Dynamics of dsRNA mycoviruses in black Aspergillus populations

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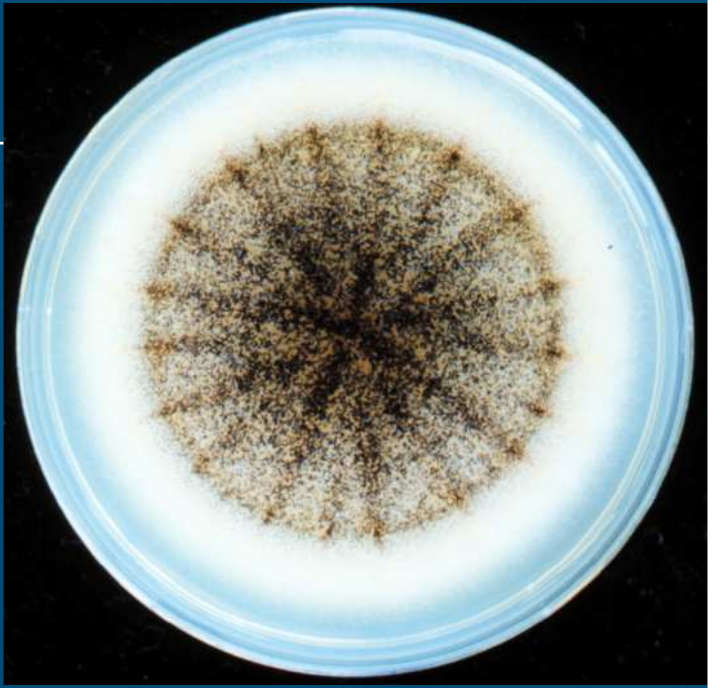
Overview

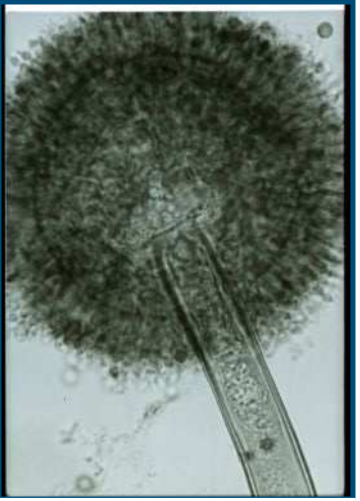
Dynamics of dsRNA mycoviruses in black Aspergillus populations:

- Isolation of black Aspergilli
- Virus presence and variance
- Model for balanced infection
- Transfer of viruses
- Effects of virus infections
- Conclusions

Isolation of black Aspergilli



- Dark brown to black conidiospores
- Mop-like conidiophores
- Asexual – (Parasexual?)
- Species complex
  - *A. niger*
  - *A. tubingensis*
  - *A. carbonarius*
  - *A. japonicus*
  - *A. aculeatus*
  - *A. foetidus*
  - *A. heteromorphus*
  - *A. brasiliensis*
  - *A. vadensis*



Isolation of black Aspergilli

- Saprophytic lifestyle
- Versatile metabolism

- Exclusive isolation on 20% tannic acid
- Growth on up to 80%!
- All culture collection strains

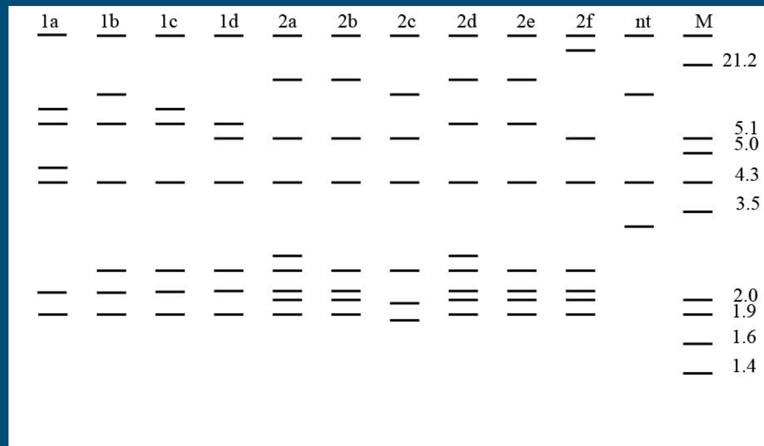
Isolation of black Aspergilli

- Soil and Humus samples collected world wide, 1990-1995

  ↓
- Isolation on 20% tannic acid

  ↓
- Single spore colonies

  ↓
- characterization on mt RFLP



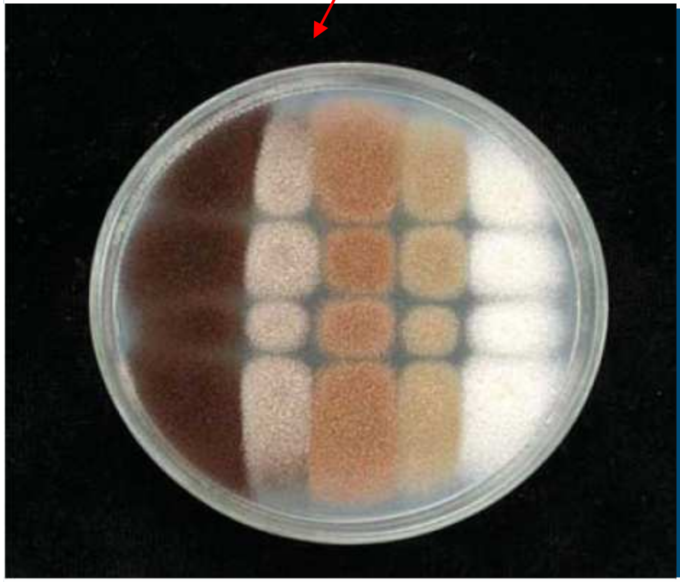
Varga *et al* 1994 Can. J. Microbiol 40:612
Kevei *et al* 1996 Ant.v.Leeuwenhoek 70:59
Hamari *et al* 1997 Ant.v.Leeuwenhoek 72:337

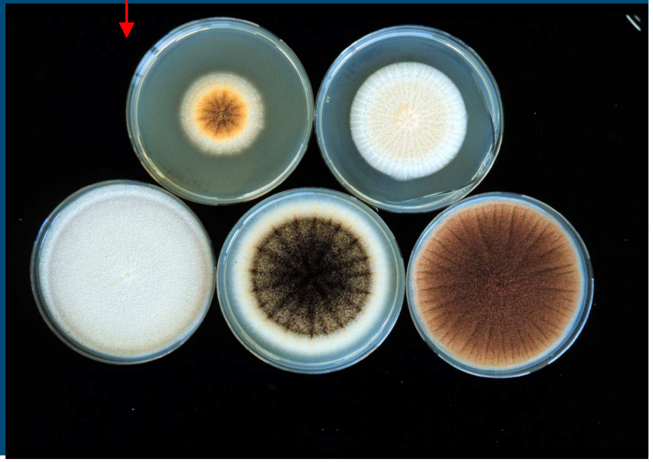
Growth on 20% tannin: not associated with black spores

Table 2. List of *Aspergillus* colour mutants tested on 20 % tannic acid medium.

Strain	Name	Colour mutants[a]	20 % tannin	Strain	Name	Colour mutants[a]	20 % tannin
N050	*A. carbonarius*	*fwn, brn*	+	N067	*A. nanus*	*fwn, brn, gry*	+
N055	*A. japonicus*	*fwn, brn, whi, gry*	+	N068	*A. usami*	*fwn, brn*	+
N057	*A. jap. aculeatus*	*fwn, brn, whi*	+	N070	*A. intermedius*	*fwn, brn*	+
N059	*A. niger*	*fwn*	+	N076	*A. foetidus*	*fwn, brn*	+
N062	*A. awamori*	*fwn, brn*	+	N400	*A. niger*	*fwn, brn, gry, olv*	+
N064	*A. phoenicis*	*fwn, brn*	+				

[a] Conidium colours: *fwn*, fawn; *brn*, brown; *whi*, white; *gry*, grey; and *olv*, olive green.





Growth on 20% tannin: 11 tannase-like genes

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Isolation of black Aspergilli: 668 natural isolates

	Mitochondrial haplotype													
	A. niger-aggregate													
	'*A. niger*'[b]				'*A. tubingensis*'[b]						*A. jap*[c]	*A. car*[d]		Density
Country[a]	1a	1b	1c	1d	2a	2b	2c	2d	2e	2f			No. of isolates	(No. of spores/g soil)
Canada (3)	–	–	4	6	–	–	–	–	–	–	–	–	10	0–2
France (4)	3	–	1	–	–	2	–	–	–	–	3	–	9	0–2
Netherlands (27)	1	6	5	–	40	6	–	–	–	–	–	–	58	0–8
Switzerland (2)	–	–	–	–	2	1	1	–	–	–	–	–	4	0–3
Morocco (2)	–	–	–	–	6	2	–	–	–	–	1	1	10	25–85
Egypt (2)	–	7	7	–	10	–	–	–	–	–	–	–	24	8–10
Israel (4)	–	1	–	–	4	–	–	–	–	–	–	1	6	2–3
Guinea (1)	–	6	3	–	–	–	–	–	–	–	–	–	9	65
Gabon (5)	3	24	5	–	4	7	1	–	1	–	40	–	85	40–60
Kameroon (6)	1	16	6	–	1	2	6	–	–	–	9	1	42	25–150
Brazil (4)	3	8	20	–	5	5	–	–	–	–	–	–	41	50–75
Indonesia (12)	27	77	74	1	50	33	4	9	14	1	23	7	320	50–250
Malaysia (1)	–	5	–	–	1	–	–	–	–	–	–	–	6	n.d.
Nepal (1)	–	6	–	–	–	–	–	–	–	–	–	–	6	4–6
Australia (1)	–	–	–	–	–	–	–	–	–	4	–	–	4	8–15
New Zealand (2)	–	7	–	–	1	–	–	–	–	–	–	–	8	6–12

[a] Per country the number of samples investigated is given between brackets.
[b] *A. niger* mitochondrial RFLP haplotypes 1a–1d and *A. tubingensis* haplotypes 2a–2f as recognised by Varga *et al.* (1993, 1994).
[c] jap, *A. japonicus* and direct relatives (Hamari *et al.* 1997).
[d] car, *A. carbonarius* and direct relatives (Kevei *et al.* 1996).

High density

Van Diepeningen *et al.* 2004 Mycol. Research 108:919

Isolation of black Aspergilli: World wide well mixed

Country[a]	Mitochondrial haplotype: *A. niger*-aggregate: '*A. niger*'[b] 1a	1b	1c	1d	'*A. tubingensis*'[b] 2a	2b	2c	2d	2e	2f	*A. jap*[c]	*A. car*[d]	No. of isolates	Density (No. of spores/g soil)
Canada (3)	–	–	4	6	–	–	–	–	–	–	–	–	10	0–2
France (4)	3	–	1	–	–	2	–	–	–	–	3	–	9	0–2
Netherlands (27)	1	6	5	–	40	6	–	–	–	–	–	–	58	0–8
Switzerland (2)	–	–	–	–	2	1	1	–	–	–	–	–	4	0–3
Morocco (2)	–	–	–	–	6	2	–	–	–	–	1	1	10	25–85
Egypt (2)	–	7	7	–	10	–	–	–	–	–	–	–	24	8–10
Israel (4)	–	1	–	–	4	–	–	–	–	–	–	1	6	2–3
Guinea (1)	–	6	3	–	–	–	–	–	–	–	–	–	9	65
Gabon (5)	3	24	5	–	4	7	1	–	1	–	40	–	85	40–60
Kameroon (6)	1	16	6	–	1	2	6	–	–	–	9	1	42	25–150
Brazil (4)	3	8	20	–	5	5	–	–	–	–	–	–	41	50–75
Indonesia (12)	27	77	74	1	50	33	4	9	14	1	23	7	320	50–250
Malaysia (1)	–	5	–	–	1	–	–	–	–	–	–	–	6	n.d.
Nepal (1)	–	6	–	–	–	–	–	–	–	–	–	–	6	4–6
Australia (1)	–	–	–	–	–	–	–	–	–	4	–	–	4	8–15
New Zealand (2)	–	7	–	–	1	–	–	–	–	–	–	–	8	6–12

[a] Per country the number of samples investigated is given between brackets.
[b] *A. niger* mitochondrial RFLP haplotypes 1a–1d and *A. tubingensis* haplotypes 2a–2f as recognised by Varga *et al.* (1993, 1994).
[c] jap, *A. japonicus* and direct relatives (Hamari *et al.* 1997).
[d] car, *A. carbonarius* and direct relatives (Kevei *et al.* 1996).

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Isolation of black Aspergilli: predominantly *A.niger + A.tubingensis*

Country[a]	Mitochondrial haplotype: *A. niger*-aggregate: '*A. niger*'[b] 1a	1b	1c	1d	'*A. tubingensis*'[b] 2a	2b	2c	2d	2e	2f	*A. jap*[c]	*A. car*[d]	No. of isolates	Density (No. of spores/g soil)
Canada (3)	–	–	4	6	–	–	–	–	–	–	–	–	10	0–2
France (4)	3	–	1	–	–	2	–	–	–	–	3	–	9	0–2
Netherlands (27)	1	6	5	–	40	6	–	–	–	–	–	–	58	0–8
Switzerland (2)	–	–	–	–	2	1	1	–	–	–	–	–	4	0–3
Morocco (2)	–	–	–	–	6	2	–	–	–	–	1	1	10	25–85
Egypt (2)	–	7	7	–	10	–	–	–	–	–	–	–	24	8–10
Israel (4)	–	1	–	–	4	–	–	–	–	–	–	1	6	2–3
Guinea (1)	–	6	3	–	–	–	–	–	–	–	–	–	9	65
Gabon (5)	3	24	5	–	4	7	1	–	1	–	40	–	85	40–60
Kameroon (6)	1	16	6	–	1	2	6	–	–	–	9	1	42	25–150
Brazil (4)	3	8	20	–	5	5	–	–	–	–	–	–	41	50–75
Indonesia (12)	27	77	74	1	50	33	4	9	14	1	23	7	320	50–250
Malaysia (1)	–	5	–	–	1	–	–	–	–	–	–	–	6	n.d.
Nepal (1)	–	6	–	–	–	–	–	–	–	–	–	–	6	4–6
Australia (1)	–	–	–	–	–	–	–	–	–	4	–	–	4	8–15
New Zealand (2)	–	7	–	–	1	–	–	–	–	–	–	–	8	6–12

[a] Per country the number of samples investigated is given between brackets.
[b] *A. niger* mitochondrial RFLP haplotypes 1a–1d and *A. tubingensis* haplotypes 2a–2f as recognised by Varga *et al.* (1993, 1994).
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[d] car, *A. carbonarius* and direct relatives (Kevei *et al.* 1996).

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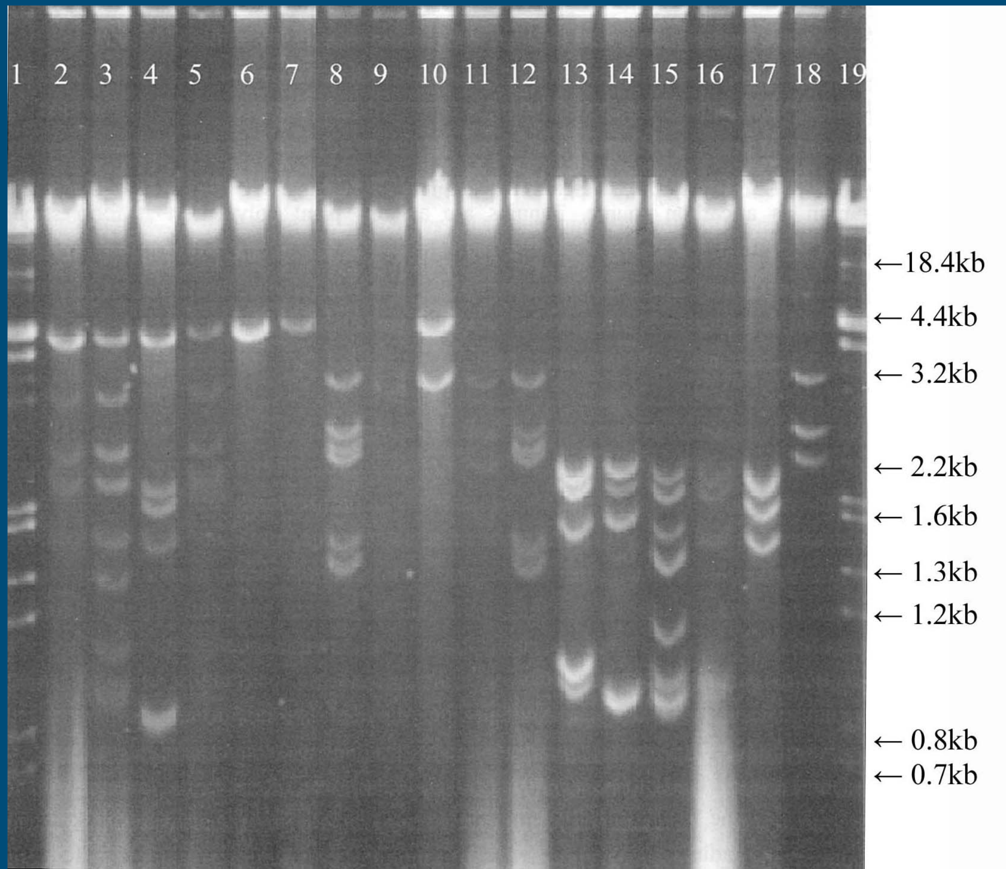
Virus presence and variance

Large variation:

- 1-8 dsRNA fragments
- 0.7 – 4.5 kb

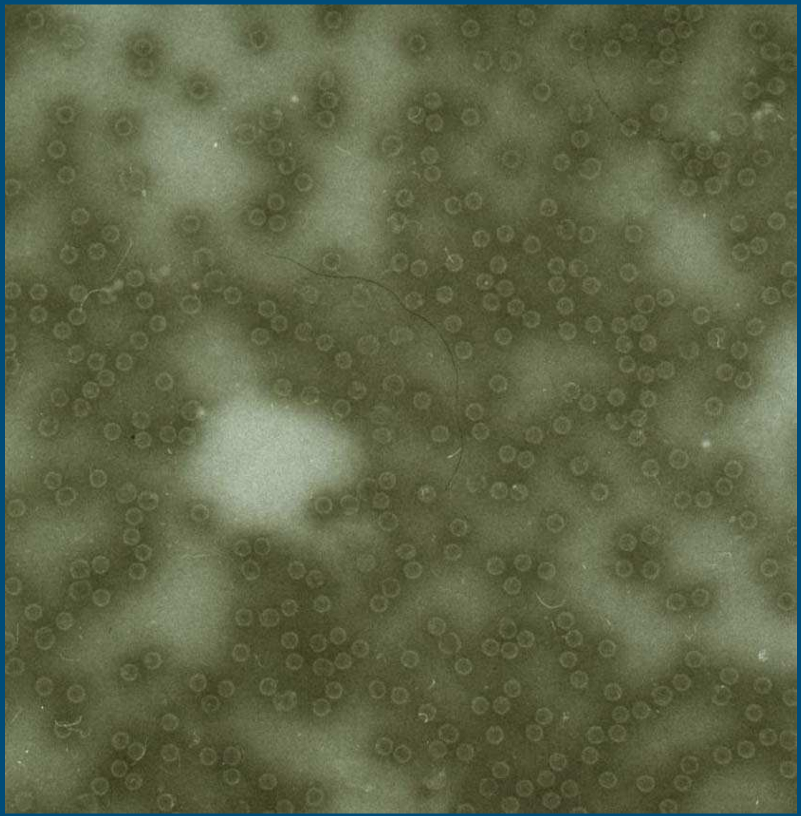
- Different viruses and/or defective interfering particles



Virus presence and variance

EM:

- isometric particles
- 25 - 40 nm



World wide virus distribution: 10% infection nearly all types and locations

Country	Mitochondrial haplotype												Total
	A. niger				*A. tubingensis*						*A. jap*[a]	*A. car*[b]	
	1a	1b	1c	1d	2a	2b	2c	2d	2e	2f			
America													
Canada	—	—	0/4	0/6	—	—	—	—	—	—	—	—	0/10
Brazil	0/3	1/8	1/20	—	0/5	0/5	—	—	—	—	—	—	2/41
Europe													
France	0/3	—	0/1	—	—	0/2	—	—	—	—	0/3	—	0/9
Netherlands	0/6	2/10	2/6	—	5/53	0/7	0/1	0/1	—	—	—	—	9/84
Switzerland	—	—	—	—	0/2	0/1	0/1	—	—	—	—	—	0/4
Africa													
Morocco	—	—	—	—	0/6	1/2	—	—	—	—	0/1	0/1	1/10
Egypt	—	0/7	0/7	—	0/10	—	—	—	—	—	—	—	0/24
Israel	—	0/1	—	—	1/4	—	—	—	—	—	—	0/1	1/6
Guinea	—	0/6	3/3	—	—	—	—	—	—	—	—	—	3/9
Gabon	0/3	4/24	1/5	—	1/4	0/7	0/1	—	1/1	—	5/40	—	12/85
Cameroon	0/1	1/16	0/6	—	0/1	0/2	0/6	—	—	—	0/9	0/1	1/42
Asia													
Indonesia	1/27	12/77	6/74	0/1	7/50	1/33	0/4	2/9	2/14	0/1	2/23	1/7	34/320
Malaysia	—	0/5	—	—	0/1	—	—	—	—	—	—	—	0/6
Nepal	—	0/6	—	—	—	—	—	—	—	—	—	—	0/6
Oceania													
Australia	—	—	—	—	—	—	—	—	—	0/4	—	—	0/4
New Zealand	—	0/7	—	—	1/1	—	—	—	—	—	—	—	1/8
Total	1/43	21/167	13/126	0/1	15/143	2/59	0/13	2/10	2/15	0/5	7/76	1/10	64/668

Virus infections are given per number of isolated strains of a certain haplotype and per country.

[a] *jap* = *japonicus* and direct relatives.

[b] *car* = *carbonarius* and direct relatives.

van Diepeningen *et al., 2006 Fungal Genet. Biol. 43:446-452*

Model: virus dynamics

- 10% mycovirus infection in the black Aspergilli

Balance ?

Loss

- ‘Curing’ (vertical transfer)
- Fitness costs to host

Gain

- Infection (horizontal transfer)
- Benefit to the host

Transfer of viruses

Vertical: to offspring

Horizontal: via hyphal anastomosis

Vertical:

- several hundreds of single conidiospores: no loss of viruses

Transfer of viruses

- Horizontal virus transfer:

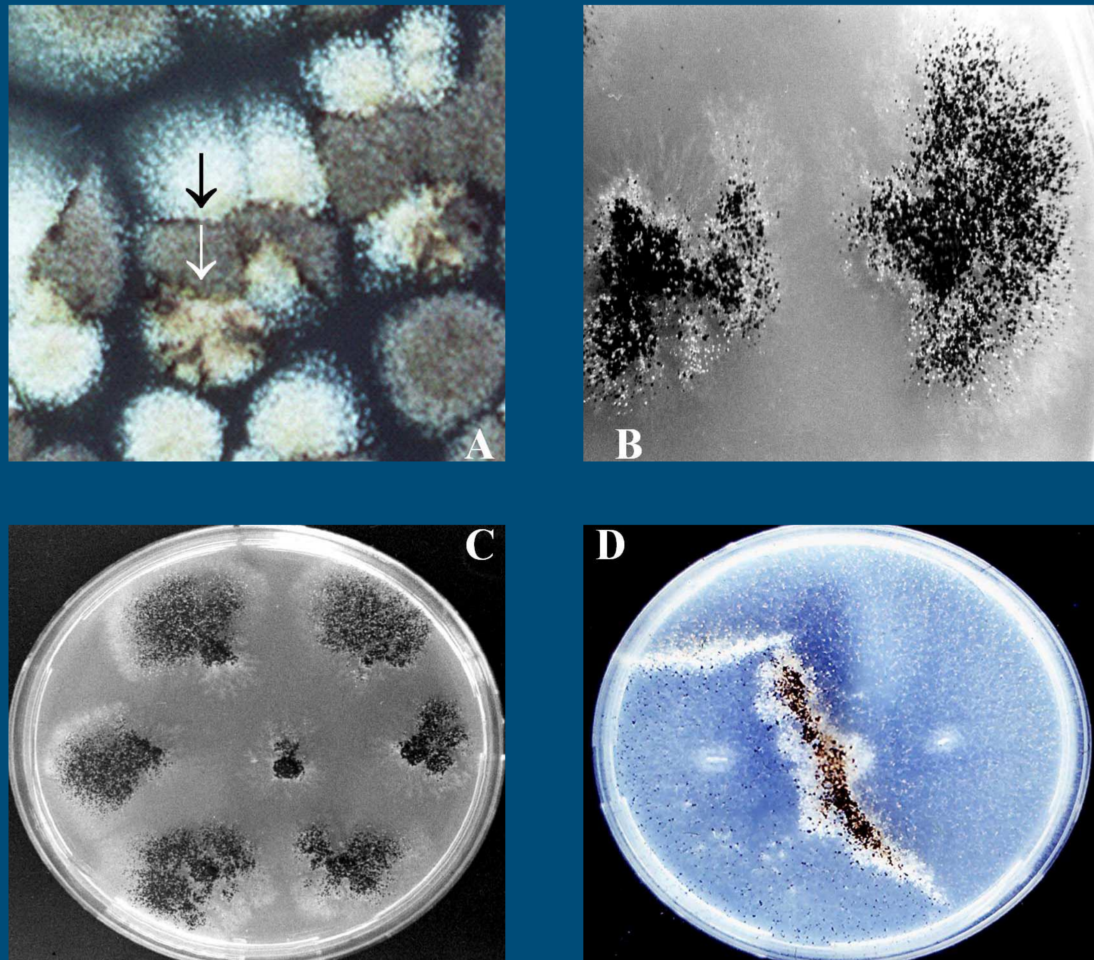
Donor → Acceptor ↓		Ind 1.8.16 * (1b)		Ind 1.8.7* (1b)		Ind 1.7.8* (1c)		Ind 1.8.22* (1c)		Ind 1.6.19* (2a)		Ind 1.8.26* (2d)	
Ind 1.8.2 *cnx*	(1a)	–	–	–	–	–	–	–	–	–	–	–	–
Ind 1.8.1 *cnx*	(1b)	–	–	–	–	–	–	–	–	–	–	–	–
Ind 1.8.13 *cnx*	(1c)	–	–	–	–	–	–	–	–	–	–	–	–
Ind 1.8.9 *cnx*	(1d)	–	*	–	–	–	–	+	+	–	–	–	–
Ind 1.8.39 *cnx*	(2a)	–	–	–	–	–	–	n.d	–	–	–	–	–
Ind 1.8.42 *cnx*	(2b)	–	–	–	–	–	–	–	–	–	–	–	n.d
Ind 1.7.6	(J)	n.d.	n.d.	–	–	n.d	–	n.d.	–	n.d.	–	n.d	–
Ind 1.5.5* *cnx*	(1b)			–	–	–	–			–	–	–	–
Ind 1.7.8* *cnx*	(1c)	–	–	–	–			–	–	–	–	–	–
Ind 1.8.22* *cnx*	(1c)			–	–	–	–			–	–		
Ind 1.6.19 **cnx*	(2a)			–	–	–	–	–	–				

After 6 weeks intensive growth/ regular damage:
Very little transfer: due to heterokaryon incompatibility?

Transfer of viruses

Tests for heterokaryon (in)compatibility:

A. Color complementation
B. Complementation auxotrophic mutations
C. Dominant resistancies
D. Chlorate resistance/ nitrate deficiency (nia and cnx)



Transfer of viruses

- heterokaryon (in)compatibility

Strain	mt	Ind 1.8 2	Ind 1.8 29	Ind 1.8 1	Ind 1.8 3	Ind 1.5 5	Ind 1.8 7	Ind 1.8 10	Ind 1.8 11	Ind 1.8 19	Ind 1.8 16 +	Ind 1.8 16 –	Ind 1.8 13	Ind 1.4 24	Ind 1.8 21	Ind 1.8 22	Ind 1.7 8	Ind 1.8 9	Ind 1.6 19	Ind 1.6 23	Ind 1.8 42	Ind 1.2 15	Ind 1.8 26	Ind 1.4 32	Ind 1.4 29	N4 00	Z 1.1	N0 62
Ind 1.8.2	1a	+																										
Ind 1.8.29	1a	–	+																									
Ind 1.8.1	1b	–	–	+																								
Ind 1.8.3	1b	–	–	–	+																							
Ind 1.5.5	1b	–	–	+	–	+																						
Ind 1.8.7	1b	–	–	–	–	–	+																					
Ind 1.8.10	1b	–	–	–	–	–	–	+																				
Ind 1.8.11	1b	–	–	–	–	–	–	+	+																			
Ind 1.8.19	1b	–	–	–	–	–	–	–	–	+																		
Ind 1.8.16 *	1b	–	–	–	–	–	–	–	–	–	+																	
Ind 1.8.16 *½	1b	–	–	–	–	–	–	–	–	–	+	+																
Ind 1.8.13	1c	–	–	–	–	–	–	–	–	–	–	–	+															
Ind 1.4.24	1c	–	–	–	–	–	–	–	–	–	–	–	–	+														
Ind 1.8.21	1c	–	–	–	–	–	–	–	–	–	–	–	–	–	+													
Ind 1.8.22	1c	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+												
Ind 1.7.8	1c	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+											
Ind 1.8.9	1d	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	+										
Ind 1.6.19	2a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+									
Ind 1.6.23	2a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+								
Ind 1.8.42	2b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+							
Ind 1.2.15	2b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+						
Ind 1.8.26	2d	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+					
Ind 1.4.32	J	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+				
Ind 1.4.29	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+			
N400	1a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+		
Z 1.1	1b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	
N062	1c	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Most are self-compatible, but incompatible to others

Transfer of viruses: can virus free isolates be infected?

Protoplast fusion

- Novozym 234

↓

- $10^{6\text{-}7}$ + $10^{6\text{-}7}$ protoplasts

↓

- PEG/osmotically stabilized medium

↓

- individual recombinant (mt-Oli^{R}) fusion products / total analysis acceptor

Transfer of viruses

- Protoplast Fusion Experiment - black Aspergilli

Independent transfer of mt-oli^r and virus

Back *Aspergillus* acceptors	Black *Aspergillus* donors							
	Ind 1.5.5		Ind 1.7.8		Ind 1.8.16		Ind 1.8.3*	
	I	II	I	II	I	II	I	II
Ind 1.8.1, *fwn, nia*	+	4/4	+	73/77	–	4/6	+	0/9
Ind 1.8.3, *fwn, nia*	–	0/10	–	0/10	+	4/10	+	10/10
Ind 1.8.9, *fwn, cnx*	–	10/10	+	4/10	+	0/10	–	12/12
Ind 1.8.42, *fwn, nia*	–	0/10	+	10/10	+	0/10	+	0/10
Ind 1.5.5, *fwn, cnx*	X	X	+	10/10	X	X	X	X
Ind 1.7.8, *fwn, cnx*	+	0/10	X	X	+	0/10	X	X
Ind 1.8.16, *fwn, cnx*	X	X	+	10/10v	X	X	X	X
N062, *fwn, nia*	–	0/10	–	0/6	–	0/2	–	0/8

- 55-60% transfer: virus free isolates can become infected

Transfer of viruses

- Protoplast Fusion - black Aspergilli to *A. nidulans*

A. nidulans acceptors	Black *Aspergillus* donors			
	341	Ind 1.7.8	Ind 1.8.7	Ind 1.8.16
701, *nia*	+	+	+	+
702, *nia*	+	+	+	+
701, *nia*	+	+	n.d.[a]	+
704, *nia*	+	+	+	+

- 100% transfer from *A.niger* to *A.nidulans*
- In nature no infections known in *A. nidulans*

Transfer of viruses

- Protoplast Fusion – *A. nidulans* to *A. nidulans*

A. nidulans acceptors	*A. nidulans* donor: 701
701. *nia*	+
702. *nia*	+
701. *nia*	+
704. *nia*	+

- Between heterokaryon incompatible *A. nidulans* strains 100% transfer

Transfer of viruses

- Protoplast Fusion - *A. nidulans* to black *Aspergilli*

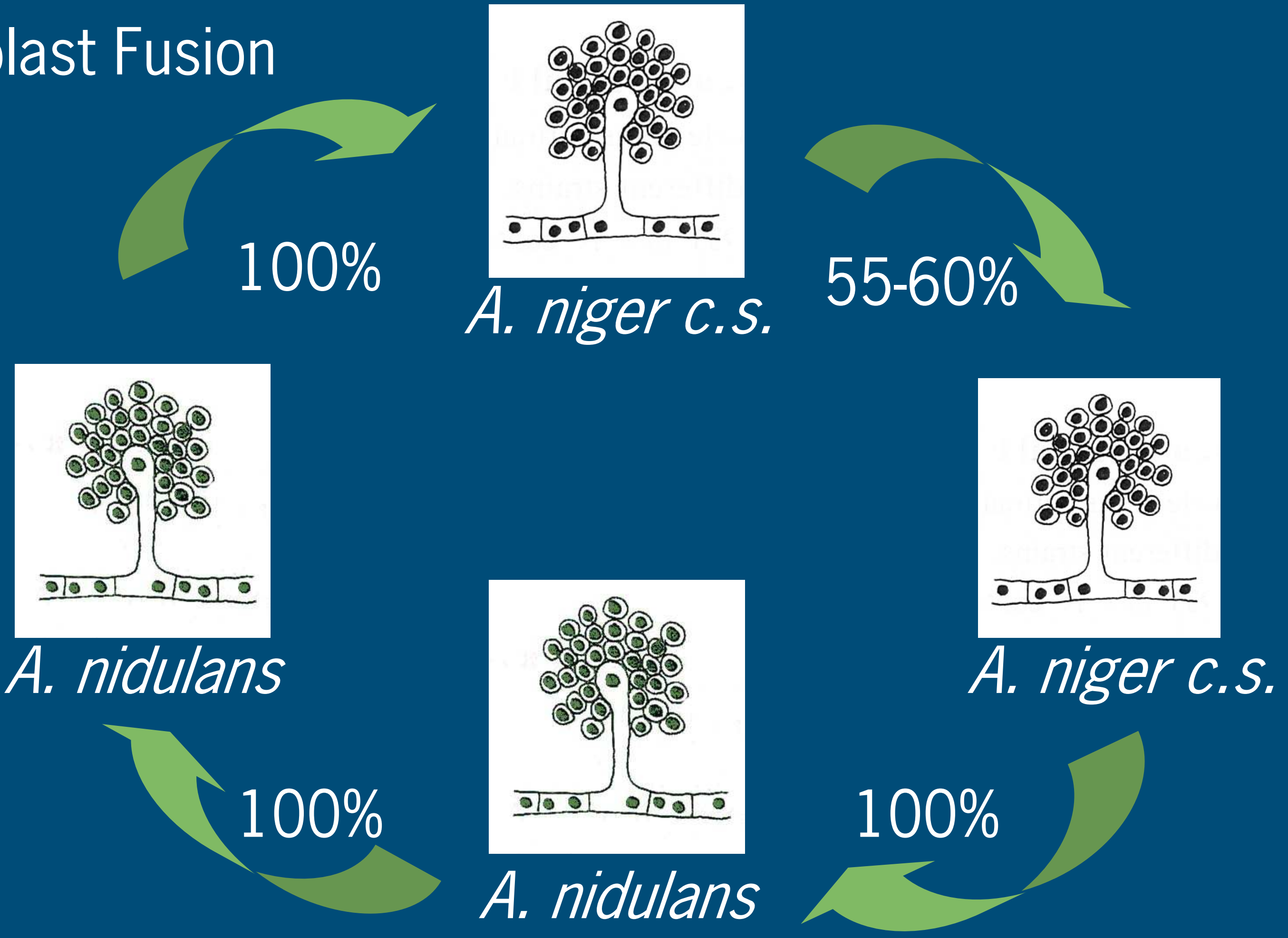
Black *Aspergillus* acceptors	*A. nidulans* donor: 701
Ind 1.8.1, *fwn, nia*	+
Ind 1.8.3, *fwn, nia*	+
Ind 1.8.9, *fwn, cnx*	+
Ind 1.8.42, *fwn, nia*	+
N062, *fwn, nia*	±

hsi

- 100% Transfer

Transfer of viruses

- Protoplast Fusion

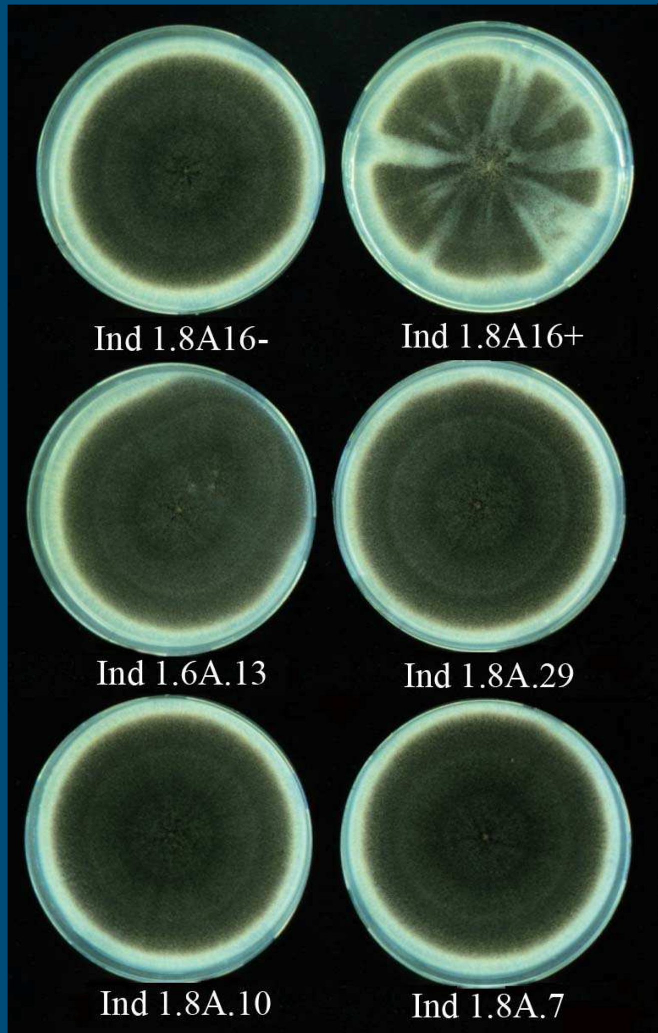


van Diepeningen *et al., 1998 Fungal Genetics & Biology 25:171*

Effects of virus infections

- Mycoviruses: Cryptic infections
- 1/68 infected strains phenotypic effect

→ virus titer effect



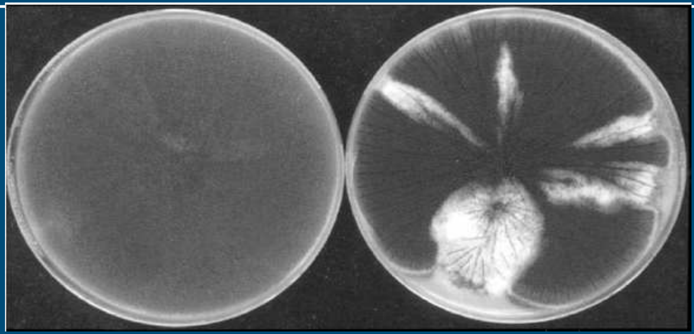
Effects of virus infections

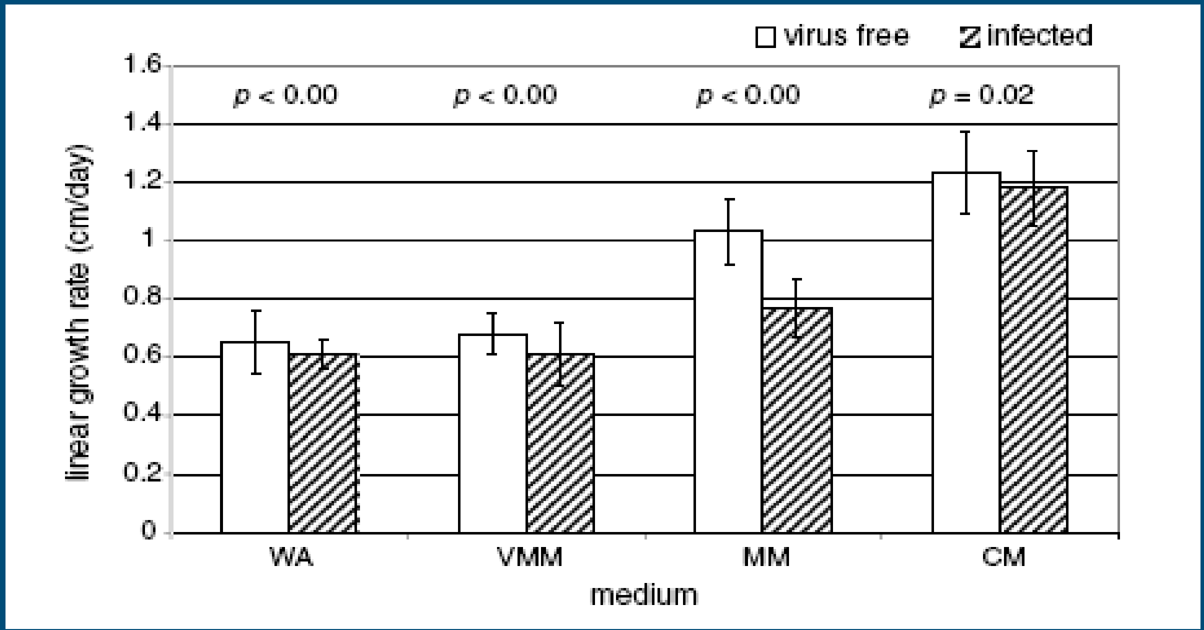
- Quantification fitness effects
  - Isogenic lines from transfer experiments

- Resource competition:
  - Linear Growth Rate
  - Spore Production
- Interference competition
  - Competition with reference strains

Effects of virus infections

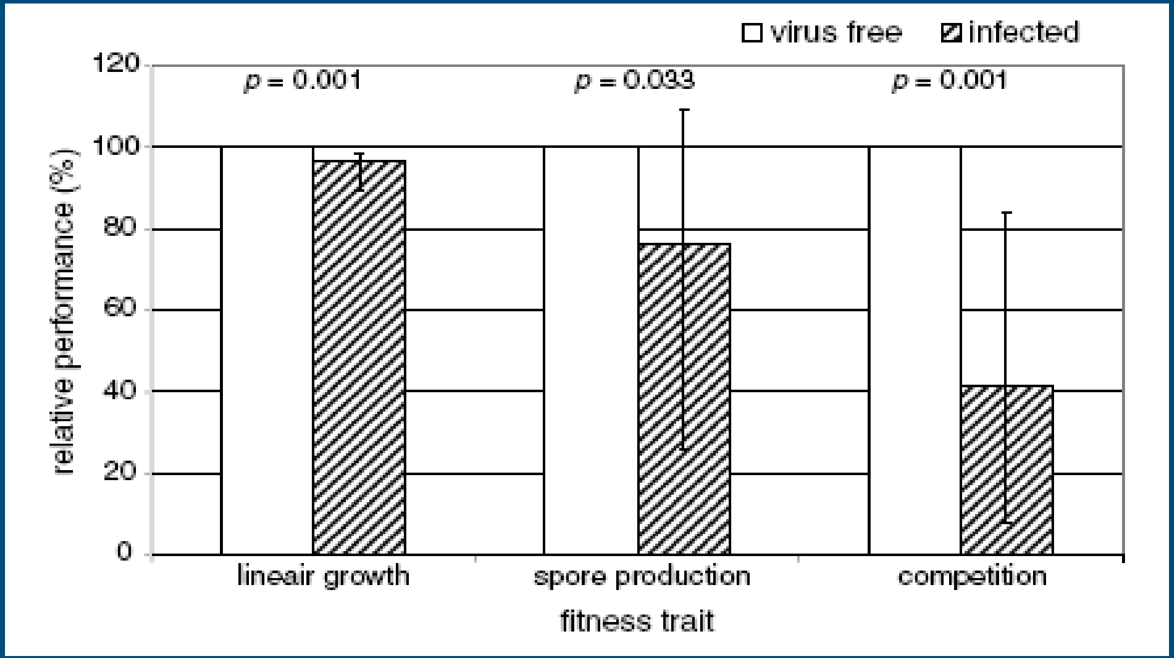
- Linear Growth Rate – Ind 1.8.16





Effects of virus infections

- Resource and interference competition average effects of 'cryptic' viruses



-3.3% -24% -59%

Conclusions: model

- 10% mycovirus infection in the black Aspergilli

Balance?

Loss

- Spontaneous loss 0%
- Fitness costs 5-50%

Gain

- Horizontal transfer ≈1%
- No benefits 0%

Conclusions

- 20% tannic acid selection all black Aspergilli
- 10% infection, high diversity, world-wide
- Transfer limited by heterokaryon incompatibility
- Large fitness consequences virus infections

- Balance fitness costs - transfer rate?

→ No balance?

→ other ways of transfer?

→ fitness effects in nature?

Dynamics of dsRNA mycoviruses in black Aspergillus populations

Questions and suggestions?

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