Control of Verticillium in tree nurseries through biological soil disinfestation

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Dutch tree nursery industry

- Production area: 15.600 Ha (CBS, 2012)
- Production value: 590 million € (PT, 2011)
    - 60 % for export
- Verticillium Wilt serious problem
    - Avenue, park & street trees
        - incl. *Acer, Fraxinus, Tilia, Ulmus*
    - Rose plants
    - Flowering shrubs








Damage by VW in nursery industry

- Verticillium wilt of trees
  - Many important tree species susceptible
  - Cause: *V. dahliae* (interaction with *P. penetrans*)
  - No resistance in susceptible species
- Control
  - Prevention best option
  - Vd present in many areas
  - Annual loss estimated: 5.5 M€

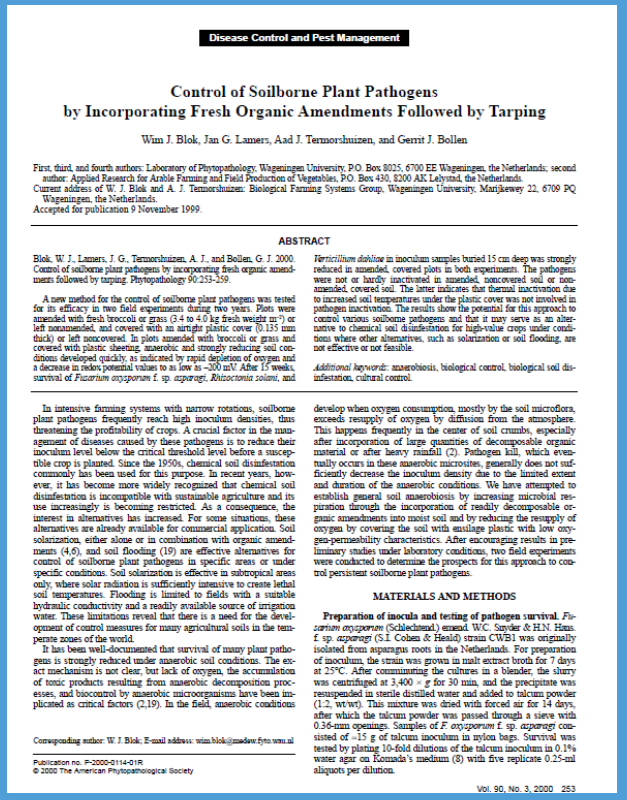
→ Eradication from soil

  - Soil fumigants: neg. environmental aspects
  - Enhanced interest non-chemical techniques

Biological Soil Disinfestation

- Alternative method to chemical soil disinfestation
  - Addition of fresh organic material
  - Cover with airtight plastic
  - → anaerobic decomposition

- Blok *et al.*, 2000: Phytopathology
  - *F. oxysporum*
  - *R. solani*
  - *V. dahliae*



Disease Control and Pest Management

Control of Soilborne Plant Pathogens by Incorporating Fresh Organic Amendments Followed by Tarping

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ABSTRACT

Blok, W. J., Lamers, J. G., Termorshuizen, A. J., and Bollen, G. J. 2000. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. Phytopathology 90:253-259.

A new method for the control of soilborne plant pathogens was tested for its efficacy in two field experiments during two years. Plots were amended with fresh broccoli or grass (3.4 to 4.0 kg fresh weight m^{-2}) or left nonamended, and covered with an airtight plastic cover (0.135 mm thick) or left noncovered. In plots amended with broccoli or grass and covered with plastic sheeting, anaerobic and strongly reducing soil conditions developed quickly, as indicated by rapid depletion of oxygen and a decrease in redox potential values to as low as −300 mV. After 15 weeks, survival of *Fusarium oxysporum* f. sp. *asparagi*, *Rhizoctonia solani*, and *Verticillium dahliae* in inoculum samples buried 15 cm deep was strongly reduced in amended, covered plots in both experiments. The pathogens were not or hardly inactivated in amended, noncovered soil or nonamended, covered soil. The latter indicates that thermal inactivation due to increased soil temperatures under the plastic cover was not involved in pathogen inactivation. The results show the potential for this approach to control various soilborne pathogens and that it may serve as an alternative to chemical soil disinfestation for high-value crops under conditions where other alternatives, such as solarization or soil flooding, are not effective or not feasible.

Additional keywords: anaerobiosis, biological control, biological soil disinfestation, cultural control.

In intensive farming systems with narrow rotations, soilborne plant pathogens frequently reach high inoculum densities, thus threatening the profitability of crops. A crucial factor in the management of diseases caused by these pathogens is to reduce their inoculum level below the critical threshold level before a susceptible crop is planted. Since the 1950s, chemical soil disinfestation commonly has been used for this purpose. In recent years, however, it has become more widely recognized that chemical soil disinfestation is incompatible with sustainable agriculture and its use increasingly is becoming restricted. As a consequence, the interest in alternatives has increased. For some situations, these alternatives are already available for commercial application. Soil solarization, either alone or in combination with organic amendments (4,6), and soil flooding (19) are effective alternatives for control of soilborne plant pathogens in specific areas or under specific conditions. Soil solarization is effective in subtropical areas only, where solar radiation is sufficiently intensive to create lethal soil temperatures. Flooding is limited to fields with a suitable hydraulic conductivity and a readily available source of irrigation water. These limitations reveal that there is a need for the development of control measures for many agricultural soils in the temperate zones of the world.

It has been well-documented that survival of many plant pathogens is strongly reduced under anaerobic soil conditions. The exact mechanism is not clear, but lack of oxygen, the accumulation of toxic products resulting from anaerobic decomposition processes, and biocontrol by anaerobic microorganisms have been implicated as critical factors (2,19). In the field, anaerobic conditions develop when oxygen consumption, mostly by the soil microflora, exceeds resupply of oxygen by diffusion from the atmosphere. This happens frequently in the center of soil crumbs, especially after incorporation of large quantities of decomposable organic material or after heavy rainfall (2). Pathogen kill, which eventually occurs in these anaerobic microsites, generally does not sufficiently decrease the inoculum density due to the limited extent and duration of the anaerobic conditions. We have attempted to establish general soil anaerobiosis by increasing microbial respiration through the incorporation of readily decomposable organic amendments into moist soil and by reducing the resupply of oxygen by covering the soil with ensilage plastic with low oxygen-permeability characteristics. After encouraging results in preliminary studies under laboratory conditions, two field experiments were conducted to determine the prospects for this approach to control persistent soilborne plant pathogens.

MATERIALS AND METHODS

Preparation of inocula and testing of pathogen survival. *Fusarium oxysporum* (Schlechtend.) emend. W.C. Snyder & H.N. Hans. f. sp. *asparagi* (S.I. Cohen & Heald) strain CWB1 was originally isolated from asparagus roots in the Netherlands. For preparation of inoculum, the strain was grown in malt extract broth for 7 days at 25°C. After comminuting the cultures in a blender, the slurry was centrifuged at 3,400 × *g* for 30 min, and the precipitate was resuspended in sterile distilled water and added to talcum powder (1:2, wt/wt). This mixture was dried with forced air for 14 days, after which the talcum powder was passed through a sieve with 0.36-mm openings. Samples of *F. oxysporum* f. sp. *asparagi* consisted of ~15 g of talcum inoculum in nylon bags. Survival was tested by plating 10-fold dilutions of the talcum inoculum in 0.1% water agar on Komada's medium (8) with five replicate 0.25-ml aliquots per dilution.

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Aim of the project

Field testing of most promising methods to control VW in tree nursery crops

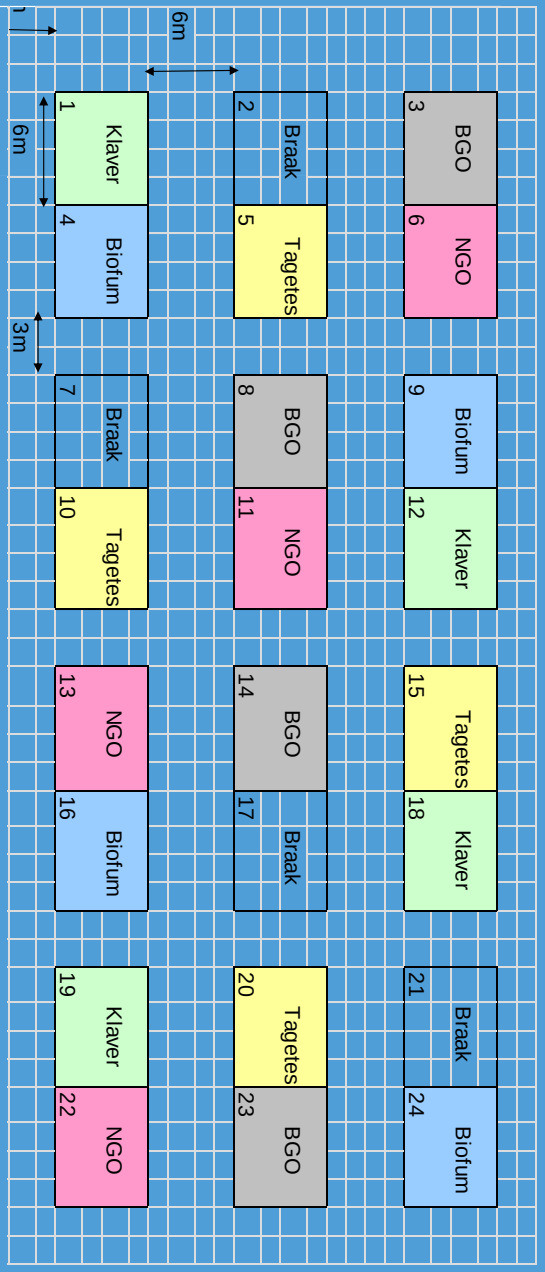
- 2 experimental fields

- Several treatments compared
  - **Biological soil disinfestation** (BGO)
  - Biofumigation
  - Tagetes + compost
  - Controls: Soil fumigation/Fallow/Clover crop

Project planning

- 2009: Experimental fields established
  - 2 locations with history of VW
    - Randwijk: clay soil
    - Vredepeel: sandy soil
  - Soil treatments
    - 6 treatments
    - 4 repeats

- 2010 - 2011: Test crops grown
  - Randwijk: *Acer platanoides*
  - Vredepeel: *Rosa canina* 'Pfander'



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1. Biological Soil Disinfestation (BGO)

- 40 ton/ha fresh cut grass (*Lolium perenne*)
- 20-25 cm deep rototilled
- 30-40 mm water added
- Tarped with plastic
- 6-10 weeks treatment → before August 1

- Toxic break-down products
- Anaerobic conditions

2: Growing Tagetes + compost

- *Tagetes patula*
  (cv. Ground control)
- 6 kg/ha
- About 3 months
- Weed-free
  +
- Compost: 75 ton/ha
- 20-25 cm rototilled

- Direct lethal action on *Pratylenchidae*
- Addition of organic material

3: Biofumigation

- Sarepta mustard (6-8 wks)
  (*Brassica juncea* cv. TerraFit)
- After reaching flowering stage
  - Fragmented/macerated (end Aug)
  - Incorparated in soil
  - Irrigated
  - Soil consolidated (light roll)

- Decomposition → Glucosinolaten (Isothiocyanate ITC)
- ITC: toxic for nematodes/soil fungi
- Increased level organic matter

4: Chemical soil disinfestation (neg. control)

- Metam Natrium (Monam)
  - Methylisothiocyanate
  - 750 L/ha
  - Mixed through soil (20-25 cm depth)

- Toxic for all soil organisms

5: Clover (positive control)

- Fixation of N

  → positive for soil life

- Increase of organic matter in soil

- Host of Vd and Pp
  - Positive control

6: Fallow (control)

- Soil kept weed free
  - Mechanically/chemically

- Natural decrease of Vd and Pp populations in soil

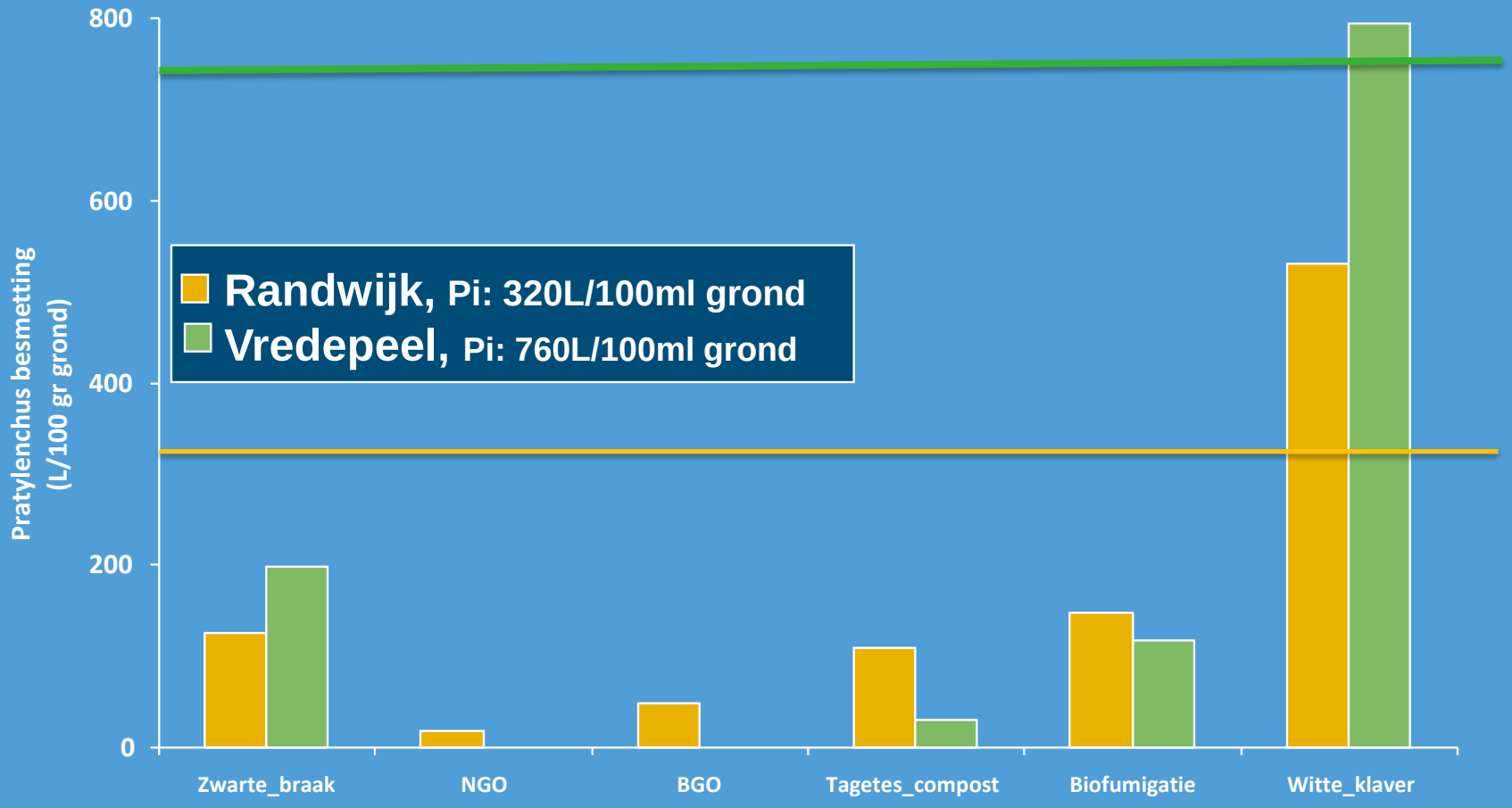
  (Negative control)

Experimental fields Randwijk & Vredepeel

Observations

- Soil samples
  - Vd & Pp
  - Pi (7/2009) & Pf (10/2009)
- Disease incidence in test crops
  - Foliar symptoms (2010-2011)
  - Vascular discolouration (2011)
- Growth of test crops

Soil samples: Pratylenchus penetrans



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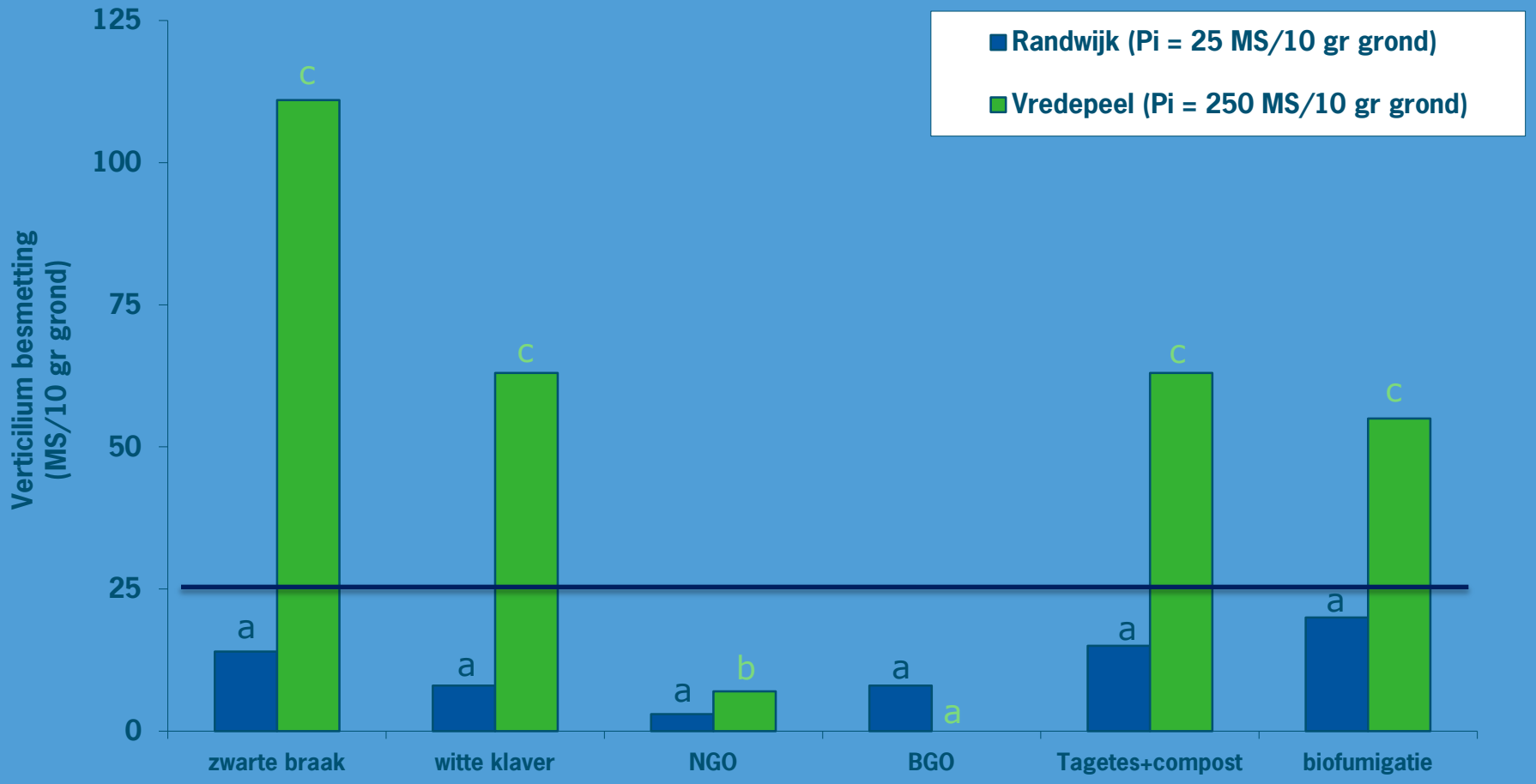
Soil samples: Verticillium dahliae



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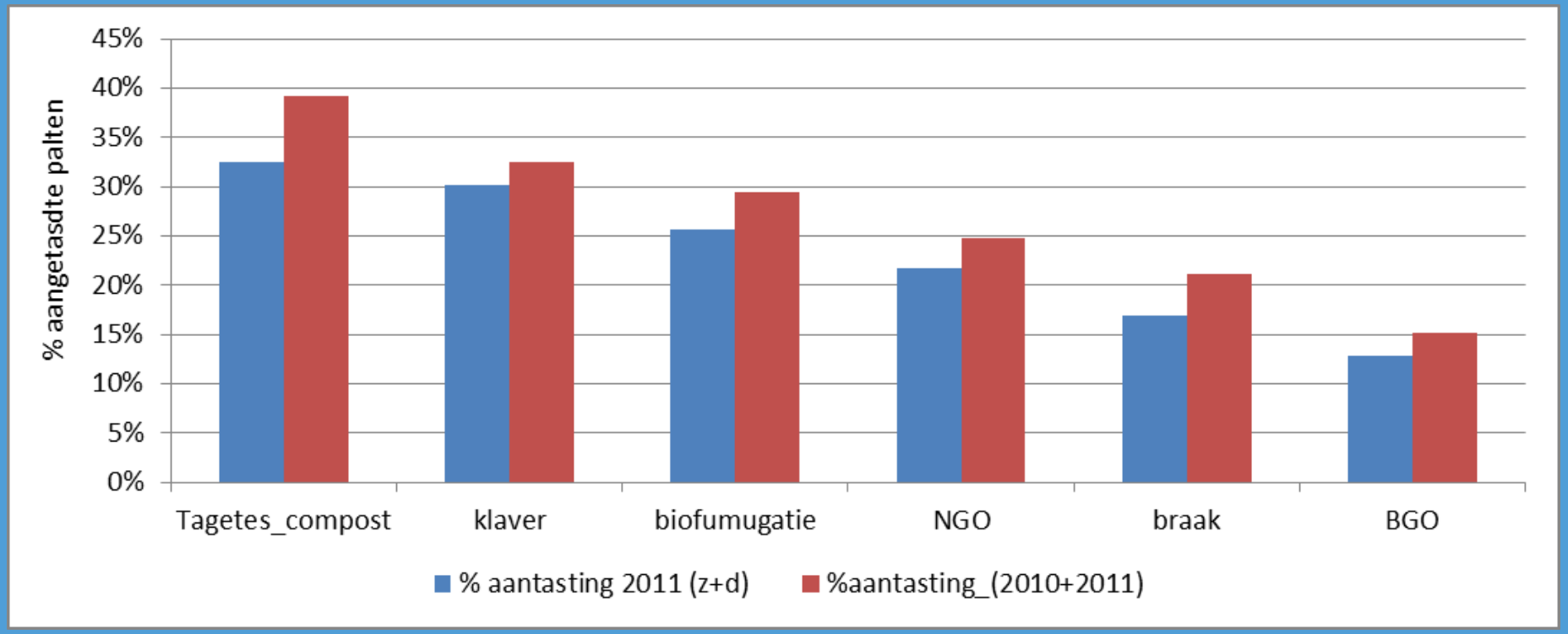
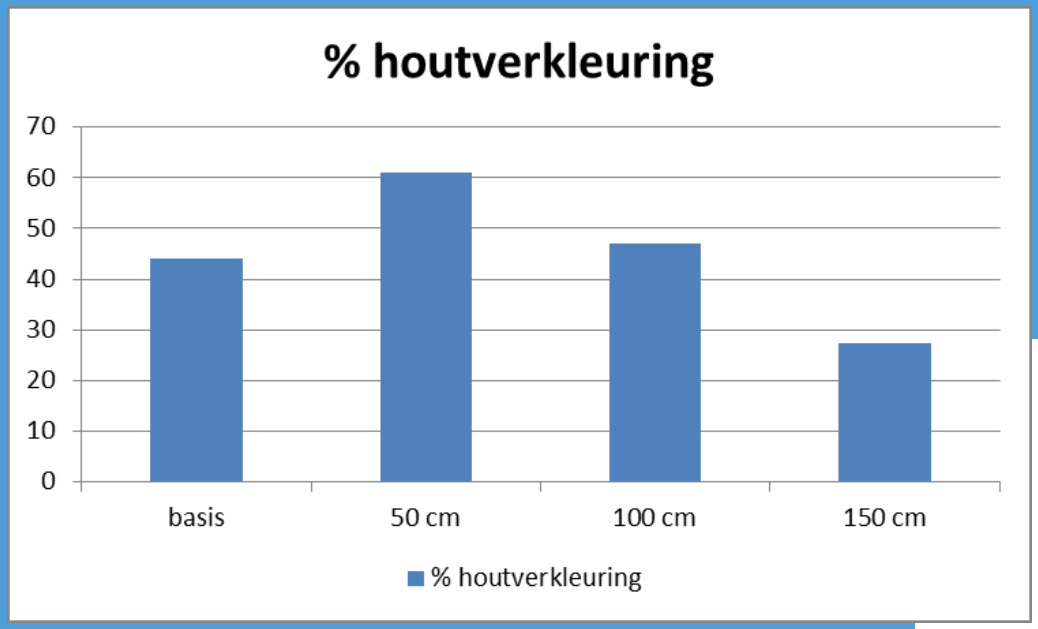
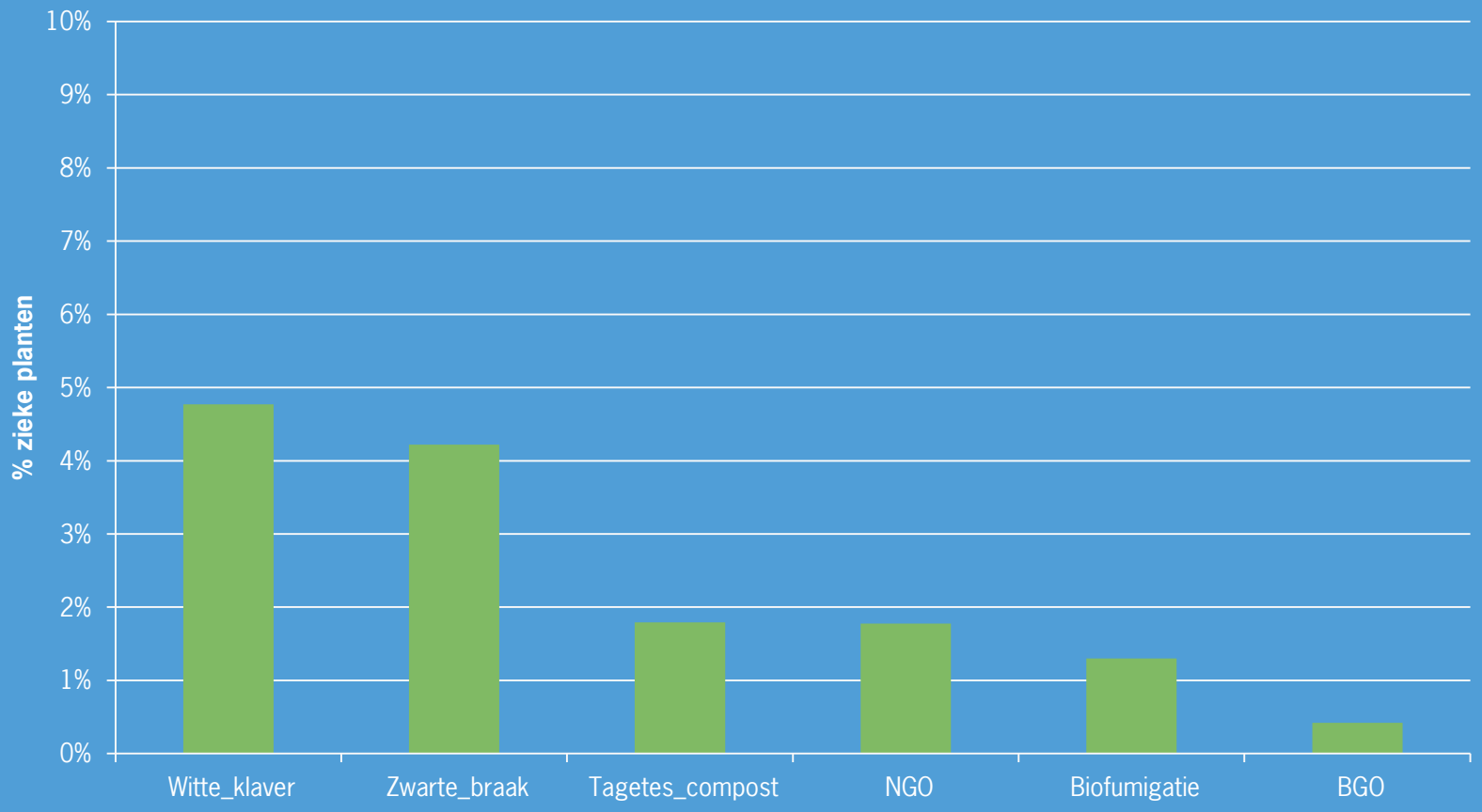
Disease incidence in maple (clay soil)



Vascular discolouration in maple



Disease incidence in rose (sandy soil)



- Final recording after harvesting and check for discolouration

Conclusions (1)

- BGO on clay soil with maple as test crop:
  - Decreased number of nematodes (large variation)
  - Decreased Vd ID in soil (→ large variation)
  - Lowest DI (→ large variation)
  - No neg. effect on growth
- However
  - Effects statistically not significant
  - Remaining ID still results in disease
  - Costs high (2500 – 4000 €/ha)

→ Without improvement (how?) no perspective for use of BGO on clay soil in practice of tree nursery

Conclusions (2)

- BGO on sandy soil with rose as test crop:
  - Very good control of nematodes (comparable to fumigation)
  - Very good control of Vd ID in soil (best of all treatments)
  - Almost no disease in next year
  - No neg. effect on growth
- However
  - Costs high (2500 – 4000 €/ha)

→ Good perspectives for control of Vd in tree nursery crops on sandy soil through BGO, but only for high value crops

Future: Test with trees on sandy soil

	Pi	Pe
Tagetes -1	7	0
Tagetes-2	3	3
BGO-1	3	0
BGO-2	10	0

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Thank you for your attention

Questions ?

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