Diversity of fungi with special reference to the fungus-insect interaction

M.V.Deshpande
National Chemical Laboratory
Pune
mv.deshpande@ncl.res.in

How many species exist on earth?

Group	Known species	Estimated number
Bacteria	4,760	40,000
Fungi*	72,036	15,00,000
Algae	40,000	60,000
Plants	2,67,750	2,95,000
Protozoa	30,800	-
Insects	8,00,000	60,00,000
Other arthropods/ Minor invertebrates	1,32,461	-
Molluscs	50,000	-
Reptiles	6,300	-
Fish	19,000	21,000
Birds	9,198	-
Mammals	4,170	-

****Every year 1500 species of fungi are taxonomically studied***

Evolution of fungus-insect associations

From contact to stable symbiosis

Agonistic - mutualistic relationships

The questions posed?

- Are entomopathogenic fungi only entomopathogens?

- Origins and nature of the use of living insects or other invertebrates by fungi
- Changes and probably future changes in the nature of insect-fungus interaction
- Are they have common origin or coming from unrelated lines of fungal evolution?
- Is it just another source of nutrients (protein rich diet) which is easily available?
- Host jumping from plants-insects- plants or fungi or mammals (??)

- Pathogenecity to non-lethal parasitism

Which came first saprophyte or entomopathogen?

Fungal econutritional group

Host range	Fungal econutritional group	Ability
Specialist	Obligate pathogen	Ability to infect a healthy host
	Facultative pathogen	
Generalist	Opportunistic pathogen	
Non-pathogen	Saprobe	Ability to live saprobically

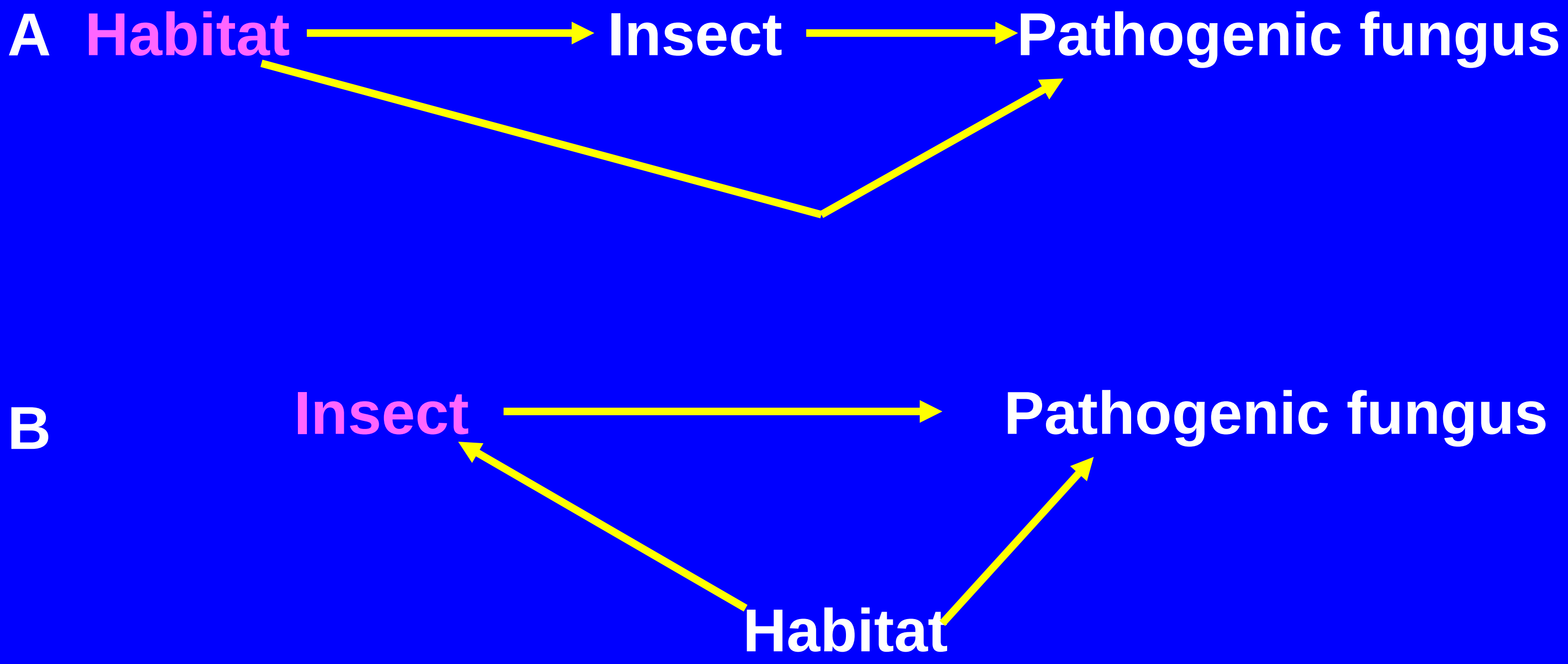
Which came first, saprophyte or entomopathogen?

Phylogenetic studies using 18S rRNA and mating (MAT) genes

Entomopathogens (*Cordyceps bassiana, Cordyceps brongniartii, Cordyceps militaris, Cordyceps sinclairii, Cordyceps takaomontana, Isaria cateniannulata, Isaria farinosa, Isaria fumosorosea,Isaria javanica, Lecanicillium muscarium* and *Torrubiella flava***) are considered as a phylogenetically defined group, and are closely related to mycopathogens (***Lecanicillium psalliotae* and *Verticillium fungicola***)**

Entomopathogens (*Cordyceps cylindrica, Cordyceps subsessilis, Metarhizium anisopliae* and *Nomuraea rileyi***) and pathogens of plants, nematodes and slime molds are relatively related to each other**

Adaptation of proteases and carbohydrases of saprophytic, phytopathogenic and entomopathogenic fungi to the requirements of their ecological niches
Cellulase-chitinase- protease change their specificities, temperature and pH optima, mode of action, etc.



Two models to show relative influence of habitat and insect host on the genetic structure of entomopathogenic fungi

Fungus- fungus and – insect interactions

Dual specificity

Rhizoctonia
Sclerotium

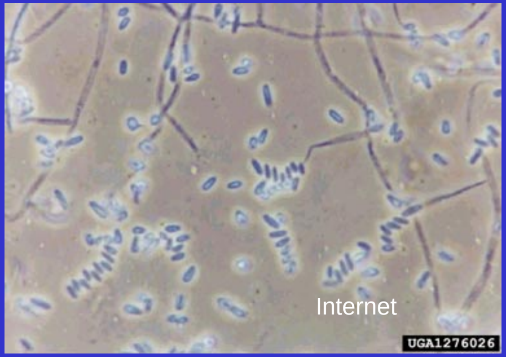
Trichoderma -

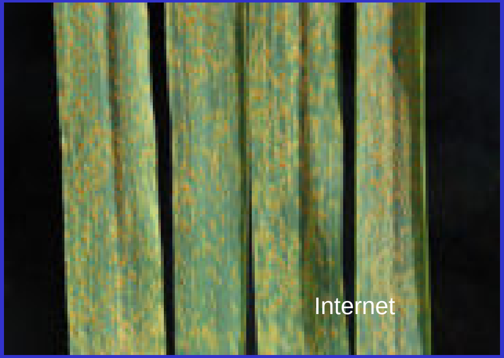
Elm bark beetlle

Fungus- insect and –fungus interactions
Dual specificity

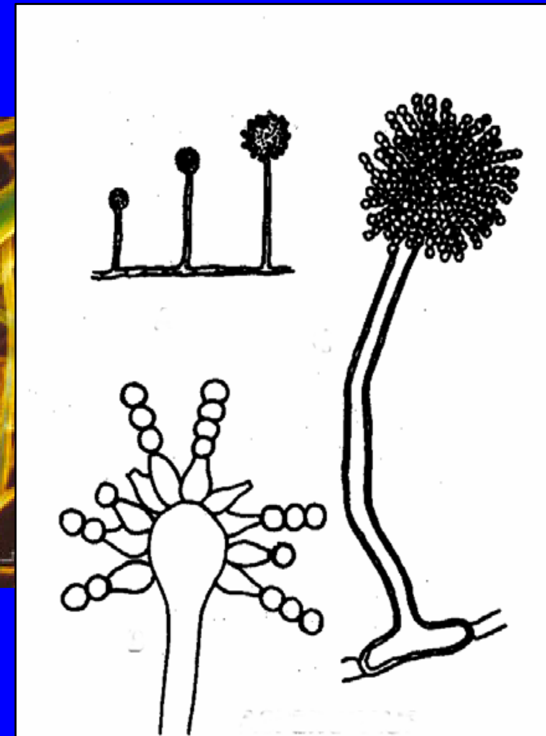
Mealy bugs, aphids and mites

Verticillium lecanii





***Uromyces appendiculatus* bean rust,**
***Uromyces dianthi* carnation rust,**
***Puccinia recondita f. sp. tritici*, wheat leaf rust**
***Puccinia striiformis* stripe rust of wheat**
***Phakopsora pachyrhizi* soybean rust**



Lack of Host Specialization in *Aspergillus*
Less specialized life style

Due to
Broad range of enzymes to attack damaged protective covers sometimes intact covers of plants, animals, insects

What is with our neighbour ?

Diversity of entomopathogens

377 fungi from 46 species and 27 genera

Known insect pathogen

Beauveria bassiana
Metarhizium anisopliae var. anisopliae
Lecanicillium lecanii
Paecilomyces farinosus, P. fumosoroseus
Tolypocladium inflatum

Opportunistic pathogen

Aspergillus flavus
A. sydowii
Cladosporium cladosporioides
Clonostachys rosea f. catenulatum
C. rosea f. rosea
Fusarium avenaceum
F.oxysporum
F. redolens
F. solani
Fusarium sp.
Geomyces pannorum
Gloeotinia temulenta
Lecythophora sp. 2.0
Mariannaea elegans
Mortierella spp.
Mucor spp.
Penicillium brasilianum
P. chrysogenum
P. corprophlium
P. thomii
Pestalotiopsis theae

Secondary colonizer

Absidia glauca
Chaetomium globosum
Clonostachys sp.
Fusarium equiseti
F. sambucinum
F. tricinctum
Penicillium simplicissimum
P. italicum
Periconiella mucunae
Pseudeurotium zonatum
Rhizopus oryzae
Talaromyces flavus
T. trachyspermus
Trichoderma aureoviride
T. koningii
T. parceramosum
T. virens
Williopsis satumus (yeast)
Unidentified yeast

Balsam fir forests infested with spruce budworm

Paecilomyces farinosus
Saprophyte or an opportunistic pathogen

- **Saprophyte**
  Abundance is proportional to the litter

- **Opportunistic pathogen**
  Abundance increases due to spruce budworm

Entomopathogenic fungi
A valuable alternative to fight against insect pests

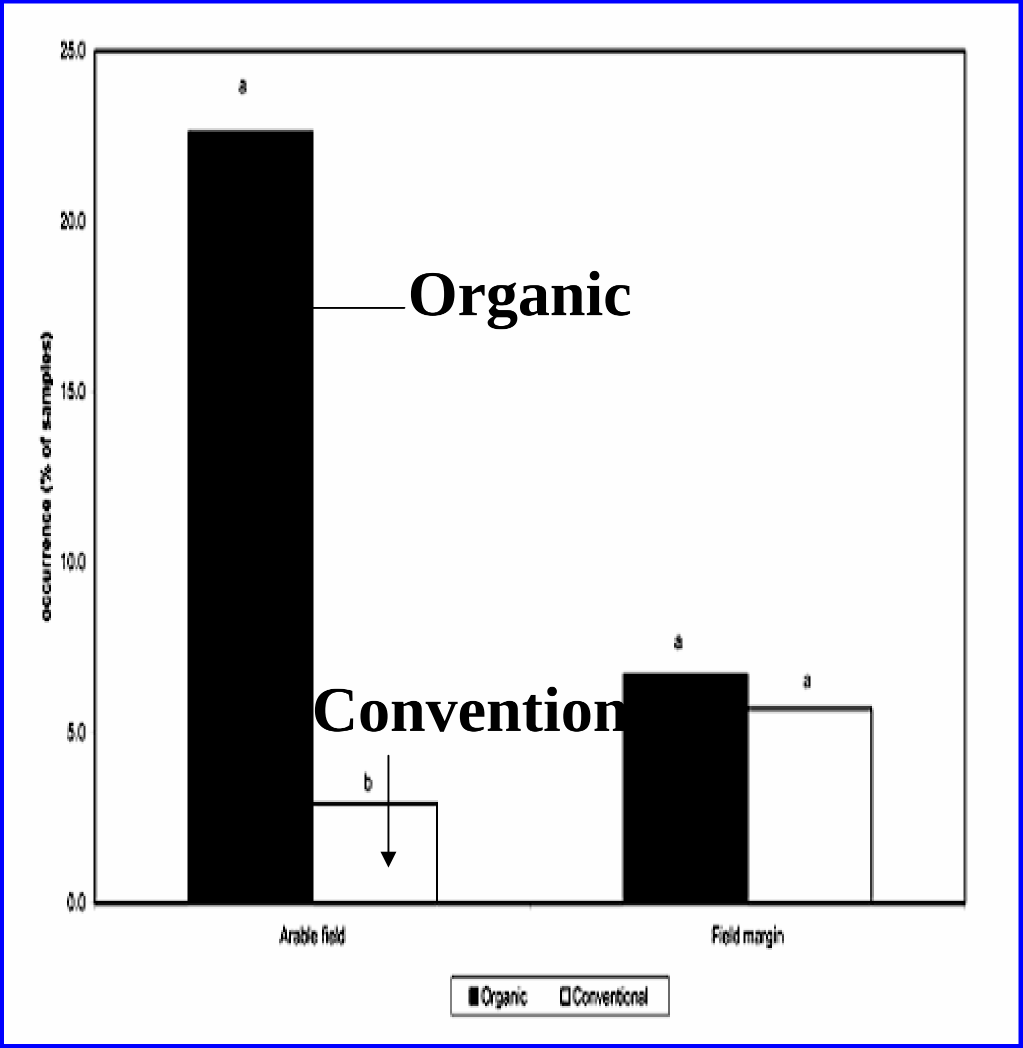
Three groups of insect-pathogenic fungi

- **Commonly encountered fungi with dry conidia and lipophylic cell walls- *Metarhizium, Beauveria, Nomuraea* and *Paecilomyces***

- **Others frequently encountered but have less potential- *Verticillium lecanii* and *Entomophaga grylli***

- **Saprophytic species- mistaken identity- *Aspergillus, Fusarium* and *Penicillium***

Which factors affect the population of insect pathogenic fungi?



Habitat association in the groups of insect-pathogenic fungi

- **Farming system**
- **Field margins**
- **Bait insects**

M. anisopliae
O (5.5%); C (2.9%)

B.bassiana
O (3.3%); C (0%)

Identification of potential strains

Habitat selection (Soil, temperature, crop, agriculture practices- irrigation, pesticides, forest density, etc**)**

and not **Insect-host selection**

drives the population structure

Different methods for the isolation of potential entomopathogens

Soil dilution method

***Galleria* bait method**

CDE activities (Chitin medium)

Where do we find entomopathogens?

Nomuraea rileyi **from the dead** ***Spodoptera*** **larvae**

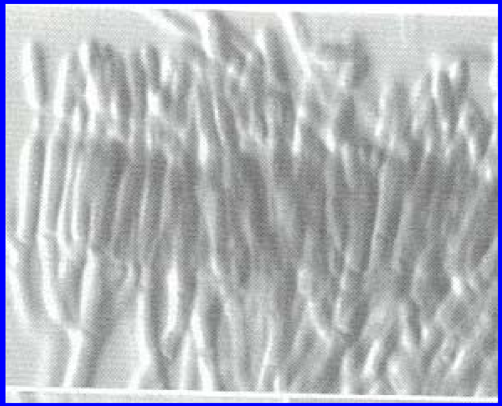
Dead insects

- **Insects hanging from plants, under trees and bushes on soil**
- **Body is soft and black- bacterial or viral infection**
- **Body is hard- fungal infection**

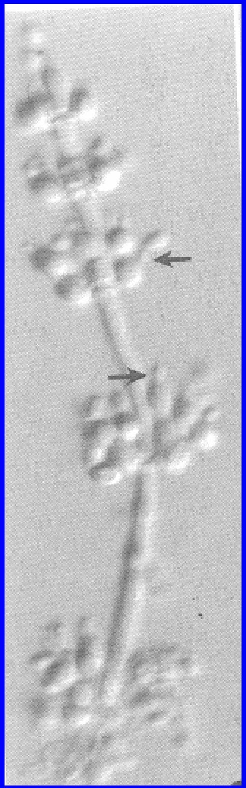
Live insects

- **Collect and keep them in the cages and feed them**
- **Abnormal Behaviour - not feeding, poor coordination,**
- **Jerky movements, excessive grooming and loss of orientation**

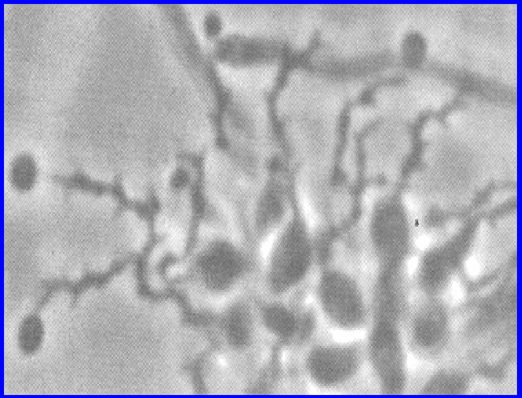
ENTOMOPATHOGENIC FUNGI ON *H. ARMIGERA*



M.anisopliae



N.rileyi



B. bassiana

Origin of *Metarhizium* isolates

Isolate	Field/Source	Total isolates
Source: Soil		
M1311, M1322, M1333, M2104, M2305, M2416, M2427, M2508, M42014, M45115, M45216, M45317, M79120, M79221, M79322	Tomato	15
M3419, M34210, M34311, M34412, M34513	Custard apple	5
M81123, M91124, M91225, M91326, M91528, M91427, M91629, M91730, M91831, M91932, M111145	Sugarcane	11
M101133, M101234, M101335, M101436, M101537, M101638, M101739, M101840, M101941, M102042, M102143, M102244	Brinjal	12
M51118, M51219	Okra	2
M131150, M141151, M141252, M151153	Pigeon pea	4
M121146, M121247, M121348, M121449	Chickpea	4
Source: Insect hosts		
M16255, M16356, M16457, M16558, M16659	Pigeon pea-Greasy cutworm	5
M16154, M16760	Sugarcane-Mealybug	2
M16861	Sugarcane-White grub	1
M16962	Sugarcane-Beetle	1
M161063	Sugarcane-*Pyrilla perpussila*	1

Soil isolates is based on crop, geographical location, plot, sub-plot if any, sample and isolate number

Insect isolates is based on crop, geographical location, host and isolate number

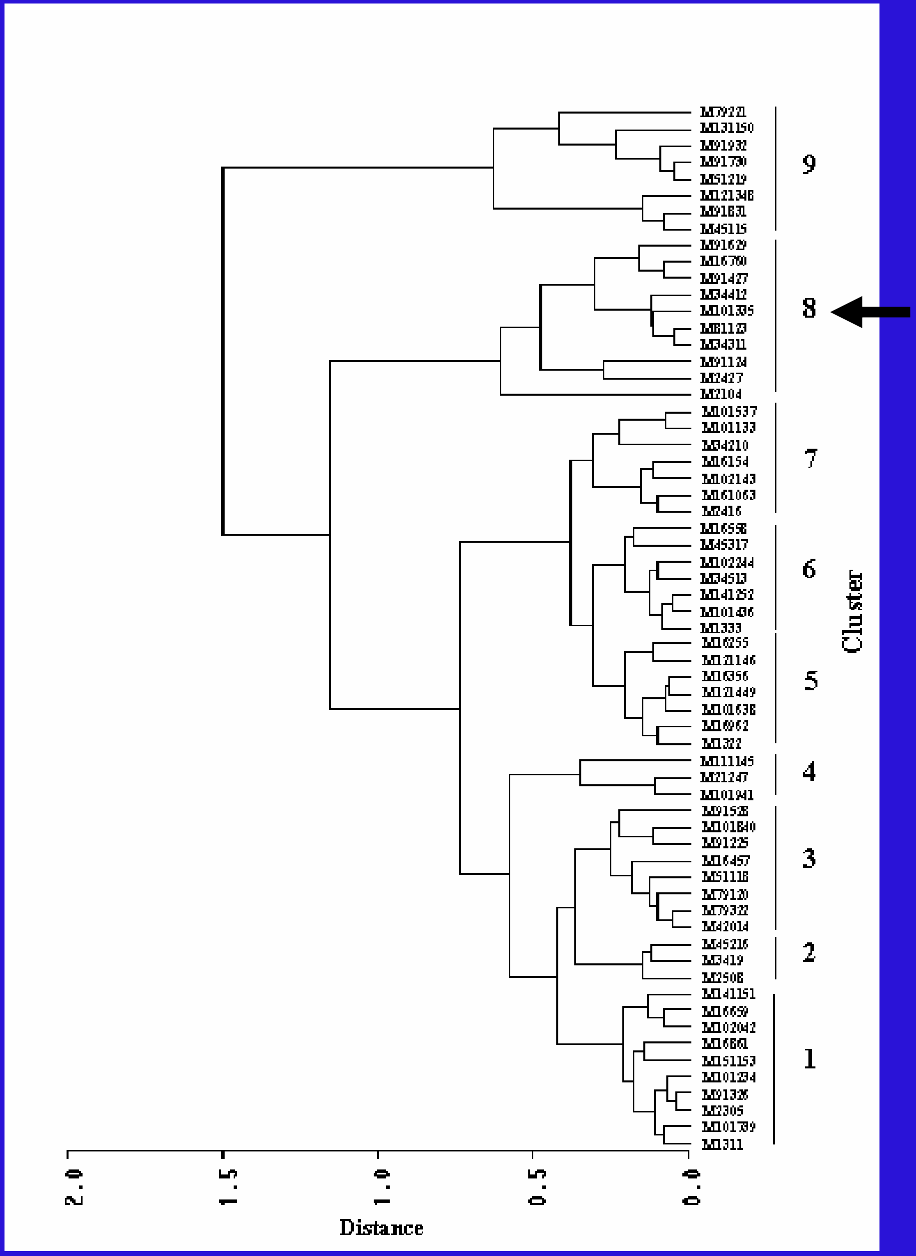
Parameters to study potential entomopathogens

- Extracellular production of CDE: chitinase, CDA, chitosanase, protease and lipase activities

- Regression analysis demonstrated the relation of CDE activities with *H. armigera* mortality

- Based on LT50 of the 10 isolates towards *H. armigera* five isolates were selected
  Cluster 8

- All the 3 isolates, M34412, M34311 and M81123 showed comparable RAPD patterns with a 935G primer

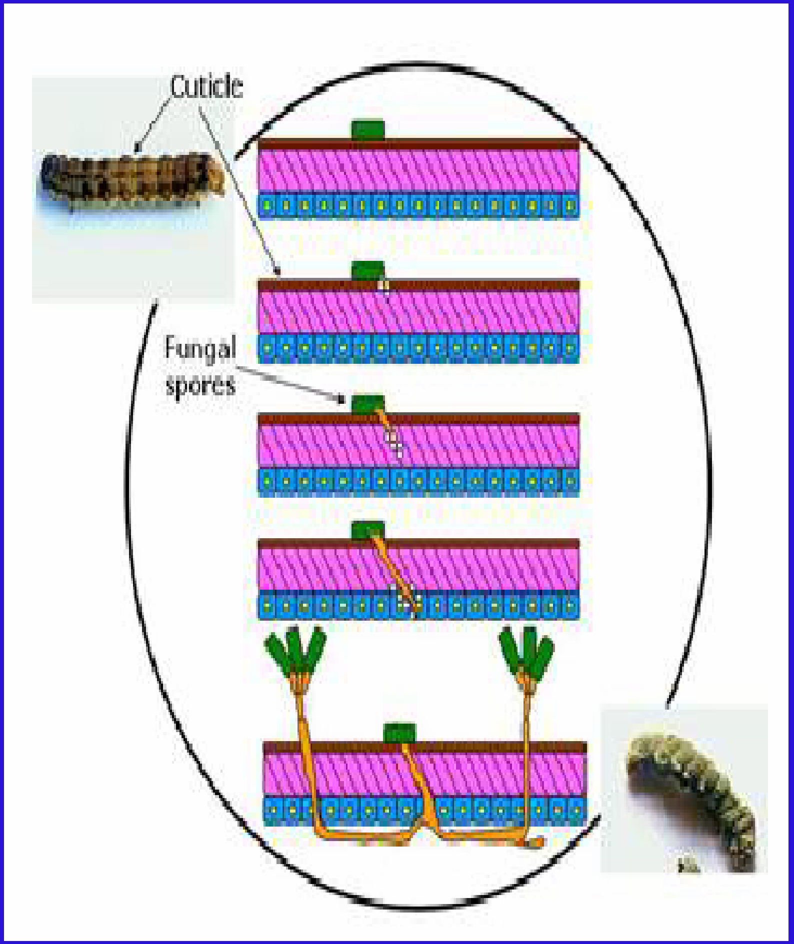


Adaptation…evolution(!) of *M. anisopliae*

Protease-lipase –chitinase act sequentially in fungus-insect interaction

Chitin deacetylase- chitosanase complex for chitin degradation in some *Metarhizium* isolates

Constitutive CDA modifies cell wall chitin to chitosan for self-defense against insect chitinase



What is the use of molecular characterization of entomopathogens?

Identity of the potential strain

[persistence after application, horizontal spread]

Diversity

[Geographical location, soil condition, crops]

Relatedness of entomopathogens

[Interspecies and intergeneric]

Markers to study diversity

- Host recognition
- Cell surface lectins and carbohydrates
- Enzymes/ toxins involved in killing process
- Isoenzymes

DNA-based

- RFLP (Restriction Fragment Length Polymorphism)
- RAPD (Random Amplified Polymorphic DNA)
- AFLP (Amplified Fragment Length Polymorphism)
- Microsatellite (Variable Nucleotide Tandem Repeat)
- SSCP (Single Strand Conformation Polymorphism)
- SNP (Single Nucleotide Polymorphism)

Microsatellite DNA analysis for *M. anisopliae* at 6 different loci

Isolate					Microsatellite loci			
	CA				GAA			Field
M. anisopliae	E	I	J	M	325	307	Area	Crop
M2305	157	117	95	95	130	150	Saswad	Tomato
M3419	157	117	95	95	130	150	Saswad	Custardapple
M2104	180	106	74	109	164	159	Saswad	Tomato
M34210	180	106	74	109	164	159	Saswad	Custardapple
M34311	172	111	72	107	166	159	Saswad	Custardapple
M45317	172	111	72	107	166	159	Lonikand	Tomato
M51219	172	111	72	107	166	159	Lonikand	Bhendi/Okra
M1322	172	111	74	109	164	159	Saswad	Tomato
M79322	172	111	74	109	164	159	Lonikand	Tomato
M34412	168	111	121	124	170	147	Saswad	Custardapple
2071	168	111	121	124	170	147	Re-isolate, 2002	
2065	168	124	111	121	147	170	Re-isolate,2002	
2133	168	111	121	124	170	147	Isolated after one year (2003)	
2191	168	111	121	124	170	147	%efficacy,CP,I sp.,Day0/2003	
2192	168	111	121	124	170	147	%efficacy,PP,I sp.,Day0/2003	
2194	168	111	121	124	170	147	%efficacy,PP,I sp.,Day0/2003	

The isolates re-isolated from infected *H. armigera* and from the soil samples collected after spraying resemble the original isolate.

Microsatellite variability in the entomopathogenic fungus *Paecilomyces fumosoroseus*

Host and geographical location influenced diversity

One lineage included genotypes from the B-biotype of *Bemisia tabaci* distributed across the America

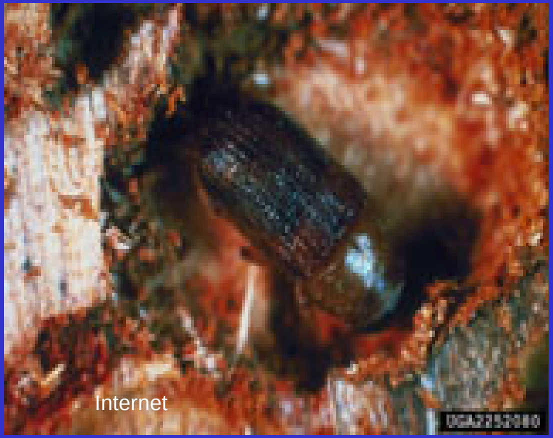
Another lineage was distributed across Asia and consisted of four distinct clusters

Mutualism

Woodwasps (Hymenoptera) attack conifers and angiosperms

Basidiomycetes *Amylostereum* and *Stereum* degrade host tissue to the assimilable form

Female larvae store arthrospores which germinate and grow.

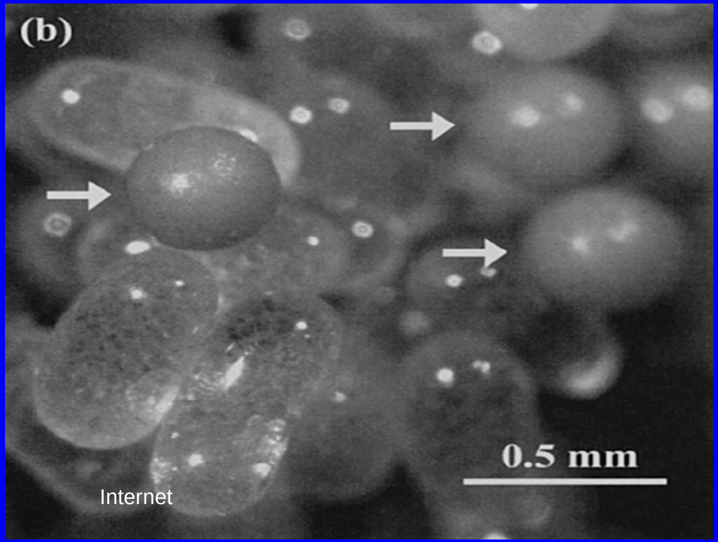


Evolutionary success of bark beetles is dependent on the symbiotic relationship with fungus *Ophiostoma novo-ulmi*

- Helpful in overcoming plant resistance
- Nutrition to the larvae
- To make host susceptible for the attack
- To modify chemicals produced by plants

Mutualism

Egg piles in the nursery nest of *R. flavipes*



Transparent eggs and spherical-shaped brown termite balls

Termite egg-mimicking fungi ("termite balls") in *Reticulitermes* spp. (Isoptera: Rhinotermitidae) nests

- ***Fibularhizoctonia* sp. forms sclerotia that morphologically and chemically mimic termite eggs**
- **Fungus gets protection and free transport by termites to a new habitat**
- **Sclerotia enhance egg survival by probably producing antimicrobial compounds against pathogens.**

<u>Insect pathogenic fungi: The Rare and the Valuable</u>

Common fungi, *Beauveria bassiana* and *Metarhizium anisopliae* are <u>valuable</u> because they are easy to grow and have a wide host range

***M. anisopliae* is the world's second most commonly encountered and second most widely used insect fungus.**

<u>Rare,</u> *Neozygites tanajoae*, a significant pathogen of cassava green mites in South America. Cassava is a staple crop for poor farmers there—and in Africa—who cannot afford to apply pesticides against the mite

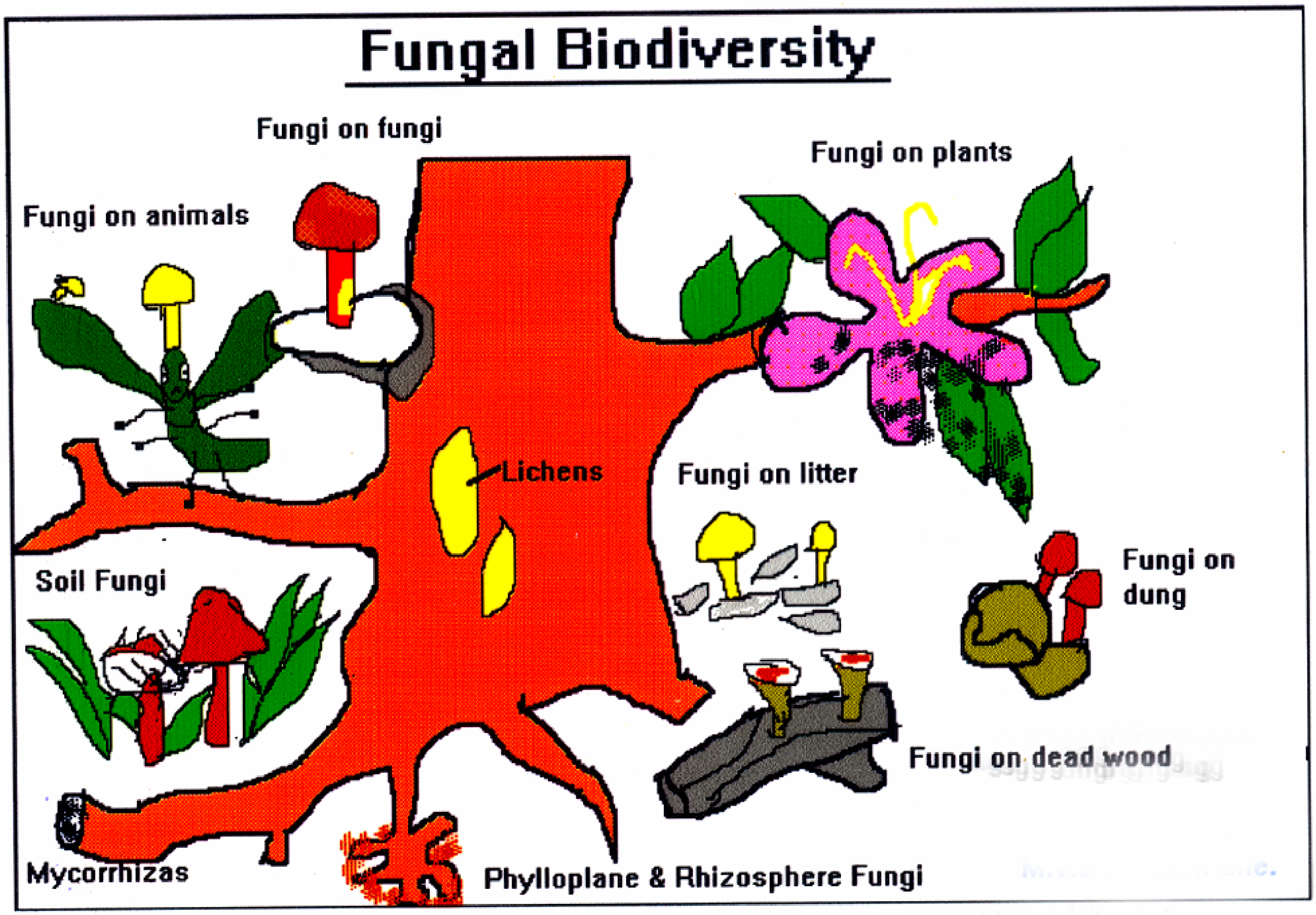
Need for the entomopathogenic fungi park

Repeated sub-culturing on artificial media affects

- **Conidia formation**
- **Conidial germination**
- **Virulence**

Maintenance in the natural habitat

……Just a Beginning



Mycologist

……and not the end