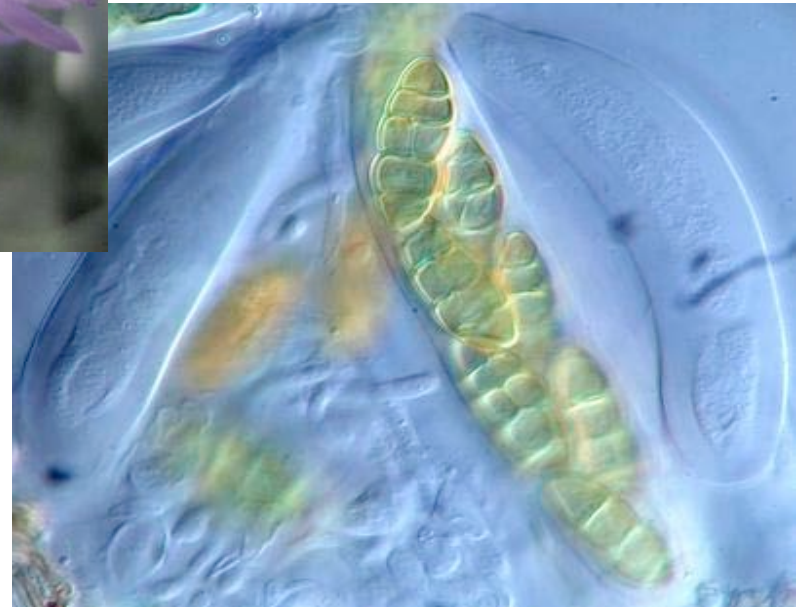


Spotted knapweed (*Centaurea stoebe* L.) and its endophytic fungi

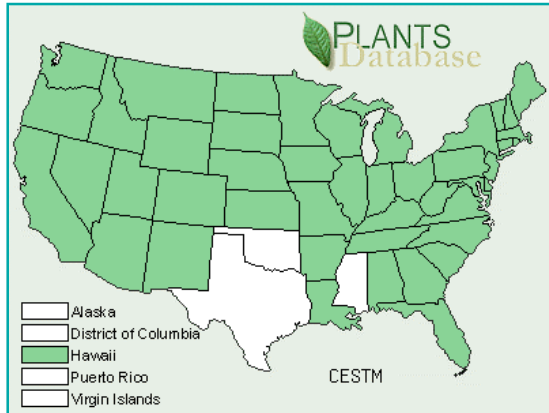


Alexey Shipunov

Center for Invasive Species
and Small Populations
(CRISSP)
University of Idaho



Spotted knapweed



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





Volga river
habitat
(LID119)



However, in native region (e.g., Russia and Ukraine),
knapweed does not demonstrate invasion ability

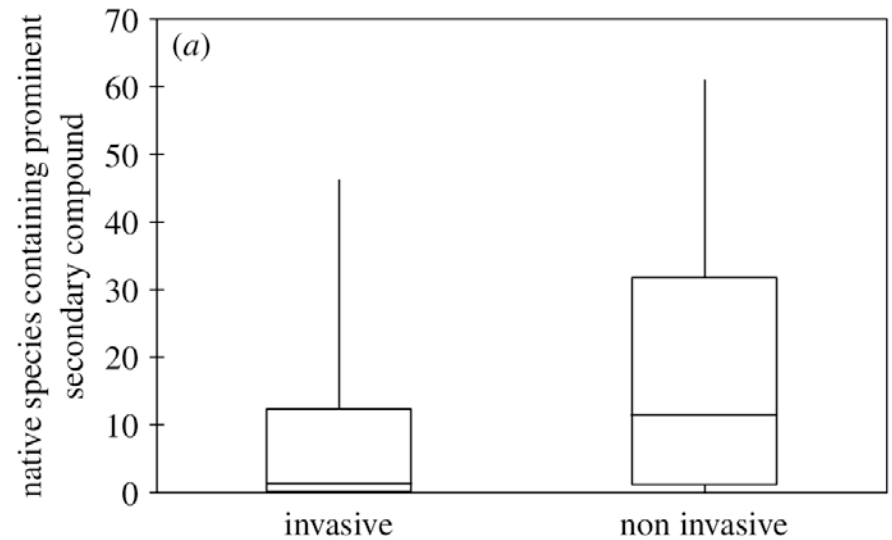
Hypotheses of invasiveness

- Invasive plants have **more fitness** (in terms of productivity and/or competition)
- Invasive plants fill “**gaps**” in ecological niches structure
- Invasive plants occur mostly in **disturbed** habitats
- Invasive plants escape from their native **enemies**
- Invasive plants have **novel weapons**

“Novel weapons”

Plants may contain chemical compounds that are new to the introduced range and may **repel herbivores** or **negatively affect surrounding plants**.

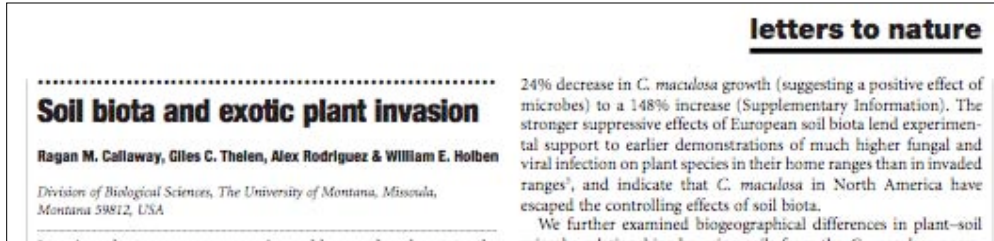
Many invasive North American plants have been reported to have antimicrobial, antiherbivore and allelopathic effects, which are most probably the consequences of unique (for American flora) secondary chemical compounds.



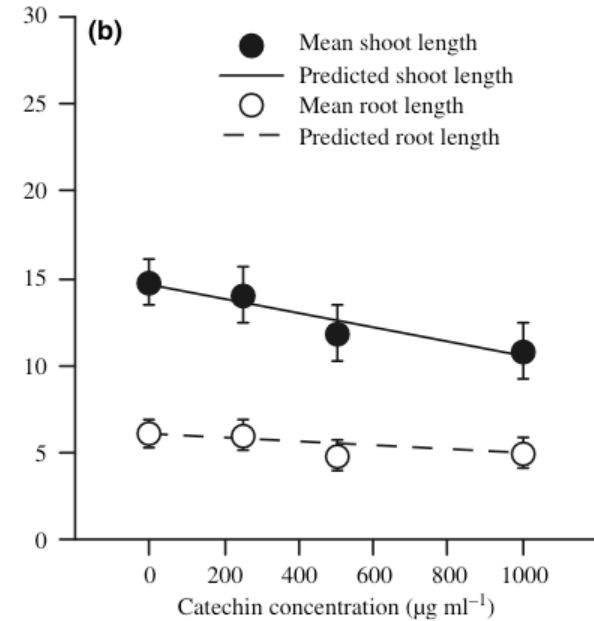
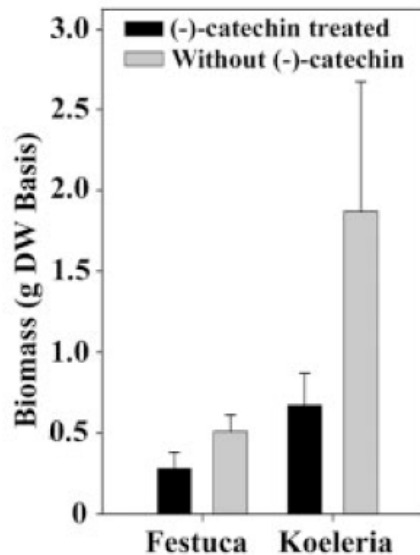
(From Cappucino & Arnason, 2006): **invasive** plants share their prominent secondary compounds with less native North American plants than **non-invasive** plants

Spotted knapweed is among plants which have significant phytotoxic (allelopathic) effect. Some secondary compounds were believed to have this effect: **cnicin** and **catechins**.

Catechin or not catechin



The most accepted opinion (Callaway et al., 1999 and many others) is that catechin-contained 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.

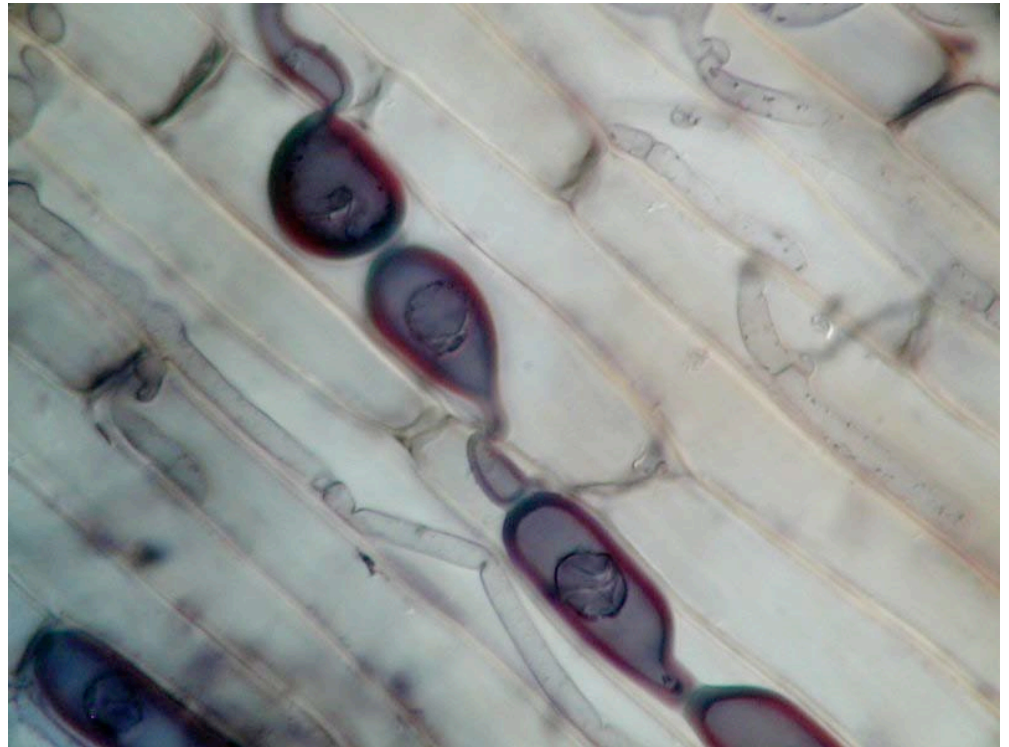
J Chem Ecol (2006) 32: 2327–2331
DOI 10.1007/s10886-006-9168-y

RAPID COMMUNICATION

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

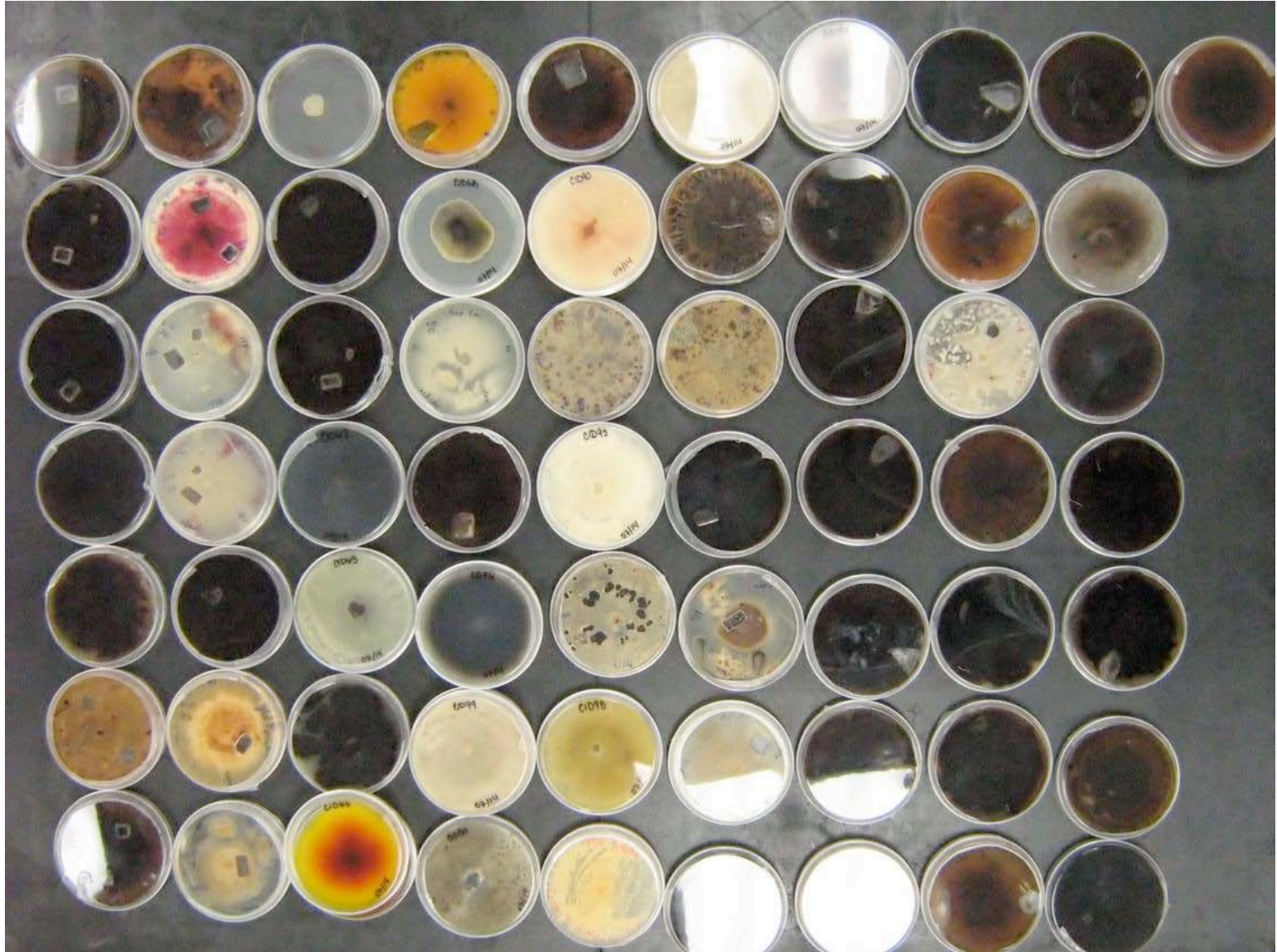
Fungal endophytes

- Inhabit **all** plants
- Can produce secondary metabolites which are beneficial to the host plant, e.g., taxol from *Taxus* trees
- Specific endophytes may play specific roles, and many plants host very diverse arrays of endophytes

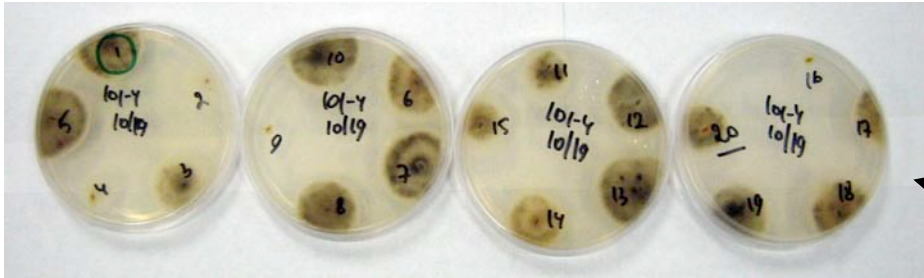


We think fungal endophytes affect how spotted knapweed interacts with neighboring plants

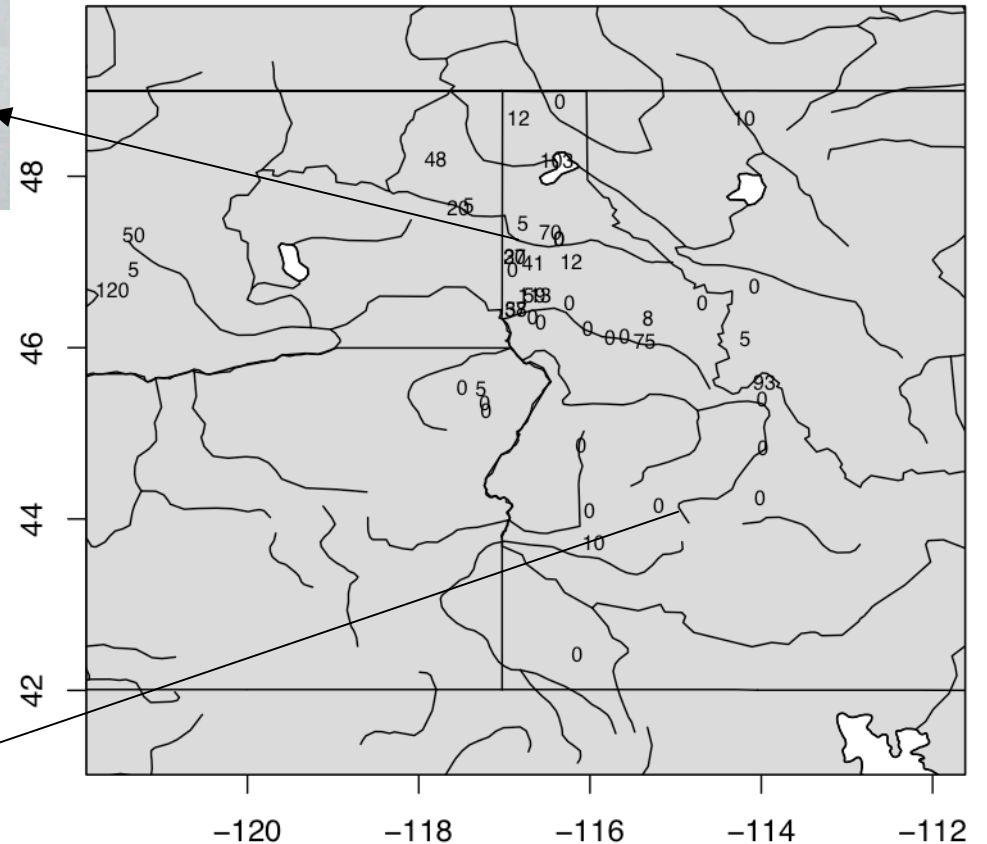
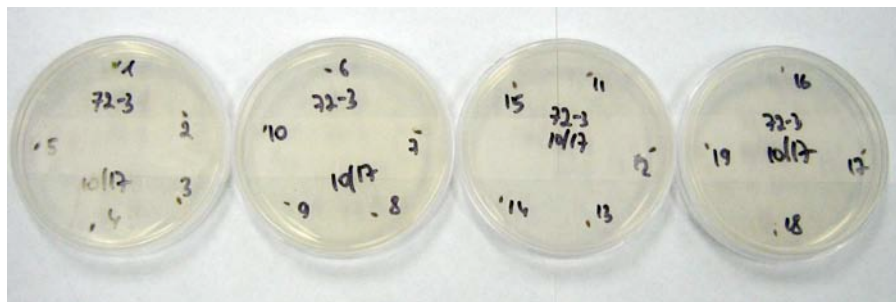
Knapweed endophyte diversity



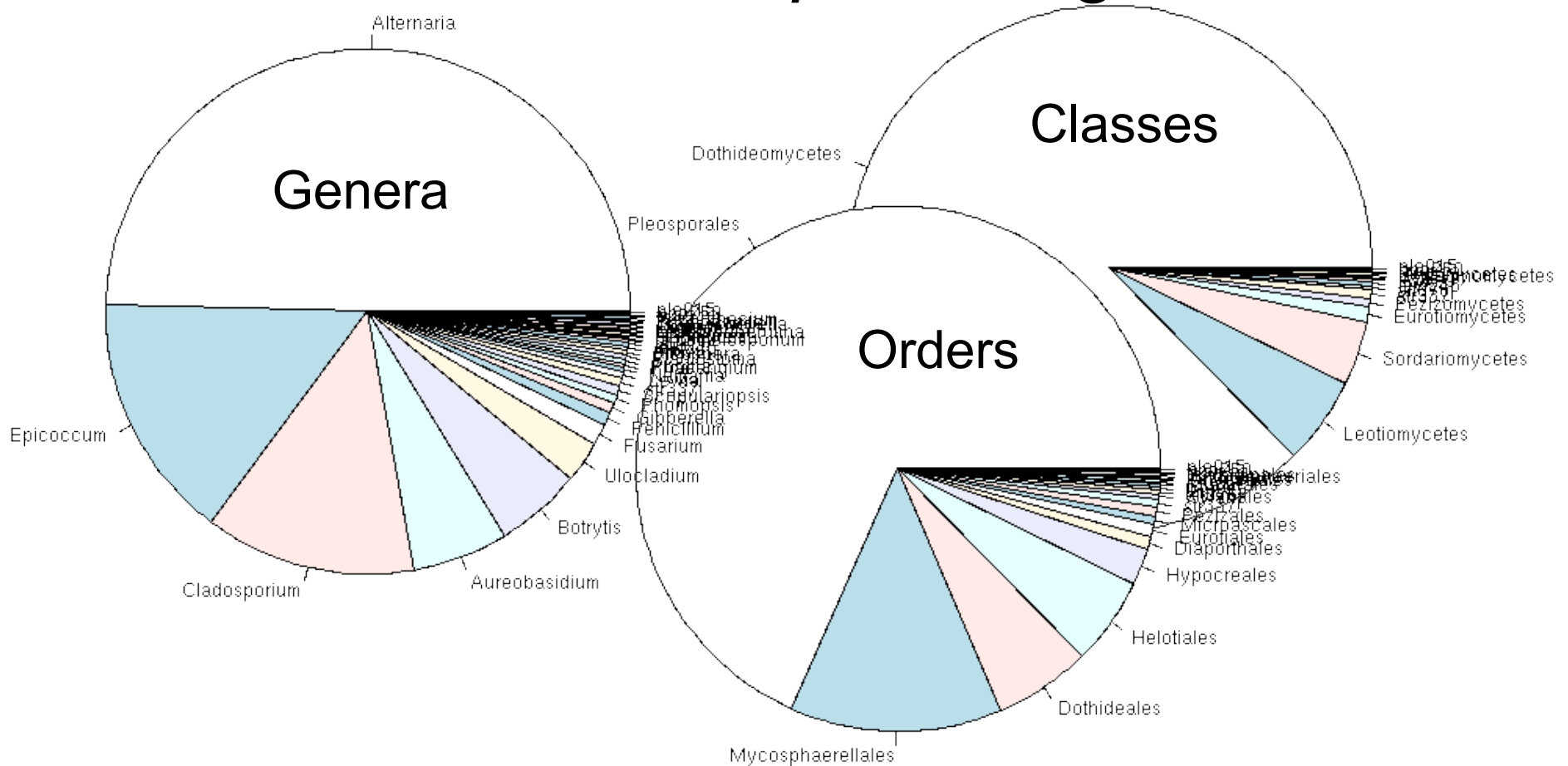
Isolation frequency varies



from
>100%
to
0%



Most endophytes belong to *anamorphic* fungi

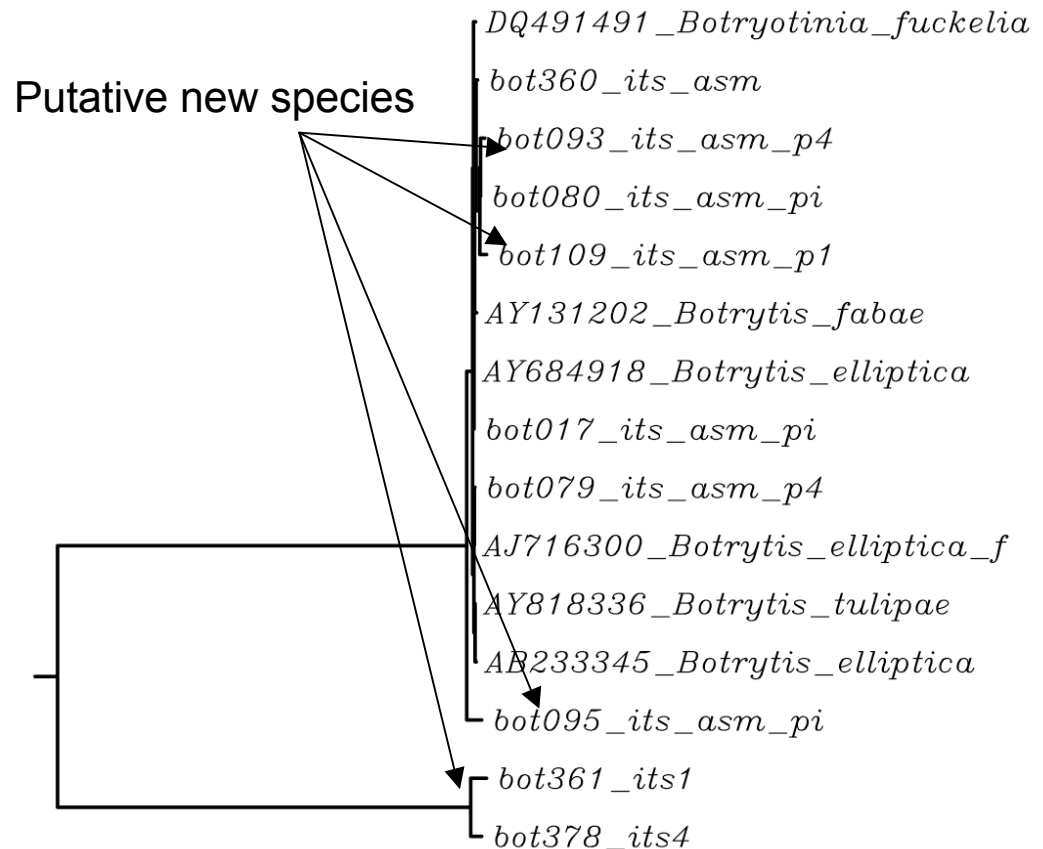


Anamorphic fungi do not have morphological characteristics to distinguish them so we used molecular tools (mostly ITS1/5.8S/ITS2 gene sequences). More than 65% of them have no exact matches in the NCBI GenBank nucleotide database.

Some endophytes may represent novel lineages



Botrytis (anamorph of *Botrytinia*, Sclerotiniaceae)



“Host-jumping” vs. co-introduction

Endophytes in introduced plants like knapweeds must either have been **co-introduced** in seeds of their host, or have ‘**jumped**’ from other plants in the invaded range of their hosts



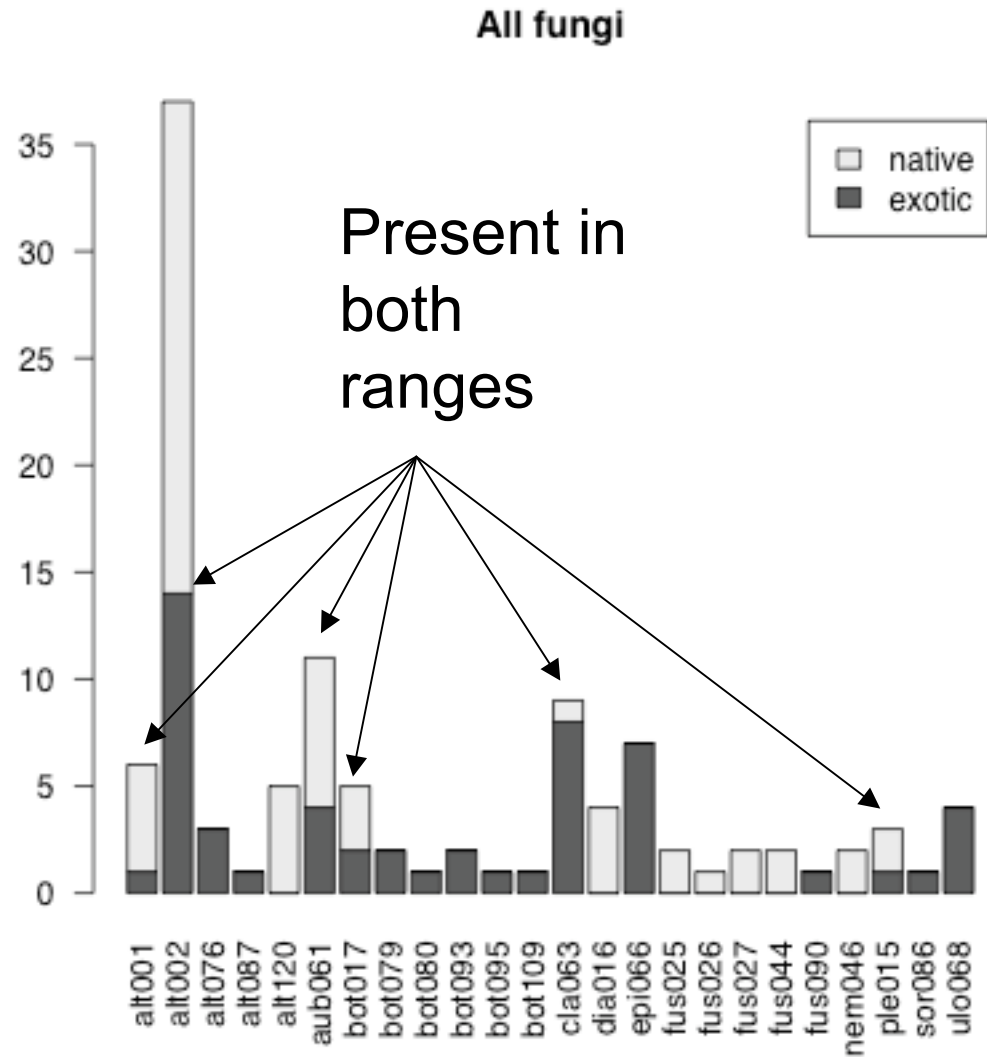
```
AY531673_Pt_C_limon_Italy
AY531689_Pt_C_sp_Italy
AY531677_Pt_C_sp_Italy
AY531678_Pt_C_limon_Italy
AY531670_Pt_C_limon_Italy
AY531669_Pt_C_limon_Italy
AY531681_Pt_C_sp_Italy
DQ792942_Pt_C_limon_Israel
DQ792939_Pt_C_sinensis_Israel
DQ792928_Pt_C_sp_Israel
AY531682_Pt_C_sp_Italy
DQ792936_Pt_C_limon_Israel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

This endophyte (CID250, from Germany) have 99% identity with GenBank sequences of *Phoma tracheiphila*, very dangerous pathogen of *Citrus* trees



SUDDEN DIEBACK CAUSED BY MAL SECCO.

Distribution among native and exotic ranges

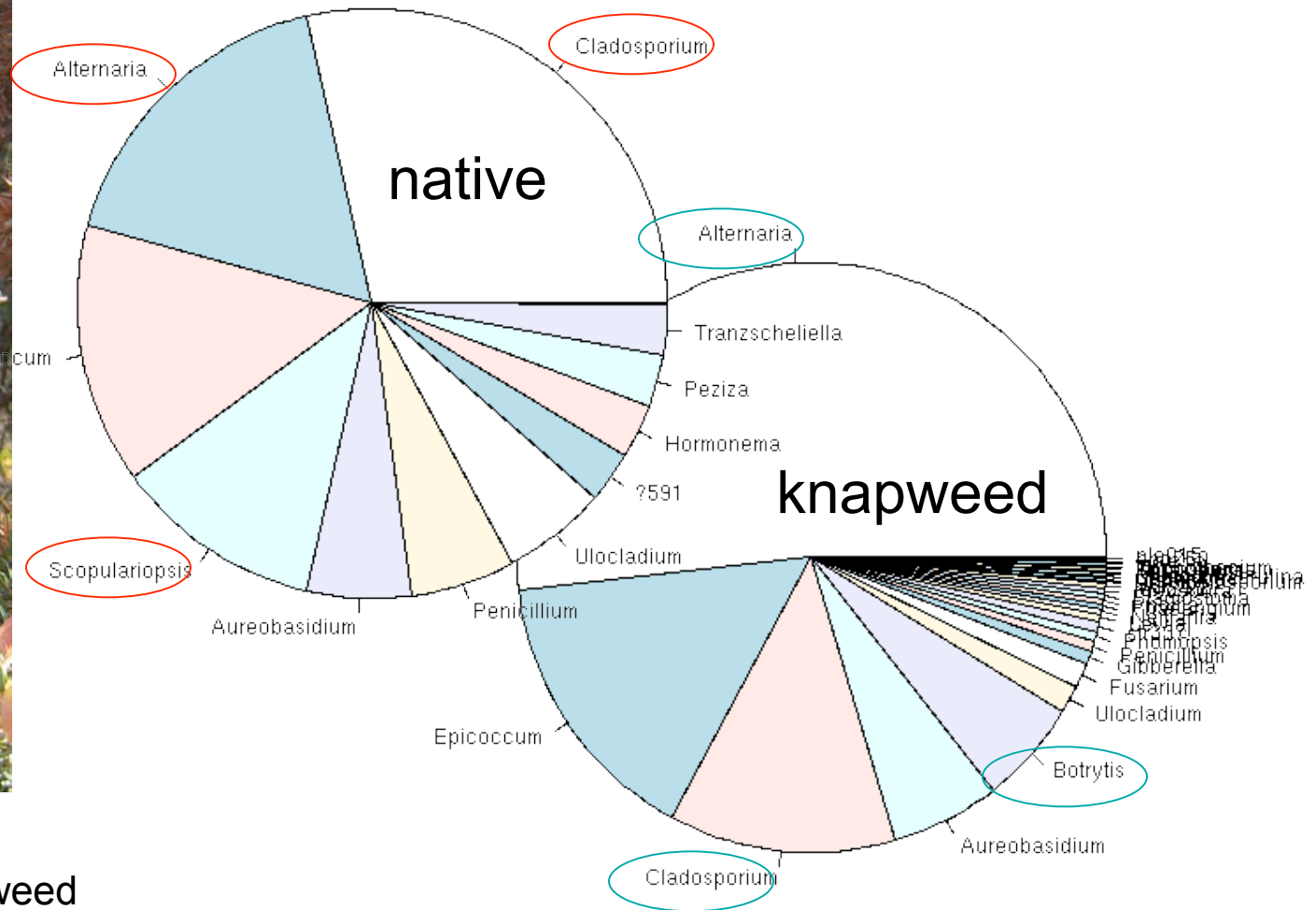


Data from 2004/2005 sampling

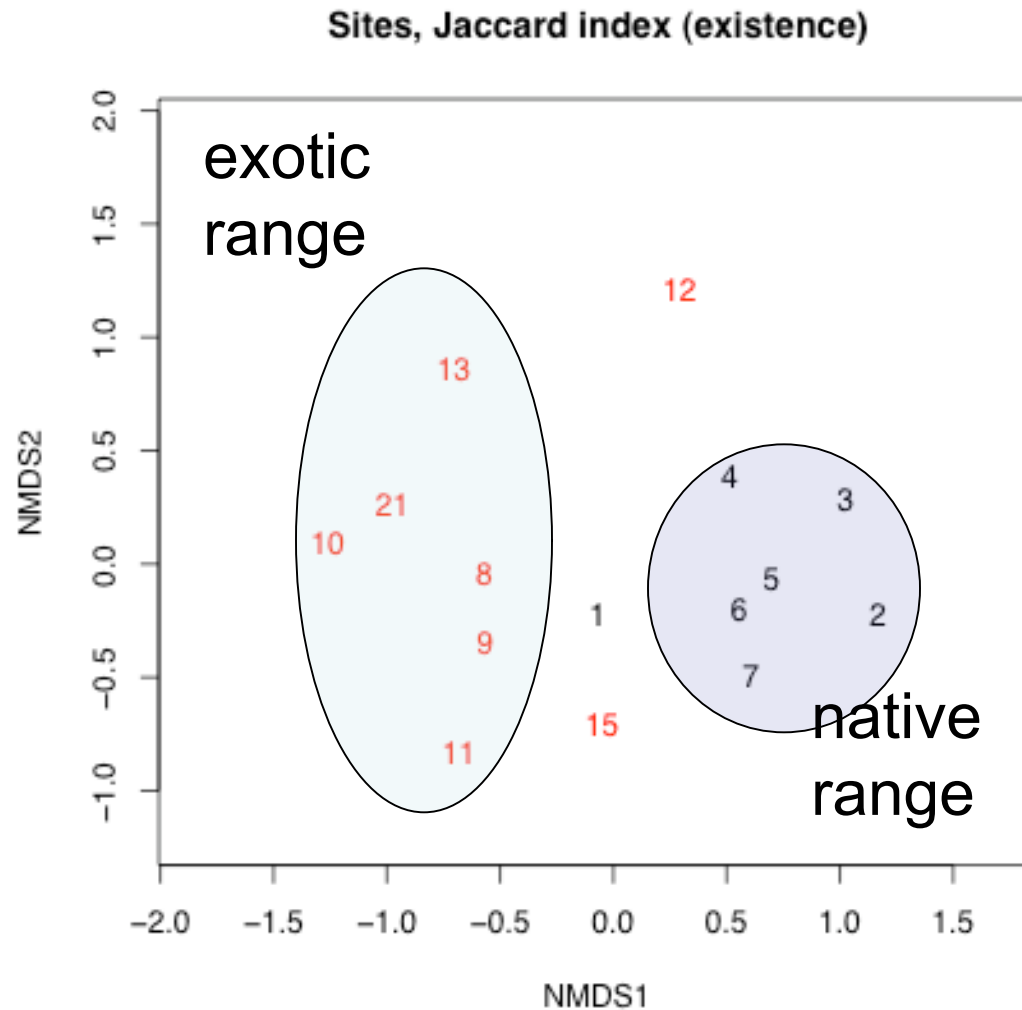
Comparing diversity with native Asteraceae



Saussurea americana,
American closest knapweed
relative



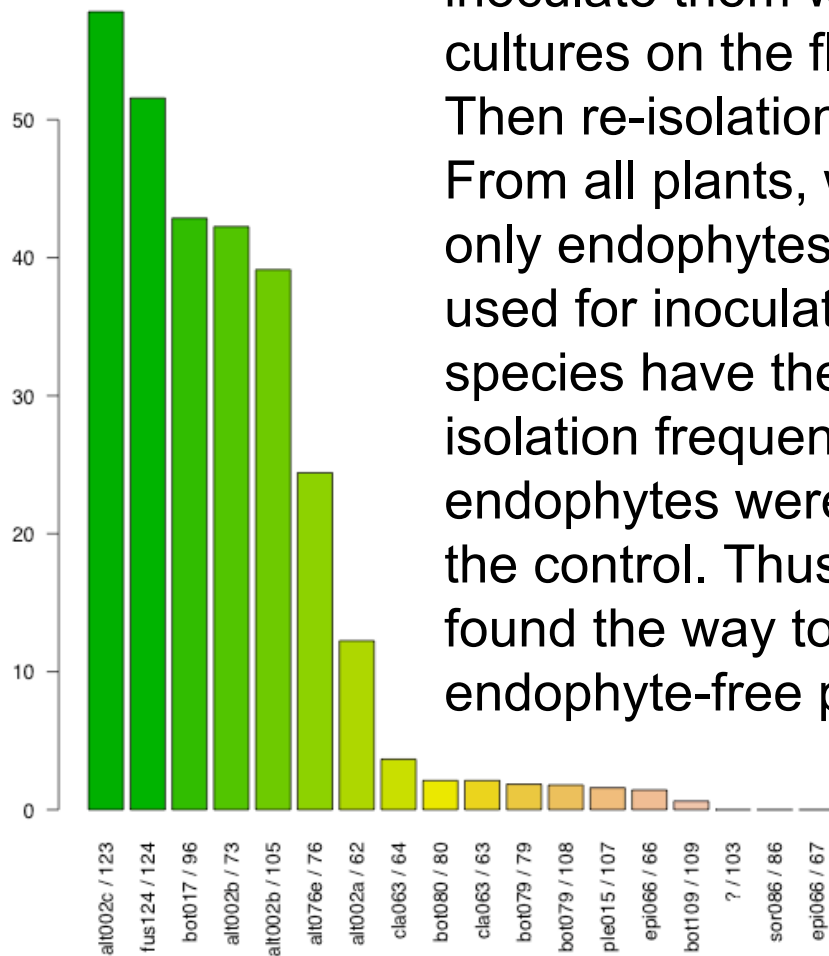
Are endophyte communities different?



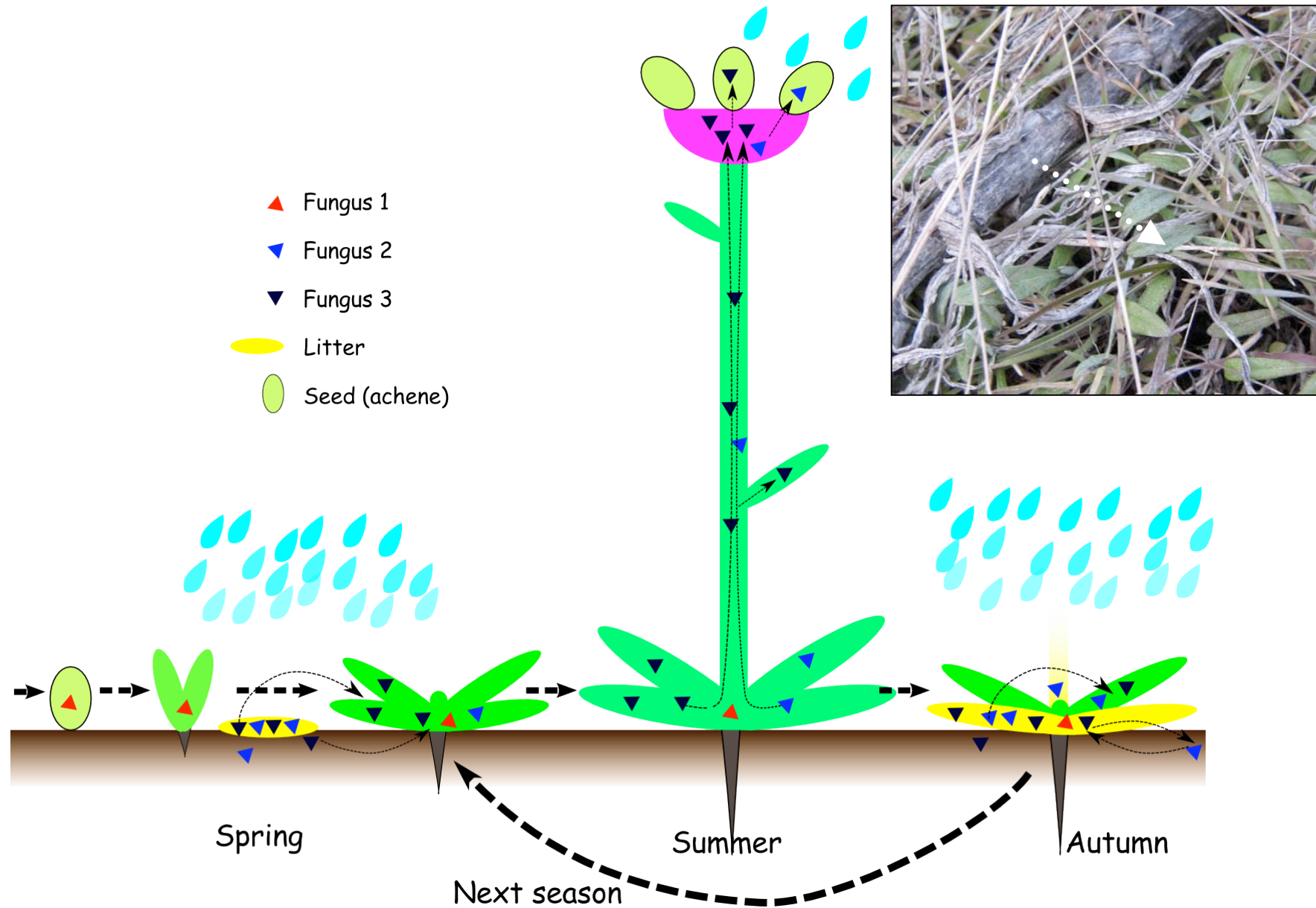
Data from 2004/2005 sampling

Endophyte-free plants

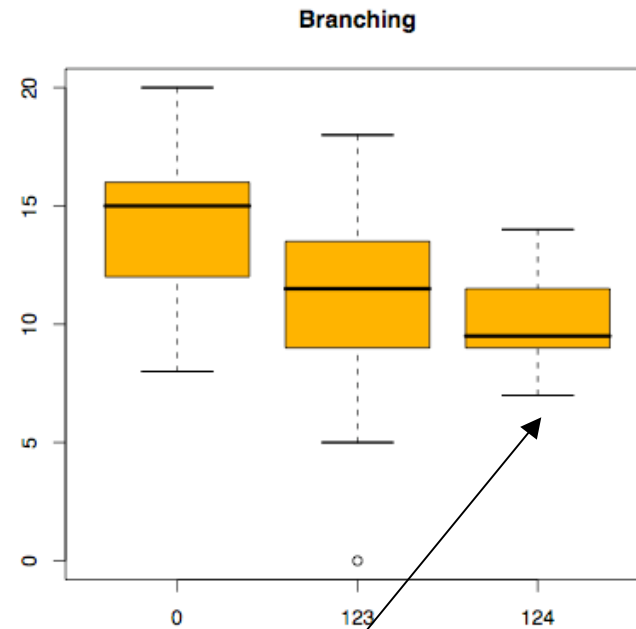
We cultivated the 2nd generation of knapweed and inoculate them with liquid fungal cultures on the flowering stage. Then re-isolation were done. From all plants, we obtained only endophytes which were used for inoculation. *Alternaria* species have the best re-isolation frequency. No endophytes were isolated from the control. Thus, we have found the way to produce endophyte-free plans.



Knapweed endophyte life cycle

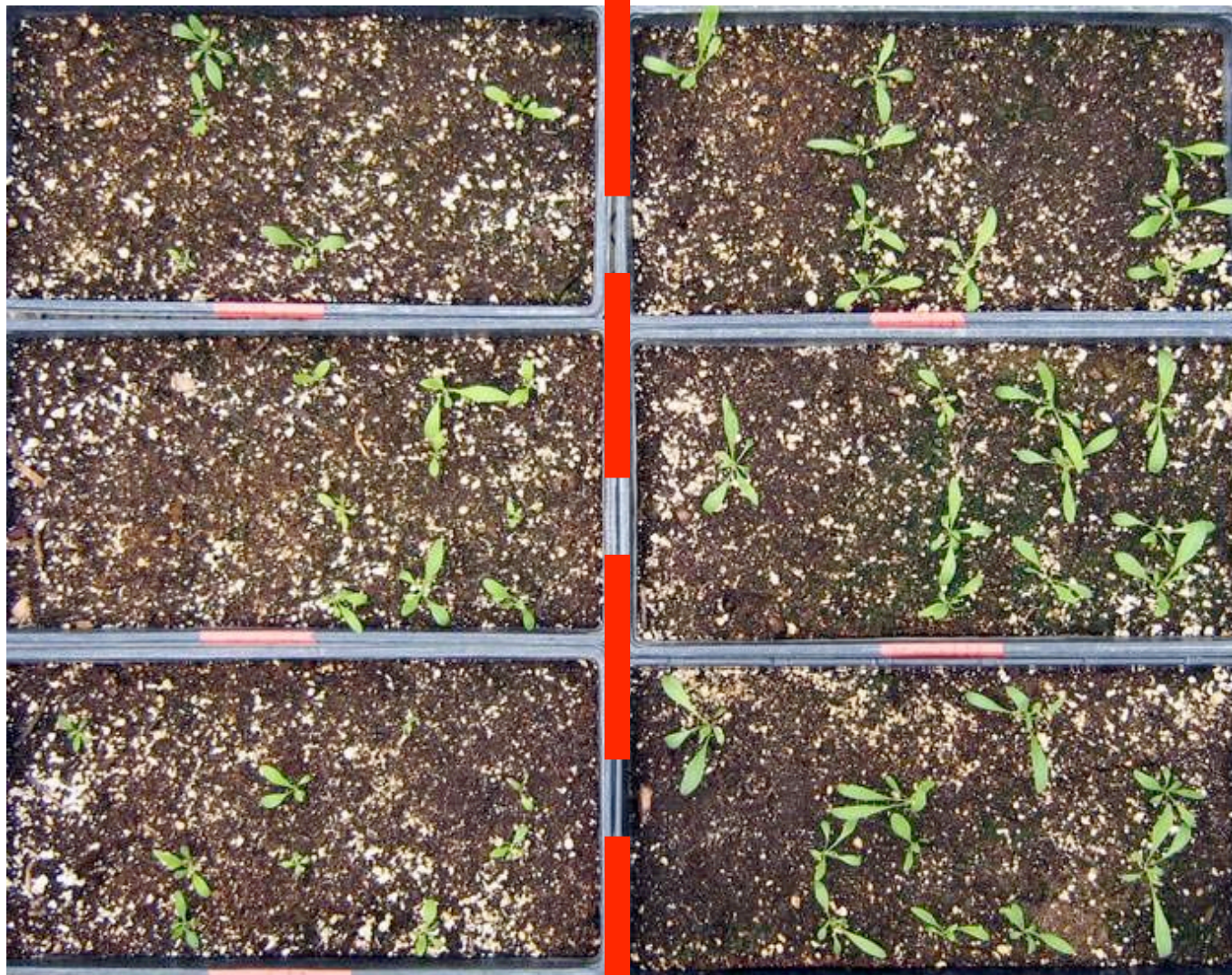


Interactions



Endophyte strain 124 (*Fusarium* sp.) suppresses the flowering of knapweed

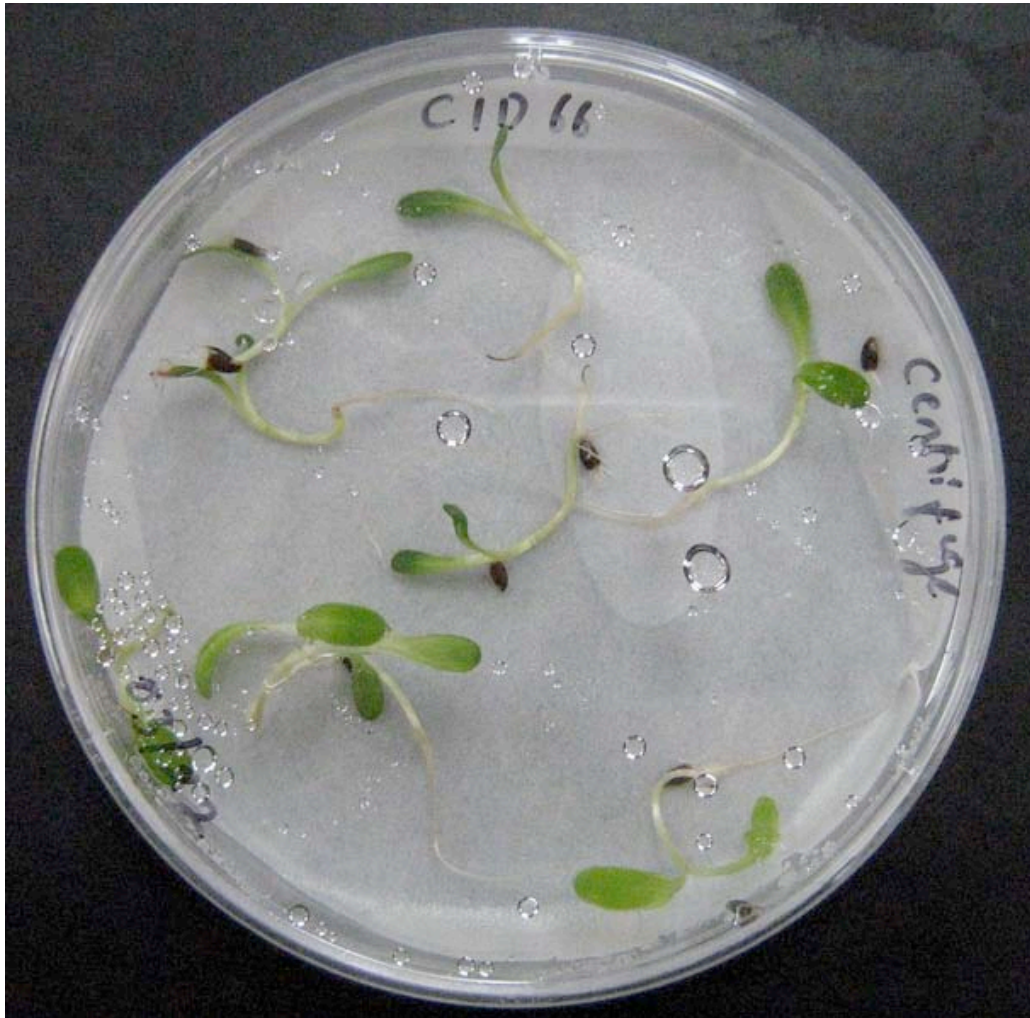
Some endophytes have even pathogenic effect



Trays with inoculated seedlings

Trays with control seedlings

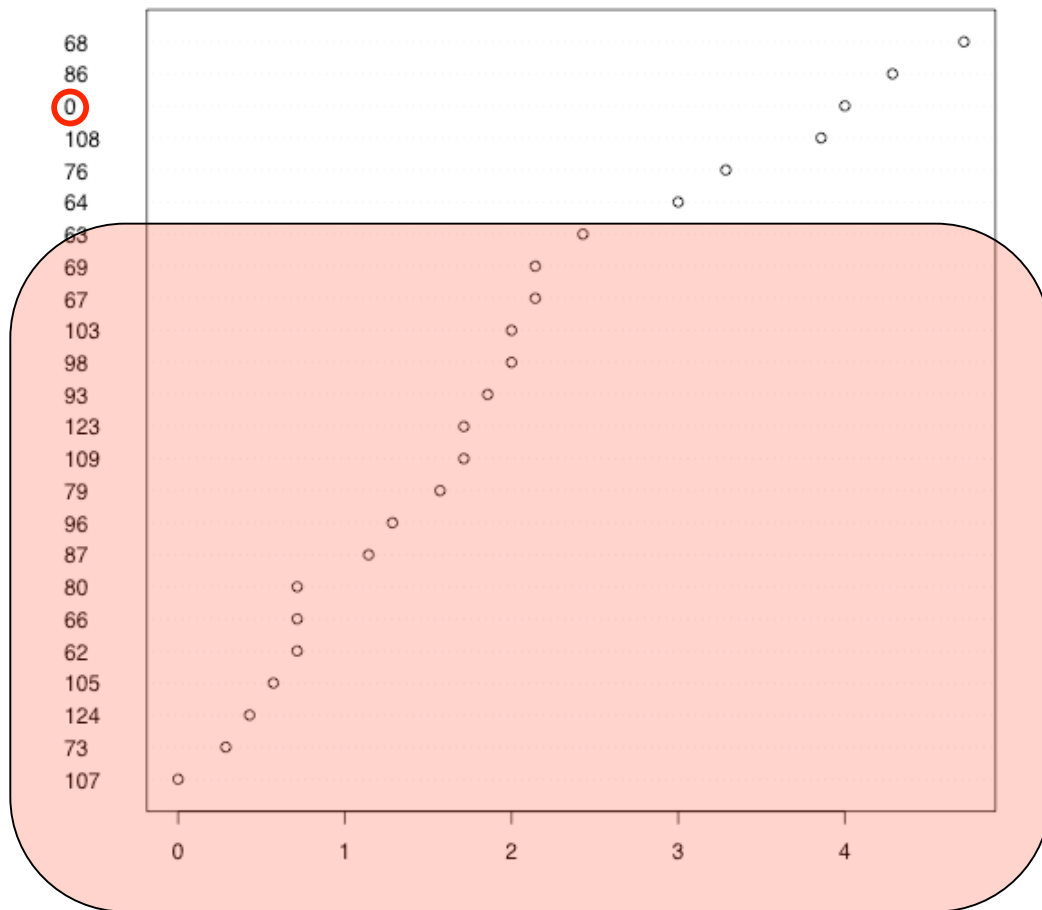
Endophytes can benefit knapweed seedlings



Experiments
with
knapweed
and *Festuca
idahoensis*
seeds

Endophytes can suppress Idaho fescue seedling growth

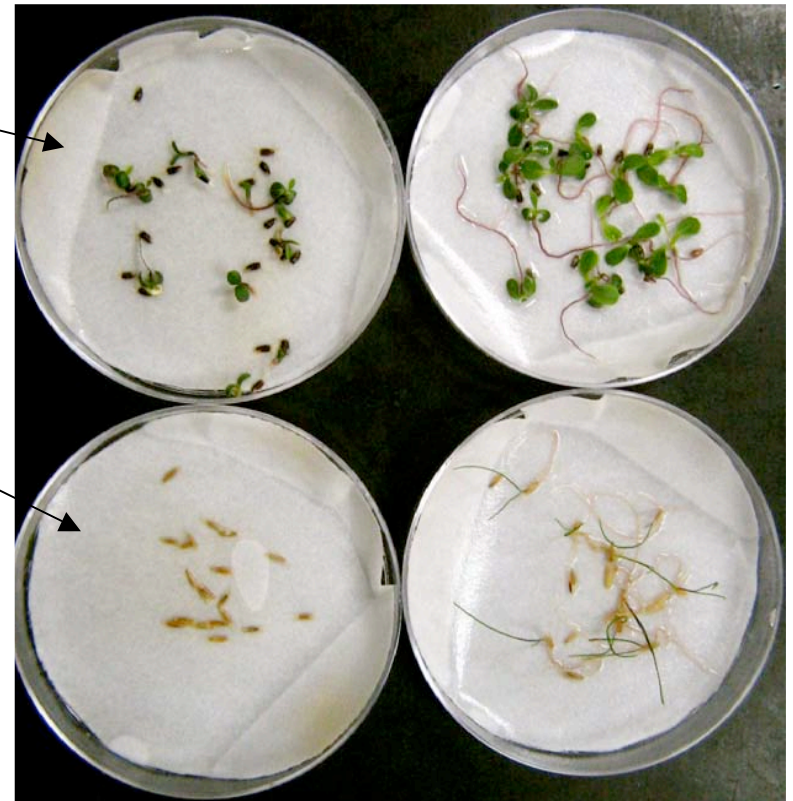
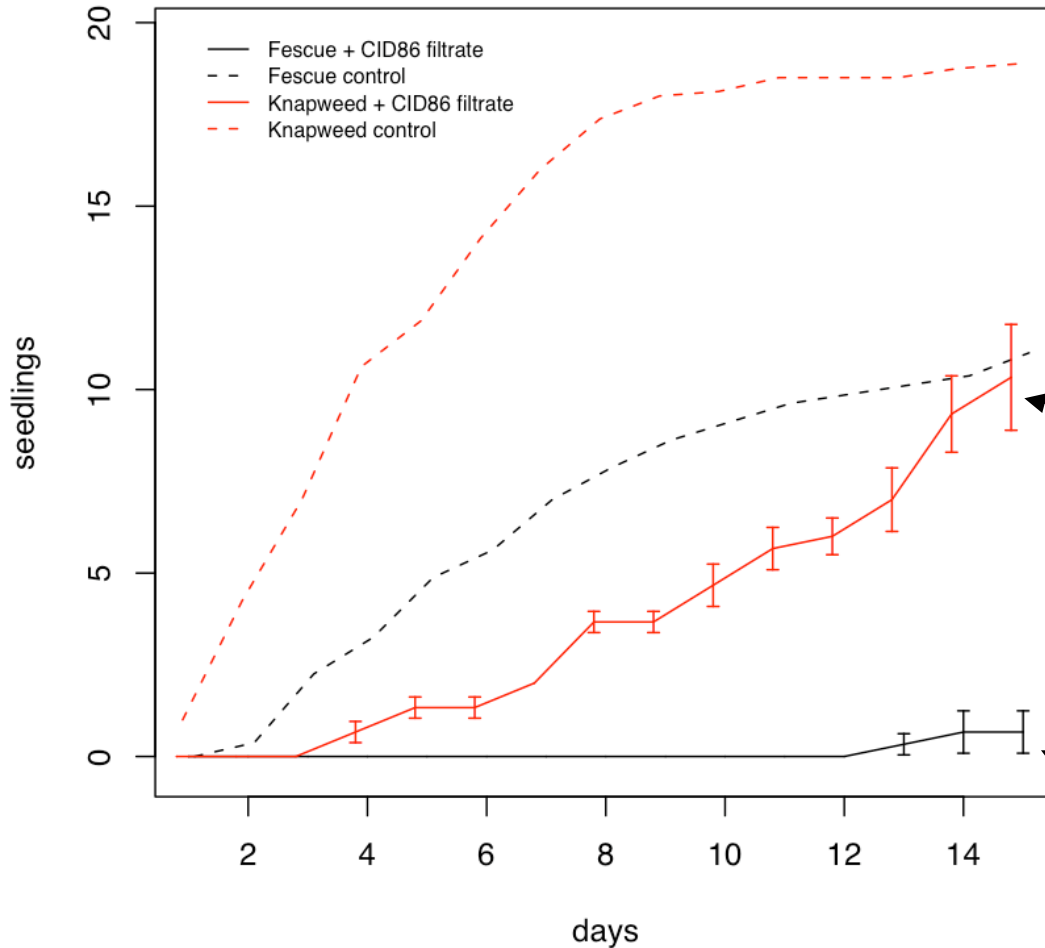
Fescue experiment, germination speed



More than 2/3 endophyte strains have **statistically significant termination effect** on *Festuca idahoensis* seeds, whereas only 1/4 of them have similar effect on knapweed seeds. Moreover, some endophytes (*Fusarium* sp.) can **kill** fescue seedlings.

Comparison of influence

As an example, *Phoma herbarum* (CID86) have selective effect: terminate fescue much more than knapweed



Competition experiment

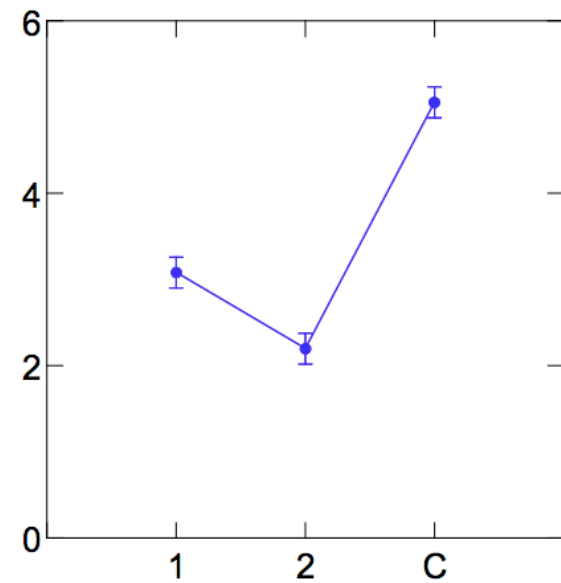


E+
knapweed
and fescue

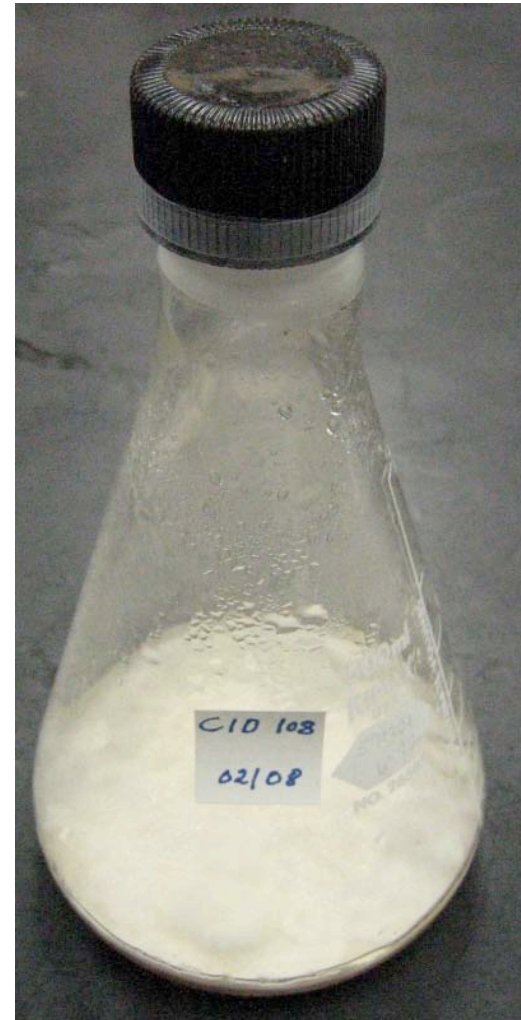
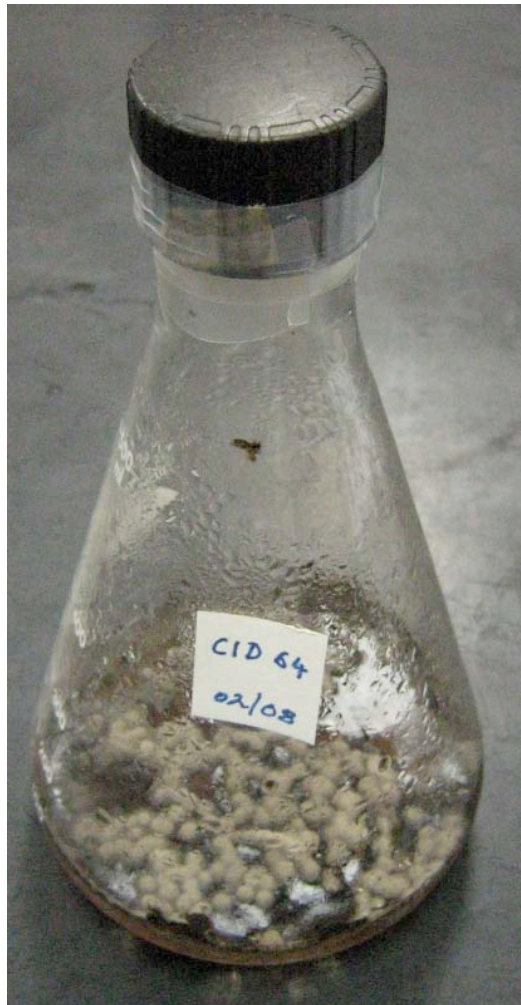
E-
knapweed
and fescue

Fescue
alone:
control

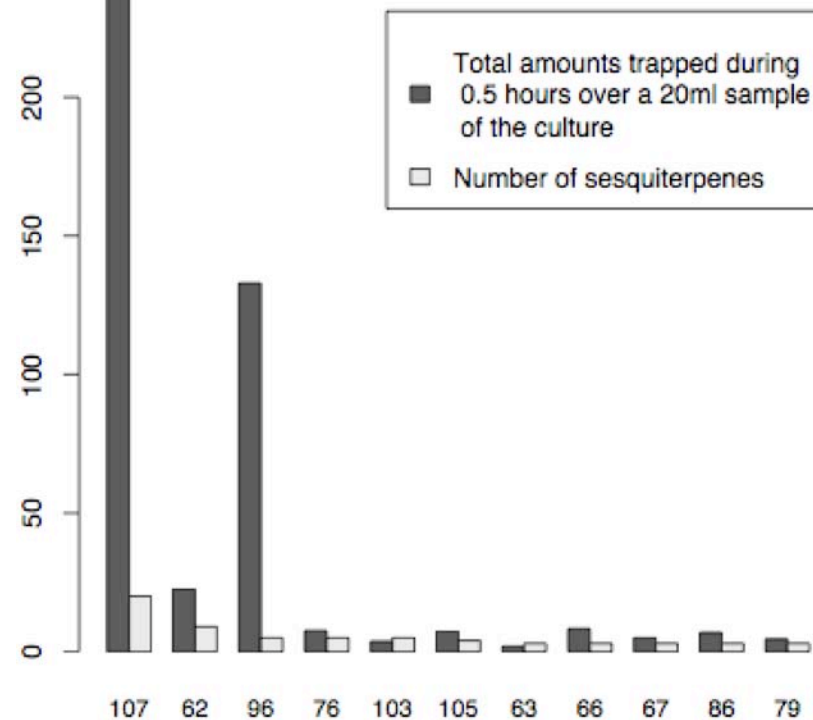
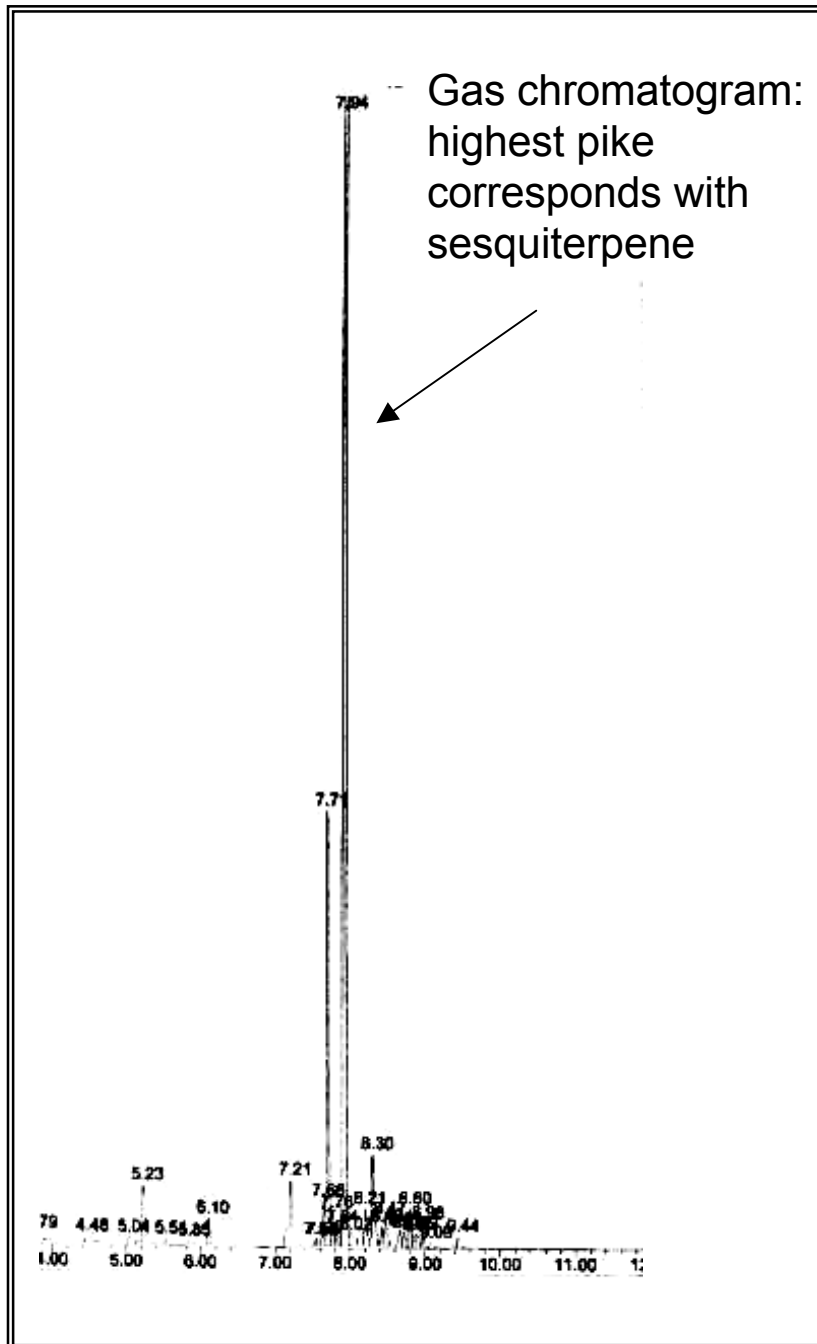
Differences in
biomass are
significant



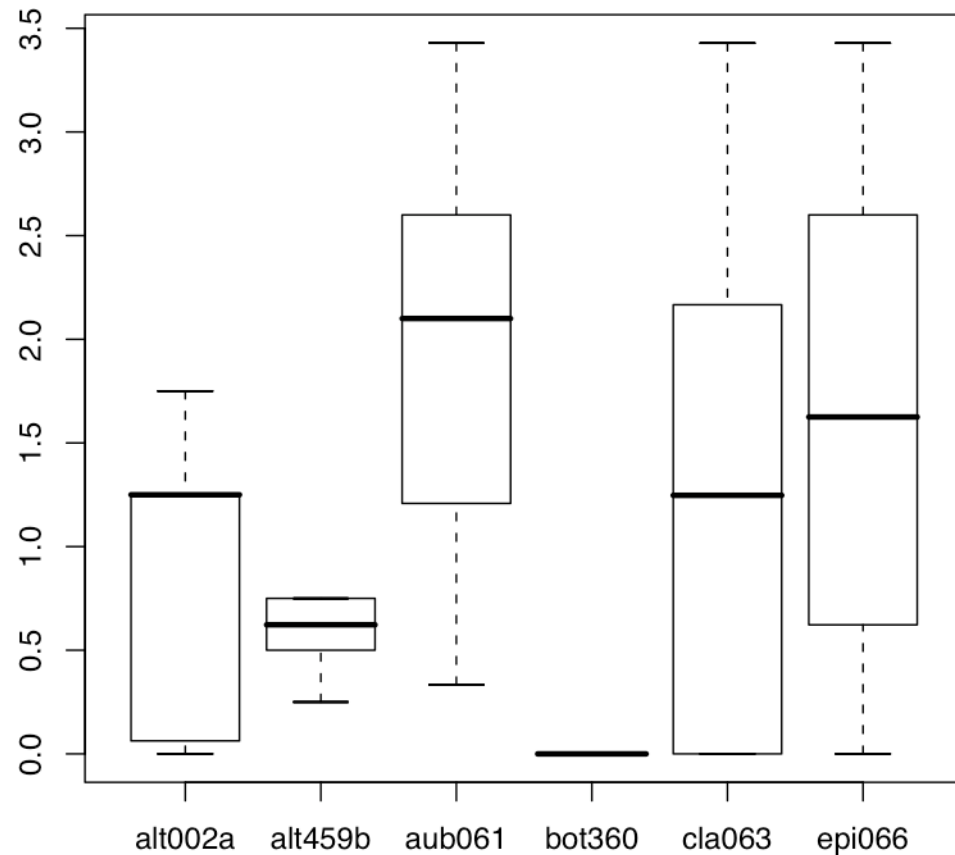
Liquid cultures and volatile compounds



At least some endophytes can produce sesquiterpenes

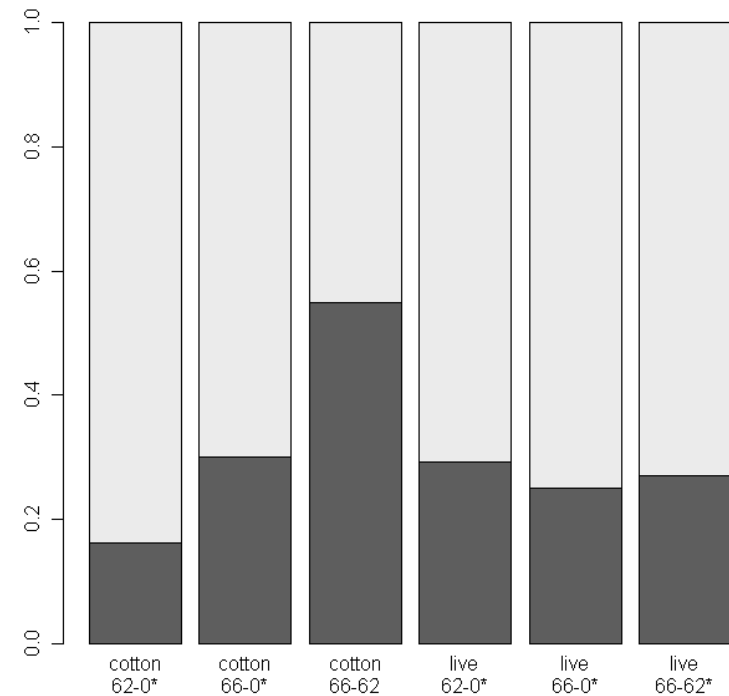


Insect larvae and fungi



Abundance of biocontrol insects *Urophora quadrifasciata* (Diptera) and *Larinus minutus* (Coleoptera) and presence of some endophytes

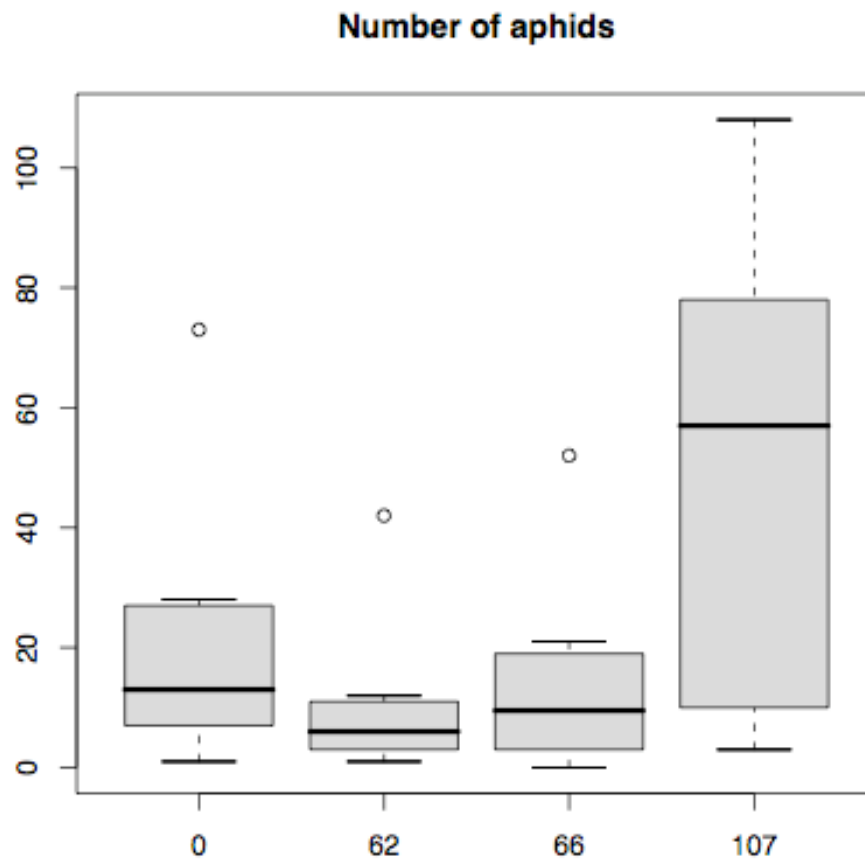
Insecticide effect



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

Conclusions

The endophyte fungi of spotted knapweed:

1. have **horizontal transmission**;
2. can have negative, neutral or positive **effect** on knapweed and fescue **seeds germination**;
3. can suppress or **change the growth** and flowering of knapweed;
4. can produce components which have an **effect on competition** with neighbours;
5. can **mediate** the interactions between knapweed and **herbivores**;
6. can produce some **volatile** chemical compounds.

Endophytes likely play a key role in spotted knapweed's ability to invade plant communities

Acknowledgements

- George Newcombe
- Anil Kumar Raghavendra
- Timothy Prather
- Cort Anderson
- Rebecca Ganley
- Sanford Eigenbrode
- Hongjian Ding
- Maryse Crawford
- The team of R project for statistical computing
- Idaho State Government



CRISSP



Web-site of the project

Knapweed project

[Russian](#) | English

Most of my materials are on the [Russian Web-site](#) (many of them are in English). Here I have put the information about my current project.

I am working now with Dr. George Newcombe and Dr. Cort Anderson in the Dept. of Forest Resources at the University of Idaho on investigating the ecology and systematics of endophytes in *Centaurea maculosa* (spotted knapweed) in its native and introduced ranges, including controlled greenhouse experiments to determine interactions among plants, endophytes, and insects and molecular systematics of endophytic fungi. I also coordinate the collaborative effort, involving faculty in ecology, entomology, mycology, and systematics (Dr. Sanford Eigenbrode, Dr. Mark Schwarzlaender, Dr. Tim Prather).

Specific objectives of the project [modified from grant proposal]:

1. Elucidation of the origin of the endophytes of *C. maculosa* (i.e., in either the native or the invaded range of *C. maculosa* itself) with sequence-based, phylogenetic tests. Origin is important because the «biogeographical source of the microbes» with which a plant interacts, can significantly affect the outcome of the host-symbiont interaction (Klironomos, 2002), and plant fitness (Callaway et al., 2004).
2. In planta determinations of interactions between endophytes of *C. maculosa* and insects, including biocontrol insects that have deliberately been released for the control of spotted knapweed.
3. In planta testing of the hypothesis of exclusive horizontal transmission of endophytes. Exclusive horizontal transmission of co-introduced fungi would have implications for plant quarantine policy and practice in the U.S. (Palm, 1999).
4. Evaluate the compositional similarity among symbiont communities from the native and invaded ranges, using a new statistical approach (Chao et al., 2005). Plant invasiveness may depend on the presence or absence, or relative abundance of key symbionts (Klironomos, 2002); host age may affect endophyte loading of *Centaurea* plants. We would employ a new aging technique for *Centaurea* (Dietz, 2002); patches have already been mapped across the Idaho landscape (Lass et al., 2002) and in eastern Washington (Roche and Roche, 1988).
5. In pursuit of generality, we would also research yellow starthistle, or *Centaurea solstitialis*, and cheatgrass, or *Bromus tectorum* (with respect to objectives 1, 3, and 4).

-
- Presentation of the first results (April 12, 2006), [PDF file, 1.4 Mb](#)
 - Abstract to the Botany 2006 conference, [PDF file, 90 kb](#)
 - [Key for the description of plants from *Centaurea stoebe/maculosa/diffusa* group](#)
 - [The sampling form for 2006](#)
 - Two additional protocols ([Cynoglossum officinale](#) and [Chondrilla juncea](#))
 - The bibliography database of the project: [BibTeX format](#), and [HTML list](#). [BibTeX](#) is the bibliography database format for TeX, you can open BibTeX files (for example) with [JabRef](#) (Mac, Linux or PC), this software could also convert BibTeX to Endnote.

[To the Russian Web-site](#)

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