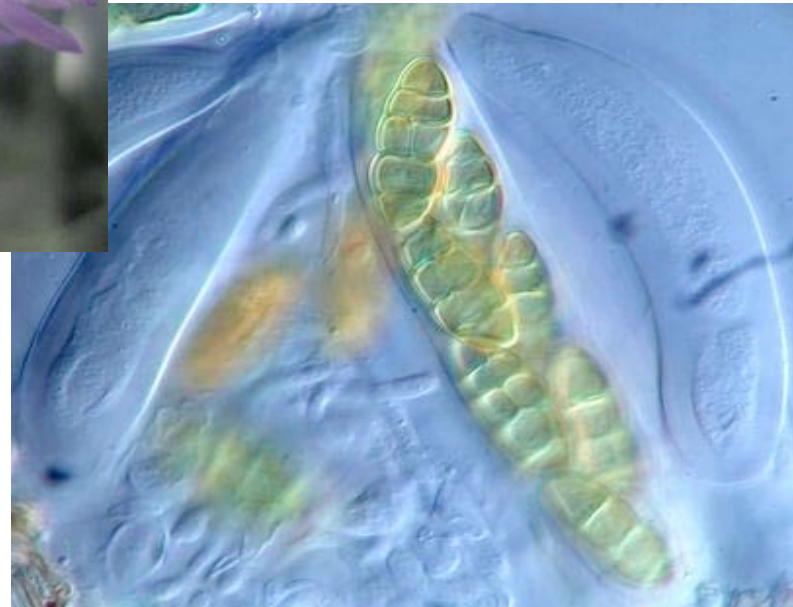


The roles and hidden diversity of endophyte fungi from spotted knapweed

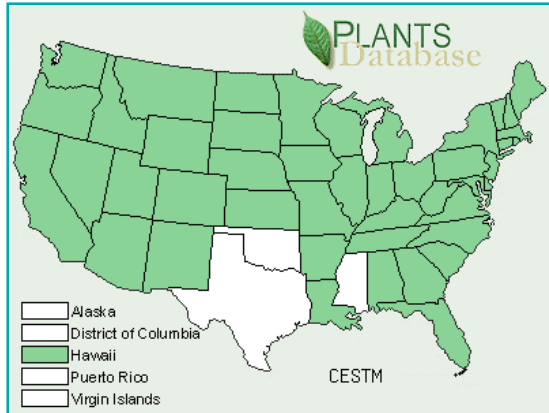


Alexey Shipunov

Post-Doctoral Research Scientist
Department of Forest Resources
University of Idaho



Spotted knapweed



Spotted knapweed
(*Centaurea stoebe* L., also known as *C. maculosa*, *C. micrantha*, *C. biebersteinii*) is a noxious, invasive plant which was introduced into North America from Eurasia. This plant is also a model in allelopathy research.



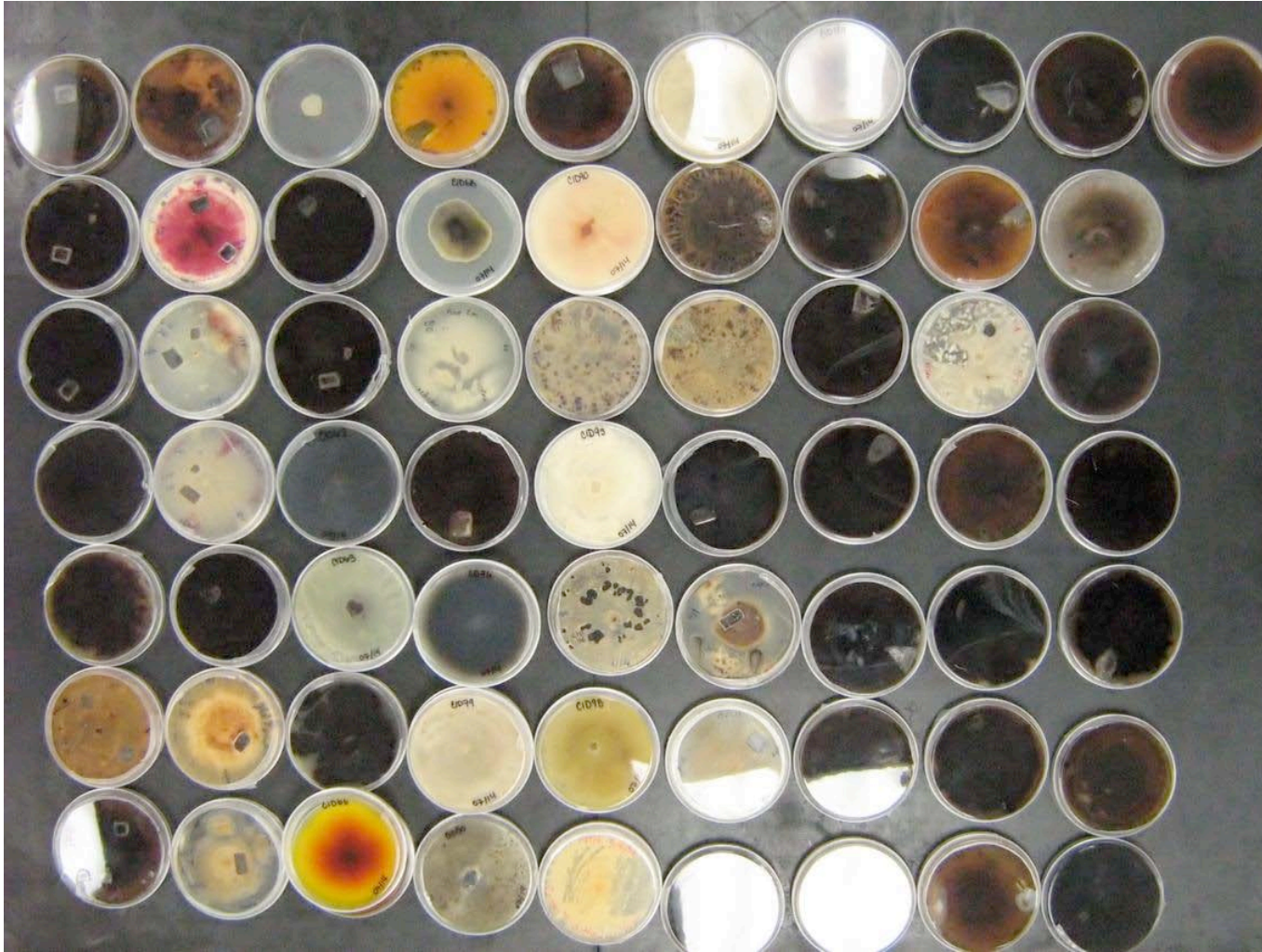


Volga river habitat



However, in native region (e.g., Russia and Ukraine),
knapweed is not invasive.

Endophyte roles

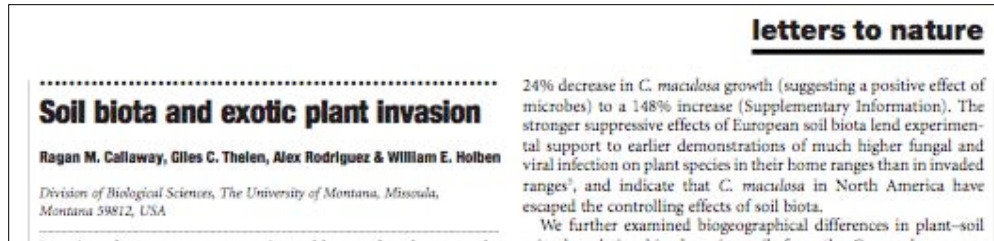


Our goal is to explain knapweed invasiveness
via plant-endophyte interactions

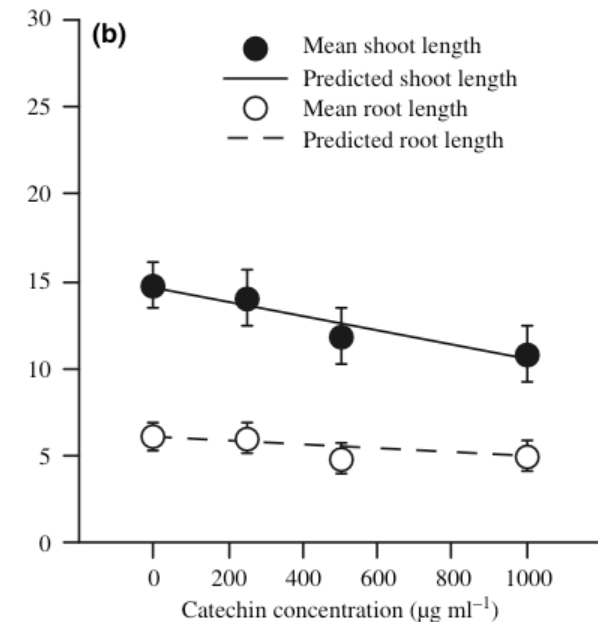
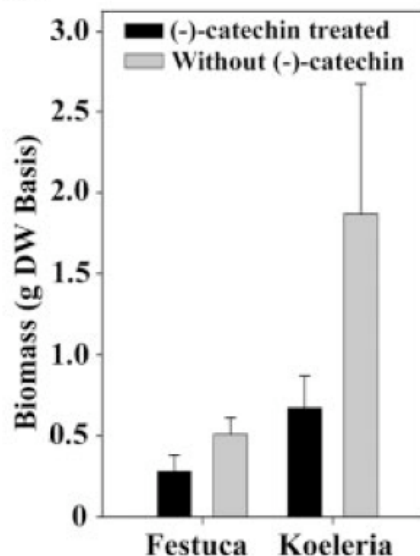
Hypotheses applicable to the knapweed invasion

- **Enemy Release Hypothesis** – enemies are left behind in the native range
- **Enhanced Mutualism Hypothesis** – new symbionts obtained in the invaded range from native hosts (“host-jumping”) and enhance abilities to compete natives, use resources etc.
- **Novel Weapons Hypothesis** – many invasive North American plants have been reported to have antimicrobial, antiherbivore and allelopathic effects. These effects could be due to chemicals “*unknown*” for naïve native plants. Co-introduced symbionts may help to produce such an effect.

Catechin or not catechin



The most accepted opinion (Callaway et al., 1999 and many others) is that *catechin*-contained knapweed root exudates are capable to suppress the growth of native grasses (*Festuca*, *Koeleria* etc.) and other plants.



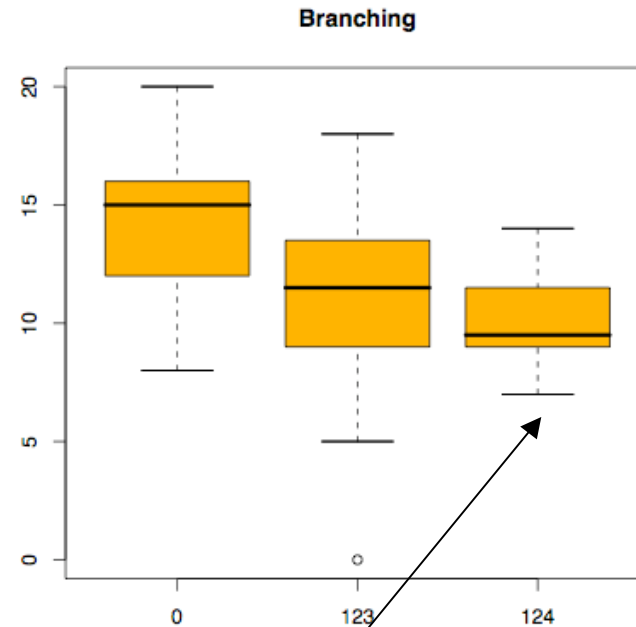
However, recent experiments (Blair et al., 2005, 2006) show the **absence** of catechin effect.

J Chem Ecol (2006) 32: 2327–2331
DOI 10.1007/s10886-006-9168-y

RAPID COMMUNICATION

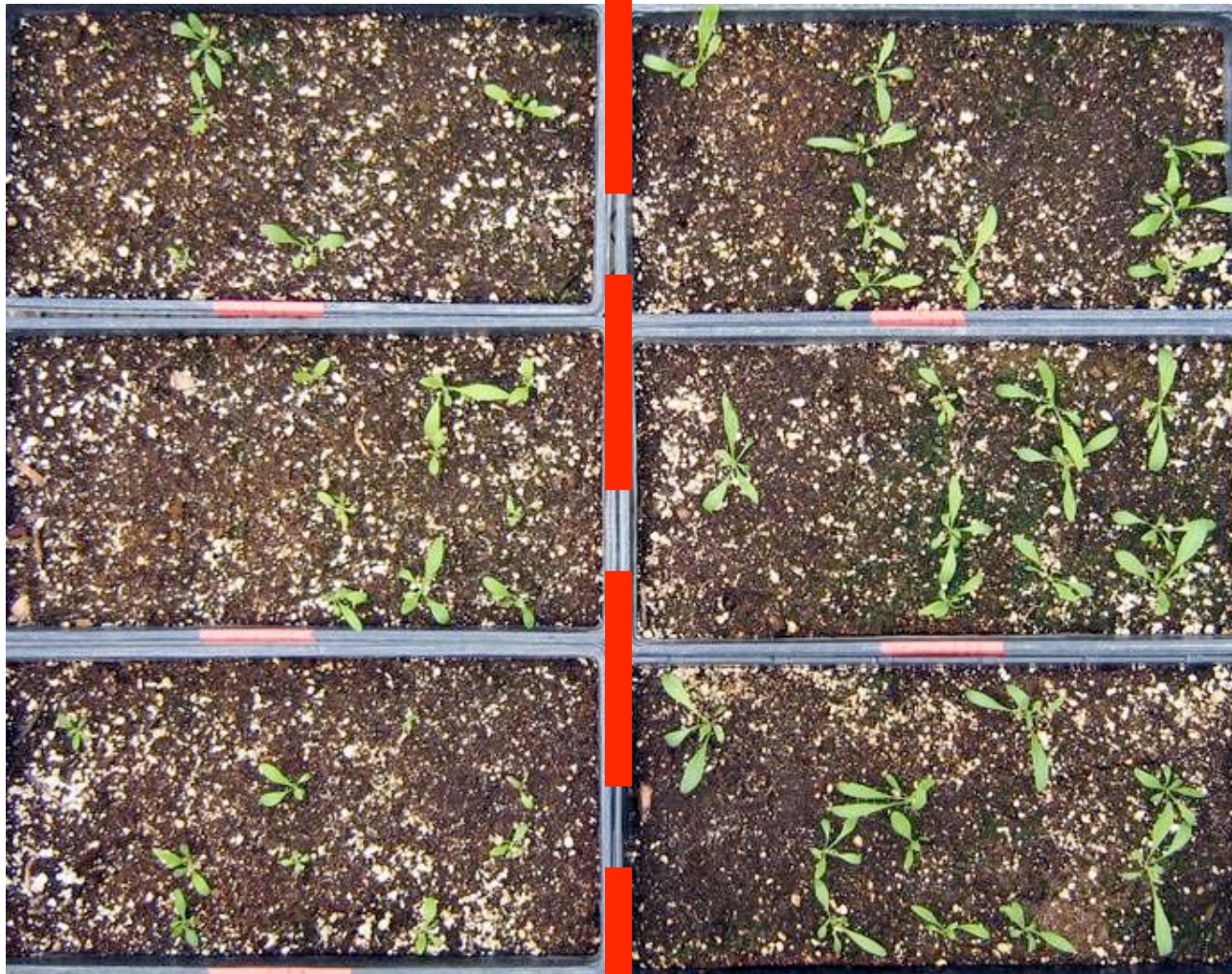
A Lack of Evidence for an Ecological Role of the Putative Allelochemical (\pm)-Catechin in Spotted Knapweed Invasion Success

Negative interactions



Endophyte strain *Fusarium* 124 suppresses the flowering of knapweed

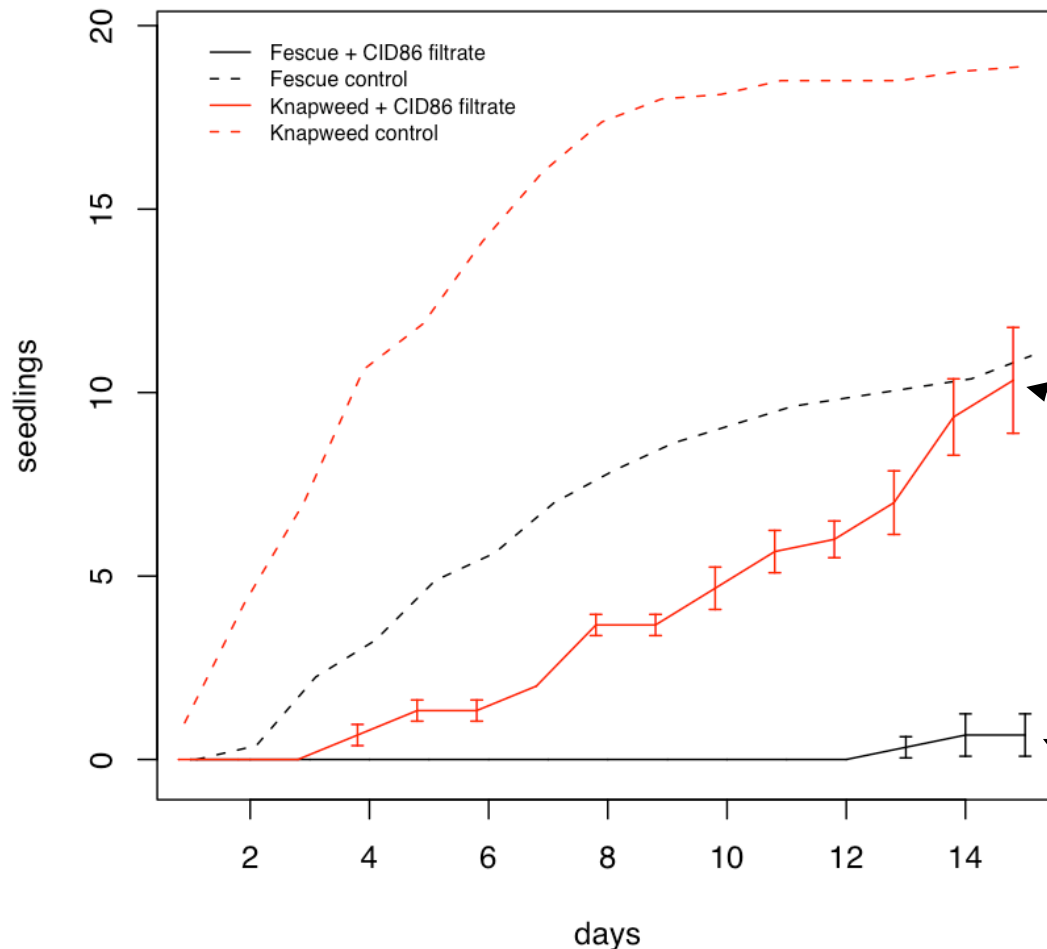
Some endophytes have almost pathogenic effect



Trays with inoculated
seedlings

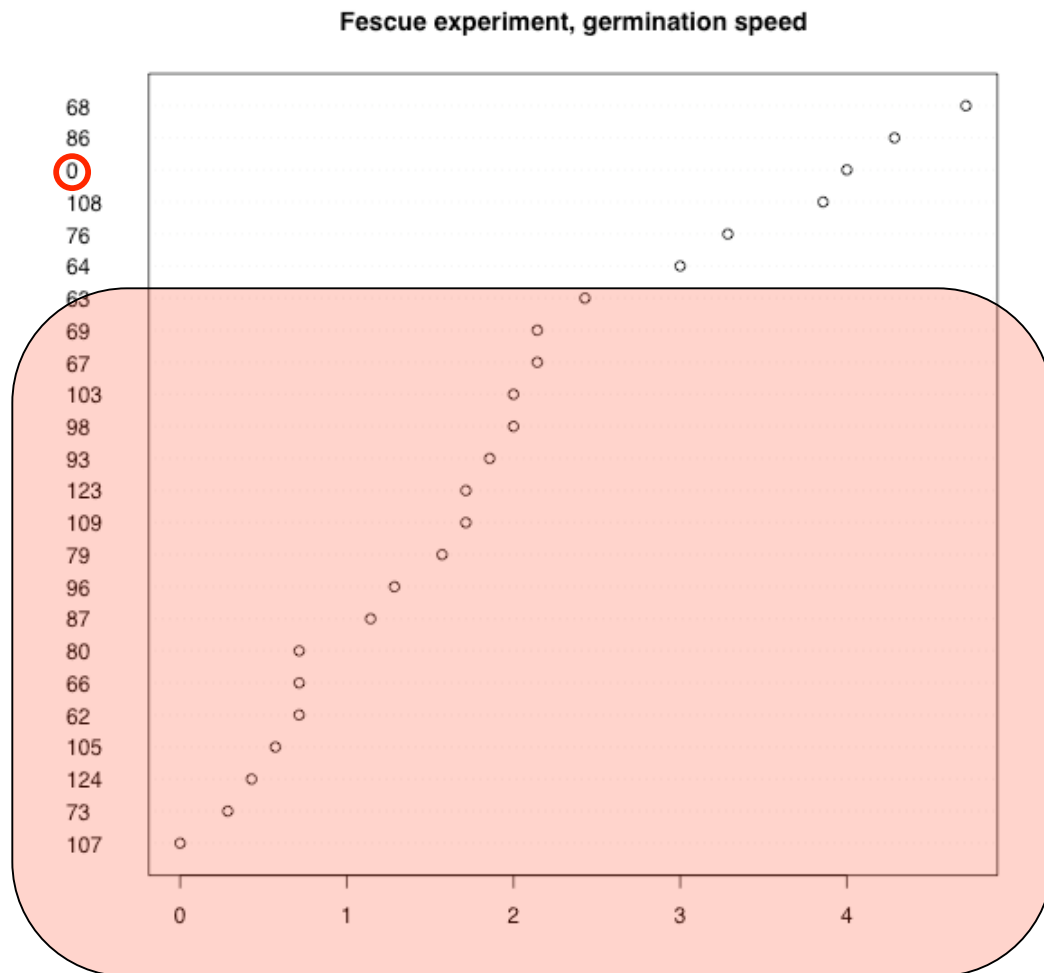
Trays with control
seedlings

Endophytes can suppress growth of Idaho fescue seedlings



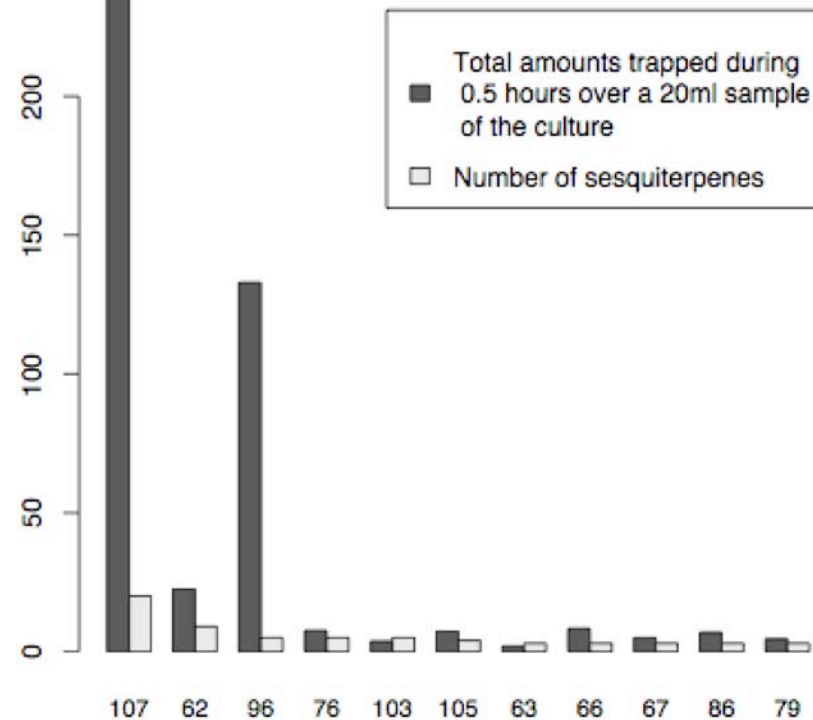
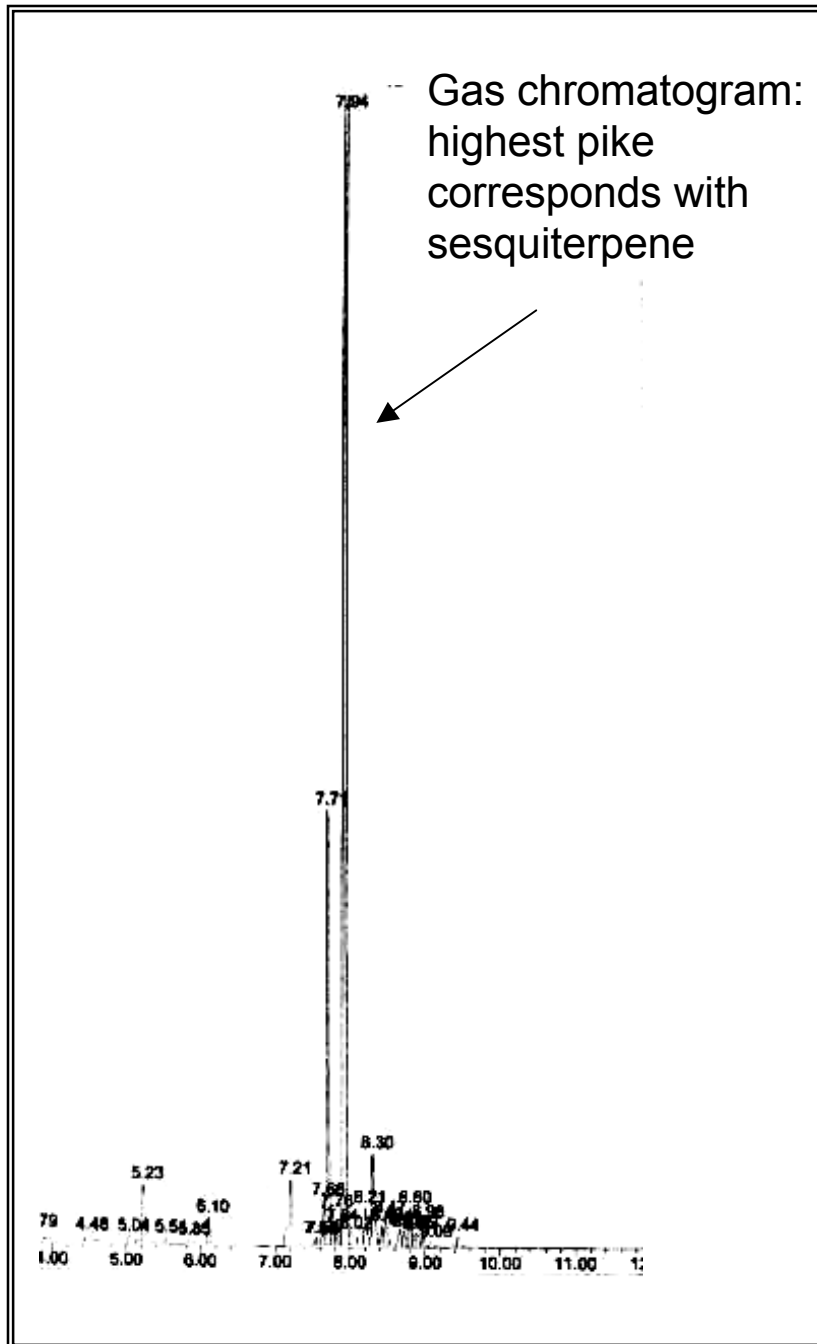
The selective effect: terminate fescue much more than knapweed

Effects in seed assays

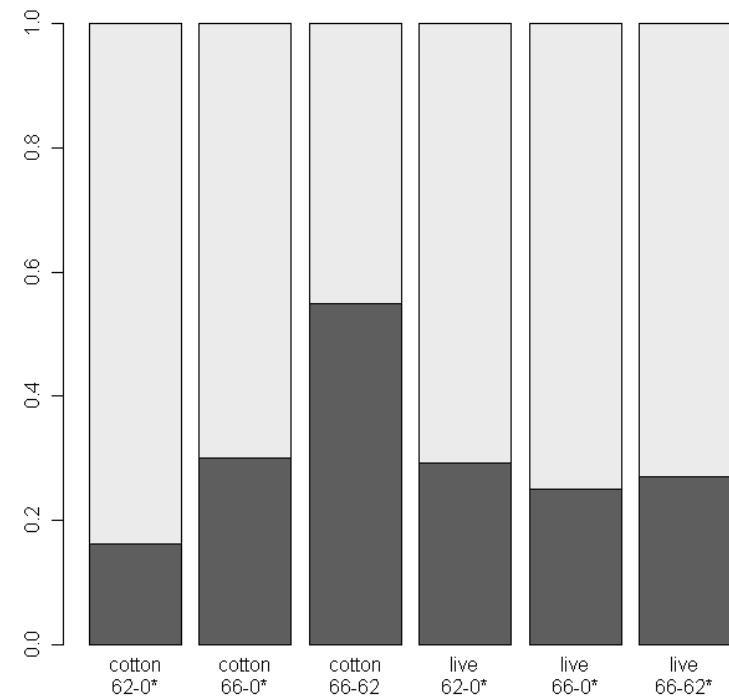


More than 2/3 endophyte strains have **statistically significant termination effect** on *Festuca idahoensis* seeds, some them have similar effect on knapweed seeds. Moreover, some endophytes can **kill** fescue seedlings.

Endophytes can produce sesquiterpenes



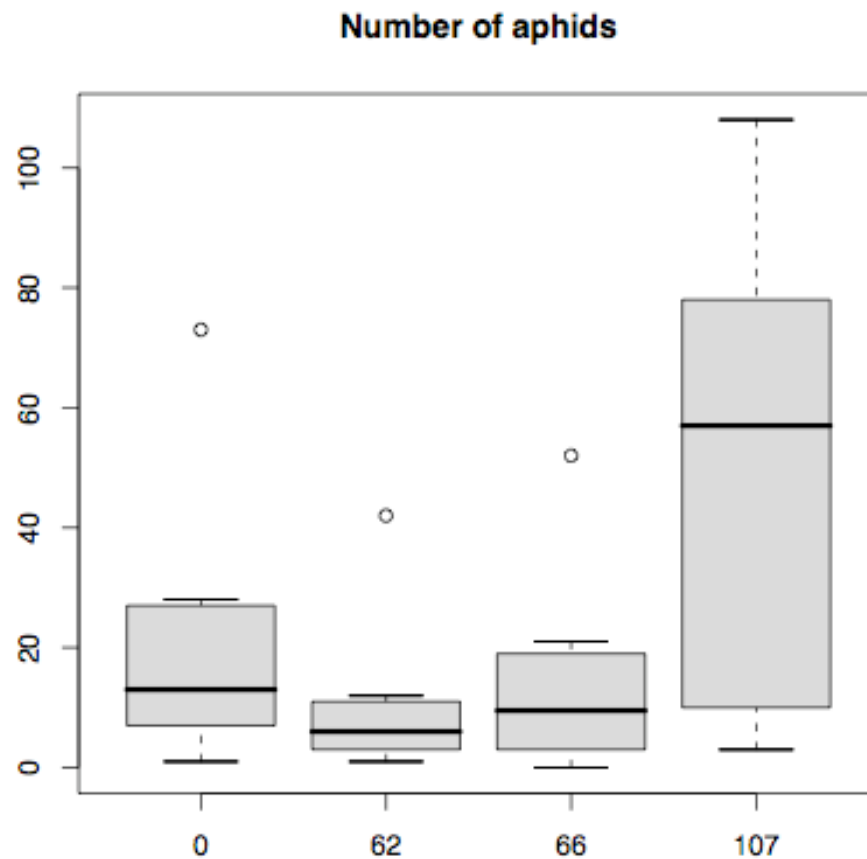
Insecticide effect



In a choice experiment, biocontrol weevils *Larinus minutus* demonstrated strong preference to non-inoculated flowers



Attraction of aphids



Some endophytes
can attract other
knapweed-eaters:
aphids



Green peach aphid
Myzus persicae

Competition experiments

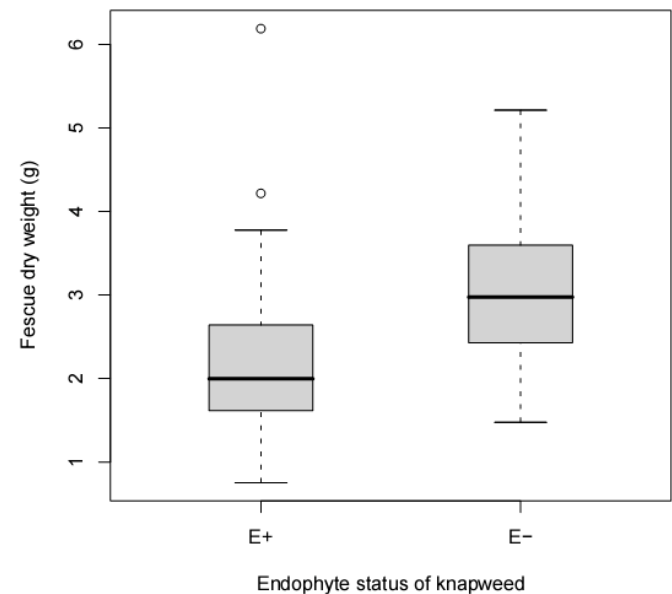


E+
knapweed
and fescue

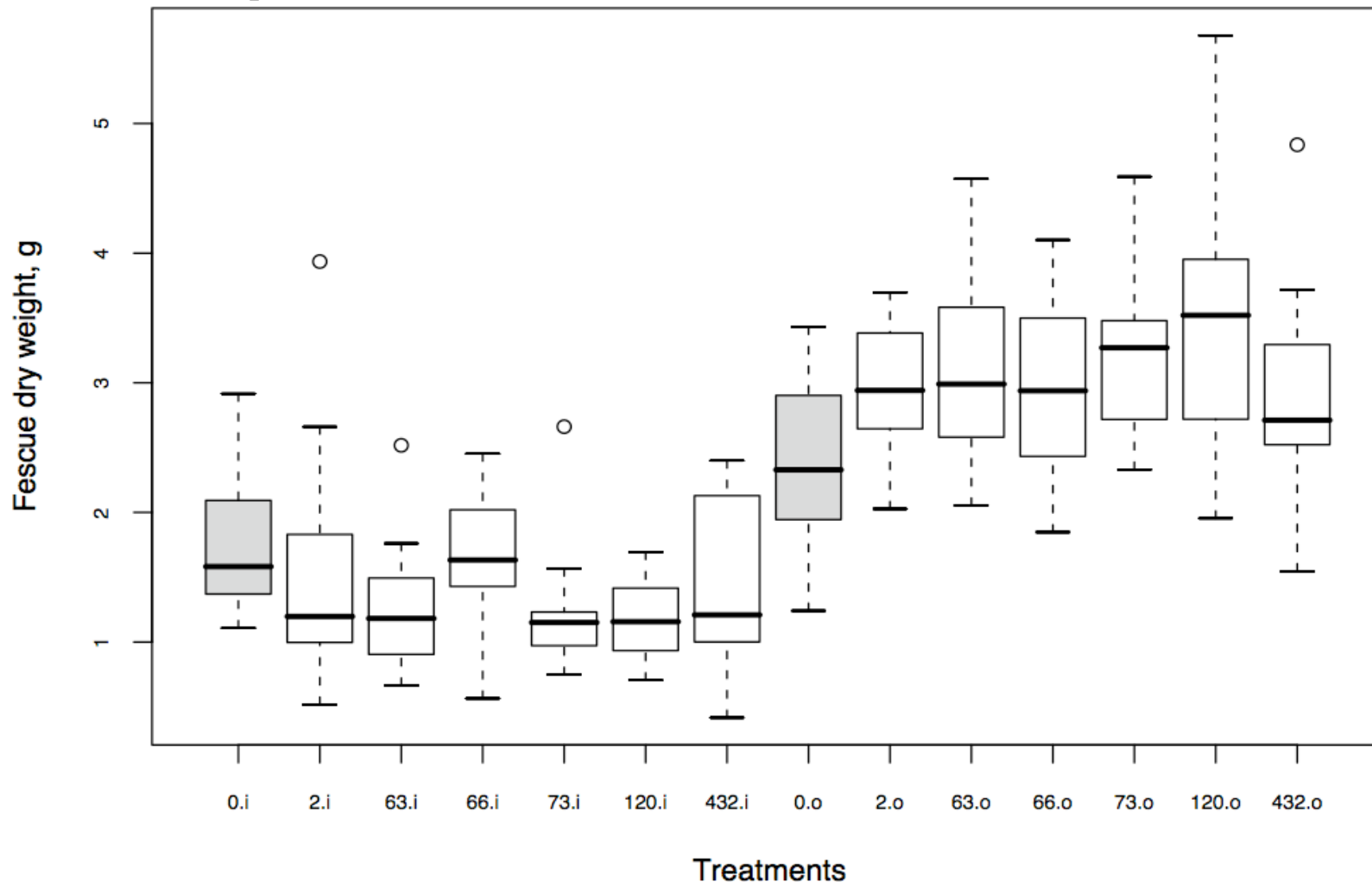
E-
knapweed
and fescue

Fescue
alone:
control

Differences in
biomass are
significant

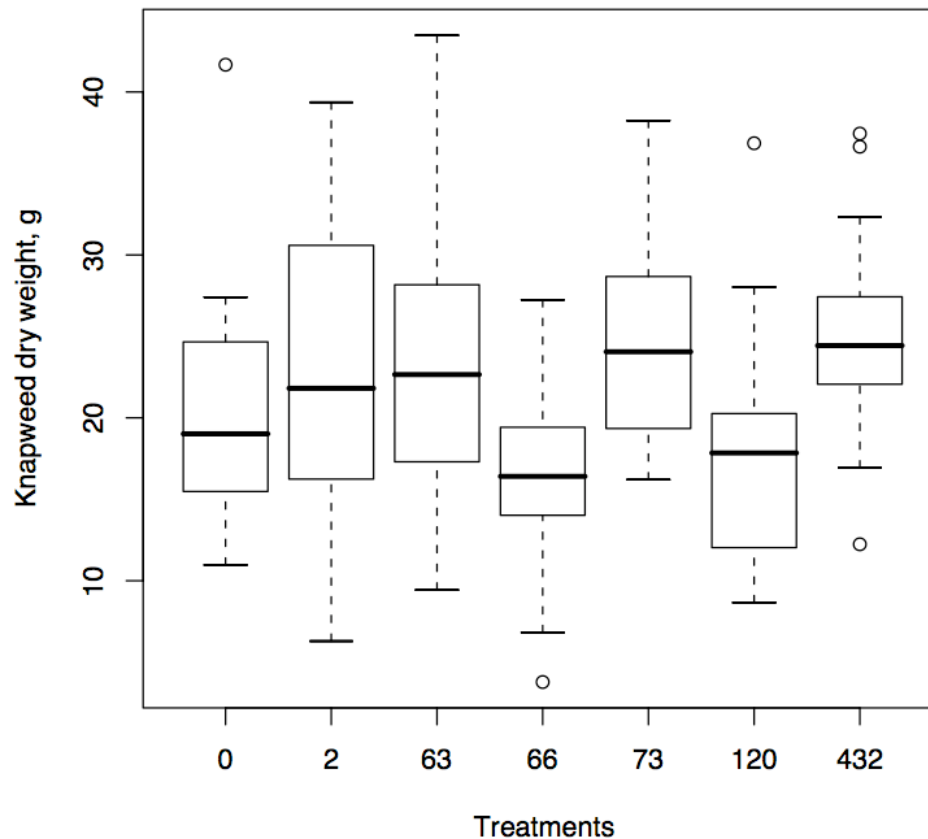


Detailed competition experiment: fescue biomass



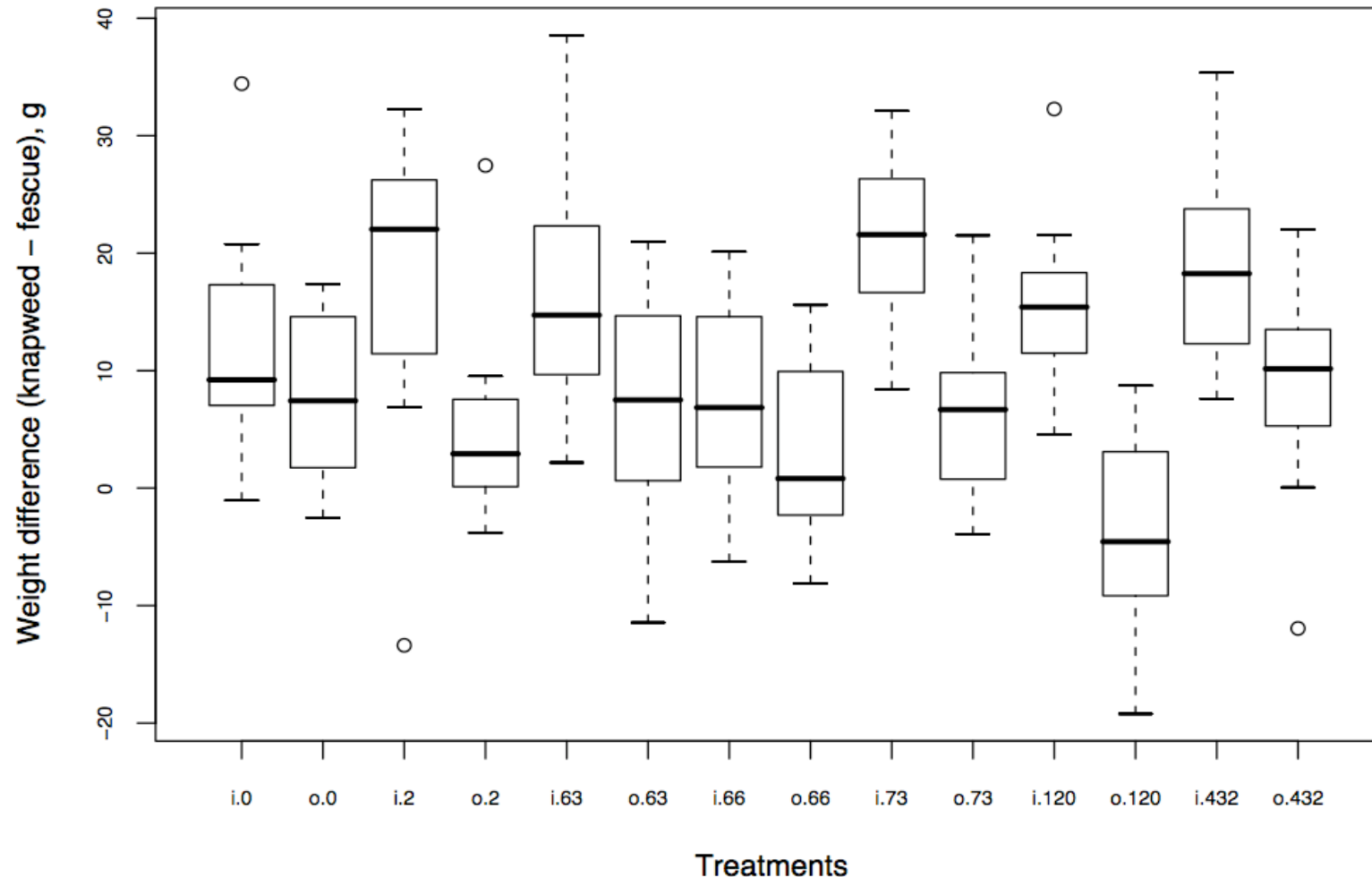
Novel weapons! Biomass of *Festuca idahoensis* decreased whereas biomass of *F. ovina* increased

Knapweed biomass



Some endophytes
(*Alternaria* 73,
Alternaria 432)
definitely increase
biomass whereas
Epicoccum 66
decreases biomass
along with plant
height and number of
flowers

Competitive advantage: the biomass difference



For nearly each pair of fescue species combined with particular endophyte, knapweed have competitive advantage over *F. idahoensis* much higher then over *F. ovina*



Spotted knapweed,
Centaurea stoebe



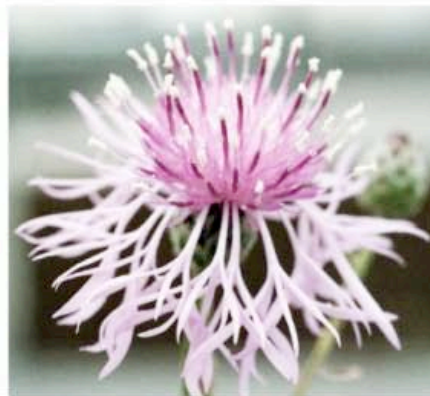
Delayed flowering:

Alternaria 123

Fusarium 124

Reduced flowering
extent:

Alternaria 62



Protection from
seedhead weevils:

Alternaria 62

Epicoccum 66

Reduced aboveground
biomass and increased
generalist herbivory:

Fusarium 107



Increase of aboveground
biomass:

Alternaria 73,

Alternaria 432

Suppressed
germination of *C. stoebe* itself:

Alternaria 120

Botrytis 360

Fusarium 107

Fusarium 396

Suppression
of germination of a competitor,
assayed in the same manner
as (-)-catechin:

Alternaria 62

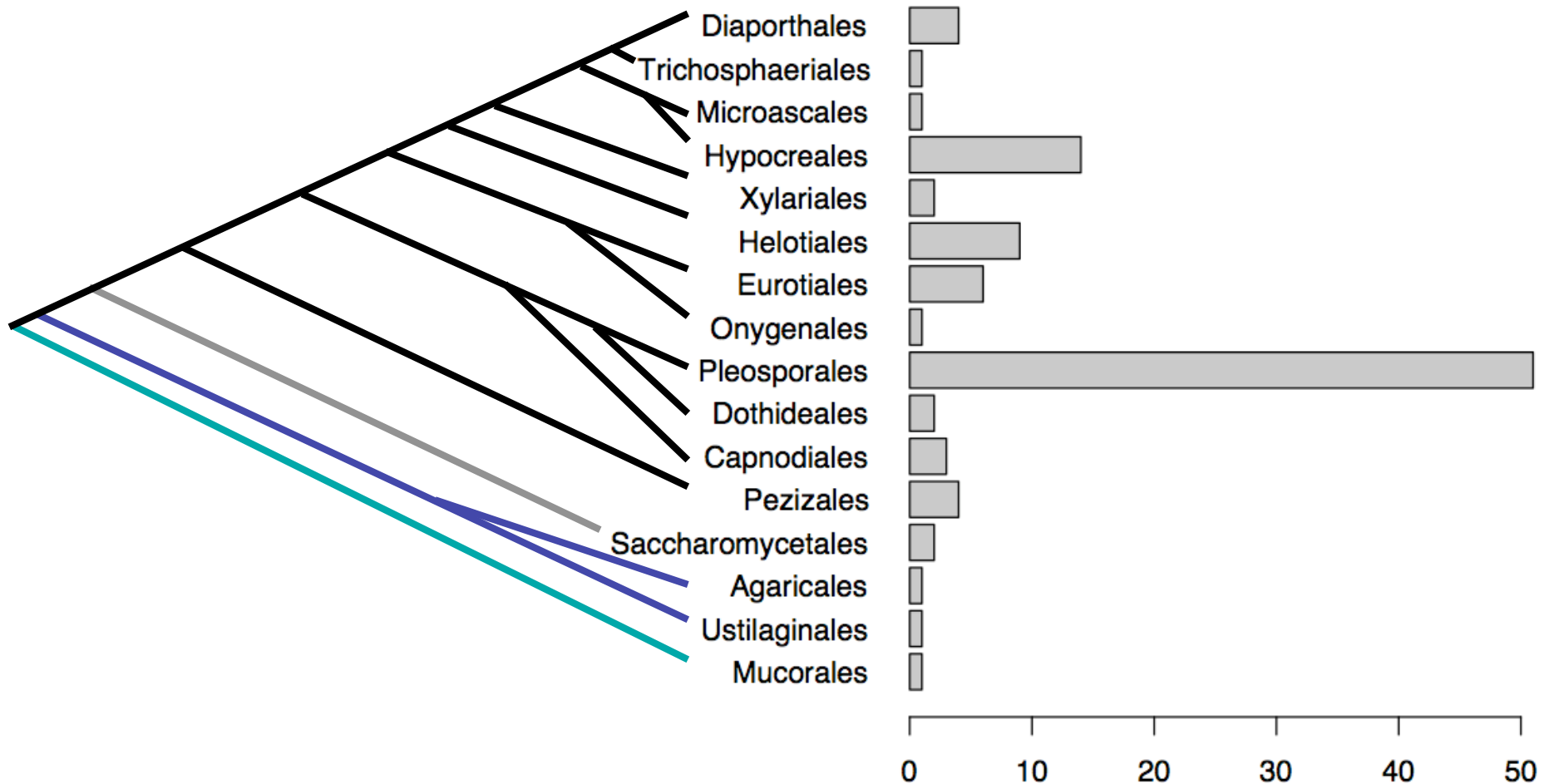
Epicoccum 66

Diversity: sampling

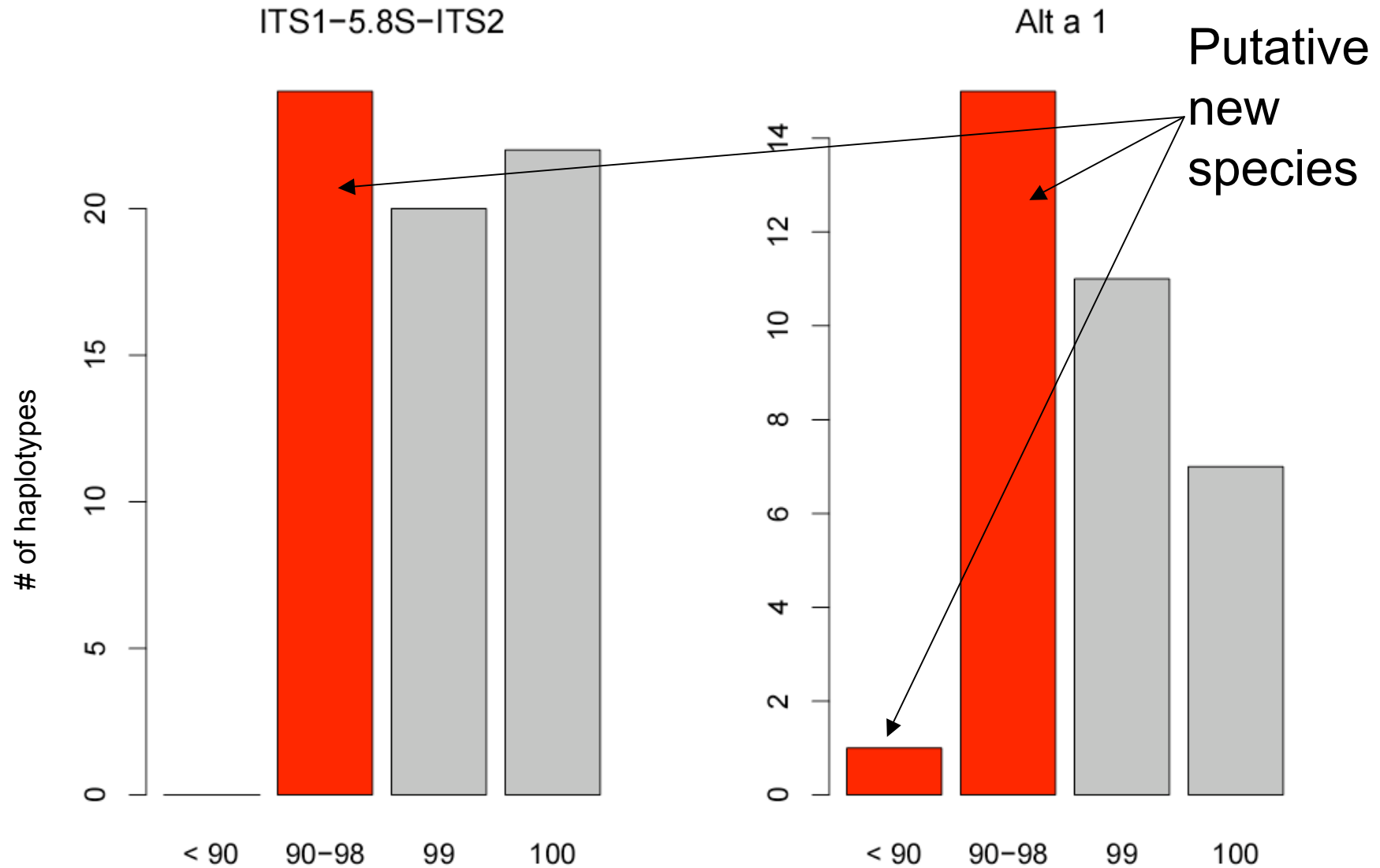


- 61 knapweed populations sampled, plus 10 populations of native North American plants (*Saussurea americana*, *Cirsium brevifolium*, *Festuca idahoensis* etc.)
- 5 plants and 100 achenes per sample
- Endophytes isolated from achenes – 2291 isolates
- Isolates grouped in 288 morphological groups (strains)
- Each group has been sequenced (ITS and “Alt a 1”), 102 haplotypes obtained

Taxonomic and phylogenetic structure



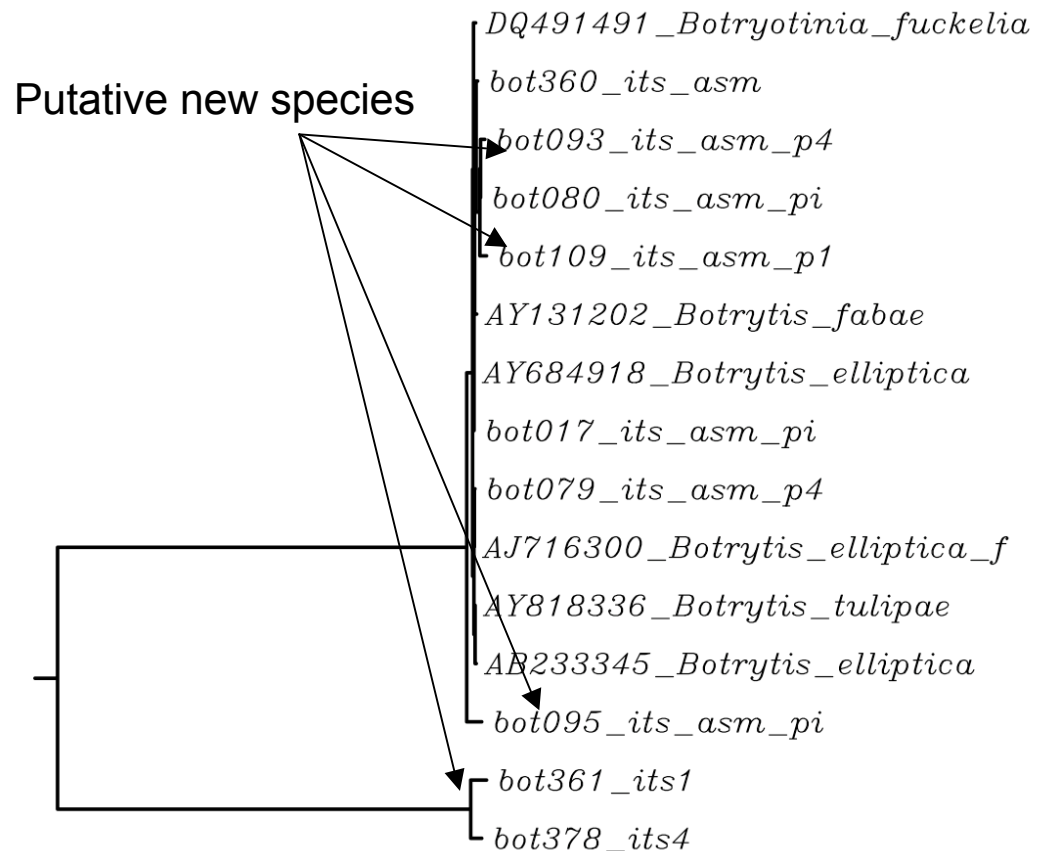
BLAST identity and new species



Some endophytes may represent novel lineages



Botrytis
(anamorph of *Botrytinia*,
Sclerotiniaceae)



The case of potential co-introduction: *Phoma tracheiphila*

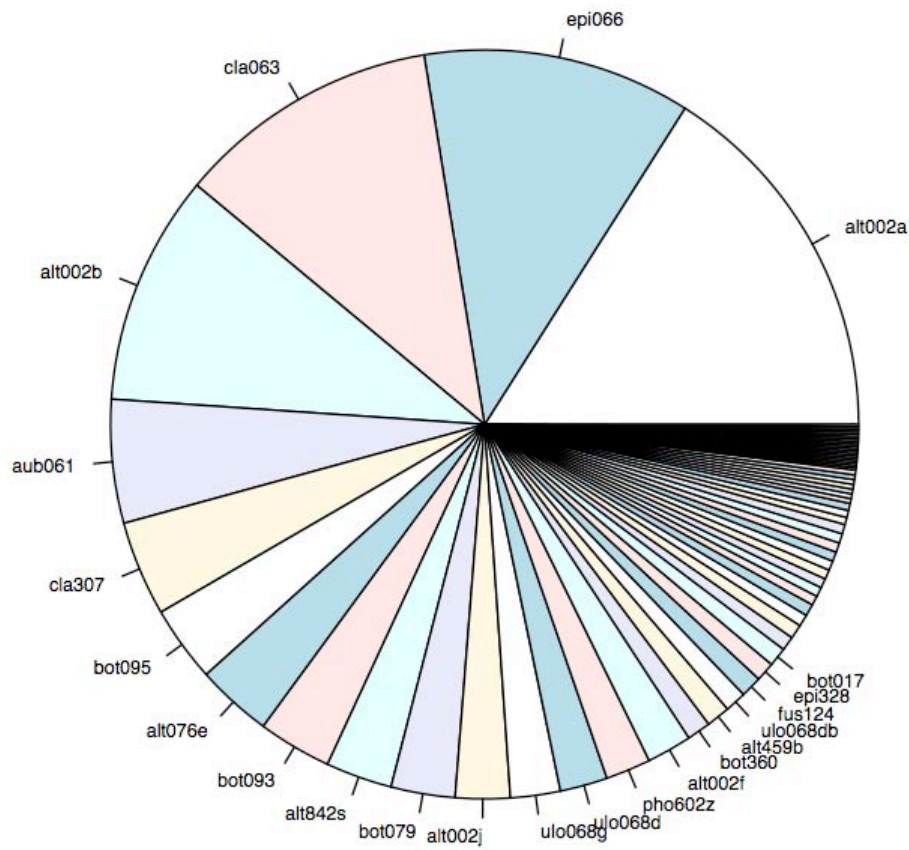
The endophyte **pho250** (South Germany) has 99% identity with GenBank sequences of *Phoma tracheiphila*, very dangerous pathogen of *Citrus* trees

AY531673_Pt_C_limon_Italy
AY531689_Pt_C_sp_Italy
AY531677_Pt_C_sp_Italy
AY531678_Pt_C_limon_Italy
AY531670_Pt_C_limon_Italy
AY531669_Pt_C_limon_Italy
AY531681_Pt_C_sp_Italy
DQ792942_Pt_C_limon_Israel
DQ792939_Pt_C_sinensis_Israel
DQ792928_Pt_C_sp_Israel
AY531682_Pt_C_sp_Italy
DQ792936_Pt_C_limon_Israel
DQ993290_Pt_C_limon_Israel
AY531672_Pt_C_limon_Italy
AY531674_Pt_C_sp_Italy
CID250_its1_2006-06-24

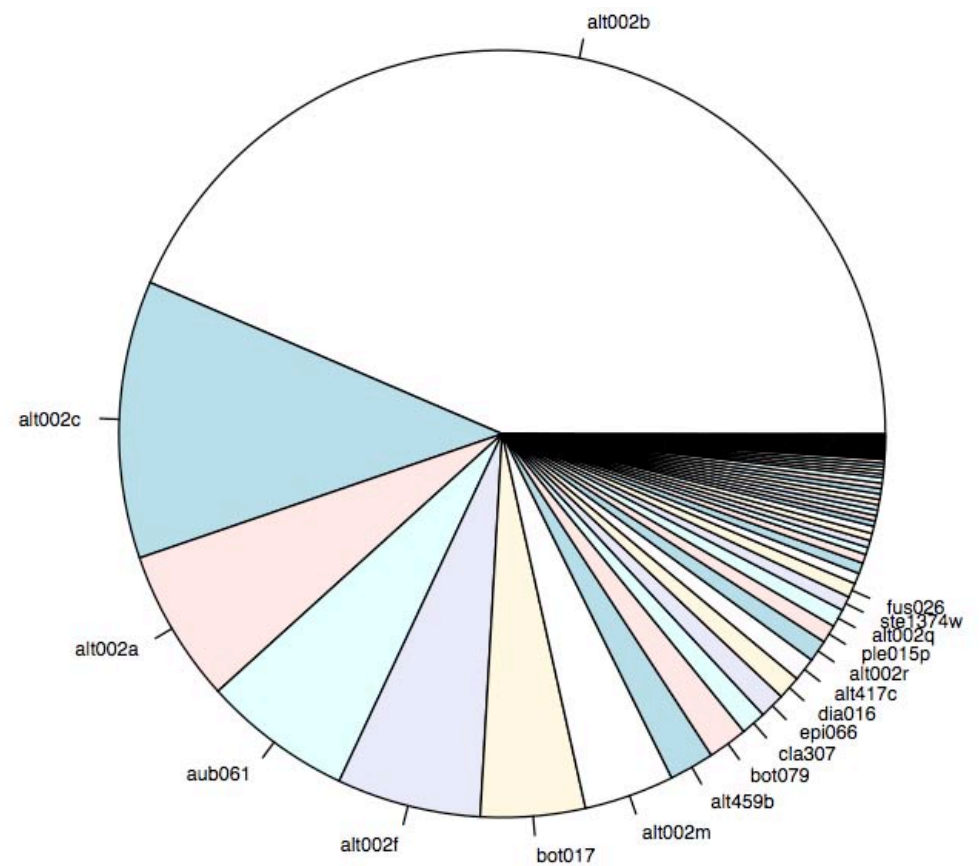


SUDDEN DIEBACK CAUSED BY
MAL SECCO.

Comparison of diversity between two ranges

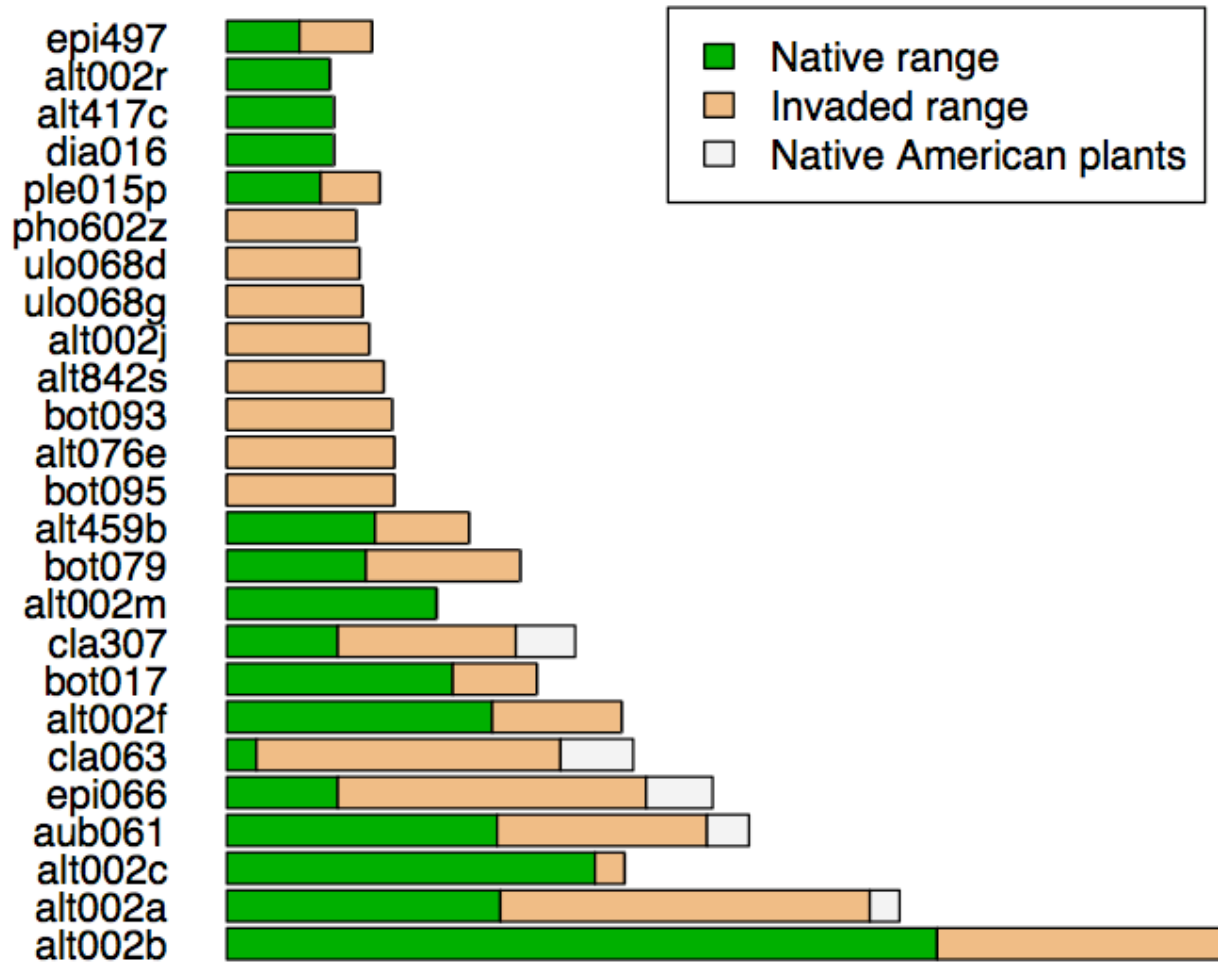


Invaded range
(N. America)



Native range
(Europe)

Endemic and cosmopolitan haplotypes

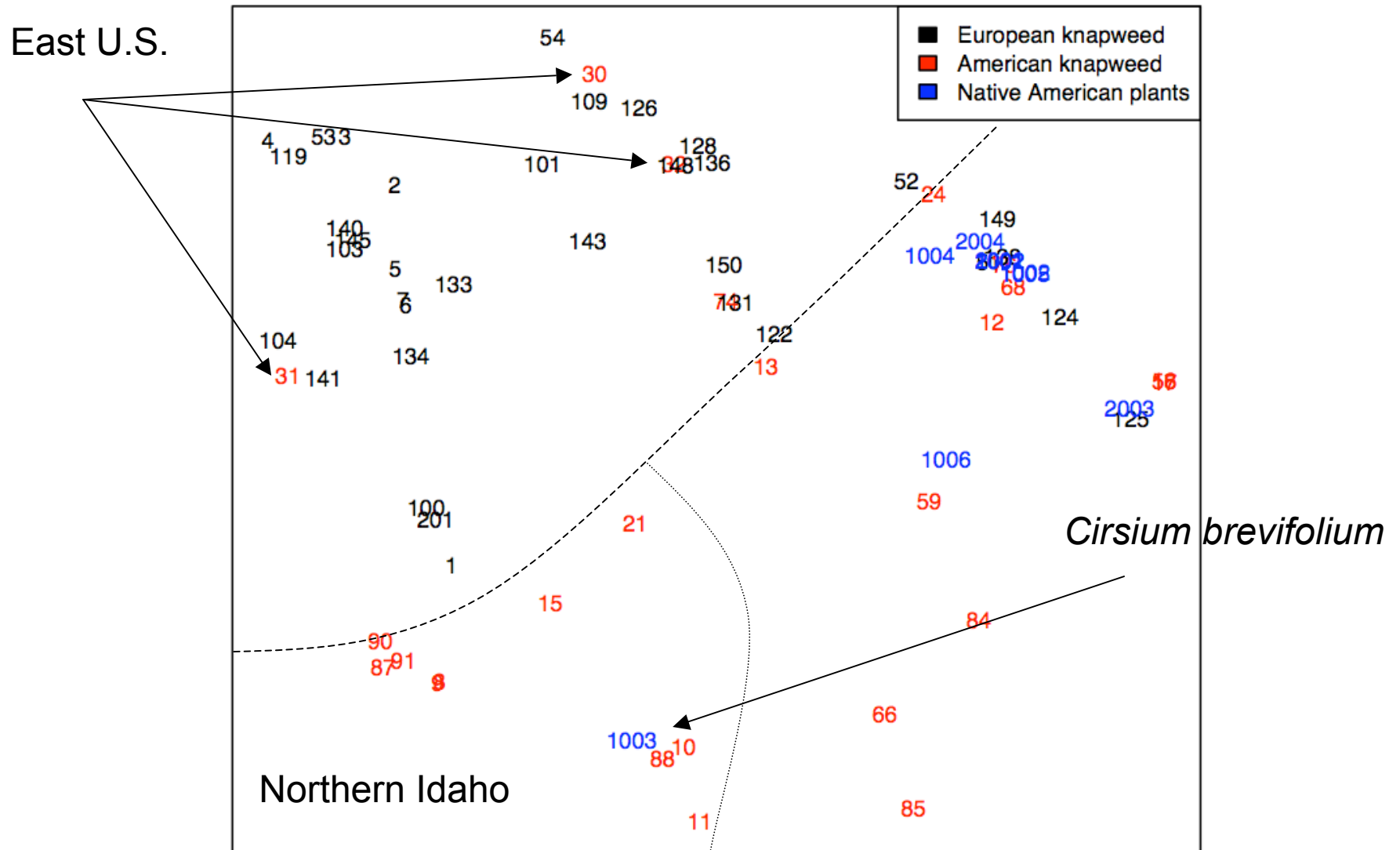


Saussurea americana,
American closest knapweed
relative

Distribution of *Botrytis* 017/095 and *Cladosporium* 063



Ordination of communities

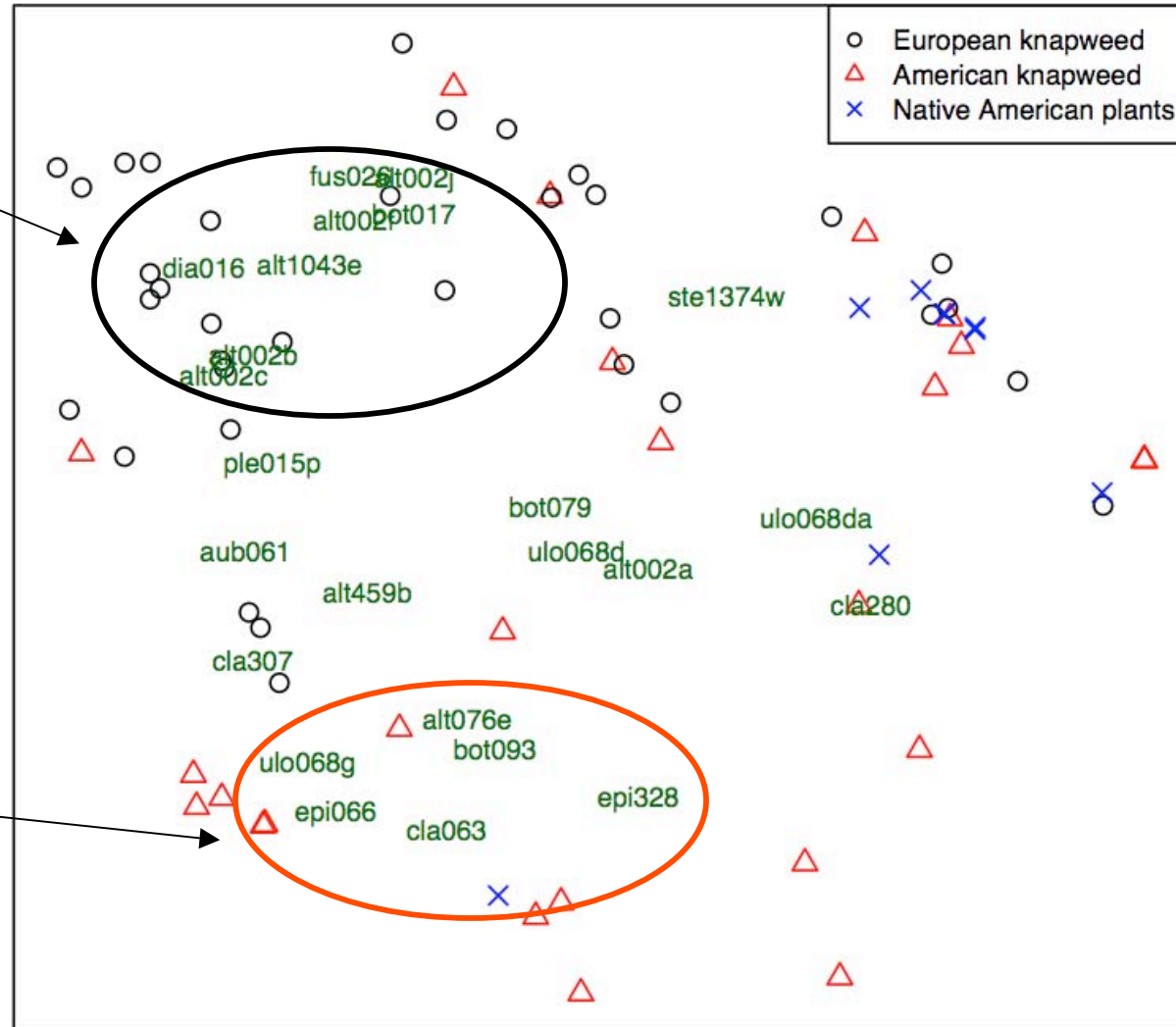


Principal coordinates analysis (PCO) based on **Chao similarity** indexes

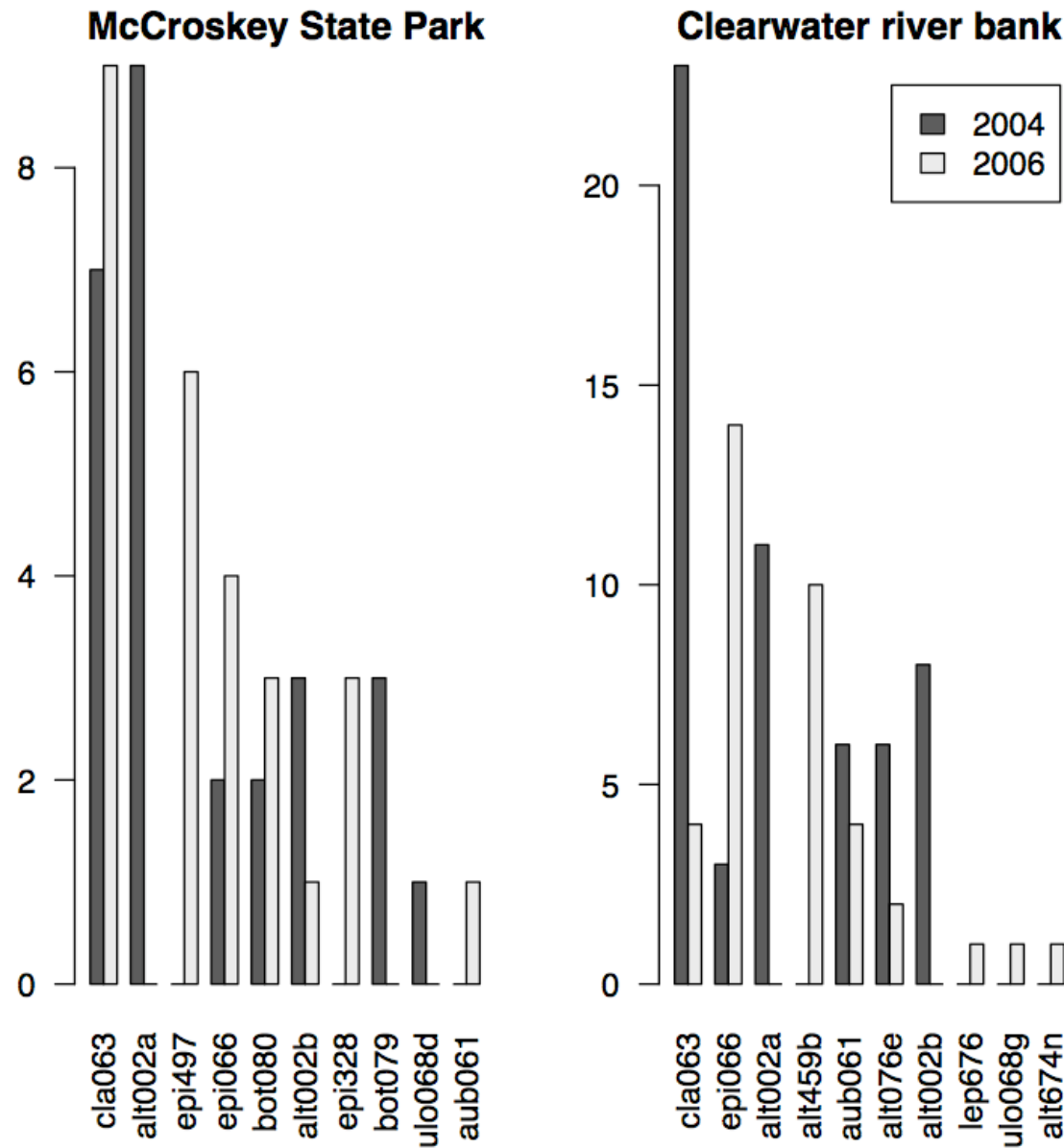
Fungal haplotypes that characterize communities

Species are common for native range

Species are common for invaded range

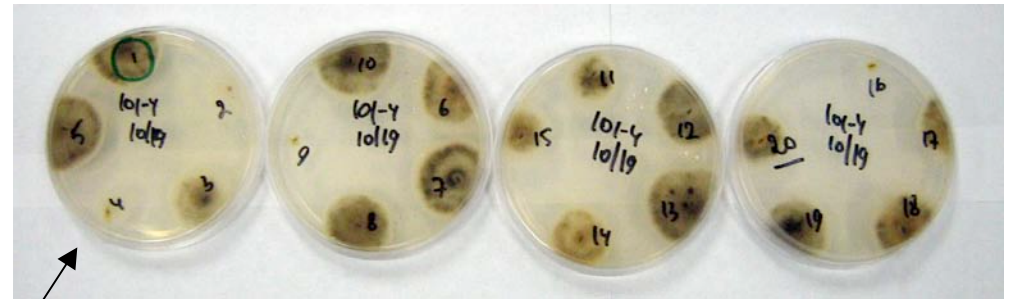
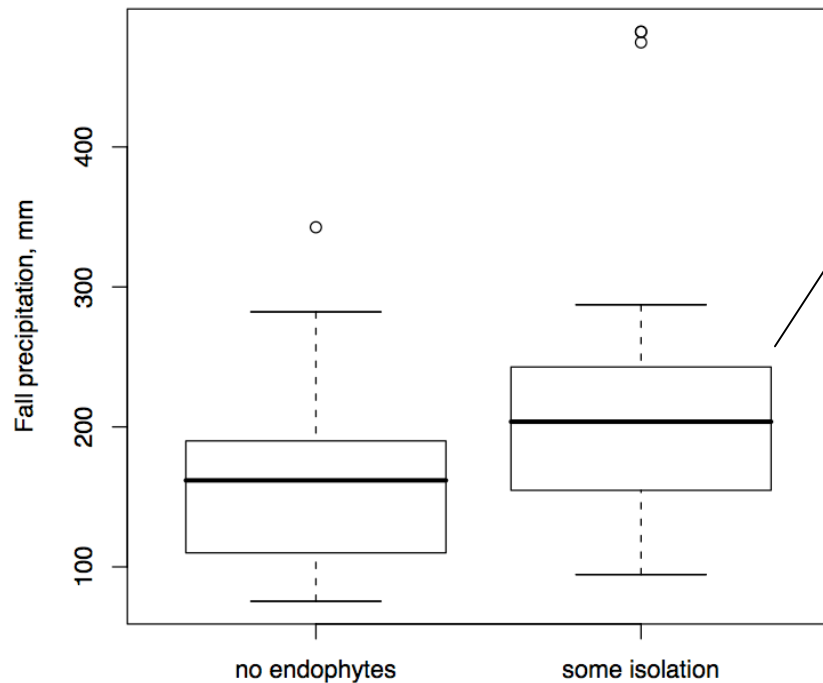


Repeated sampling

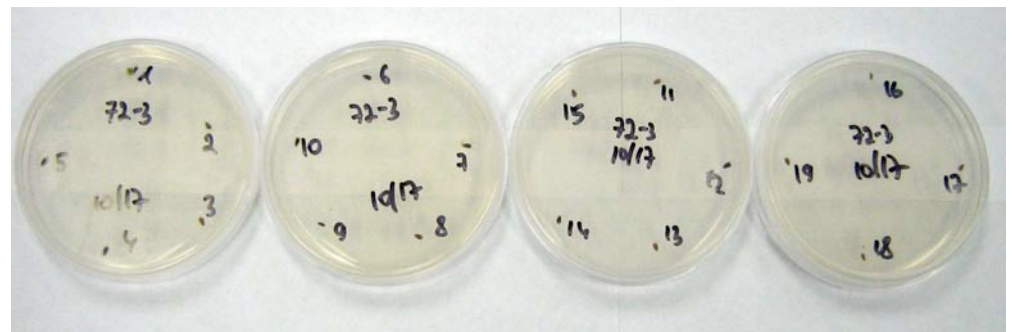


Some of most widespread fungi are repeatedly isolated whereas some *Alternaria* species are not easy to re-isolate

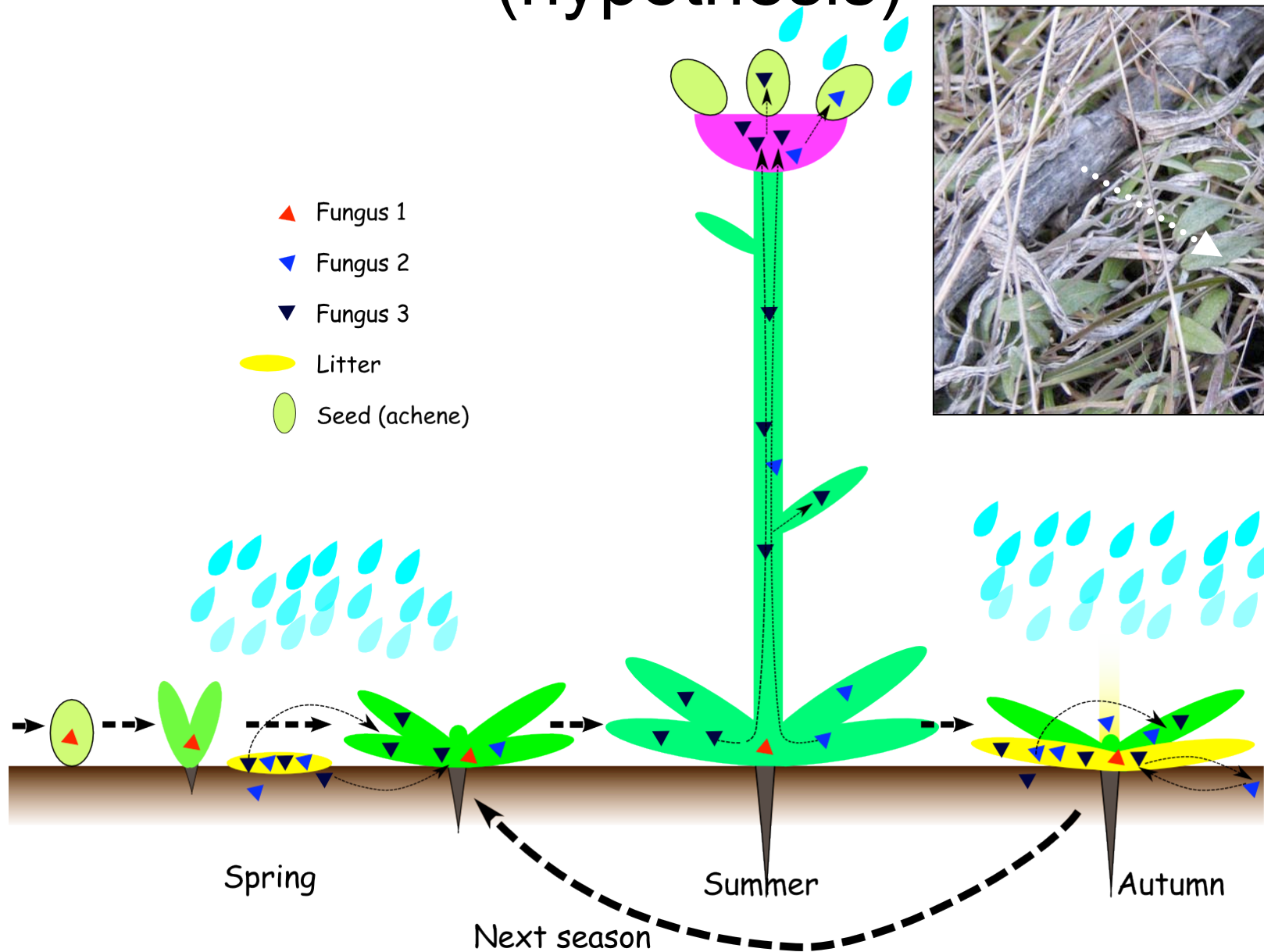
Diversity and climate



We have higher isolation frequencies from sites where late summer and fall (August-November) precipitation is higher



Life cycle of knapweed endophytes (hypothesis)



Endophytes and invasion

Hypotheses of invasion	Our research supports
Enemy Release Hypothesis	+
Enhanced Mutualism Hypothesis	+
Novel Weapons Hypothesis	+

Acknowledgements

- George Newcombe
- Anil Kumar Raghavendra
- Timothy Prather
- Cort Anderson
- Rebecca Ganley
- Sanford Eigenbrode
- Hongjian Ding
- Maryse Crawford



CRISSP



Web-site of the project:

<http://uidaho.edu/~shipunov>