene virginica)
- CASST (Alternaria cassiae)
  - Sicklepod (Cassia obtusifolia)
- Myco-Tech Paste, Chontrol Paste, Biochon (Chondrostereum purpureum)
  - Controls black cherry (Prunus serotina)
- Smolder (Alternaria destruens)
  - Controls dodder (Cuscuta pentagona)
- BioMal (C. gloeosporioides f. sp. malvae)
  - Controls rounded-leaved mallow (Malva pusilla)

Biocontrol based on Fungi

Fungicides

Trichoderma products

- Trichodex (company Makhteshim)
- Xedavir (company Xeda)
- SoilGard (company Certis USA)
- RootShield (company BioWorks)
- Esquive (company Agrauxine)
- Trianum (company Koppert)
- Remedier (company Isagro)
- Many more, also without registration

Biocontrol based on Fungi

Fungicides

Other fungicides

- AQ10 based on Ampelomyces quiqualis (company CBC)
  - WG formulation (5 x 10^9 conidia per gram)
- Prestop based on Clonostachys rosea (company Verdera)
  - WP formulation (2 x 10^8 conidia per gram)
- BOTRY-Zen based on Ulocladium audemansii (company Botry-Zen Ltd.)
  - WG formulation (2.5 x 10^8 conidia per gram)
- Tough Block based on Talaromyces flavus (company Idemitsu)
  - WP formulation (1 x 10^8 conidia per gram)
- Contans WG (Coniothyrium minitans)
  - WG formulation (1 x 10^9 conidia per gram)

Biocontrol based on Fungi

Insecticides

Fungi used to produce biological insecticides

- Beauveria bassiana (Mycontrol, Botanigard, Naturalis)
- Metarhizium anisopliae (Met52, Green Muscle, BioGreen)
- Lecanicillium longisporum (Vertalec)
- Isaria fumosorosea (Preferal, NoFly, PFR 97)

Biocontrol based on Fungi

Nematicides

Fungi used to produce biological nematicides

- Paecilomyces lilacinus (**MeloCon WG and WP**, Biostat/Rootgard, PLGold)
- Pochonia chlamydosporia (KlamiC, PcMR-1)
- Myrothecium verrucaria (DiTera)
- Other fungi like Arthrobotrys spp, Dactylella spp., and Duddingtonia flagrans are known as highly efficient, but are currently not commercially used.

Biocontrol based on Fungi

Paecilomyces lilacinus

Biocontrol based on Fungi

Paecilomyces lilacinus

Product features

	Biostat WP	PL Gold WP	MeloCon WG
Spore concentration per g/ml	2 x 10^7	4 x 10^9	1 x 10^{10}
ai per Kilogram	10 %	no info	4 %
recommended rate	0.25 Kg/ha	2 Kg/ha	4 Kg/ha

Biocontrol based on Fungi

Pochonia chlamydosporia

Former **Verticillium chlamydosporium**

- First reported as a parasite of nematodes by Wilcox & Tribe (1974)
- Since then several patents have been filed (Kerry BR, 1990; Franco Carlos Manuel Antunes, 2004; Qiau M , 2011)
- Only 2 products are available
  - KlamiC (Cuba)
  - PcMR-1 (Portugal)

Biocontrol based on Fungi

Myrothecium verrucaria

Product: DiTera (Valent)

TABLE 1

Crop Groups/ Representative Commodities	Nematode	Broadcast* Rate (pounds/ acre/appl)
CITRUS FRUITS such as sweet orange, lemon, grapefruit **FLOWERING, BEDDING PLANTS, ORNAMENTALS,** such as fern and hosta **LEAFY VEGETABLES AND COLE CROPS** such as celery, head & leaf lettuce, spinach, broccoli, cabbage **POME FRUITS** such as apple, pear **STONE FRUITS** such as peach, plum, prune **TREE NUTS** such as almond, pecan, walnut **GRAPE** **PINEAPPLE**	Burrowing Citrus Cyst Dagger Lance Lesion Needle Pin Reniform Ring Spiral Root-knot Sting Stubby-root Stunt	13-100

TABLE 2 – CONVERSION TABLE FOR BAND APPLICATIONS

Band Width Inches	Pounds of *DiTera* DF per 1,000 Feet of Row at Equivalent Broadcast Rate of Indicated Pounds per Acre			
	Equivalent Broadcast Rate Per Acre			
	13	25	50	100
12	0.31	0.60	1.2	2.4
18	0.47	0.91	1.8	3.6
24	0.63	1.20	2.4	4.8
30	0.79	1.51	3.0	6.0
36	0.94	1.82	3.6	7.2
48	1.26	2.40	4.8	9.6
60	1.58	3.00	6.0	12.0

To be used on a wide range of plants

But at a fairly high rate

The Technology

Solid-state fermentation

Fermenter to be autoclaved

The Technology

Solid-state fermentation

Incubation

The Technology

Solid-state fermentation

Fermenter after incubation

The Technology

Solid-state fermentation

Fermentation in spawn bags

The Technology

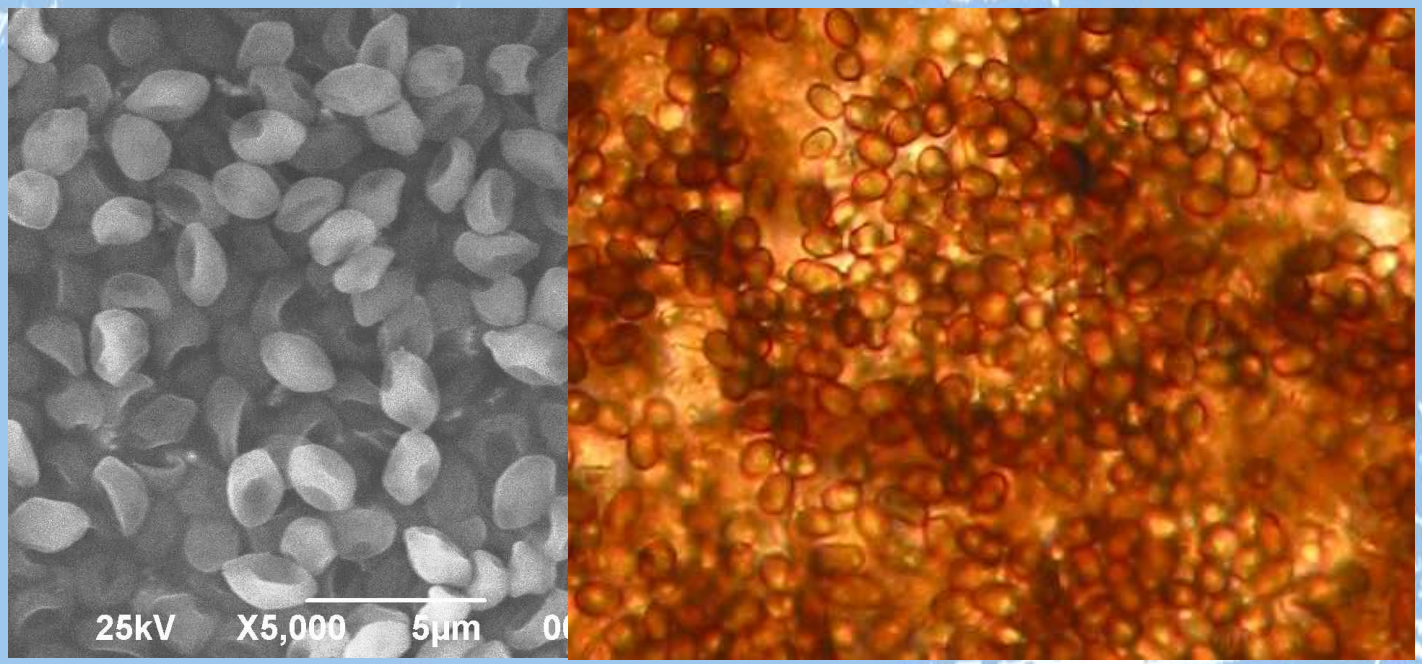
Solid-state fermentation

Fermentation in spawn bags

The Technology

Spore separion

Conidia of Paecilomyces lilacinus and Coniothyrium minitans



The Technology

Formulation

Lyophilization

The Technology

Formulation

Fluidized bed drying

The Technology

BCS Biologics - Product

Contans WG:

- Active ingredient: Coniothyrium minitans
- Water dispersible granule (WG)
- Carrier: Inert, soluble
- 1 x 10^9 viable conidia per gram
- Rate: 0.5 – 4.0 kg pro hectare
- shelf life: 24 months at +4 ℃
  and even 5 years –18 ℃
- Applicable to control
  - Sclerotinia sclerotiorum
  - Sclerotinia minor
  - Sclerotinia trifoliorum
  - Sclerotium cepivorum
- Produced on a pure biological basis

Strain Selection

The Efficacy

In regards to the screening of effective fungi the industry works closely together with universities and other scientific institutes or companies.

- Good disease or pest control in comparison to the UTC and the chemical standard
- Good yield response in comparison to the UTC and the chemical standard

→ Both at a reasonable low rate

→ Confirmation of the results in own field trials.

Strain Selection

The Applicability

Product Application

- Suitability of the product to be drenched into the soil
  - Small conidia
  - No aglomerations of the conidia in the formulation
  - No impurities from the culture substrate
  - Mixing with a surfactant possible

- Or: Application as a seed treatment
  - Good rhizosphere competitiveness
  - Compatibility with chemicals used to treat the seed

- Or: The used fungus can be applied as an endophyte

Strain Selection

The Applicability

Low rate

- Maximum 4 kg/l per hectare
- To be applied with common equipment

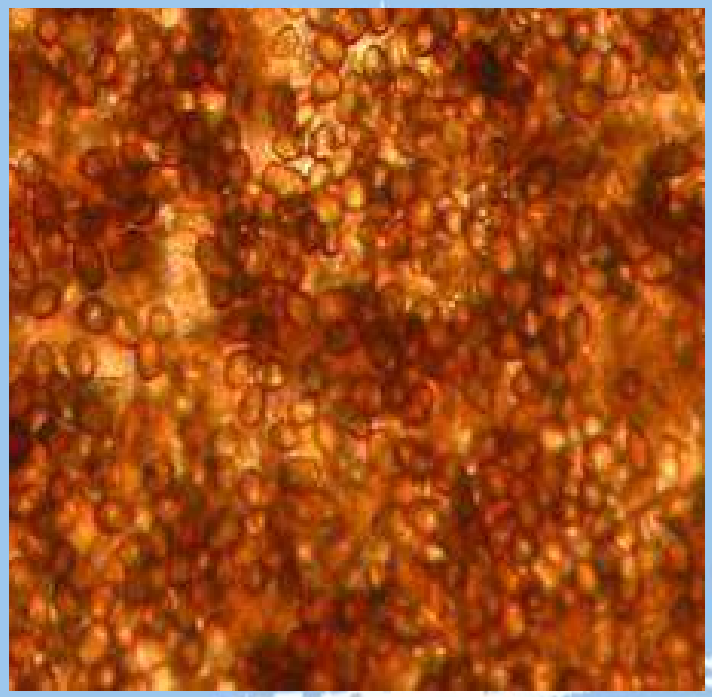
Shelf life

- Optimum
  - More than 2 years at ambient temperatures
  - More than 6 months at 30 ℃
- Just acceptable
  - 1 Year at ambient temperature
  - 3 Months at 30 ℃
- Difficult
  - Refrigerated transport and storage

Strain Selection

Economics

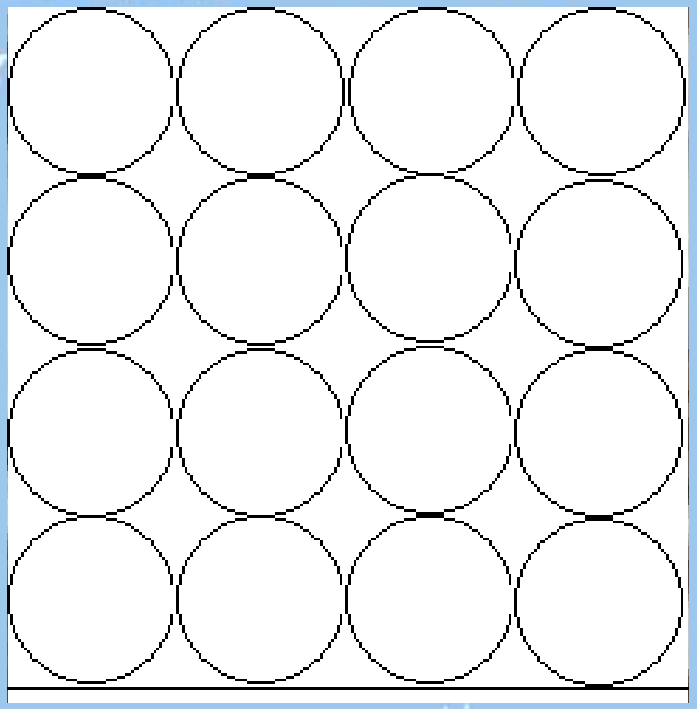
Influence of the spore size

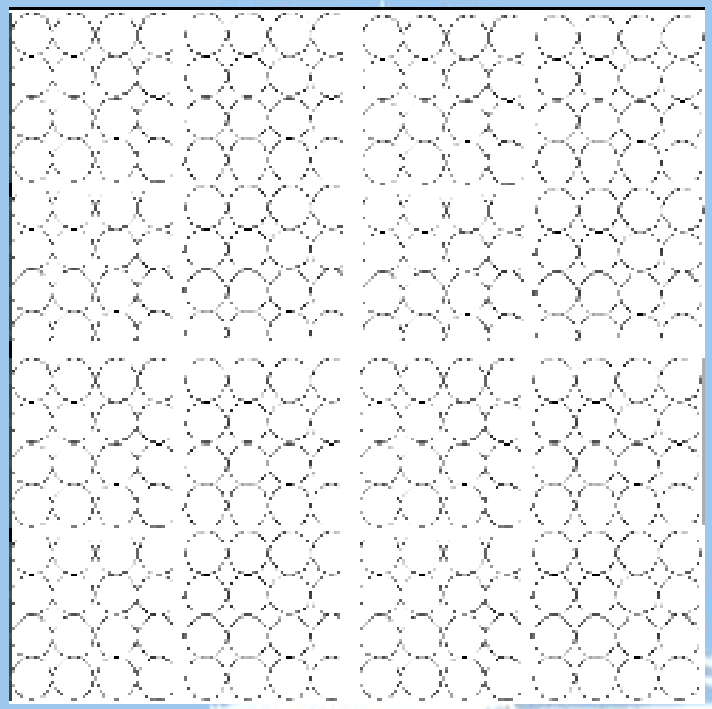


Strain Selection

Economics

Influence of the spore size





Strain Selection

Economics

Influence of the spore size

Maximum spore yield

	maximum content in 1 ml	maximum content in a 1000 l fermenter
Conidia diameter 20 µm	**1.25×10^8**	**1.25×10^{14}**
Conidia diameter 5 µm	**8.0×10^9**	**8.0×10^{15}** **(about 60x more)**

Strain Selection

Economics

Dependence of the profitability from spore yield and rate per hectare

	Number of cfu needed per sq m					
Conidia per gram culture substrate	2.5×10^9	1.0×10^9	5.0×10^8	2.5×10^8	1.0×10^8	5.0×10^7
5.0×10^{10}	**20 sq m / gram**	**50 sq m / gram**	**100 sq m / gram**	**200 sq m / gram**	**500 sq m / gram**	**1000 sq m / gram**
1.0×10^{10}	**4 sq m / gram**	**10 sq m / gram**	**20 sq m / gram**	**40 sq m / gram**	**100 sq m / gram**	**200 sq m / gram**
5.0×10^9	**2 sq m / gram**	**5 sq m / gram**	**10 sq m / gram**	**20 sq m / gram**	**50 sq m / gram**	**100 sq m / gram**
1.0×10^9	**0.4 sq m / gram**	**1 sq m / gram**	**2 sq m / gram**	**4 sq m / gram**	**10 sq m / gram**	**20 sq m / gram**
5.0×10^8	**0.2 sq m / gram**	**0.5 sq m / gram**	**1 sq m / gram**	**2 sq m / gram**	**5 sq m / gram**	**10 sq m / gram**

→ The producer knows how much he must earn manufacturing the product from one fermenter. So, he knows whether or not the whole process can be profitable for him or not.

Product Example

The Product MeloCon WG

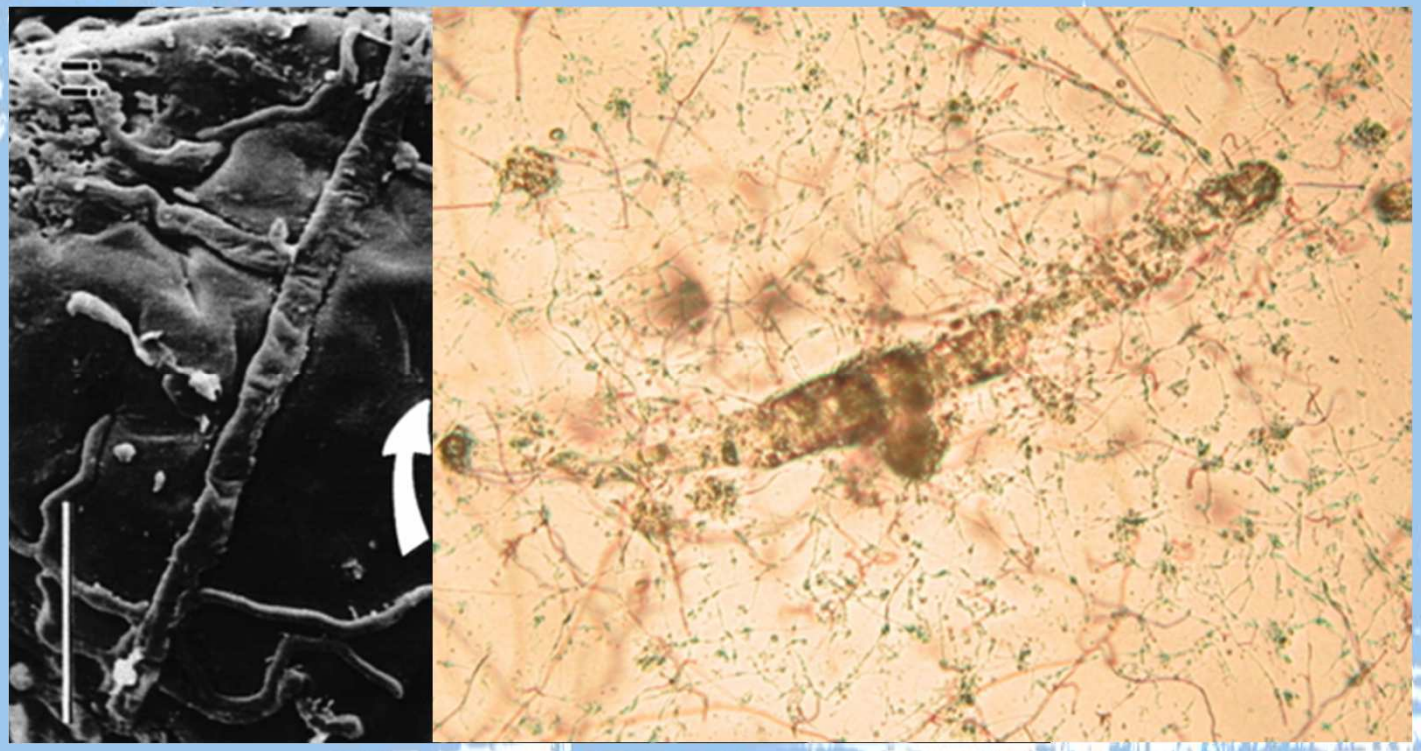
MeloCon WG:

- Contains the spores of the fungus Paecilomyces lilacinus
- Water dispersible granule (WG)
- Carrier: Inert, soluble
- 1 x 10^{10} living conidia per gram product
- Rate: 0.2 gram per plant, but not more than 4 kg per hectare per season
- Shelf life: 6 months at +4 ℃ and 24 months at –10 ℃
- Applicable to control:

  Root-knot nematodes, lesion nematodes, burrowing nematodes, reniform nematodes, citrus nematodes, sting nematodes and others
- The product is manufactured on a pure biological basis.

Product Example

The biological Nematicide MeloCon WG



Product Example

The biological Nematicide MeloCon WG

Different strategies to use MeloCon WG

Prerequisite: The spores of *P. lilacinus* have to get in contact with the nematodes.

- Application by drip irrigation
- Application by mechanical incorporation
- Application by spraying followed by sprinkler irrigation or rain

Product Example

The biological Nematicide MeloCon WG

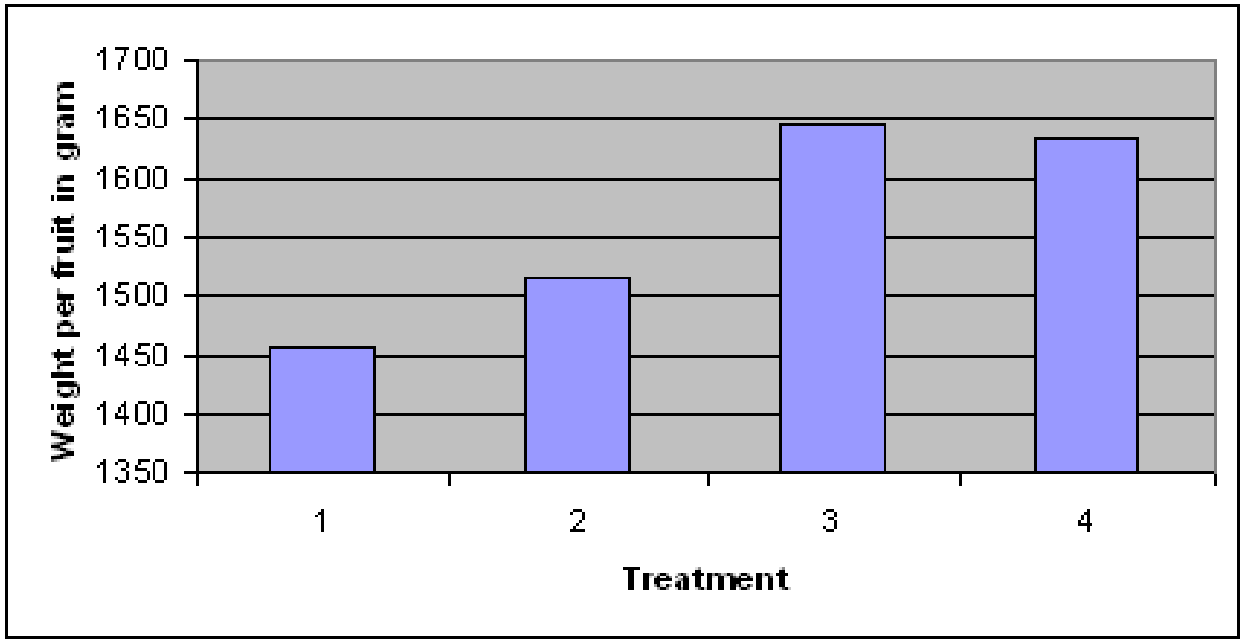
Application by drip irrigation

Product Example

The biological Nematicide MeloCon WG

Application by drip irrigation

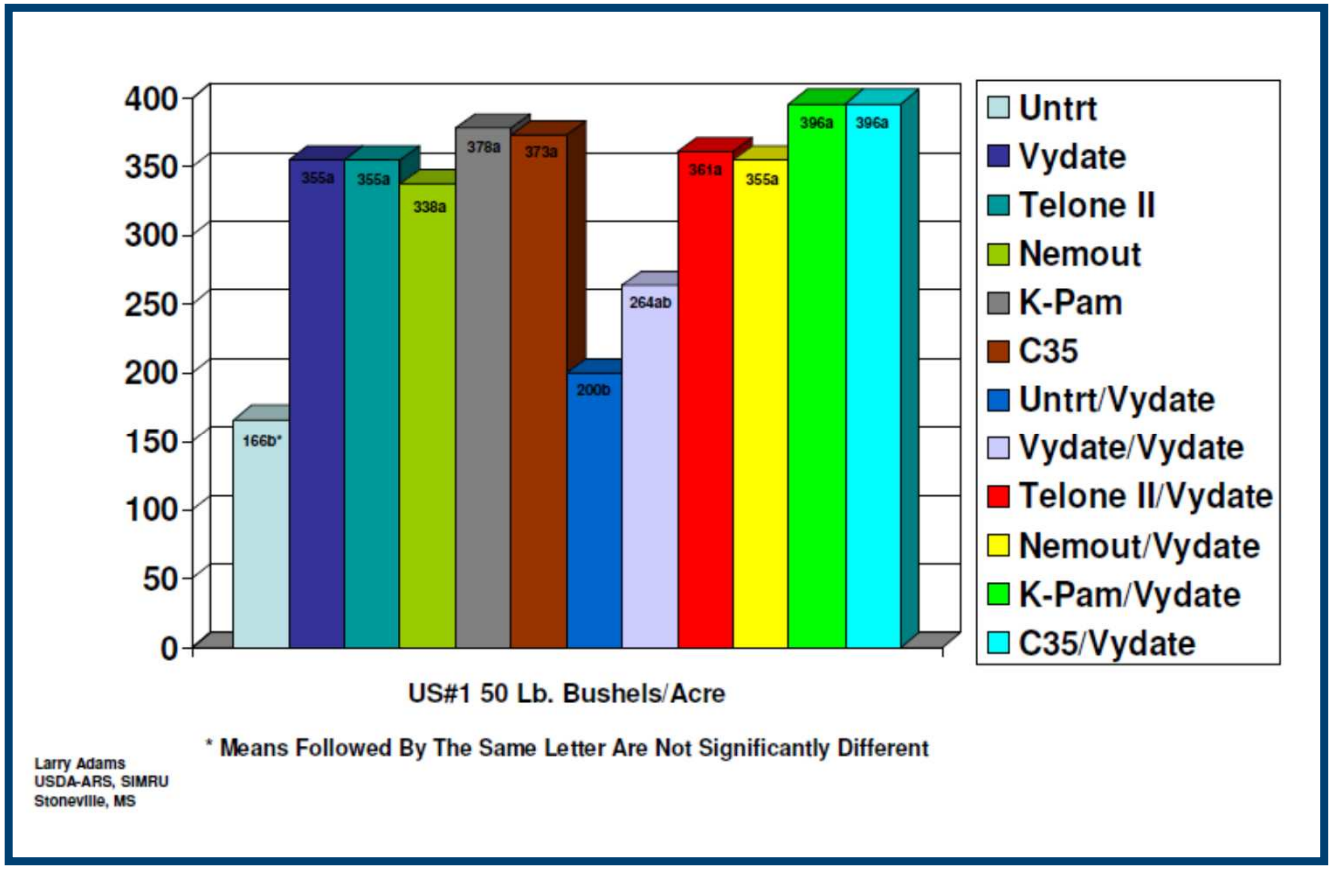
1. 1,3-D preplant fumigant
2. 1,3-D preplant and Nemacur at 24 weeks
3. 1,3-D preplant and Melocon at 12,16,20, and 24 weeks
4. 1,3-D preplant and LCF at 0, 12, 24, and 36 weeks



Product Example

The biological Nematicide MeloCon WG (Nemout)

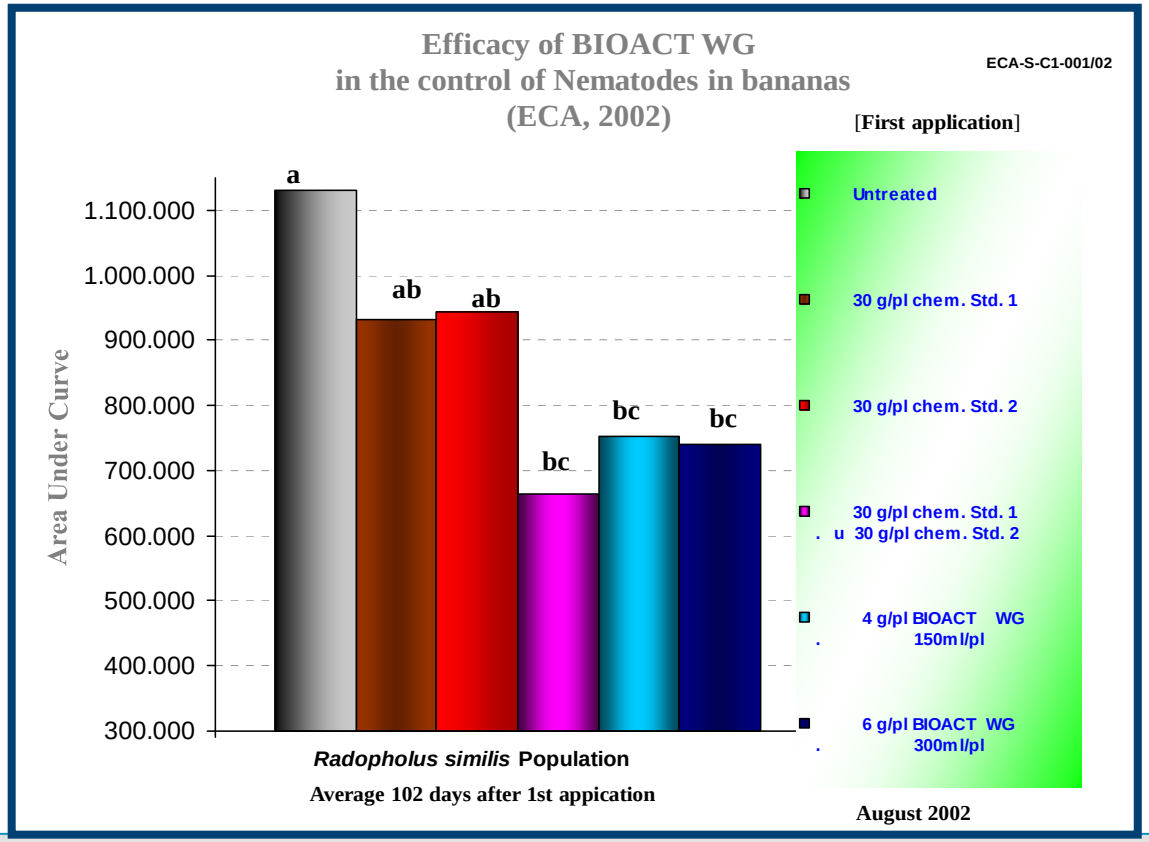
Application by mechanical incorporation



Product Example

The biological Nematicide MeloCon WG

Spray application and drench by rain in banana production

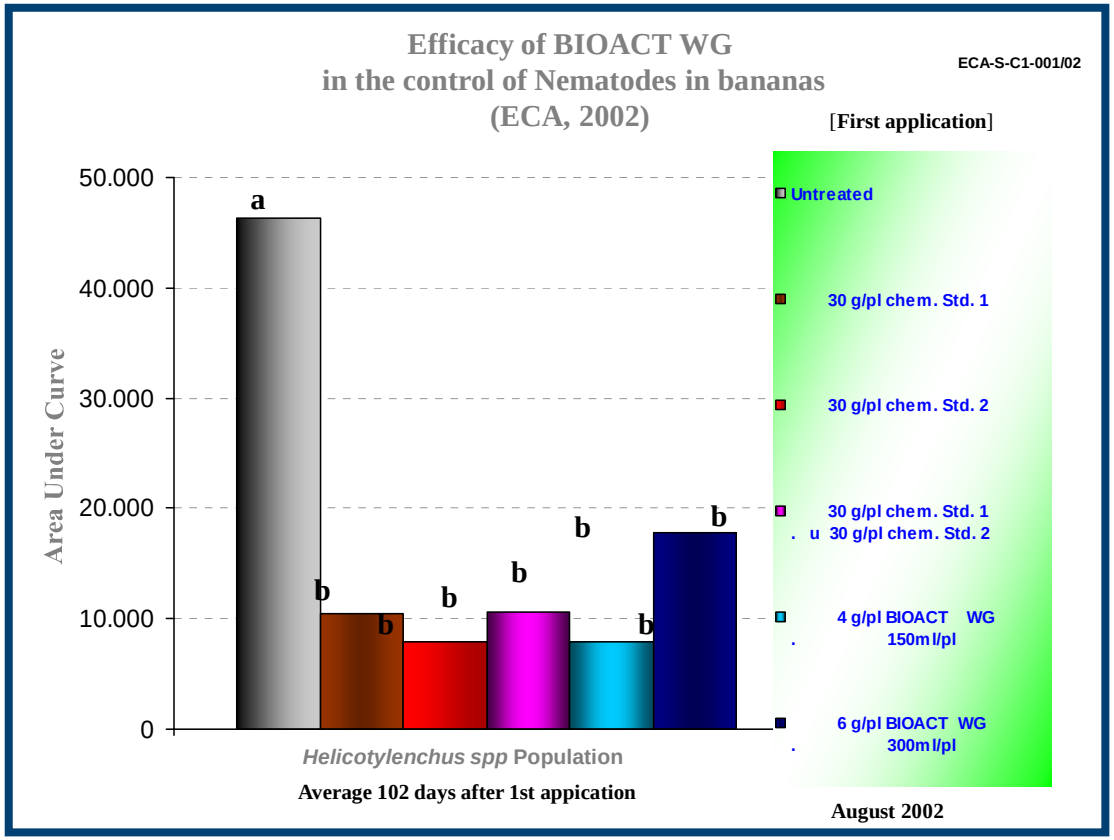


Development of Radopholus similis at different treatments

Product Example

The biological Nematicide MeloCon WG

Spray application and drench by rain in banana production

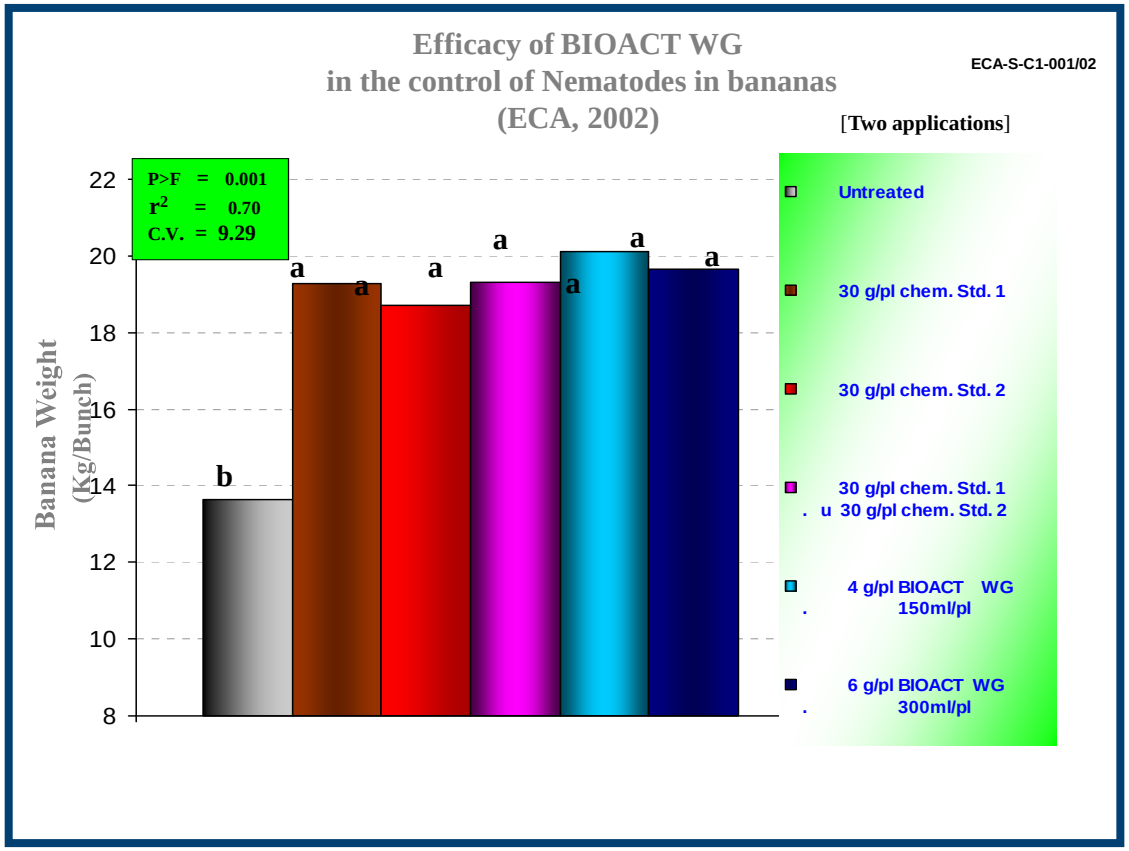


Development of Helicotylenchus spp. at different treatments

Product Example

The biological Nematicide MeloCon WG

Spray application and drench by rain in banana production



Banana yield after dfferent treatments

Product Perception

The problem

All requirements for an economic performance are fulfilled

- An interesting market, e.g. aphids in cereals
- A good effectiveness, e.g. as good as the best chemical insecticide
- A good formulation, e.g. a WG with 1 x 10^{10} conidia per gram
- A good price ensuring a good profit and acceptable for the grower

Nevertheless the product does not enter the market as expected.

→ **Advantages of biocontrol agents vis-a-vis certain chemical products are not immediately visible to the grower.**

Product Perception

The reason

- insufficient technical support
- wrong expectation of the users
- special care is needed to keep the active ingredient alive
- unauthorised products (snake oils) in the market

Where and how to apply the products?

In conventional agriculture and horticulture

- as a resistance management tool
- as a tool in product life cycle management
- at the end of the crop season to prevent residue level above MRL
- as a tool complementary to synthetic solutions
- where no other solutions are available
- in the rainy season at danger of leaching of synthetic a.i.
- where the product works better or more sustainable than the synthetic a.i.

Science For A Better Life

Thank you!