Endophyte mediated plant-herbivore interactions or cross resistance to fungi and insect herbivores?

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DEFINITION

- **Wilson 1995: “**Endophytes are fungi or bacteria which, for all or part of their life cycle, invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues but cause no symptoms of disease”

=> Includes * Latent pathogens
*** Dormant saprophytes**

FUNGAL ENDOPHYTES

Bacon, C. W., Porter, J. K., Robbins, J. D. and Luttrell, E. S. (1977). *Epichloë typhina* from toxic tall fescue grasses. Applied and Environmental Microbiology 34: 576-581.

MUTUALISM AS PREVAILING CONCEPTUAL FRAMEWORK

Endophytes increase plant resistance against herbivores???

<=>

Clay, K. 2009. Defensive mutualism and grass endophytes: still valid after all these years? In: Defensive mutualism in symbiotic association (eds. M. Torres. and J.F. Jr. White). Taylor and Francis Publications: 9-20.

FUNGAL ENDOPHYTES

Also tree endophytes attracted increasing attention with a ten years delay (see e.g. Special Feature of Ecology in 1988, vol 69: “Endophyte Mutualism and Plant Protection from Herbivores”).

Horizontally transmitted tree endophytes were described as “inducible mutualists” whilst vertically transmitted grass enodophytes were considered “constitutive mutualists” (Carroll 1988)

Fungal endophytes limit pathogen damage in a tropical tree

A. Elizabeth Arnold*†, Luis Carlos Mejía‡, Damond Kyllo‡, Enith I. Rojas‡, Zuleyka Maynard‡, Nancy Robbins‡, and Edward Allen Herre‡

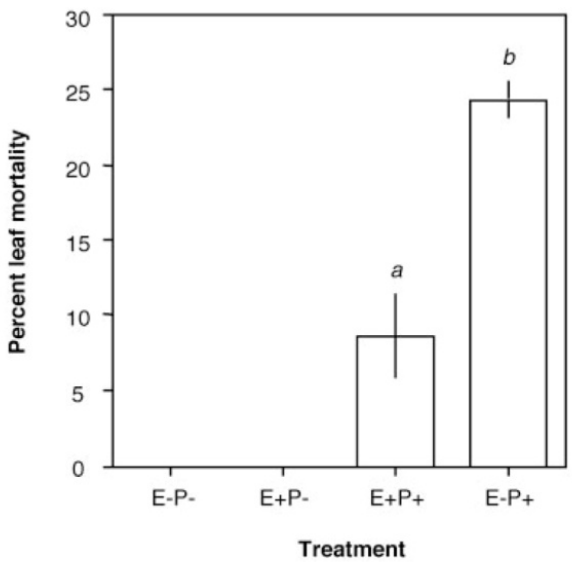

Fig. 4. Endophyte colonization reduces leaf mortality due to a foliar pathogen in seedlings of *T. cacao.* No mortality occurred among control leaves lacking endophytes and the pathogen (E−P−), nor among leaves inoculated only with endophytes (E+P−). In the presence of the pathogen, mortality occurred 2.8 times more frequently among leaves lacking endophytes (E−P+) than among leaves with endophytes (E+P+) ($P = 0.0317$).

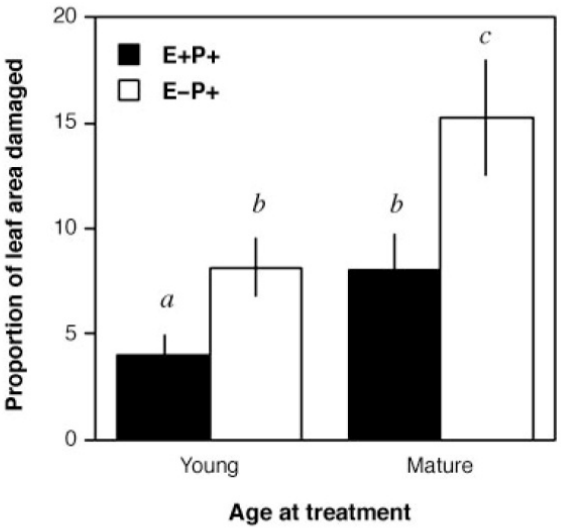

Fig. 5. Fungal endophytes reduce leaf area lost to a foliar pathogen for young and mature leaves of *T. cacao*, and the scale of beneficial effects by endophytes is specific to leaf age. For both young and mature leaves, inoculation with endophytes was associated with a decrease in mean proportion of leaf area damaged by *Phytophthora* ($P = 0.0130$, $P = 0.0241$). For young foliage, E−P+ leaves lost ≈3.5% more leaf area than did E+P+ leaves. Mature leaves lacking endophytes lost ≈7.1% more leaf area than did mature leaves with endophytes.

Together, these data demonstrate the capacity of diverse, horizontally transmitted endophytes of woody angiosperms to play an important but previously unappreciated role in host defense.

PNAS | December 23, 2003 | vol. 100 | no. 26 | 15649–15654

Fungi and the food of the gods

Keith Clay

Plants protect themselves against attacks by microorganisms in various ways. In some circumstances at least, it turns out that they enlist the help of mutualistic fungi in defence of the home front.

Chocaholics will be pleased with the results of Arnold *et al.*[1], published last month in *Proceedings of the National Academy of Sciences*. In the long term, the findings promise a strategy for increasing production of the crop that gives rise to this delectable treat. More immediately, however, they will come as a revelation to many plant biologists. They show that a poorly understood group of microbes provide their host plants with an effective defence against pathogens. The microbes are 'endophytes', fungi that live inside leaves without causing disease, and they appear to be ubiquitous associates of all plants.

Cacao provides the raw material for chocolate, the botanical name for the source tree (Fig. 1) being *Theobroma cacao* (which literally means 'food of the gods'). Production occurs primarily in the tropics of the Old World, owing to the high disease prevalence in the cacao tree's native American tropics. Arnold *et al.* report on endophytes and disease incidence in cacao trees in Panama, and find that the trees support diverse communities of fungi that are distinct from the endophytes inhabiting other tree species. The team also experimentally manipulated fungal endo-

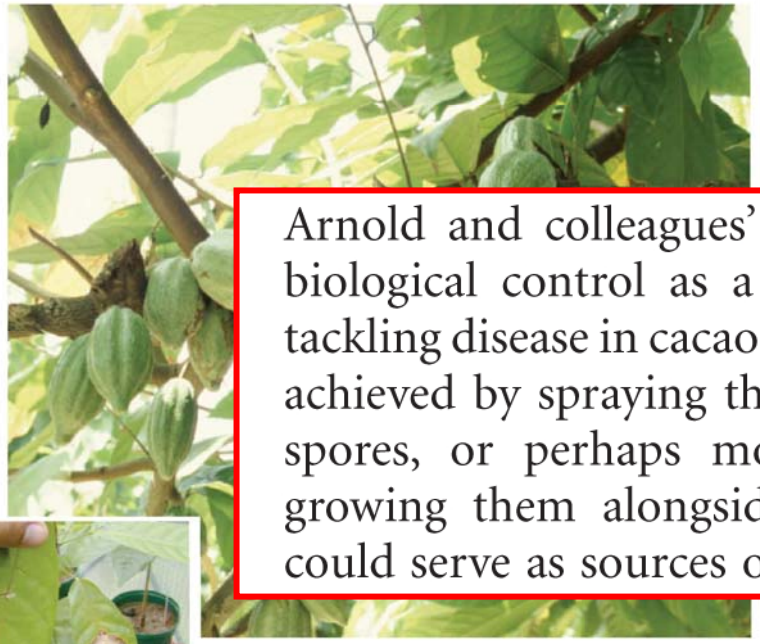

Figure 1 **The cacao tree, *Theobroma cacao*. The pods evident in this photo contain the beans from which chocolate is made. Inset, experimental cacao plants showing the effects of disease on the leaves of plants that have been inoculated with endophytes (centre) and the much greater damage to leaves of control plants.**

Arnold and colleagues' findings[1] point to biological control as a promising way of tackling disease in cacao trees. This could be achieved by spraying the trees with fungal spores, or perhaps more realistically by growing them alongside other trees that could serve as sources of fungal inoculum.

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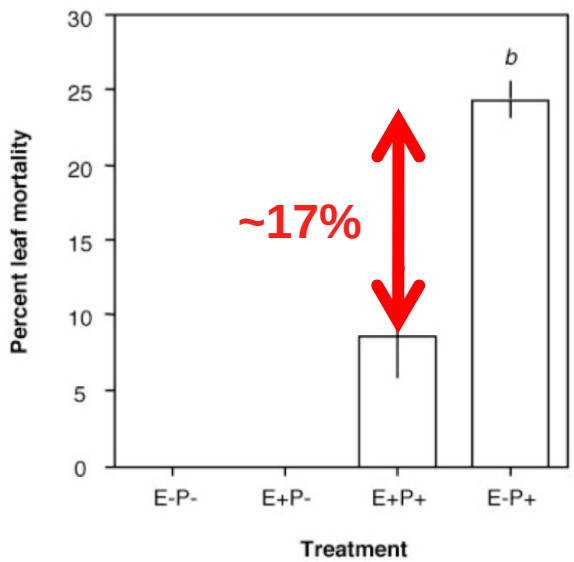

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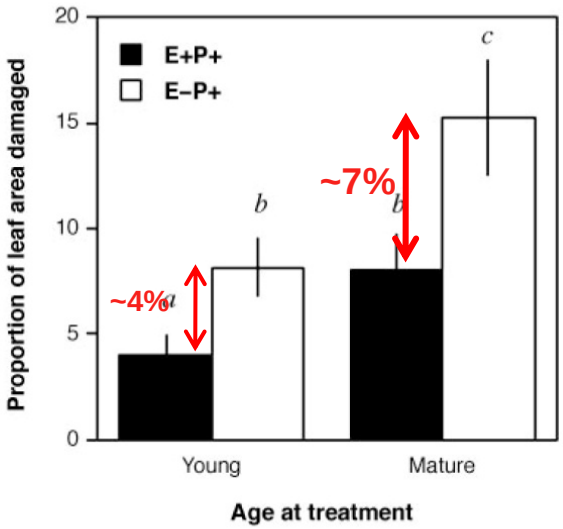

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Trees can not escape endophyte infections ⇨ No E- trees in nature!

Biologically significant?

Saikkonen (2007)
Fungal Biology Reviews 21/2-3: 67-74

Have we overemphasized the importance of endophyte mediated plant-herbivore interactions?

Fungal perspective!

Saikkonen et al. (2006) *Trends in Plant Science 11: 428-433*
Saikkonen et al. (2010) *Fungal Diversity 41: 101-113*

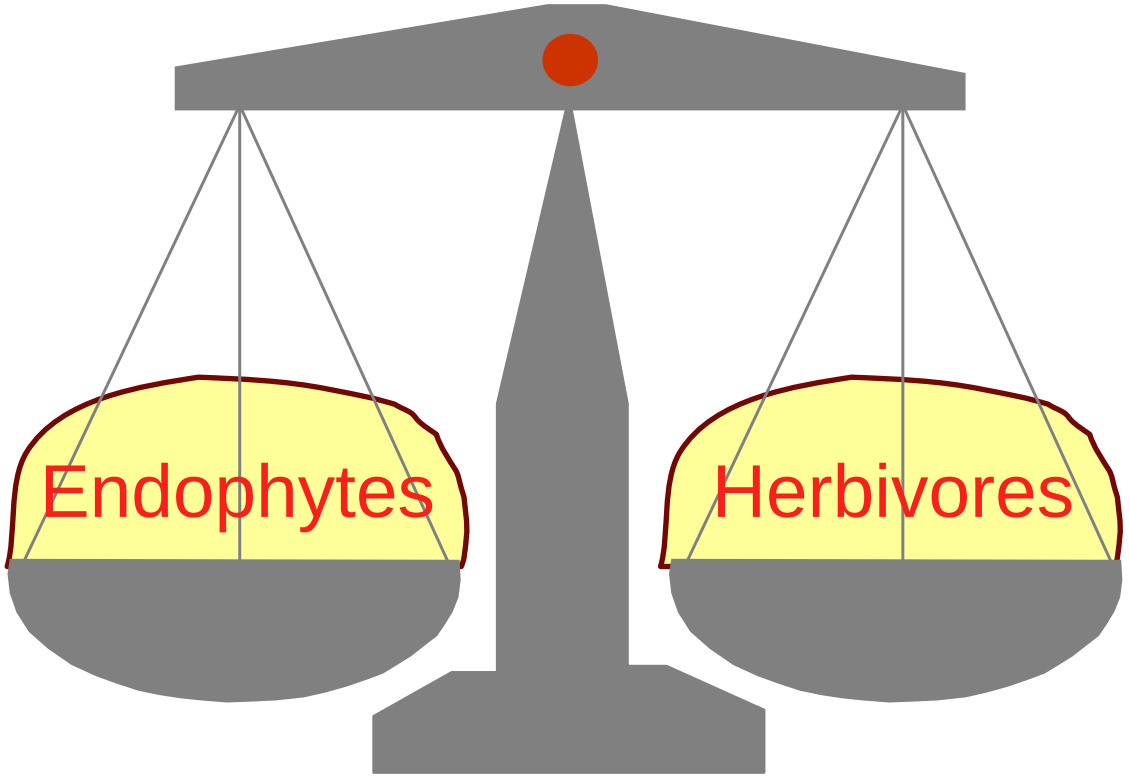
Transmission Mode and Reproduction!

Non-systemic tree-endophytes are transmitted horizontally by (either asexual or sexual) spores from plant to plant

Grass endophytes that are transmitted vertically from maternal plant to offspring via seeds probably show stronger mutualism with the host plant by deterring herbivores

?

Saikkonen et al. (1998) *Annu. Rev. Ecol. & Syst. 29: 319-343*
Saikkonen et al. (2004) *Trends in Plant Science 9: 275-280*
Saikkonen (2007) *Fungal Biology Reviews 21/2-3: 67-74*



Response to host plant <=> **Endophyte-herbivore interaction**

Ahlholm et al. (2002) *Ecology Letters 5: 648-655*

Tree-line gardens (Kevo)

Founded in 1970's

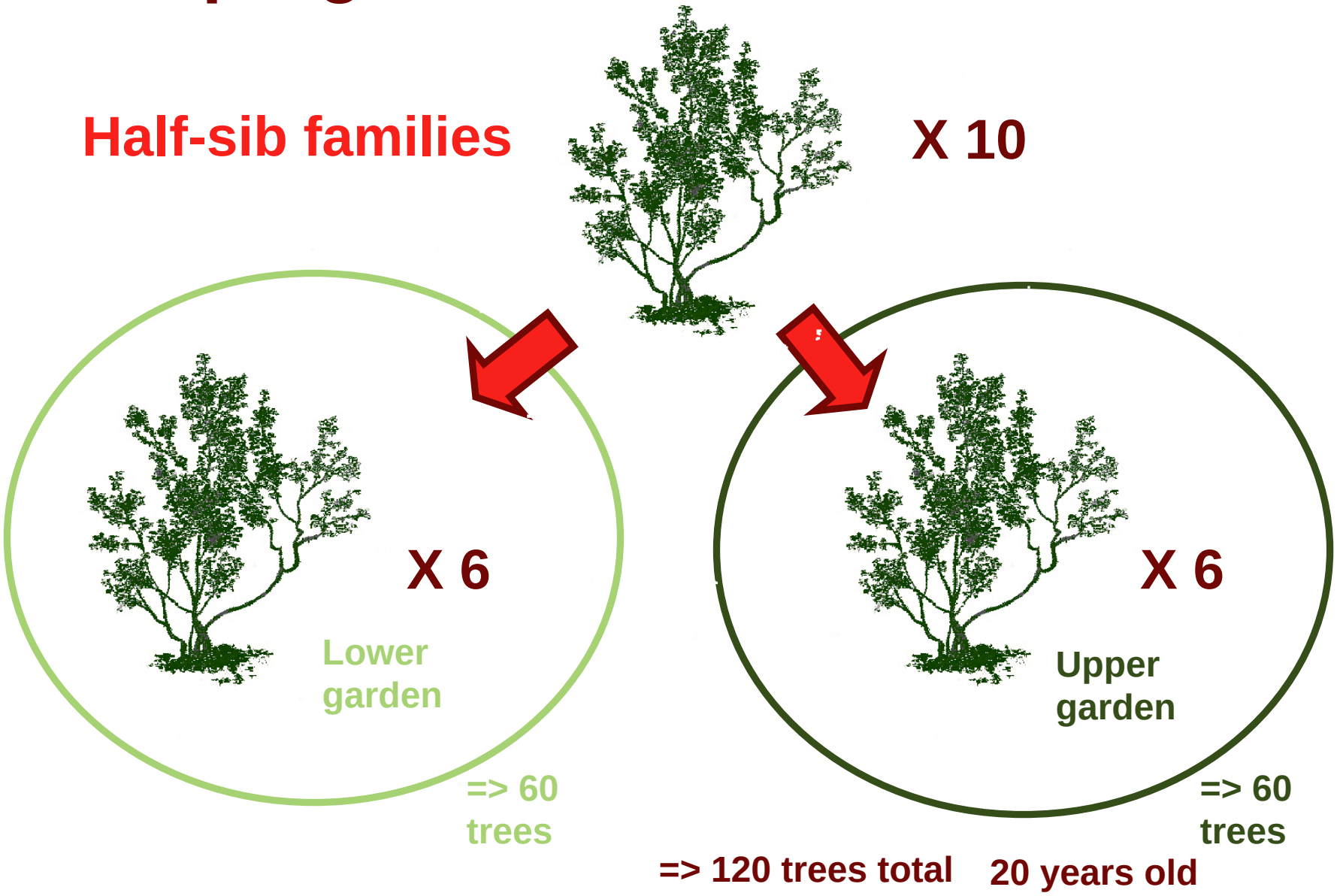
Lower garden

Upper garden

	River valley	Tundra
Altitude (a.s.l.)	90 m	270 m
Vegetation zone	birch (some pines)	tree-line

1) Mean daily summer temperatures are ~1.5-2ºC higher in the Lower garden
2) the growing season starts ~9 days earlier in the Lower garden

Birch progenies



Endophytic fungi in birch leaves

Elamo et al. (1999) Oecologia 118:151-156

Year	leaf infection %	
	Lower garden	Upper garden
1994	98	94
1995	99	98
1996	95	95

Species	% of all infections		P (ANOVA)		
	Lower	Upper	Garden	Year	Half-sib family
Fusicladium sp.	78	63	0.000	0.000	0.043
Melanconium sp.	4	15	0.000	0.000	0.068

Phenotypic correlations (r, n=60) among frequencies of foliar microfungi and performance of insect species on mountain birch in two environments (Lower and Upper garden) in northern Finland. *** = p<0.001, ** = p<.0.01, * = p<0.05, ° = p<0.10. Ahlholm et al. (2002) Ecology Letters 5:1-8.

Fungus		*Fusicladium* sp. (Endophyte)		*Melanconium* sp. (Endophyte)		*Melampsoridium betulinum* (Rust)	
		Garden					
Insect / Parameters / Year		Lower	Upper	Lower	Upper	Lower	Upper
Early season							
Middle season							
Deporaus betulae	Densities of D-rolls						
	1994	0.223°	-0.053	0.221°	0.234°	0.247°	0.219°
	1994	-0.146	-0.266*	-	-	0.250°	-0.036
	1995	-0.297*	-0.099	0.192	0.211	0.059	0.125
	1995	-0.184	-0.039	-0.016	-0.067	0.142	-0.152
	1996	0.216°	-0.342**	-0.074	0.004	0.136	0.068
Eriophyes rudis	Frequency						
	1995	0.058	0.024	-0.108	-0.048	0.001	0.178
Arge fuscinervis	Cocoon weight						
	1994	0.243°	-0.183	-	-	-0.033	0.152
	1994	0.032	0.051	-	-	-0.085	0.201
	1995	-0.234°	0.131	0.218°	0.175	-0.181	0.175
	1995	-0.021	0.112	0.038	0.082	-0.023	0.035
	1996	0.201	-0.231°	0.056	-0.076	0.078	0.131
Priophorus pallipes	RGR						
	1994	-0.028	0.051	-	-	-0.066	-0.030
	1995	0.097	0.146	-0.036	0.113	0.063	-0.013

Genetic correlations (r, n=10) among frequencies of foliar microfungi and performance of insect species on mountain birch in two environments (Lower and Upper garden) in northern Finland. *** = p<0.001, ** = p<.0.01, * = p<0.05, ° = p<0.10. Ahlholm et al. (2002) Ecology Letters 5:1-8.

Insect / Parameters / Year \ Fungus		*Fusicladium* sp. (Endophyte)		*Melanconium* sp. (Endophyte)		*Melampsoridium betulinum* (Rust)	
		Garden					
		Lower	Upper	Lower	Upper	Lower	Upper
Early season							
Mid-season							
Deporaus betulae	Densities of larvae	0.410	-0.630°	-	-	0.457	-0.284
Dineura viridorsata	RGR 1994	-0.160	-0.758*	-0.041	-0.751*	0.197	-0.065
	1995	-0.322	-0.536	0.774**	0.489	-0.003	-0.477
	1996	0.092	-0.614°	0.176	0.846**	-0.130	-0.724*
Eriophyes rudis	Frequency						
	Cocoon weight 1995	-0.128	0.516	-0.453	-0.322	0.179	0.291
Arge fuscinervis	RGR 1994	0.286	-0.565°	-	-	0.172	-0.522
	1995	-0.338	-0.580°	0.469	0.561°	-0.275	-0.583°
	1996	-0.583°	0.078	-	-	-0.526	0.244
Priophorus pallipes	RGR 1994	-0.051	0.097	-	-	0.179	-0.378
	1995	0.119	0.421	-0.342	0.185	0.217	0.258

Conclusions:

1) Potential causal association only between fungal frequencies and growth performance of one late season herbivore species, *Dineura virididorsata*

2) Occasional apparent endophyte mediated herbivory performance may actually better indicate:

- genetic differences in plant quality for fungi and herbivores, or

- responses to environmental conditions,

without causal relationship between fungal infection and herbivore performance.

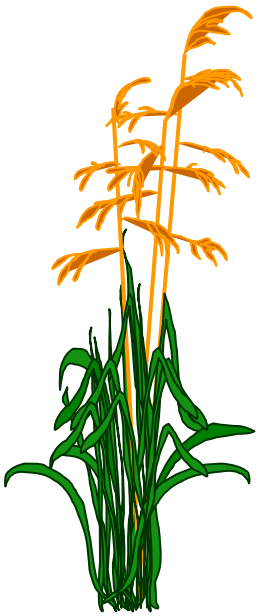
Ahlholm et al. (2002) *Ecology Letters 5: 648-655*

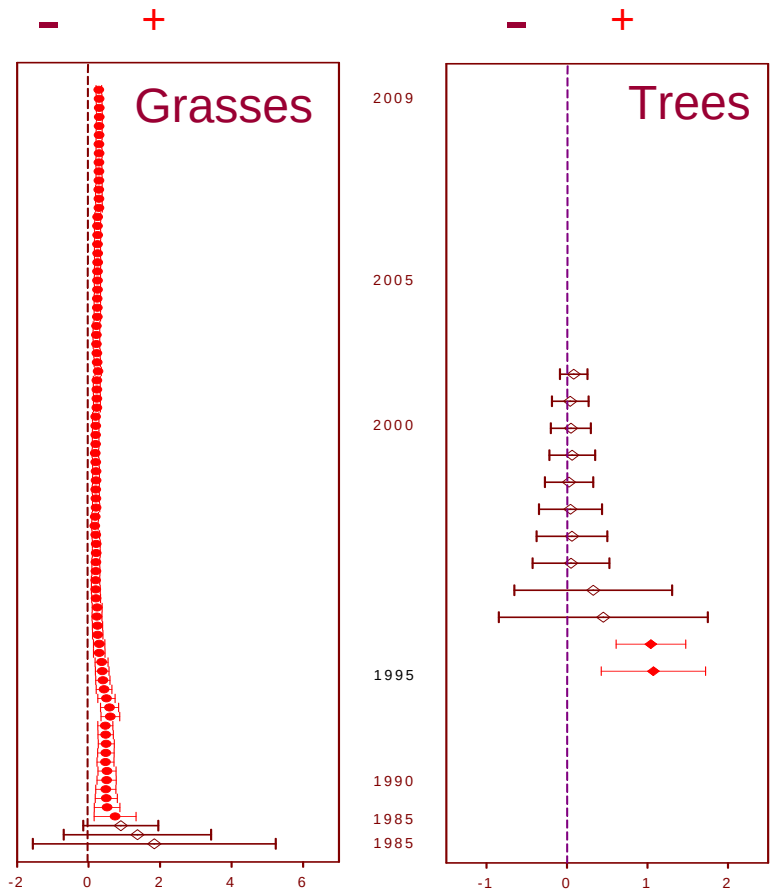
Ahlholm et al. (2002) *Evolution 56: 1566-1573*

Cumulative meta-analysis

99 studies out of 157 primary publications (36 plant, 62 herbivore and 17 predator or parasitoid taxons)

Effect of endophyte on resistance to herbivores





Effect size = 0,2993
N = 135
95% CI: 0,2109 - 0,3877

Effect size = 0,0864
N = 24
95% CI: -0,1137 - 0,2866

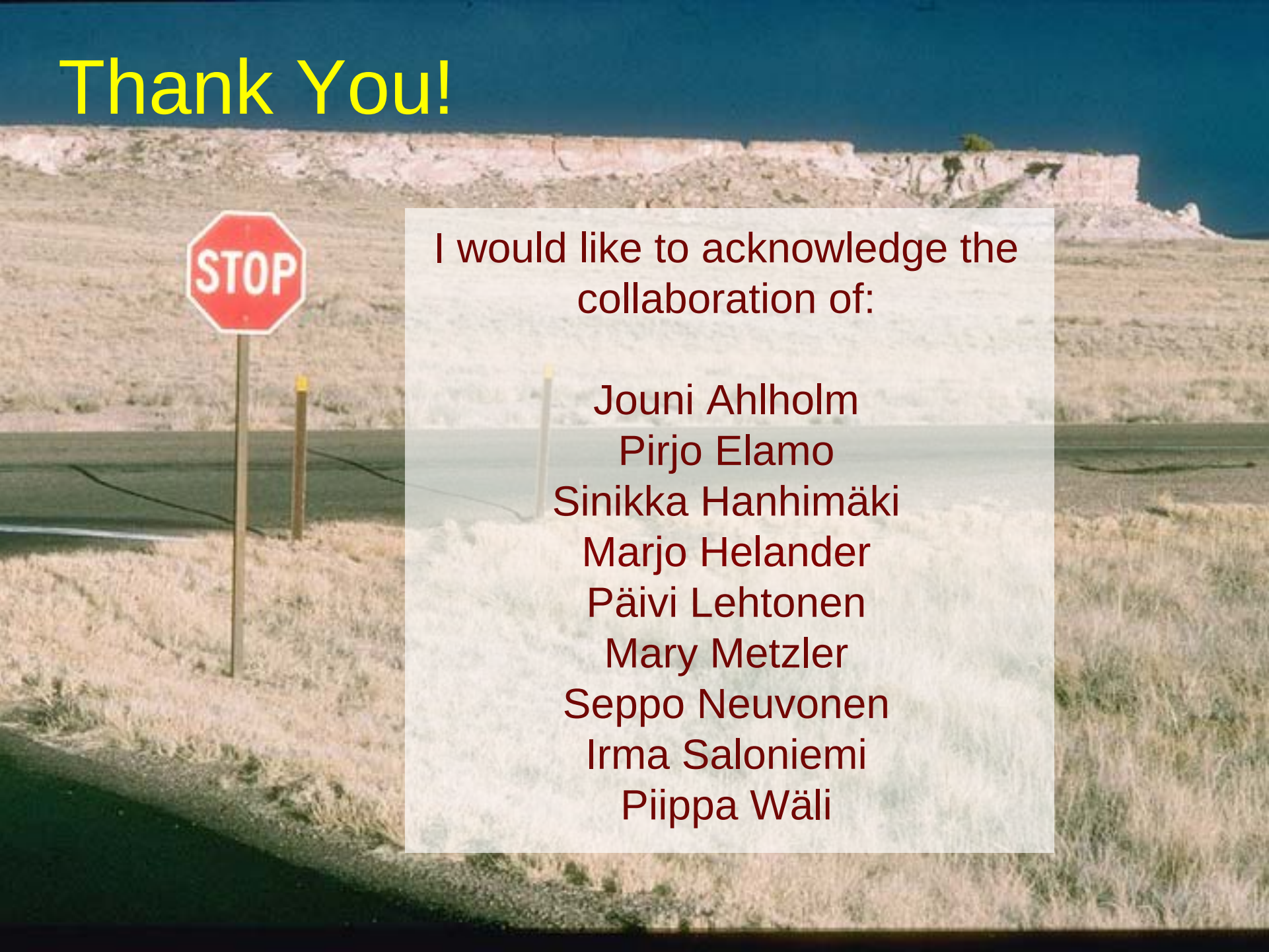
Grass vs. tree endophytes: Q_b = 3.99, p = 0.046, df = 1

Saikkonen et al. (2010) *Fungal Diversity 41: 101-113.*

Conclusions

- Seemingly endophyte-mediated herbivore performance may actually better indicate tree quality to fungi and herbivores without a causal relationship between them.
- Genetic correlation structure may have importance for the evolution of tree resistance to fungi and herbivores; e.g. negative correlation between tree resistance to pathogens and herbivores may constrain tree populations from reaching optimal species-specific resistance.
- This should be considered also in forest tree improvement attempting to overcome pests and pathogens.
- More knowledge about multispecies coevolution is necessary to fully understand bilateral interactions between plants and the organisms living on them.

Thank You!



I would like to acknowledge the collaboration of:

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Piippa Wäli

Why genetic correlations tended to be stronger in upper garden?

1) Plasticity in genetically determined birch properties (changes in gene expression)

2) Genetic differences in fungi between the two environments

Genetic diversity of *Venturia ditricha*

(anamorph *Fusicladium betulae*)

Random amplified microsatellites (RAMS)

AMOVA

Source	% of variation	*P*
Among gardens	0.45	0.17
Among half-sib families within gardens	9.73	0.0001
Within half-sib families	89.79	0.0001

ANOVA

	F (*df*)	*P*
Garden*Half-sib family	2.18 (9)	0.0322

Ahlholm et al. (2002) *Evolution 56: 1566-1573*

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Conclusions

Genetic analysis revealed that

(1) birch genotypes influence the probability of infection by particular endophyte genotypes,

(2) genetic variation correlated negatively with infection frequencies of the fungus, and

(3) the susceptibility of the birch to a particular endophyte genotype may change when environmental conditions are changed (environment-host genotype interaction).