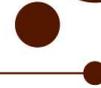




Department of Agriculture and Ecology



# **Distribution patterns of fungal entomopathogens in soil habitats:**

## **Natural occurrence, diversity, dynamics**

**Nicolai V. Meyling**



## Assessing diversity in soils

- Isolation methods

## Patterns of distribution

- Agricultural vs. natural habitats
- Horizontal distribution

## Dynamics of soil reservoir

- Cycling between below and above ground environments

## Molecular characterization

- Species identification
- Emergent patterns and implications



## **Natural occurrence on fungal entomopathogens in soil habitats – why ?**

### **Reservoir and buffer environment**

- **Natural enemies – targets for conservation  
biological control strategies**
- **Effects of management practices on fungal  
populations**
- **Find indigenous isolates for biological control**
- **Predict effects of augmented biocontrol  
strains**



## Isolation from soil environment

### Insect bait methods

- Entomopathogenic isolates
- Standardized approach?
- Which insects?



### Selective *in vitro* media

- Detection levels?
- How selective?



*"... the Galleria bait method tends to be more sensitive than the (in vitro) isolation method."*

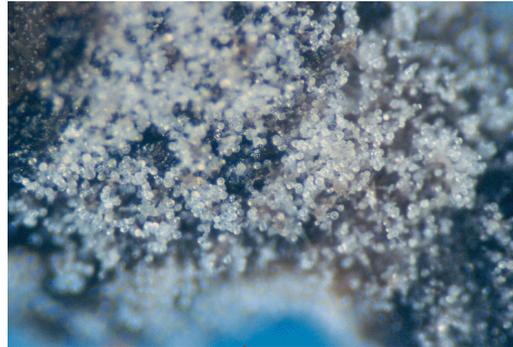
From Keller *et al.* (2003) *BioControl*, 48, 307-319





*Isaria fumosorosea*

*Conidiobolus coronatus*



*Metarhizium anisopliae*



*Metarhizium flavoviride*



*Beauveria bassiana*



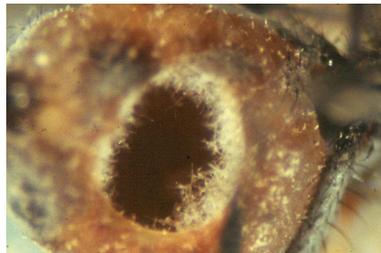
*Hirsutella nodulosa*



*Isaria farinosa*



# Host range and specialization



## Host range: using the target pest

		<i>Tolypocladium cylindrosporium</i>	<i>Metarhizium anisopliae</i>	<i>Beauveria bassiana</i>
				
<i>Delia floralis</i> (Diptera)		+	+	-
<i>Galleria mellonella</i> (Lepidoptera)		-	+	+

Based on Klingen *et al.* (2002) *Agriculture, Ecosystem and Environment*, 91, 191-198



## Host range: baiting for Entomophthorales

	<i>Cereal field</i>	<i>Grass</i>	<i>Beneath Bird Cherry trees</i>
<b><i>Pandora neoaphidis</i></b>	++	+	+
<b><i>Conidiobolus obscurus</i></b>	+	+	+



Based on Nielsen *et al.* (2003) *Biological Control*, 28, 92-100



# Temperature: baiting conditions



Habitat and Fungus	18°C	25°C
<u>Forest soil</u>		
<i>M. anisopliae</i>	0 %	7 %
<i>B. bassiana</i>	1 %	12 %
<u>Agricultural field</u>		
<i>M. anisopliae</i>	5 %	38 %
<i>B. bassiana</i>	53 %	10 %
<u>Fallow field</u>		
<i>M. anisopliae</i>	35 %	80 %
<i>B. bassiana</i>	18 %	0 %

Based on data from Mietkiewski and Tkaczuk (1998) *IOBC/WPRS Bulletin*, 21 (4), 41-44



## Distribution of fungal entomopathogens: habitat associations

- Which species in which habitat?
- We define the habitats and characteristics
- We define the parameters –
  - are they important from the fungus point-of-view?
- Correlations = causation?



Frequency of occurrence (%)

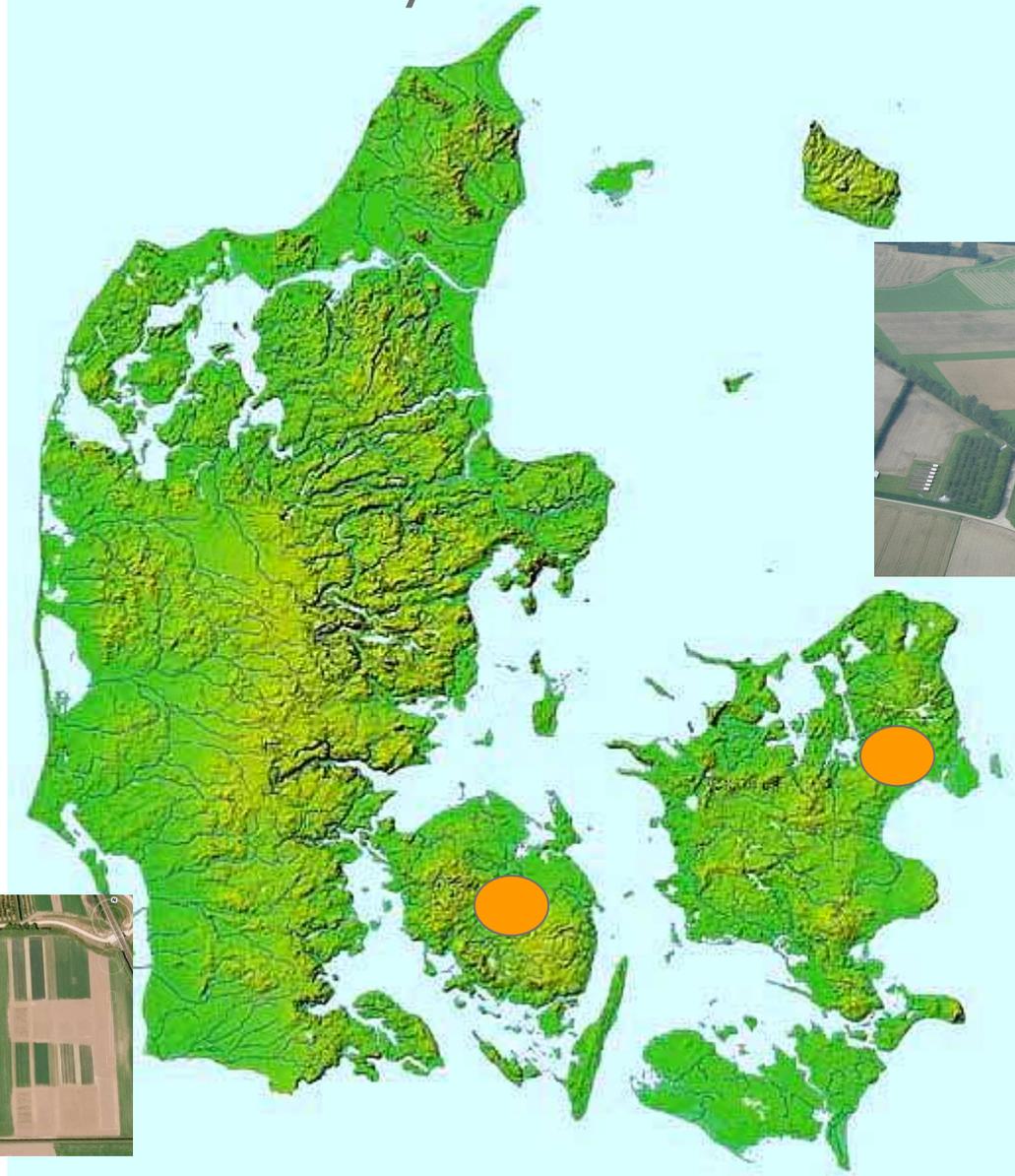
<b>Country</b>	<b>Fungus</b>	<b>Cultivated habitat</b>	<b>"Natural" habitat</b>	<b>Reference</b>
<b>Denmark</b> 55°N	<i>B. bassiana</i>	<b>38.5</b>	<b>52.9</b>	Steenberg (1995)
	<i>M. anisopliae</i>	<b>51.3</b>	<b>7.8</b>	
	<i>I. fumosorosea</i>	<b>2.6</b>	<b>9.8</b>	
<b>Finland</b> 62°N	<i>B. bassiana</i>	<b>5.6</b>	<b>28.1</b>	Vänninen (1995)
	<i>M. anisopliae</i>	<b>14.9</b>	<b>24.2</b>	
	<i>I. fumosorosea</i>	<b>0.5</b>	<b>1.7</b>	
<b>UK</b> 52°N	<i>B. bassiana</i>	<b>1.0</b>	<b>7.7</b>	Chandler et al. (1997)
	<i>M. anisopliae</i>	<b>1.0</b>	<b>1.3</b>	
	<i>I. fumosorosea</i>	<b>0.0</b>	<b>3.3</b>	
<b>Canada</b> 45°N	<i>B. bassiana</i>	<b>~35</b>	<b>~65</b>	Bidochka et al. (1998)
	<i>M. anisopliae</i>	<b>~63</b>	<b>~36</b>	
<b>China</b> 40°N	<i>B. bassiana</i>	<b>27.4</b>	<b>86.3</b>	Sun et al. (2008)
	<i>M. anisopliae</i>	<b>60.0</b>	<b>26.4</b>	
	<i>I. fumosorosea</i>	<b>15.6</b>	<b>37.5</b>	
<b>Spain</b> 40°N	<i>B. bassiana</i>	<b>~34</b>	<b>~53</b>	Quesada-Moraga et al. (2007)
	<i>M. anisopliae</i>	<b>~10</b>	<b>~4</b>	

## Habitat associations: regional scales

- "Natural" habitats
  - *B. bassiana* and *I. fumosorosea*
- Agricultural fields
  - *M. anisopliae*
- Many "similar" habitats sampled
  - Comparability assumed
- Generalizations from regional scales?
  - Local scale diversity?



# Locality specific diversity and distribution: Denmark



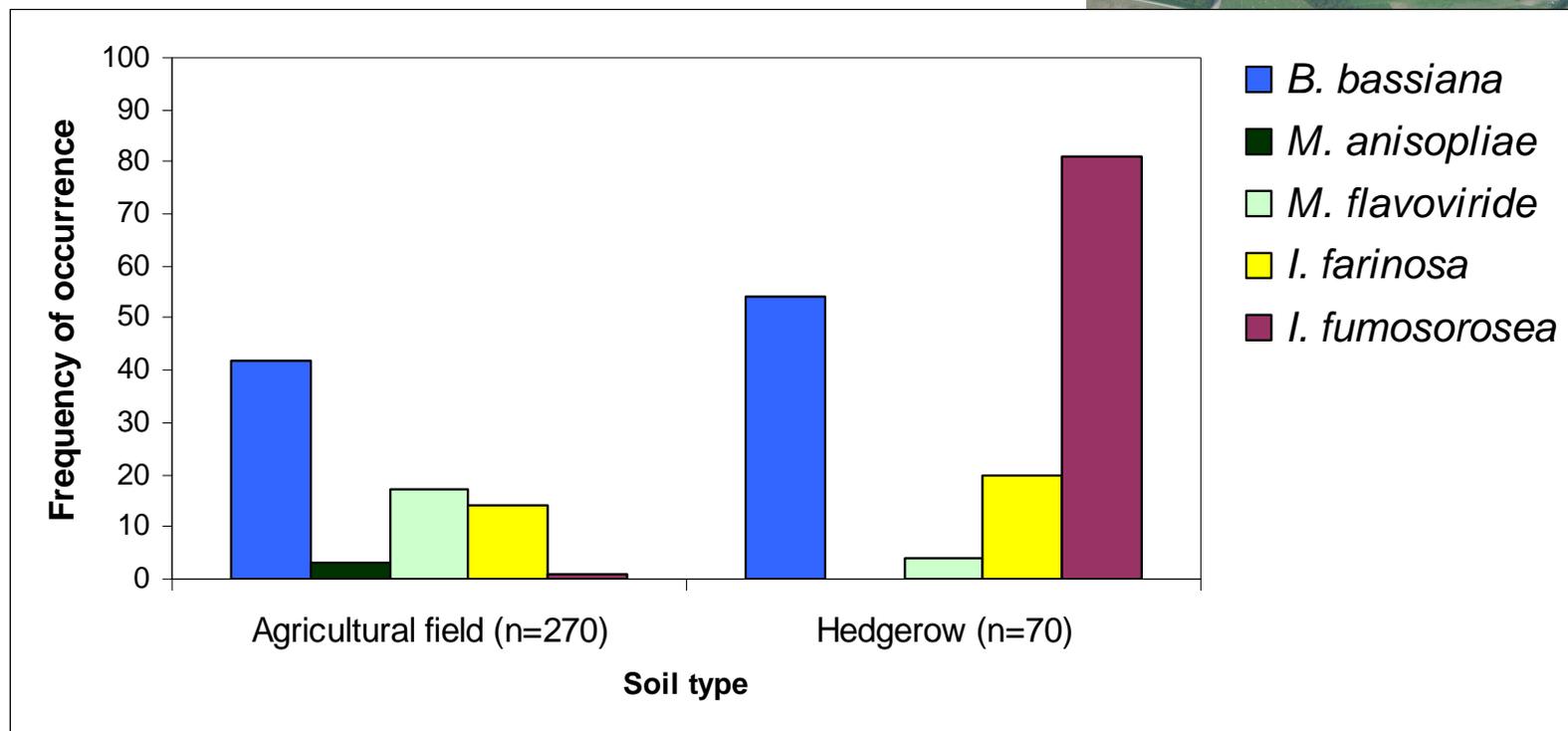
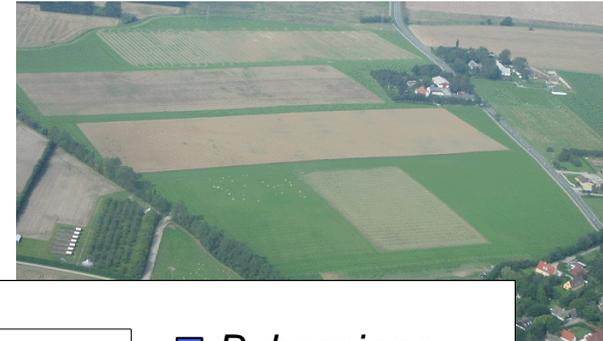
Taastrup  
(Bakkegården)

Årslev



# Taastrup, Bakkegården 2002

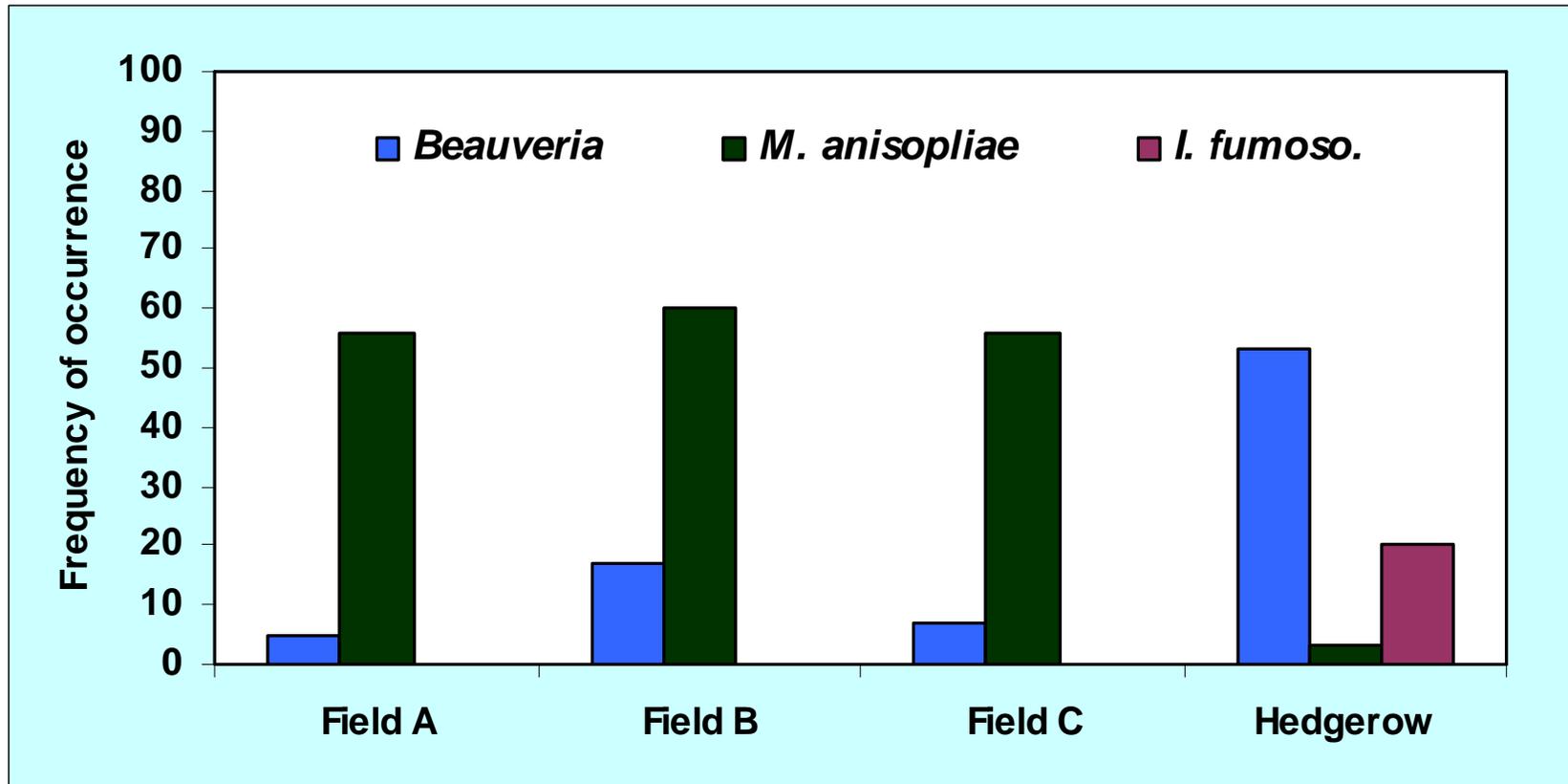
*B. bassiana* dominating in field



From Meyling and Eilenberg (2006) *Agriculture, Ecosystem and Environment*, 113, 336-341



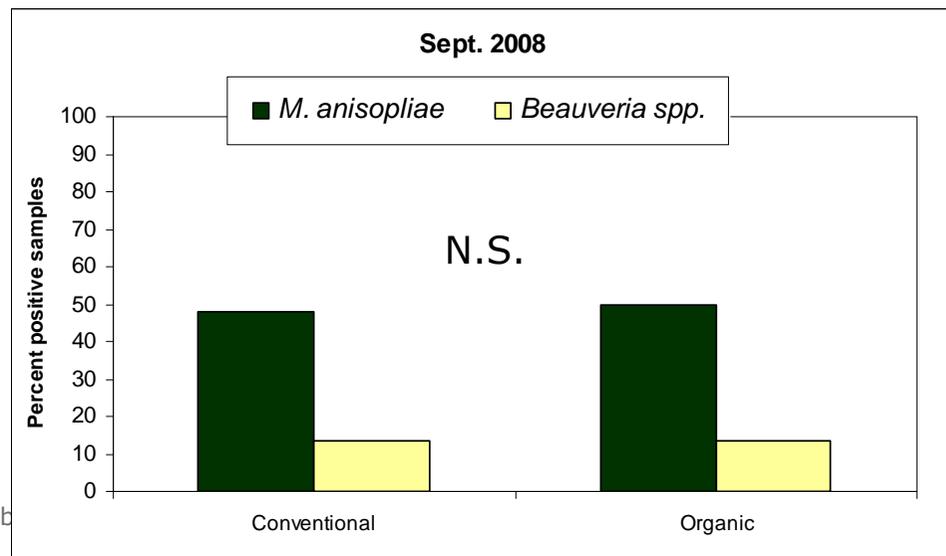
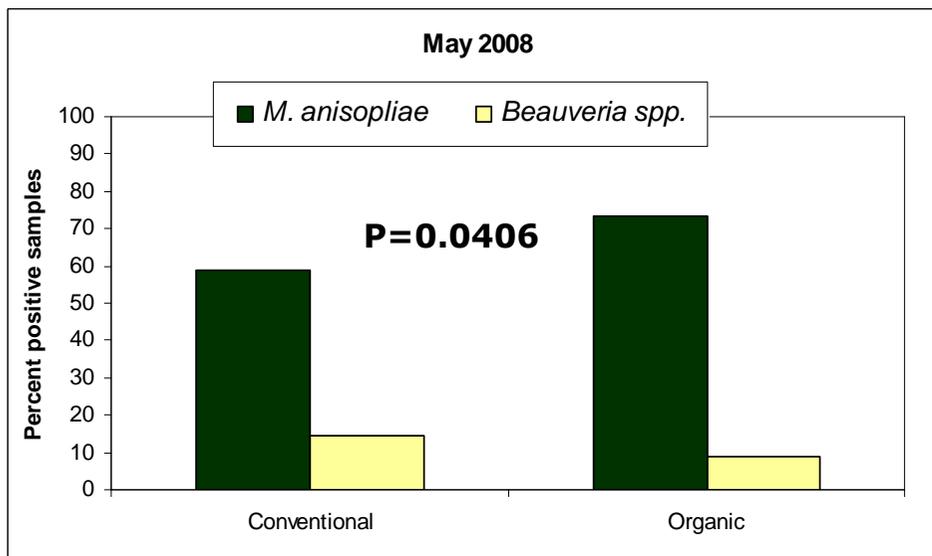
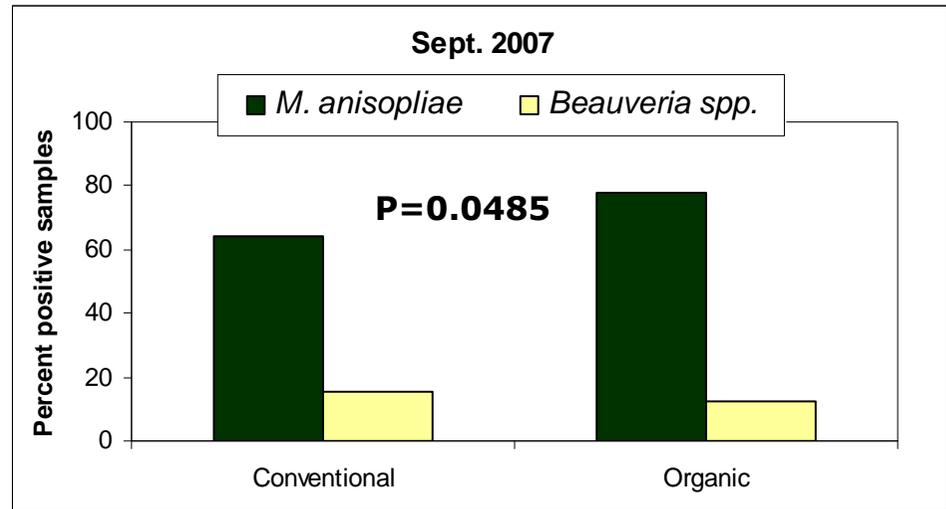
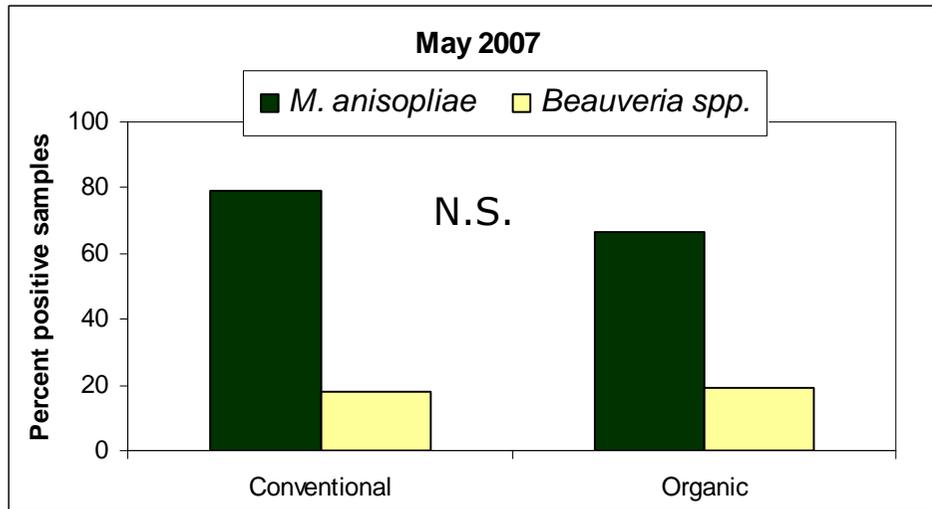
## Årslev 2006



Unpublished data

*M. anisopliae* dominating in field

# Agricultural practices: conventional vs. organic (Årslev)

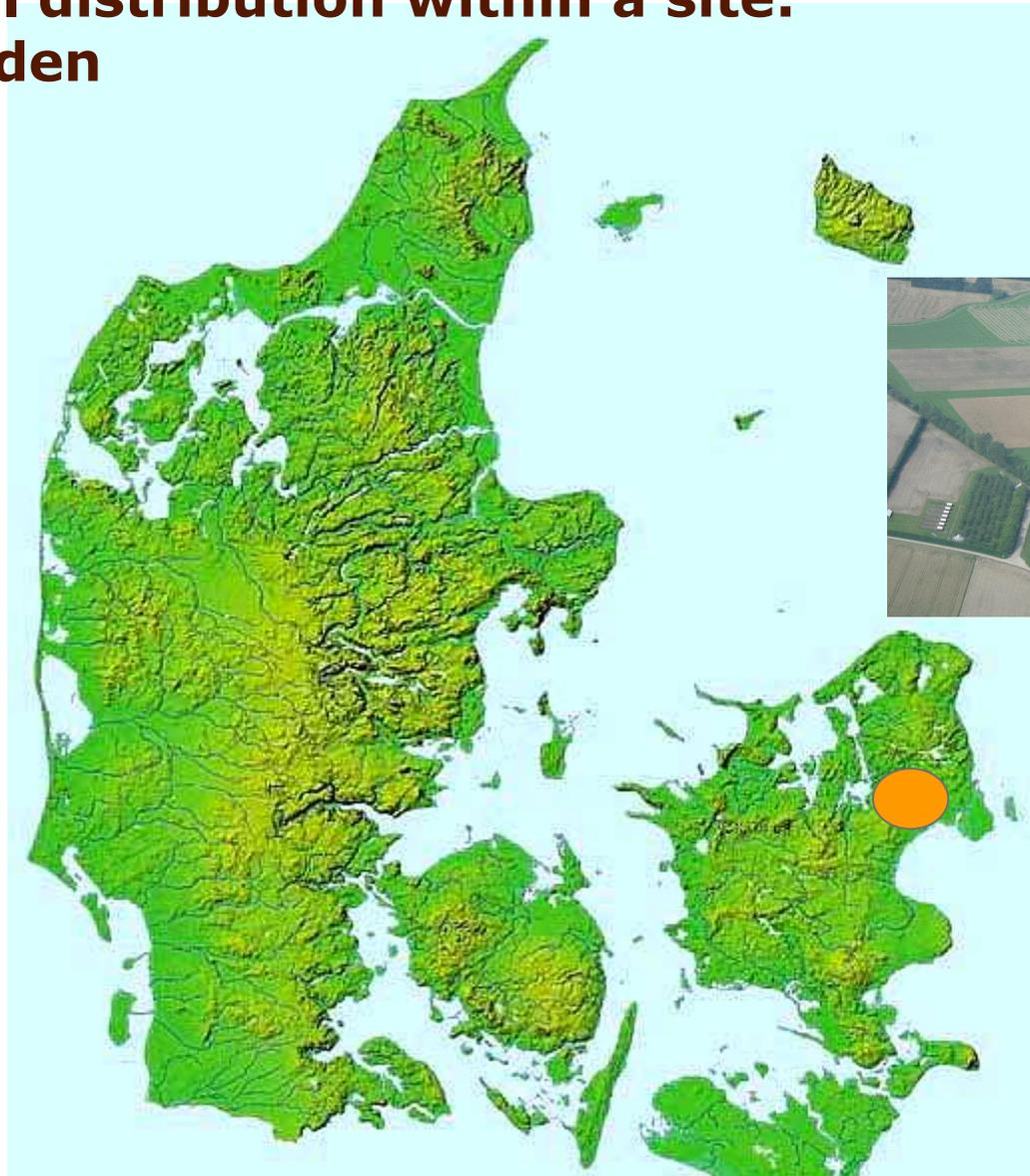


## Horizontal distribution within a site

- Where to sample ?
- Identification of patches
- Size of patches: distance between samples
- When do we have enough samples ?
- Patch dynamics ?



## Horizontal distribution within a site: Bakkegården

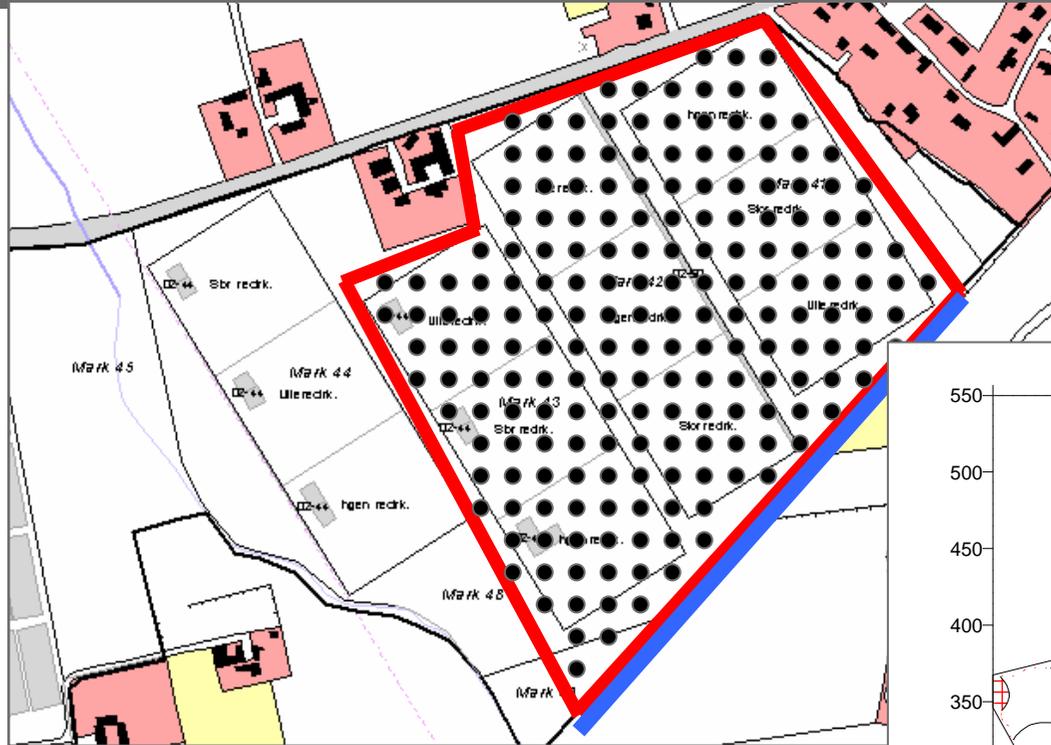


Taastrup  
(Bakkegården)

**Meyling and Eilenberg (2006) *Agriculture, Ecosystem and Environment*, 113, 336-341**

SIP Utah 2009. Fungus Division Symposium 'Fungi in Soil Habitats'  
Slide 18





GIS coordinates  
(n=274)

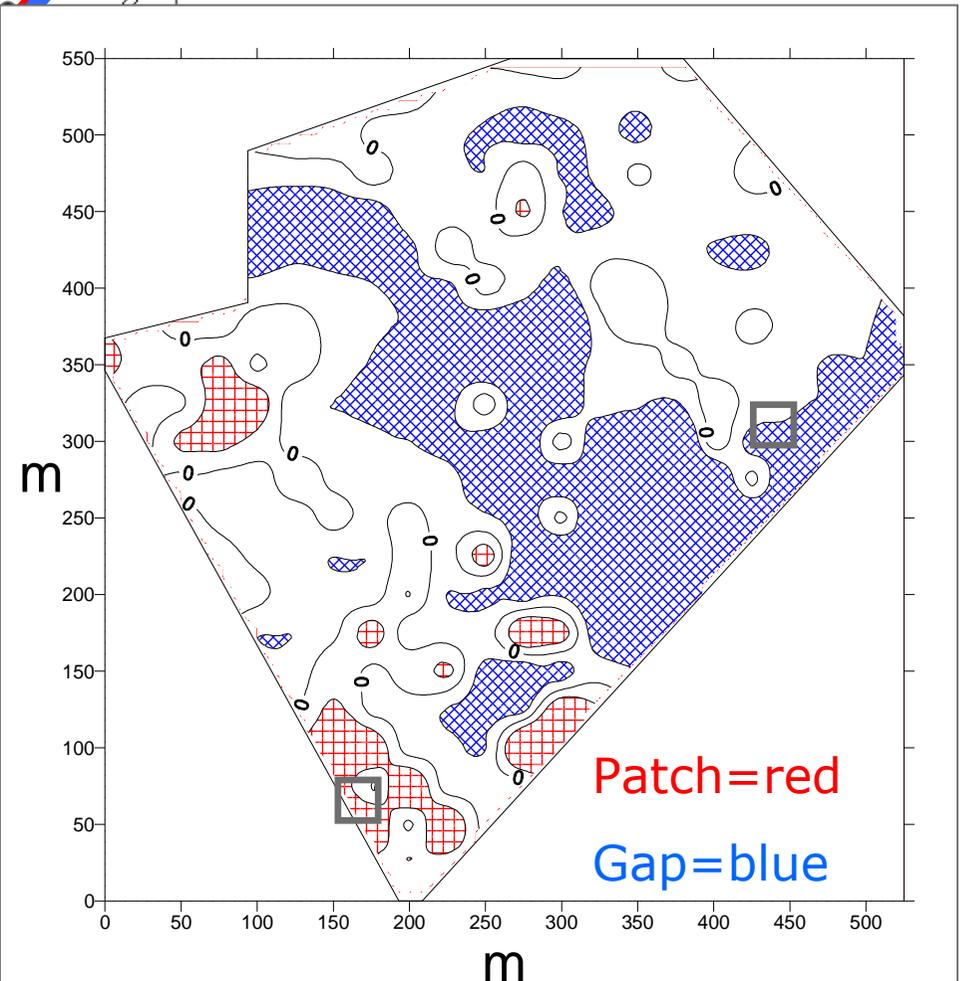
Location by GPS

***B. bassiana*: spatial statistics**

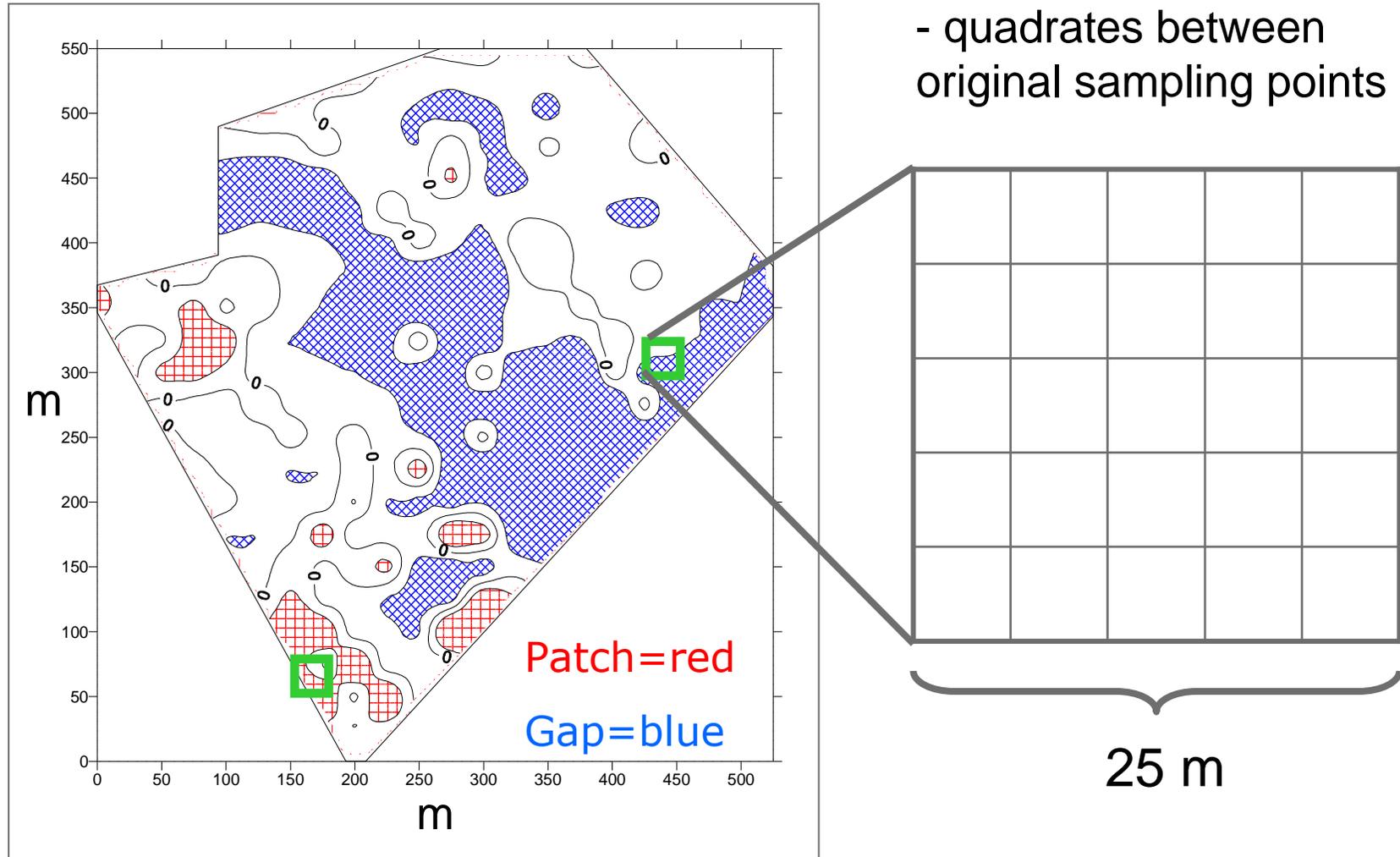
$$V_j = -1.686; p = 0.0012$$

$$V_i = 1.772; p = 0.0003$$

Significant clustering



## Have we found the distribution pattern ?



From Meyling and Eilenberg (2006) *Agriculture, Ecosystem and Environment*, 113, 336-341



## Percent positive samples from 'Patch' and 'Gap' quadrates

	'Patch'	'Gap'	Chi <sup>2</sup>	P
25x25 (n=25)				
All fungi	84	36	12.00	0.0005
<i>B. bassiana</i>	68	16	13.88	0.0002

From Meyling and Eilenberg (2006) *Agriculture, Ecosystem and Environment*, 113, 336-341

Reducing distance between sampling points confirmed results from the whole field assessment



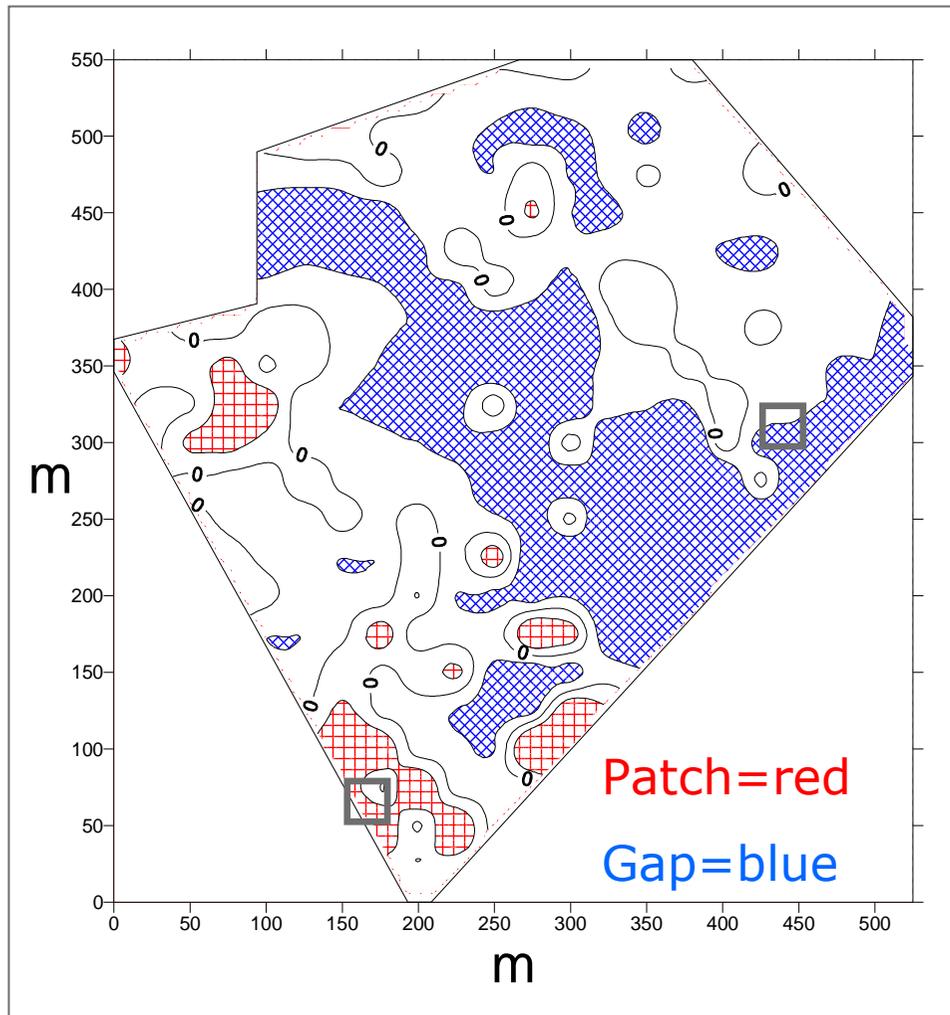
## Continuity of 'Patch' and 'Gap' quadrates 2003-2006

(n=25)	2003	2006	Chi <sup>2</sup>	P
All fungi				
'Patch'	84	96	2.00	0.1573
'Gap'	36	48	0.74	0.39
<i>B. bassiana</i>				
'Patch'	68	80	0.9356	0.3334
'Gap'	16	32	1.7544	0.1853

Based on MSc thesis 2007 by Vibeke Ærø Hansen



## Horizontal distribution at Bakkegården



- *B. bassiana* patches and gaps:
  - identified
  - persistent in time
- Why patches ?
- Where to get representable sample from this site?



## **Fungal entomopathogens in soils: do they go above ground?**

### Same fungal species in soil and insects

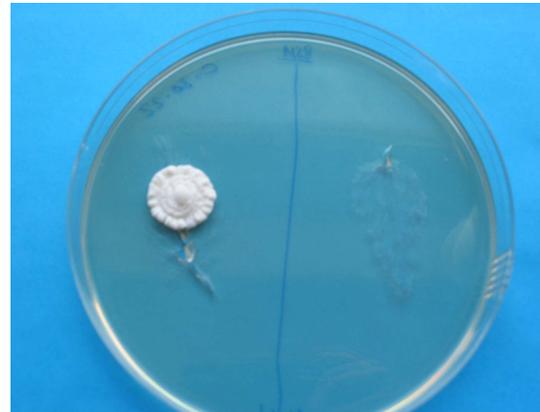
- Are they really the same ?
- Do similar genotypes cycle below and above ground ?

### Molecular identification

### Co-occurrence in time and space



## Bakkegården – *Beauveria* spp. below and above ground

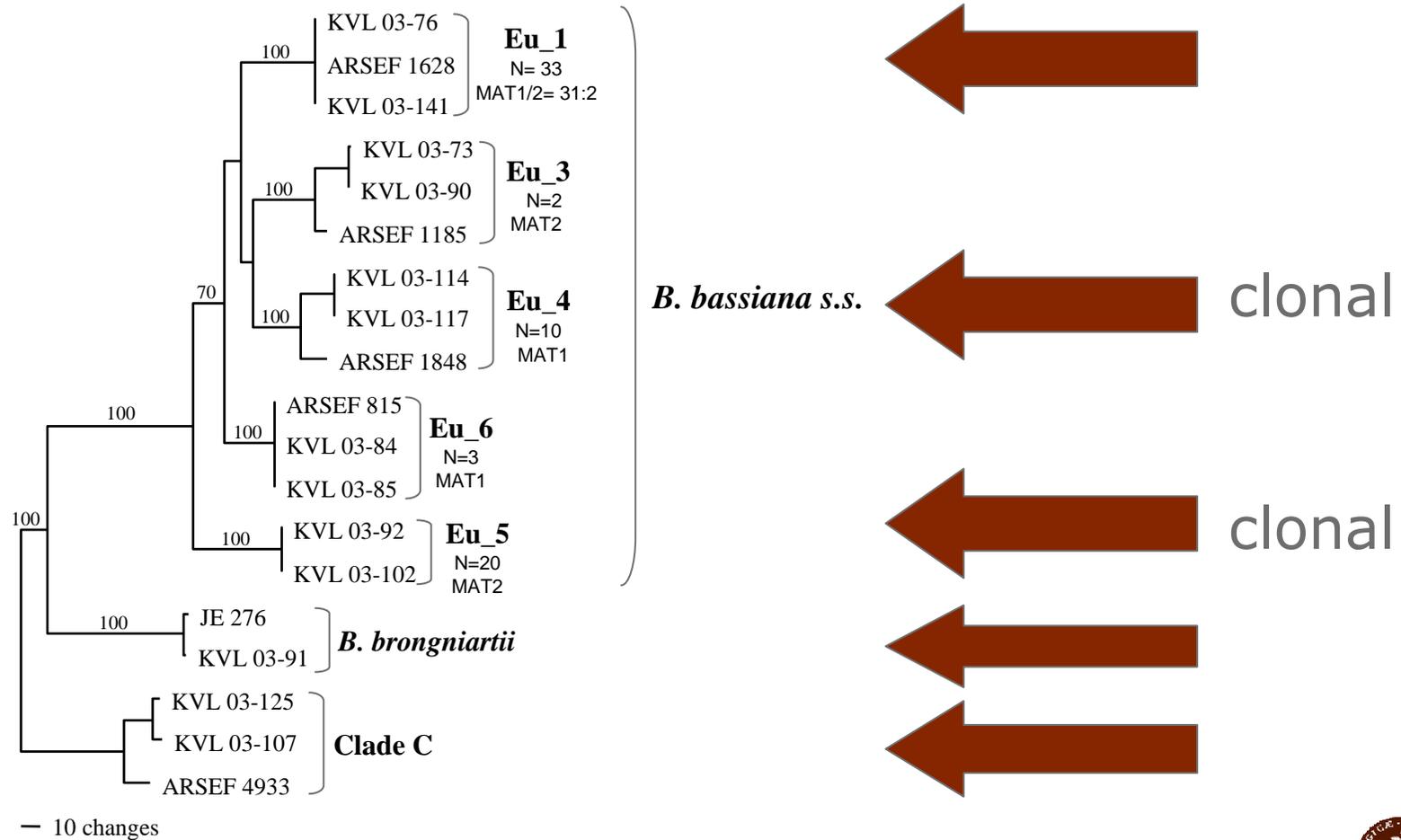


*Beauveria bassiana* morphospecies = cryptic species complex

- Single locality (hedgerow)
- Local insect community
- Host plants in hedgerow
- Soil of hedgerow



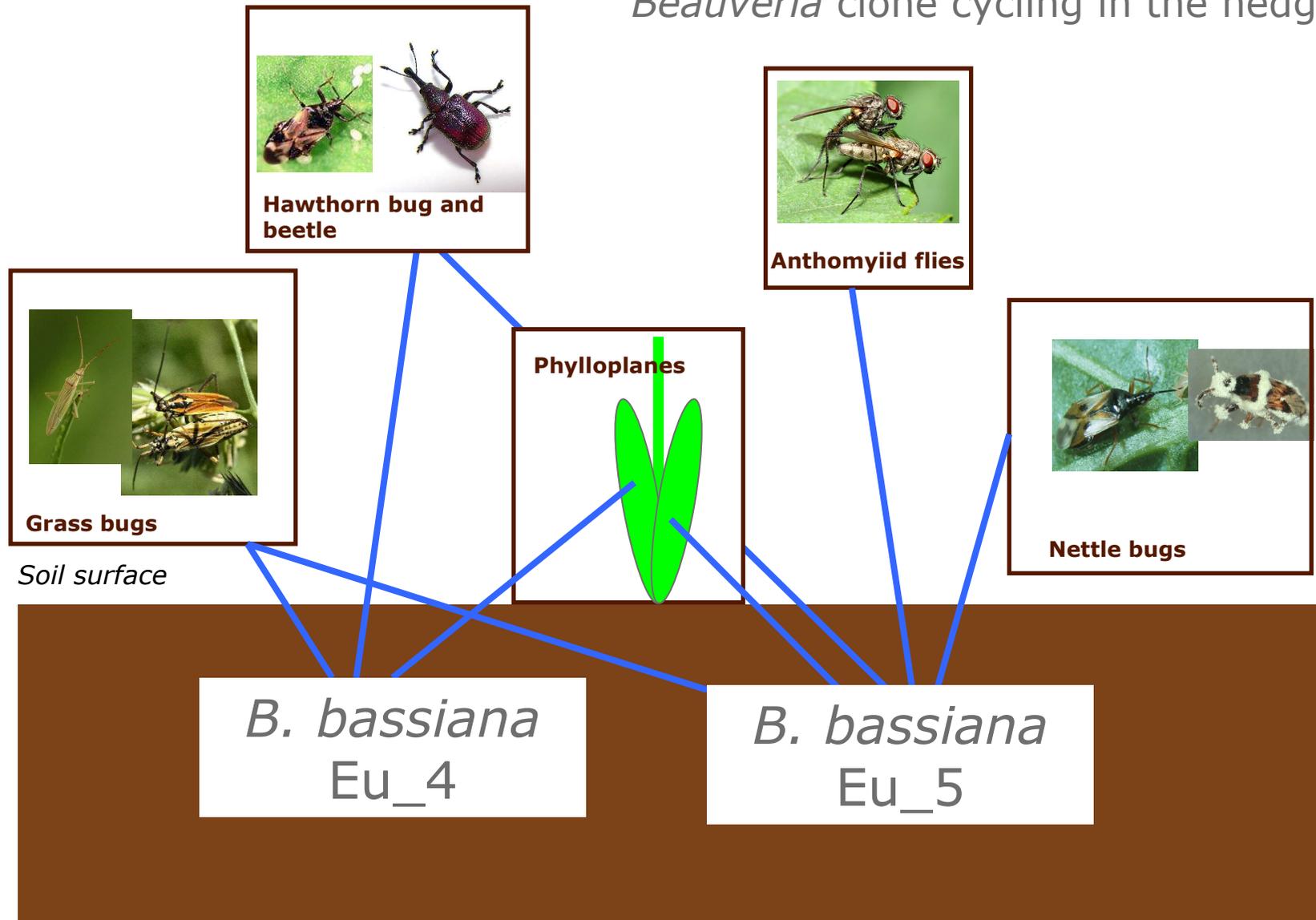
# Molecular diversity of *Beauveria* community



Meyling *et al.* (2009) *Molecular Ecology*, 18, 1282-1293



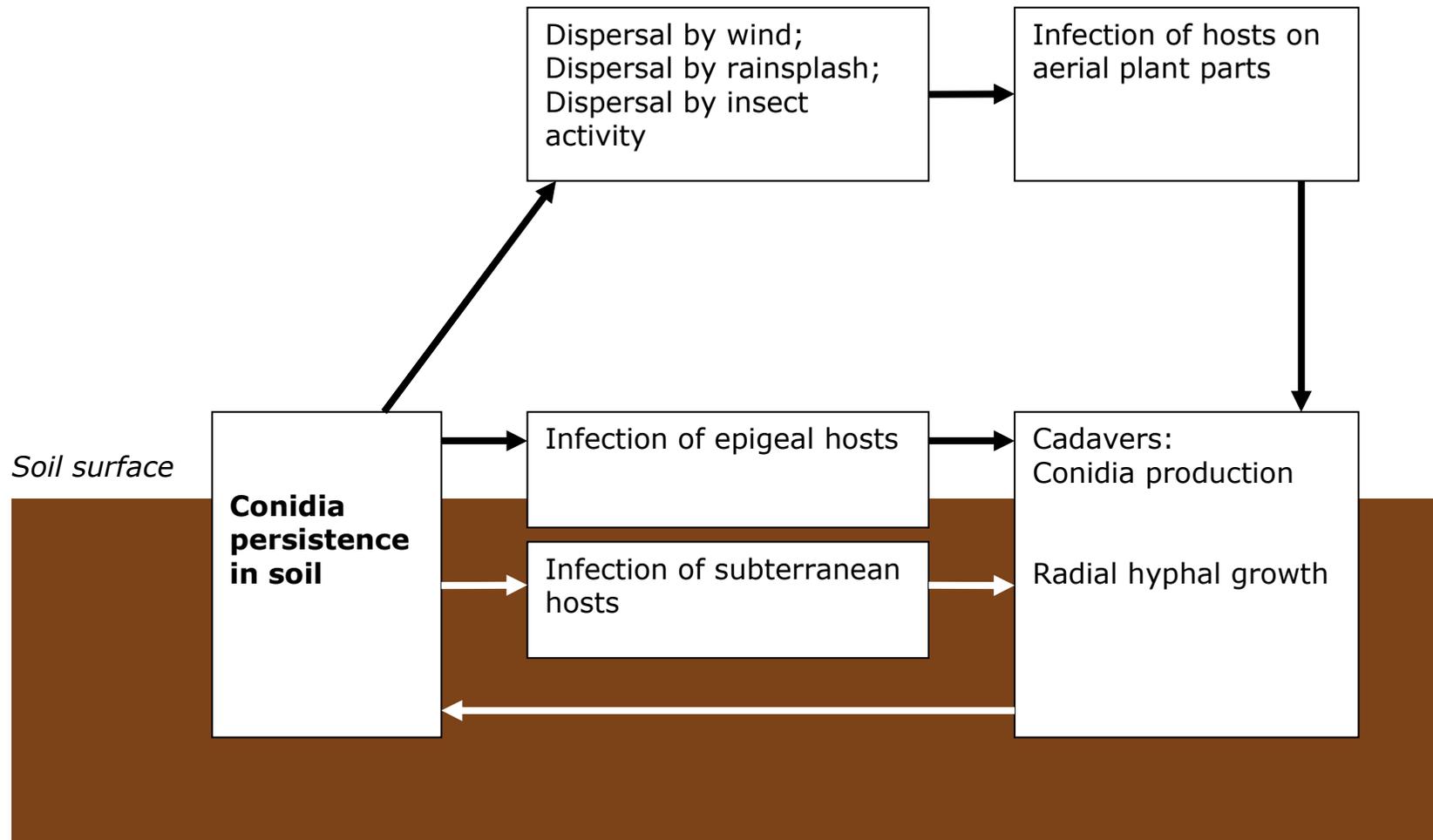
# Beauveria clone cycling in the hedgerow



Based on Meyling *et al.* (2009) *Molecular Ecology*, 18, 1282-1293



## *Beauveria bassiana* dynamics

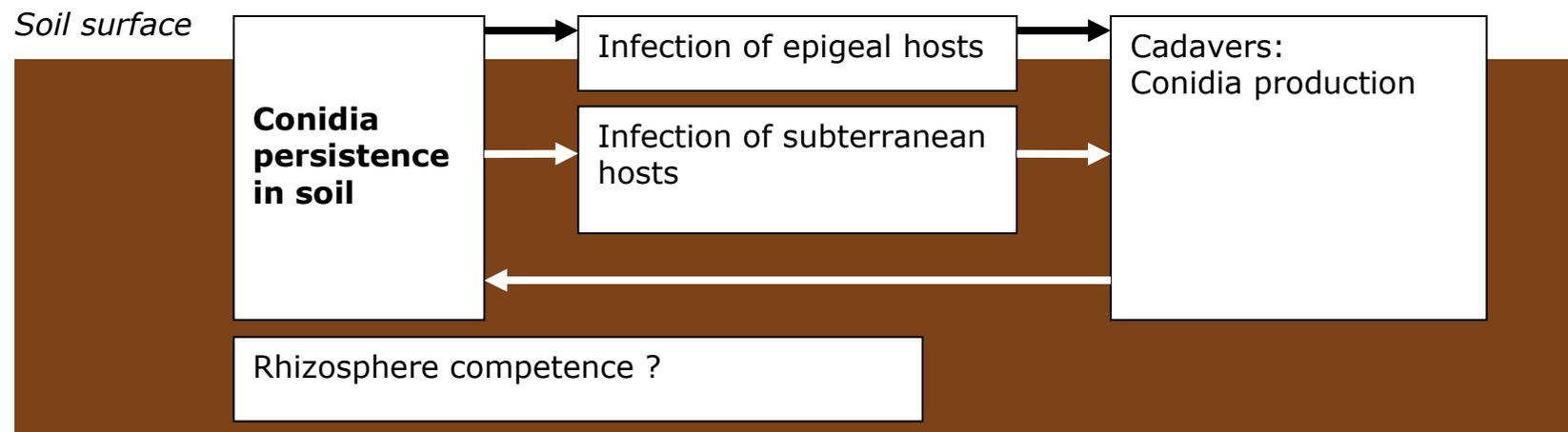


Meyling & Eilenberg (2007). *Biological Control* 43: 145-155



## *Metarhizium anisopliae* dynamics

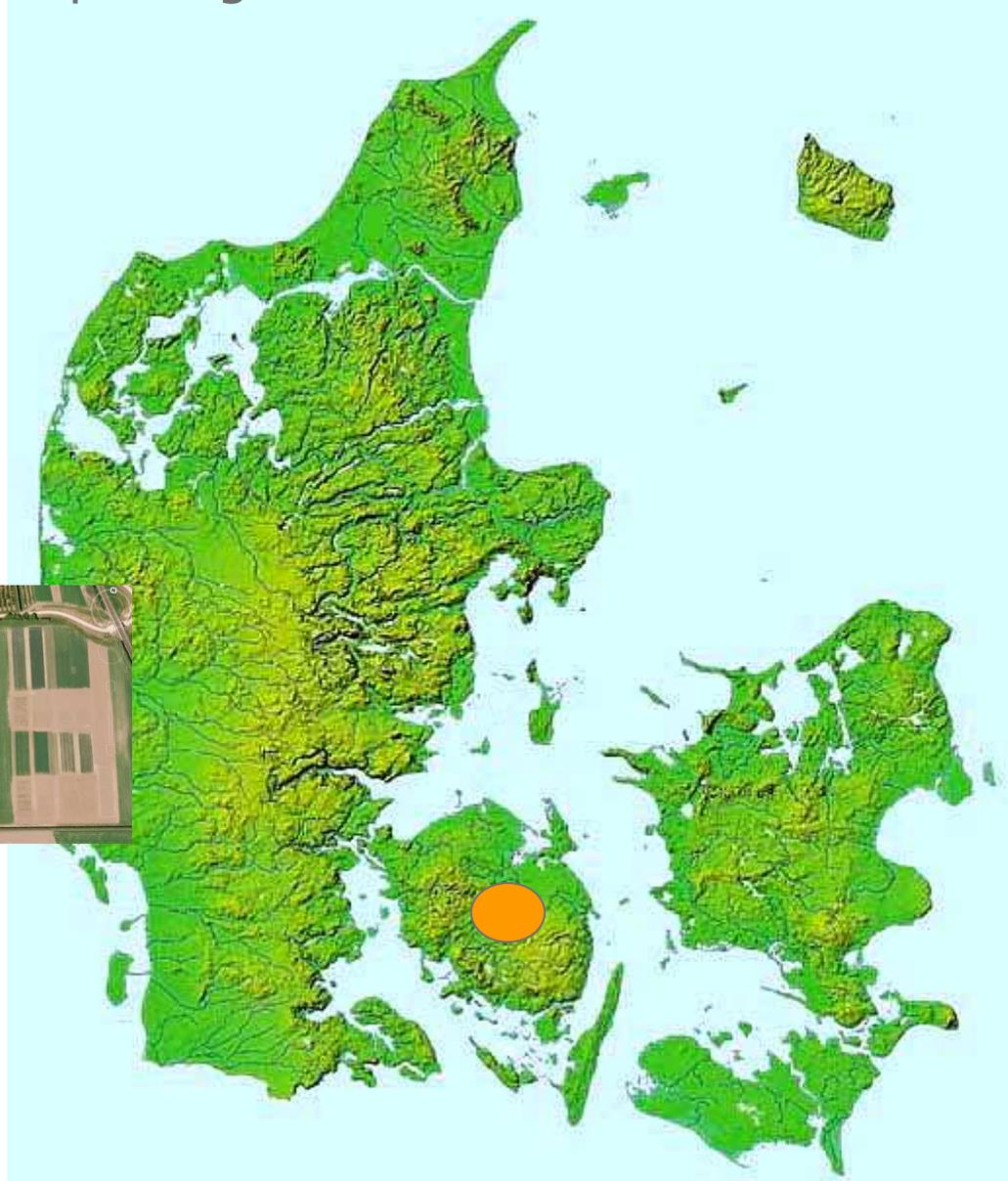
- no above ground cycling?



**Meyling & Eilenberg (2007). *Biological Control* 43: 145-155**



# Fungal entomopathogen communities above and below ground



Årslev



## Soil sampling and collection of arthropod fungus-cadavers



Below ground =  
above ground ?



# High arctic environments - Greenland

## Disko:

*I. farinosa*  
*B. bassiana*  
*T. inflatum*

## Ritenbenk:

*I. fumosorosea*  
*I. farinosa*  
*B. bassiana*



## Zackenberk:

*I. fumosorosea*  
*I. farinosa*

## Danmarkshavn:

*I. fumosorosea*  
*I. farinosa*  
*B. bassiana*



# Acknowledgements



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