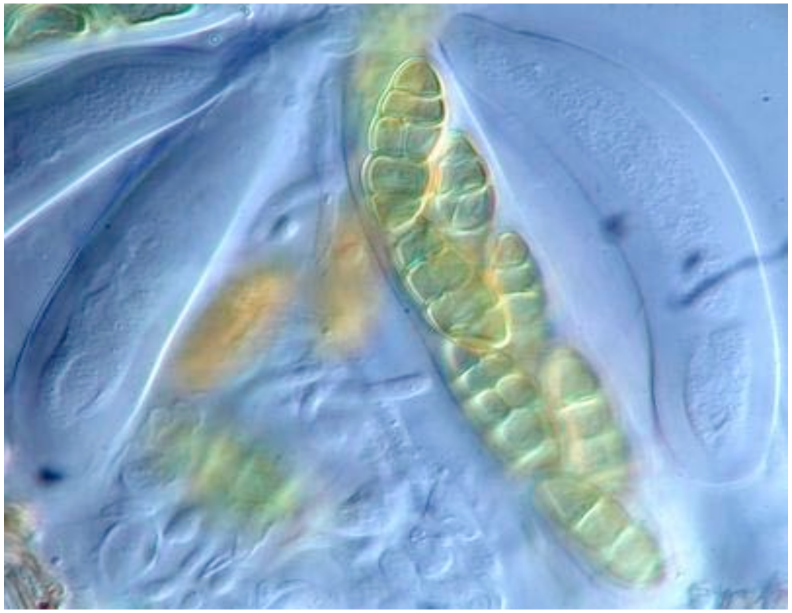
The endophyte community of spotted knapweed (*Centaurea maculosa* Lam., Asteraceae)



Alexey Shipunov, Anil Kumar Raghavendra, George Newcombe

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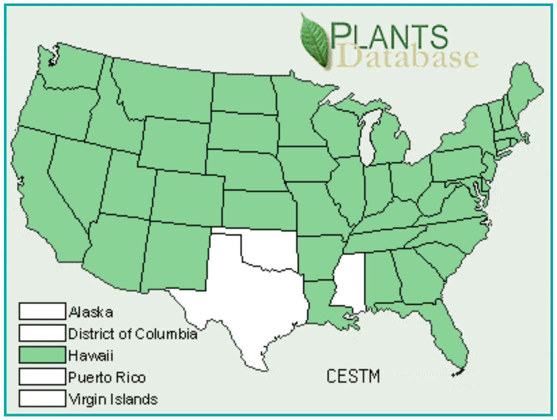
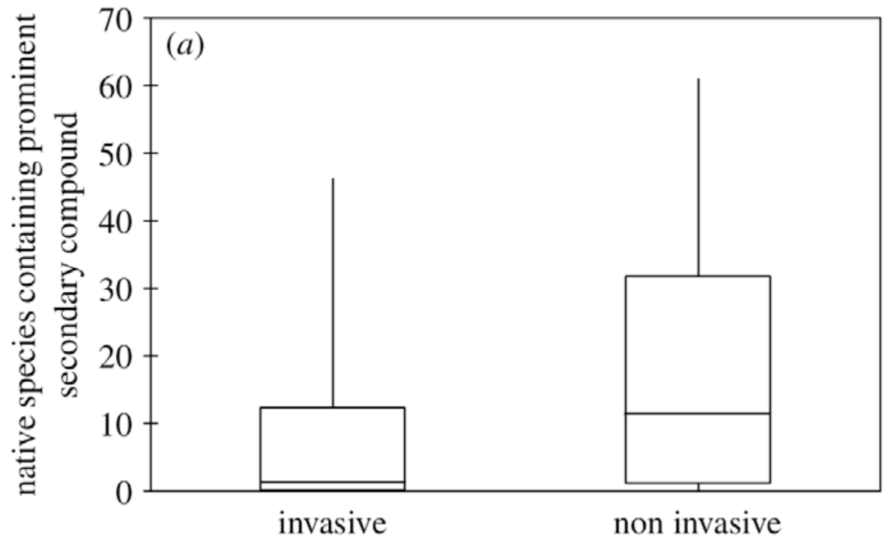
Spotted knapweed



Spotted knapweed (*Centaurea maculosa* Lam., but more acceptable name is *Centaurea stoebe* L.) is a noxious, invasive plant which was introduced into North America from Eurasia in the 19th century. There are two races: diploids (biennials) and tetraploids (perennials), only the last is present in North America.

“Novel weapons”

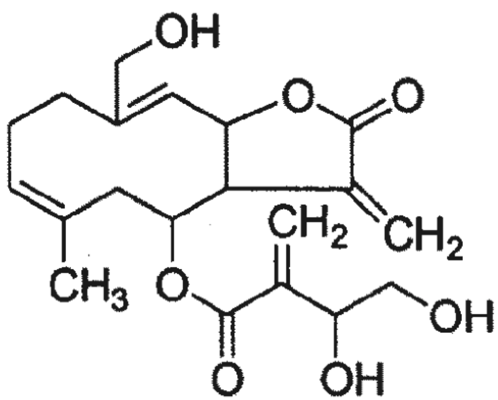
Many invasive North American plants have been reported to have antimicrobial, antiherbivore and allelopathic effects, which are most probably the consequences of unique (for American flora) secondary chemical compounds.



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

Catechin or not catechin

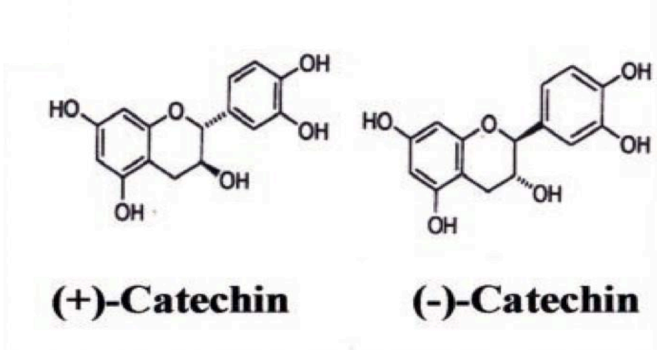
Spotted knapweed is among plants which have significant phytotoxic (allelopathic) effect. Some secondary compounds were believed to have this effect: **cnicin** and **catechins**.

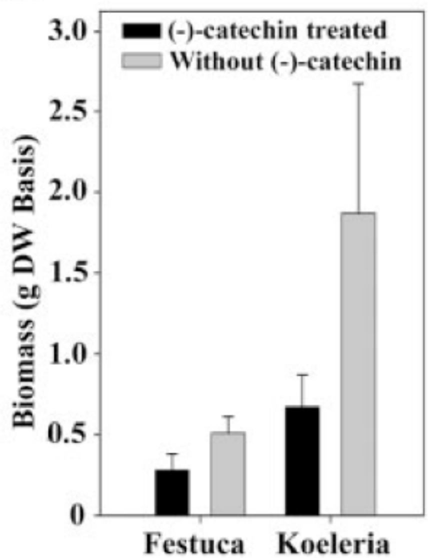


Cnicin (sesquiterpene lactone) was extracted from aerial parts of knapweed in 1967 and has been thought as main inhibitor of neighbor plants growth (Kelsey & Locken, 1987). However, some reporters told about little inhibitor effect of cnicin (Muir & Majak, 1983)

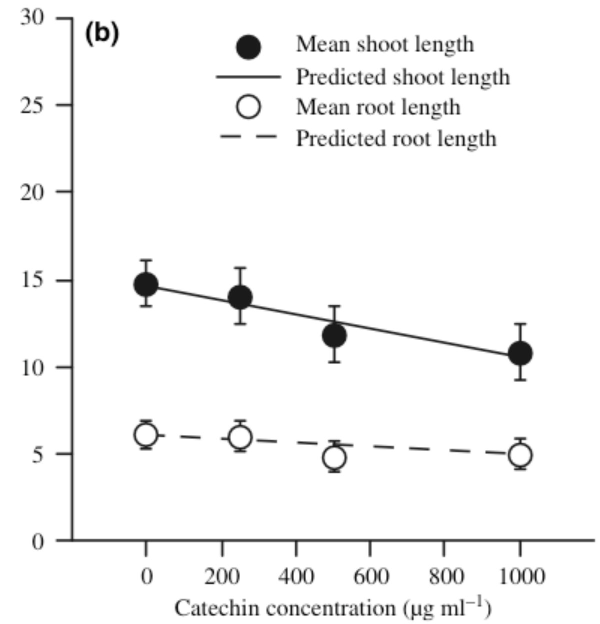
Catechins

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

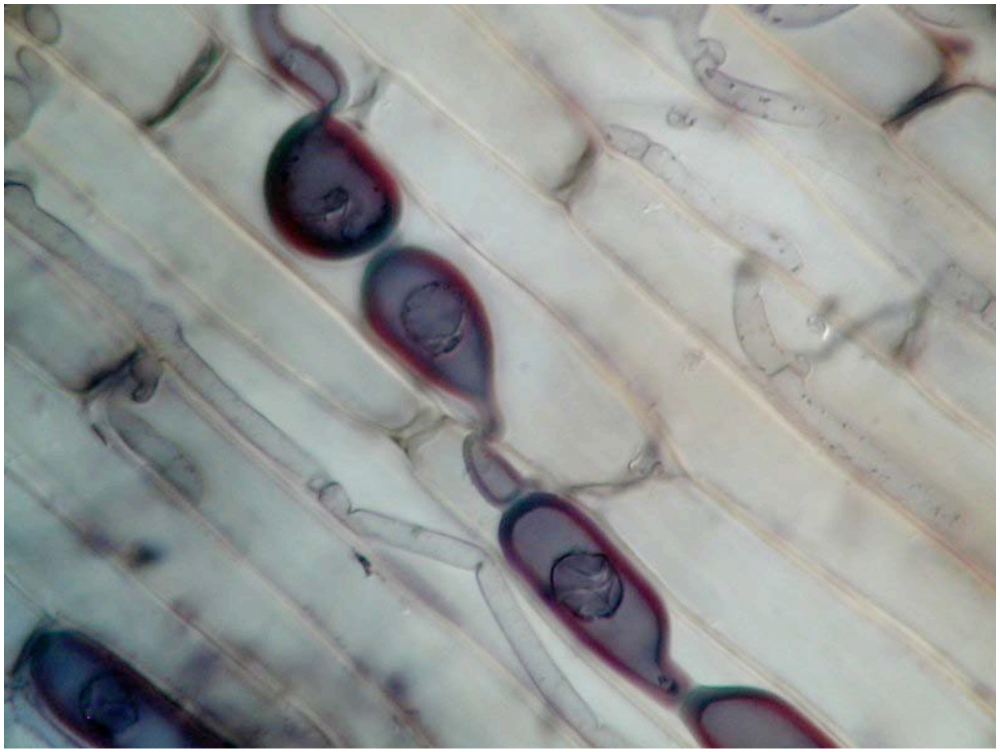




However, recent experiments (Blair et al., 2005) show the absence of catechin effect.



Fungal endophytes



1) Inhabit every plant

2) Some endophytes are known to produce secondary metabolites which are beneficial to the host plant

3) Have full spectrum from parasitism to commensalism

Therefore, the controversy could be explained if **investigated plants have different endophyte communities** and, as a consequence, different secondary compounds

Competition experiment

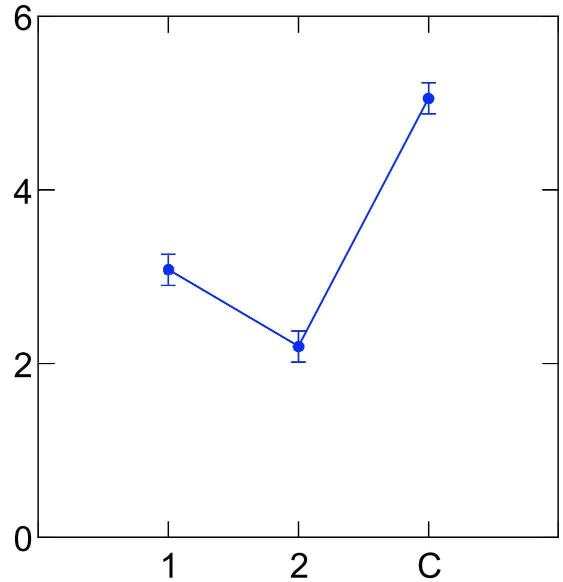
E+
knapweed
and fescue

Fescue
alone:
control

Endophyte-free (E–)
knapweed and fescue

The differences in fescue biomass are statistically significant

Least Squares Means

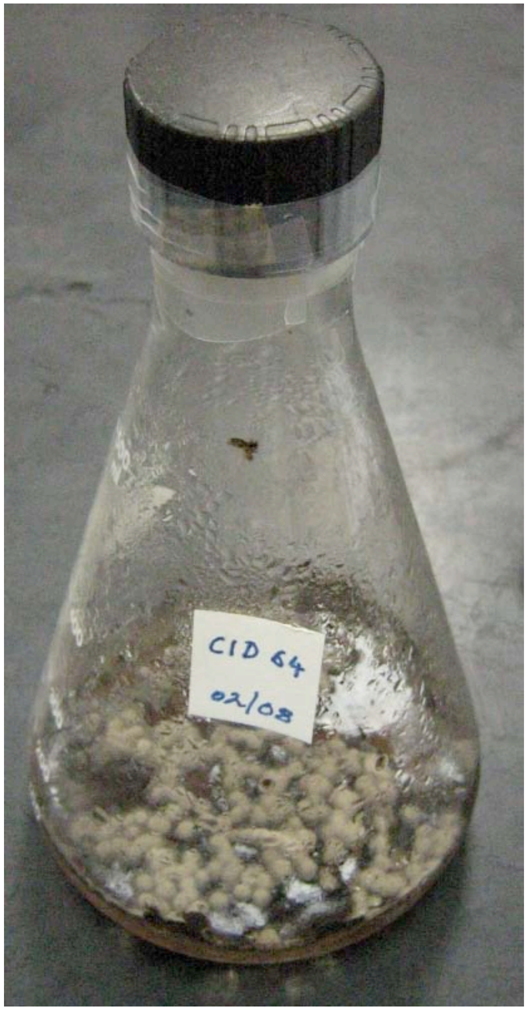


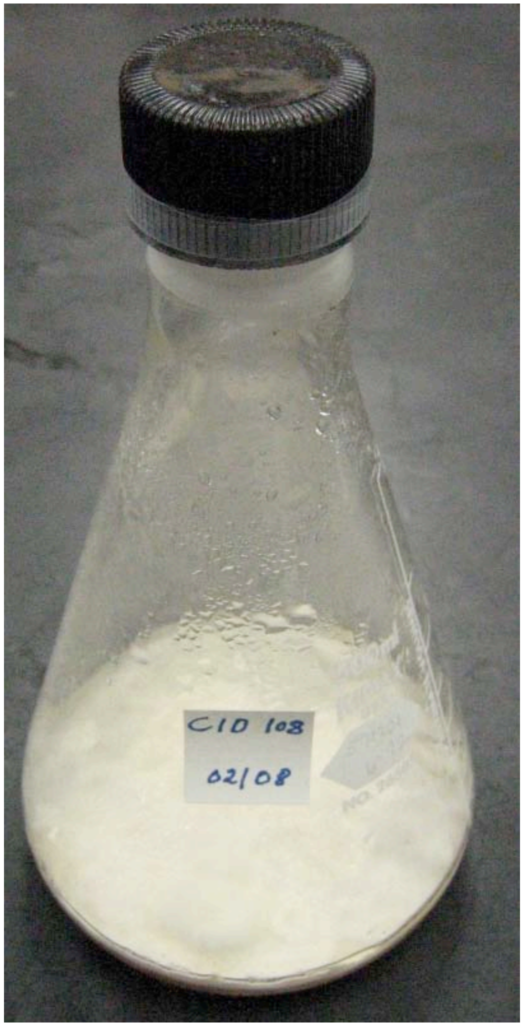
1 E– plants

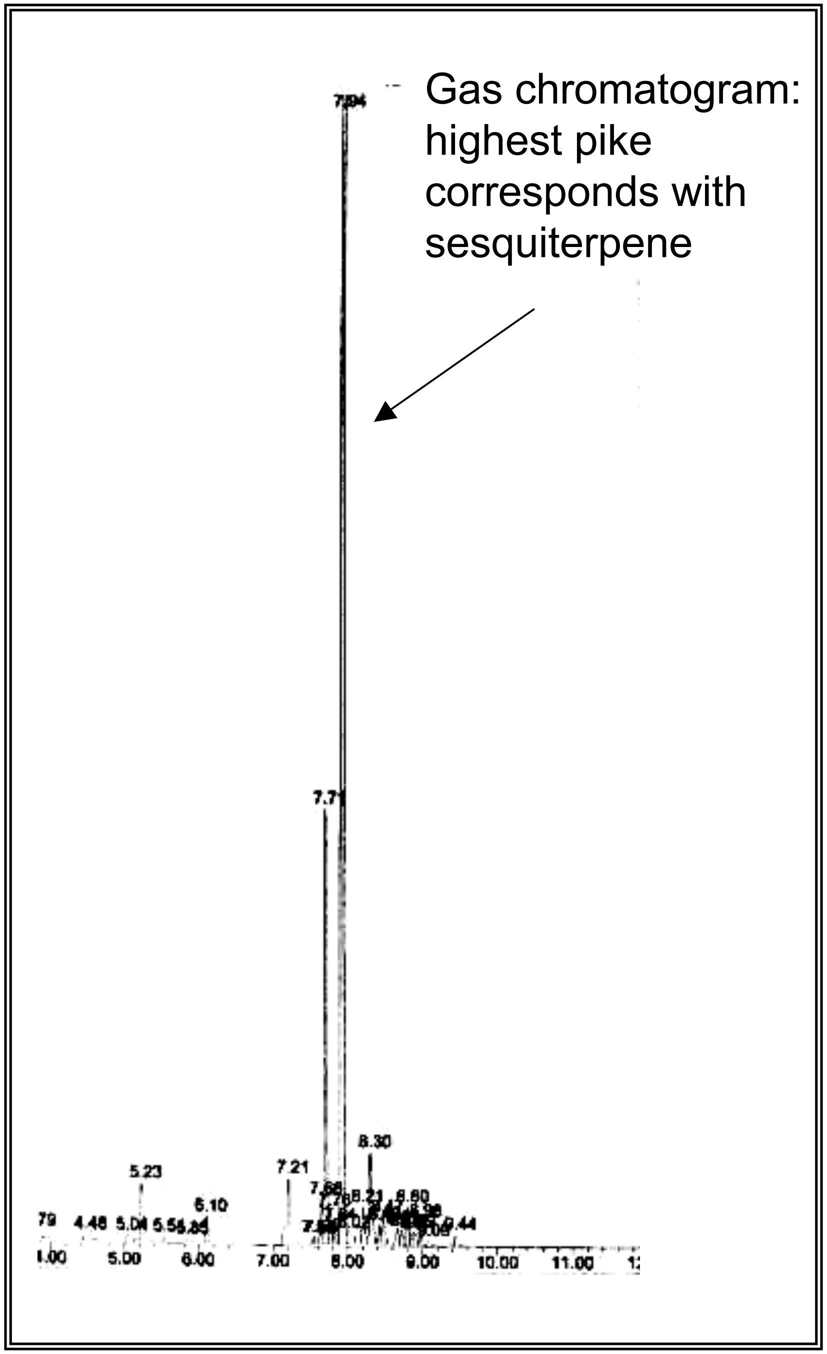
2 E+ plants

C Control (*Festuca idahoensis* alone)

Liquid cultures and volatile compounds





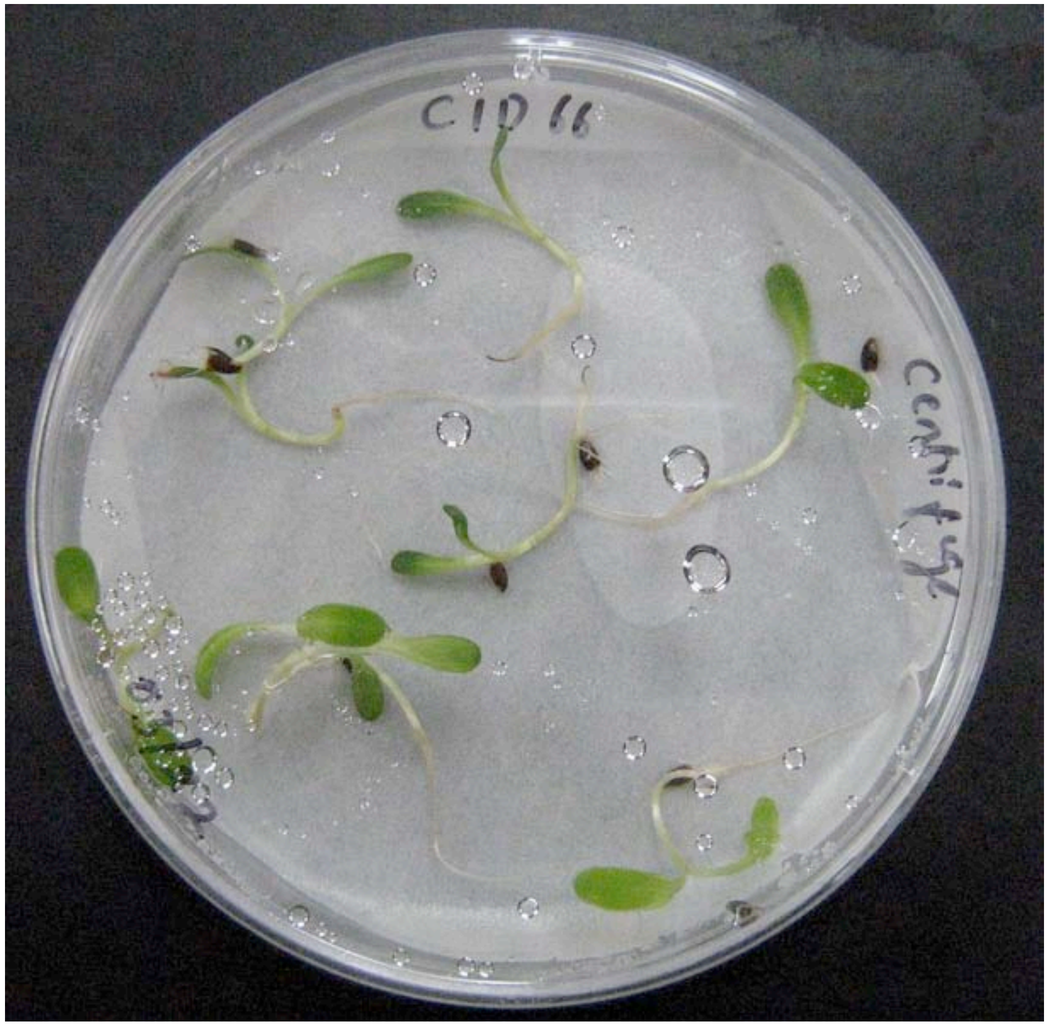


At least some endophytes can produce sesquiterpenes

We have also found that this particular endophyte strain has the insecticide effect to weevils.

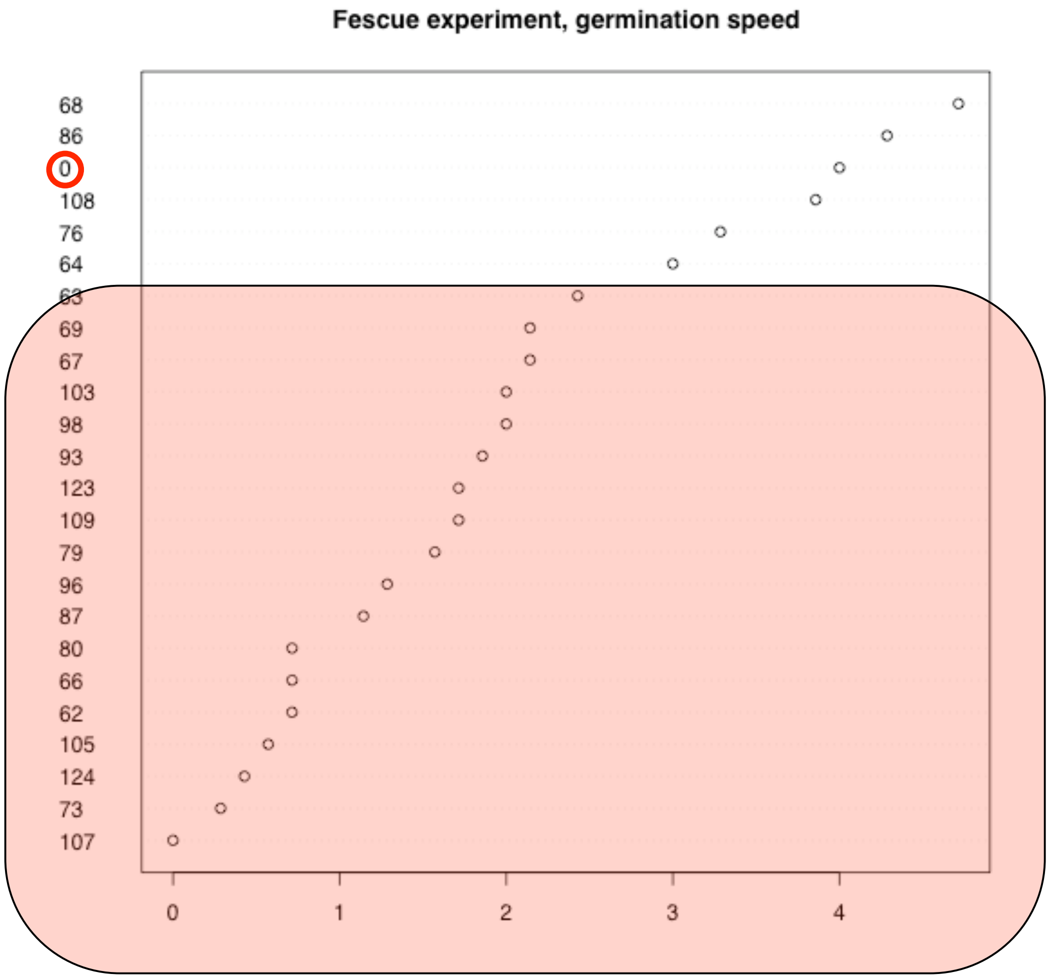
Endophytes and seed germination

Experiment with knapweed seeds
(fungal cultures were used)



Experiment with *Festuca idahoensis* seeds
(liquid culture filtrates were used, we tried to imitate Blair et al., 2005 experiment conditions)

Some endophytes are capable to suppress seed growth

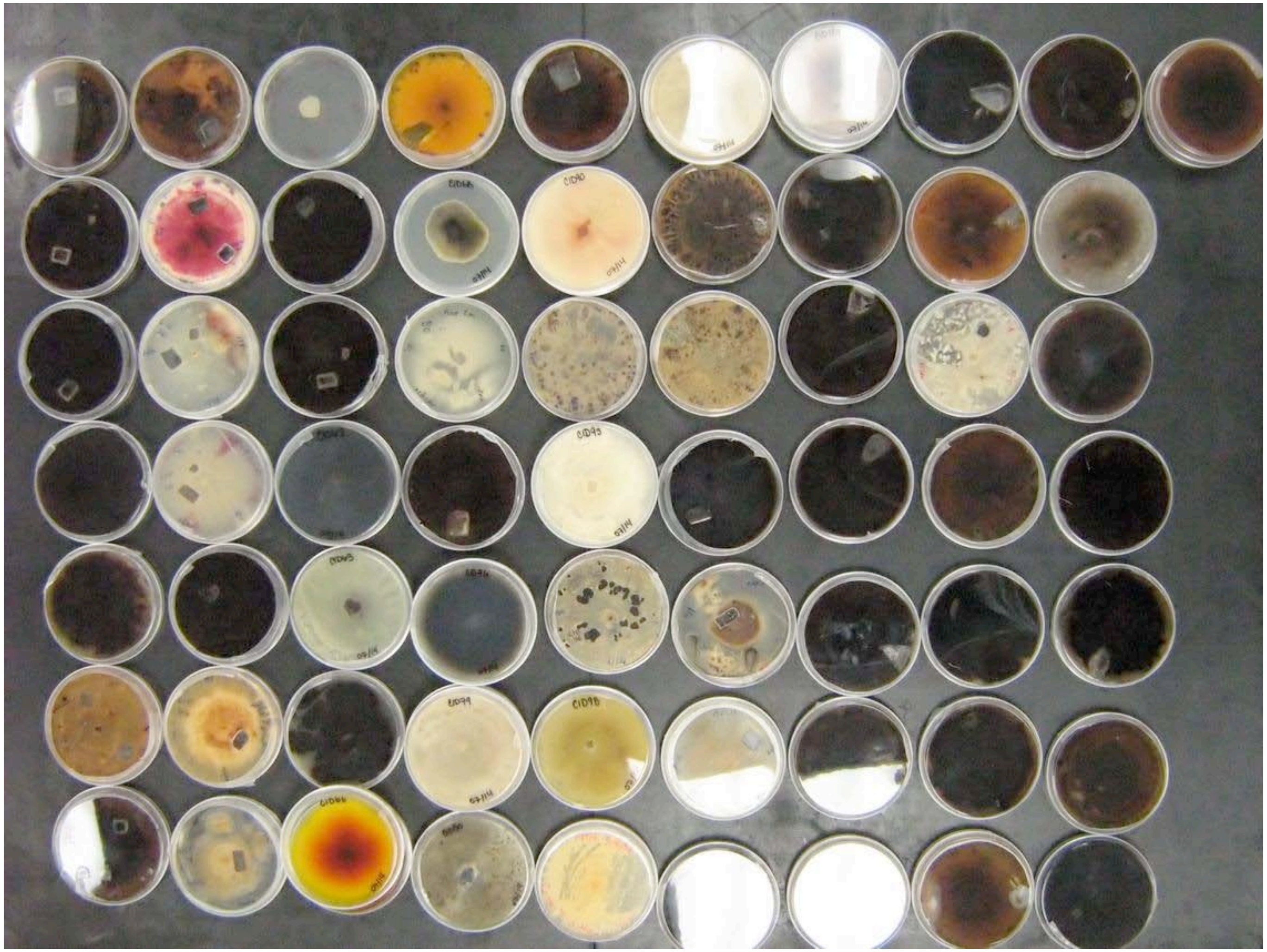


More than 2/3 endophyte strains have **statistically significant termination effect** on *Festuca idahoensis* seeds, whereas only 1/4 of them have similar effect on knapweed seeds. Moreover, some endophytes (*Pleospora* sp.) can **kill** fescue seeds.

Endophytes have different effects on knapweed

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

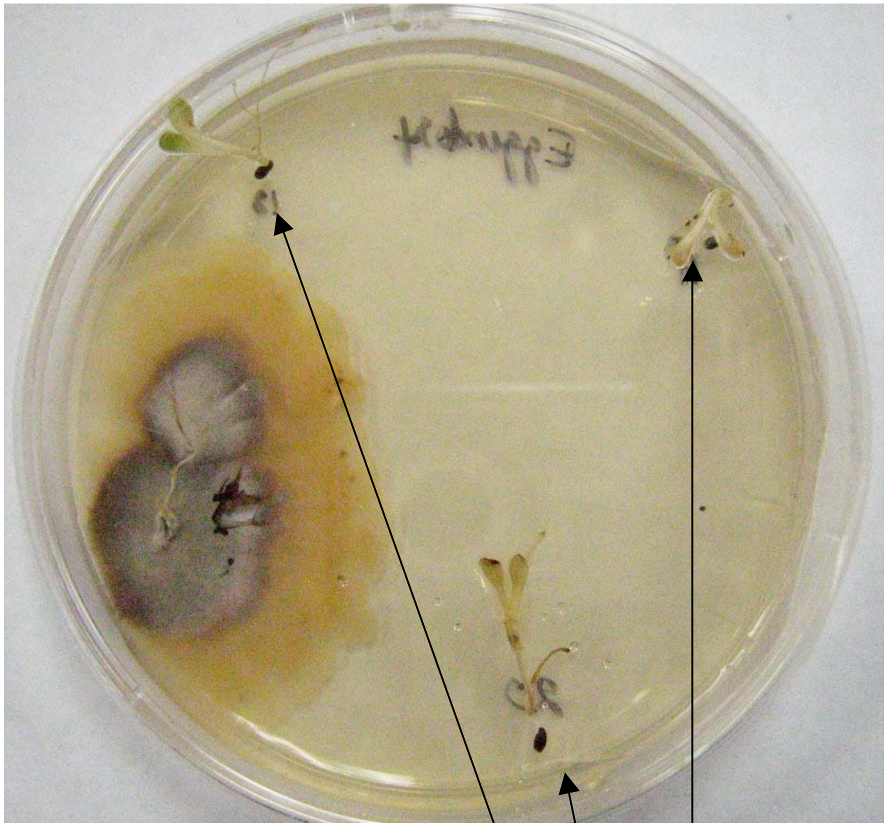
Did fungi come with their hosts?

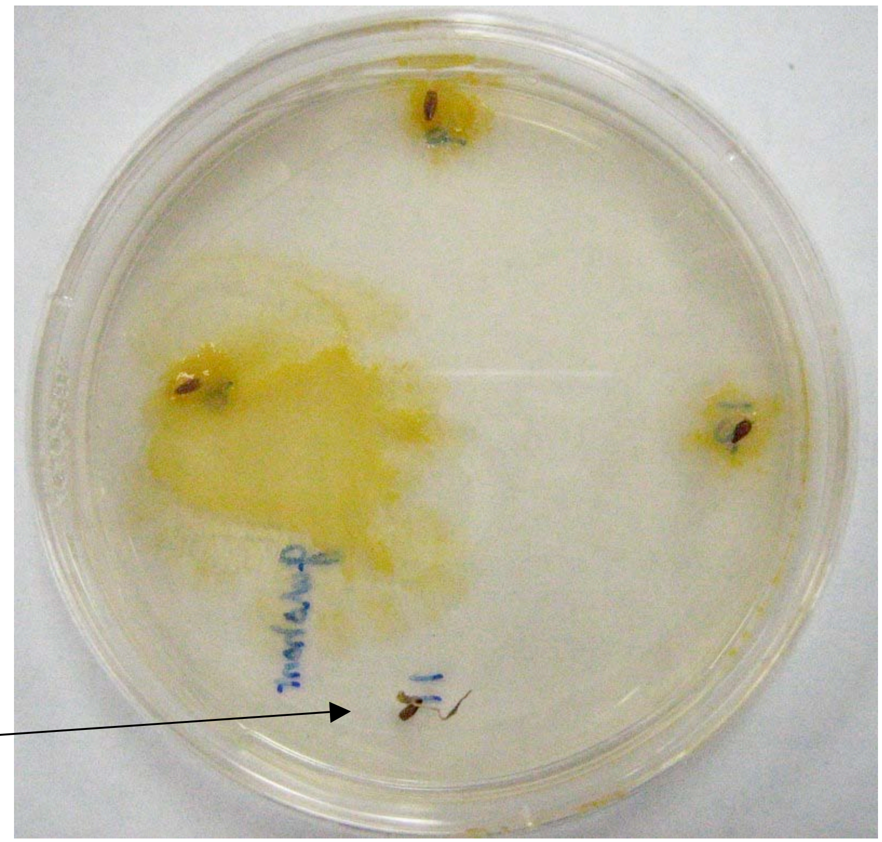


Two possibilities: “host-jumping” or co-introduction

Isolation

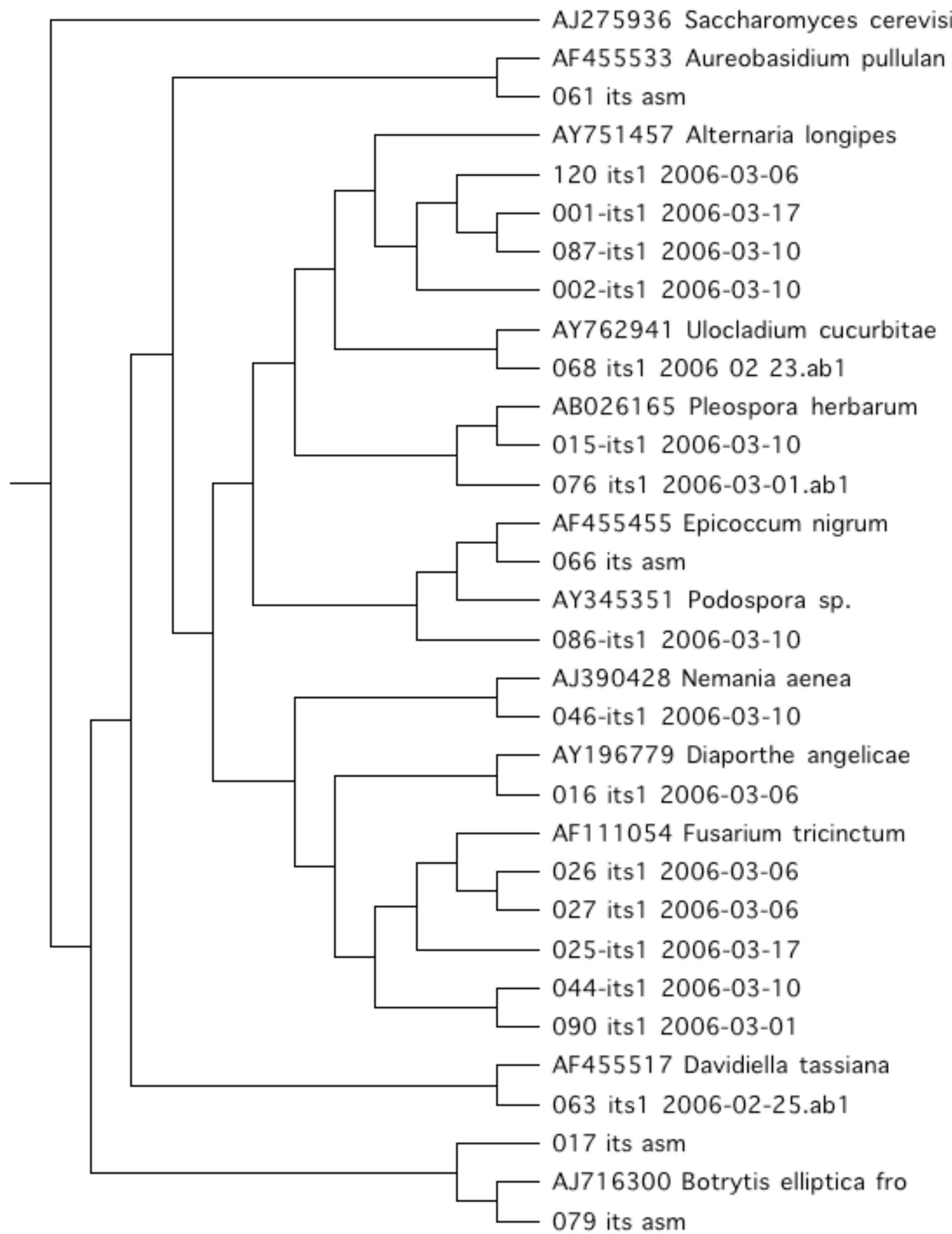
Endophytes are usually isolated from the achenes of knapweed





endophyte-free achenes

How diverse are knapweed endophytes?

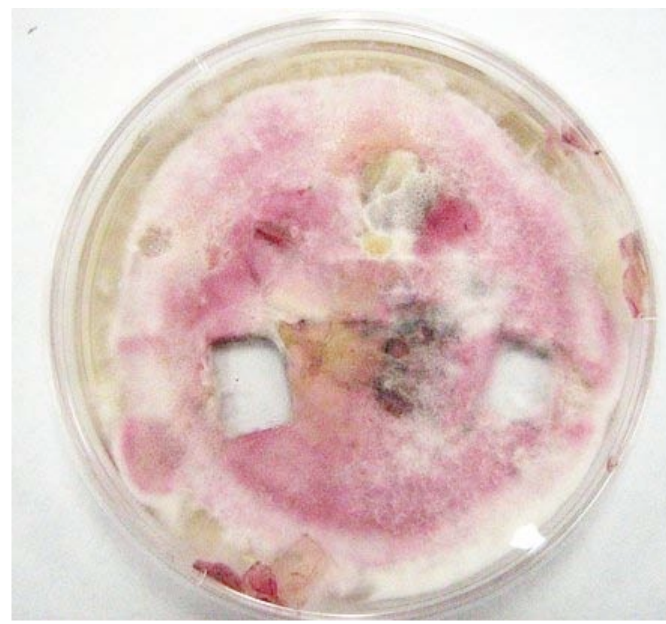


One of best MP trees from phylogenetic analysis of ITS1, 5.8S and ITS2 gene sequences.
More then 65% of them have no exact matches in the NCBI GenBank nucleotide database.

Most frequent ITS haplotypes

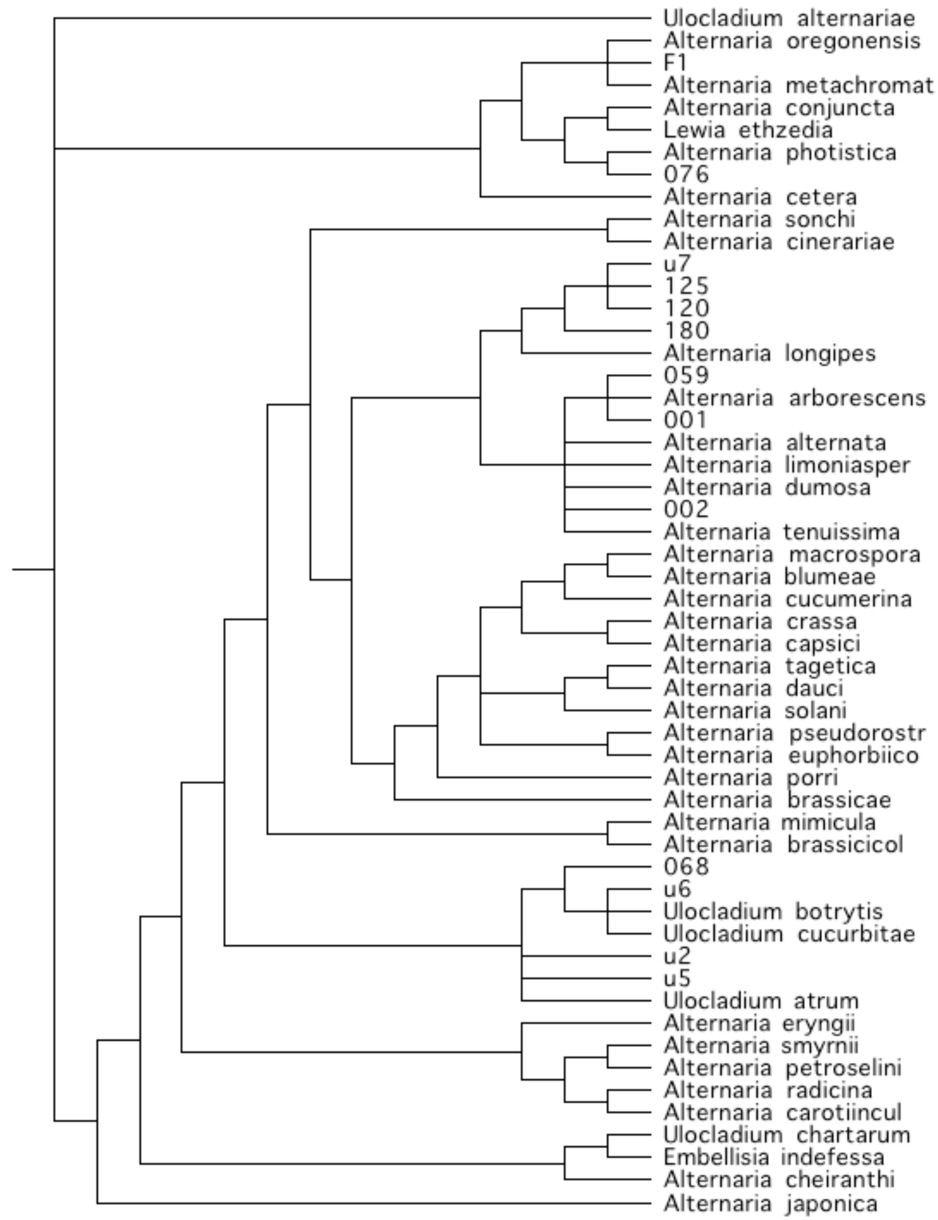
Botrytis spp., 6 ITS haplotypes

Alternaria spp., 5



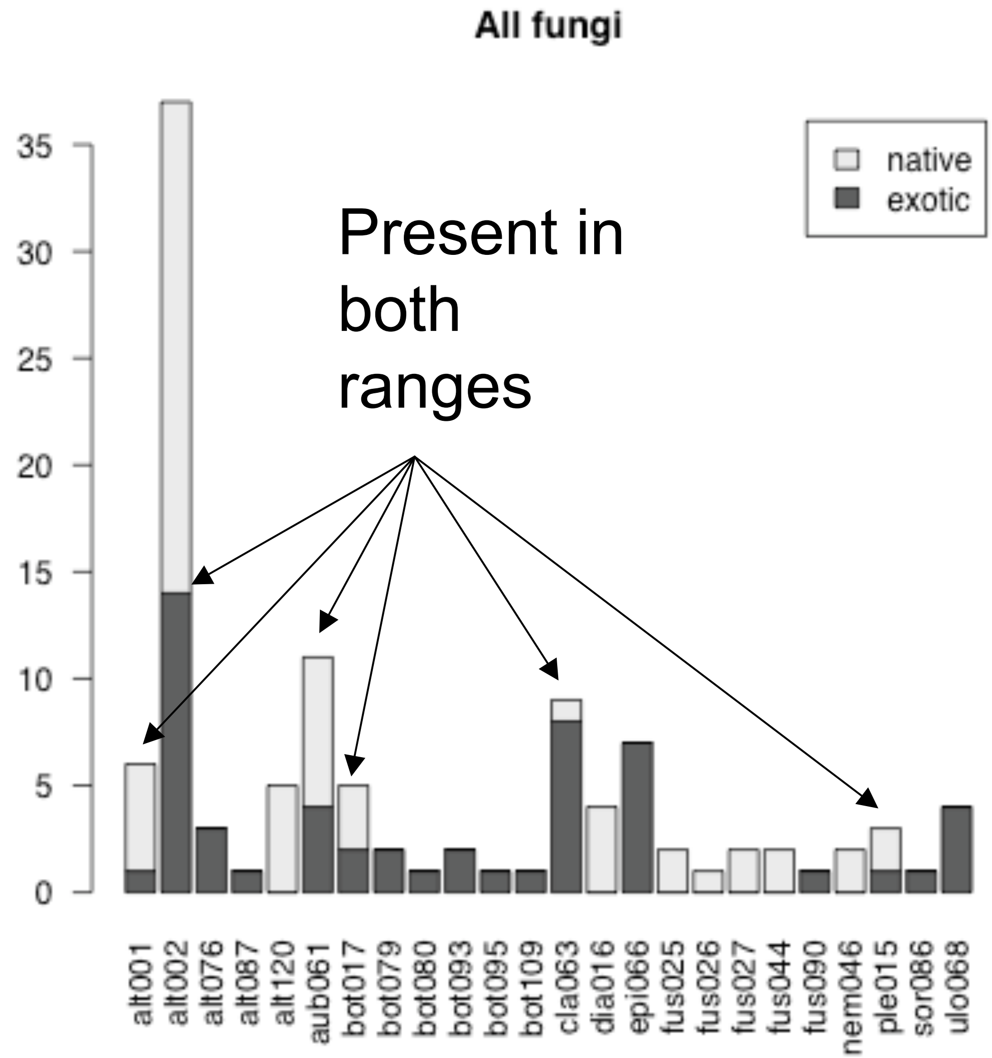
Fusarium spp., 5 (all are new to GenBank)

Alternaria allergene gene (alt a 1) was used to identify *Alternaria* and *Ulocladium* species

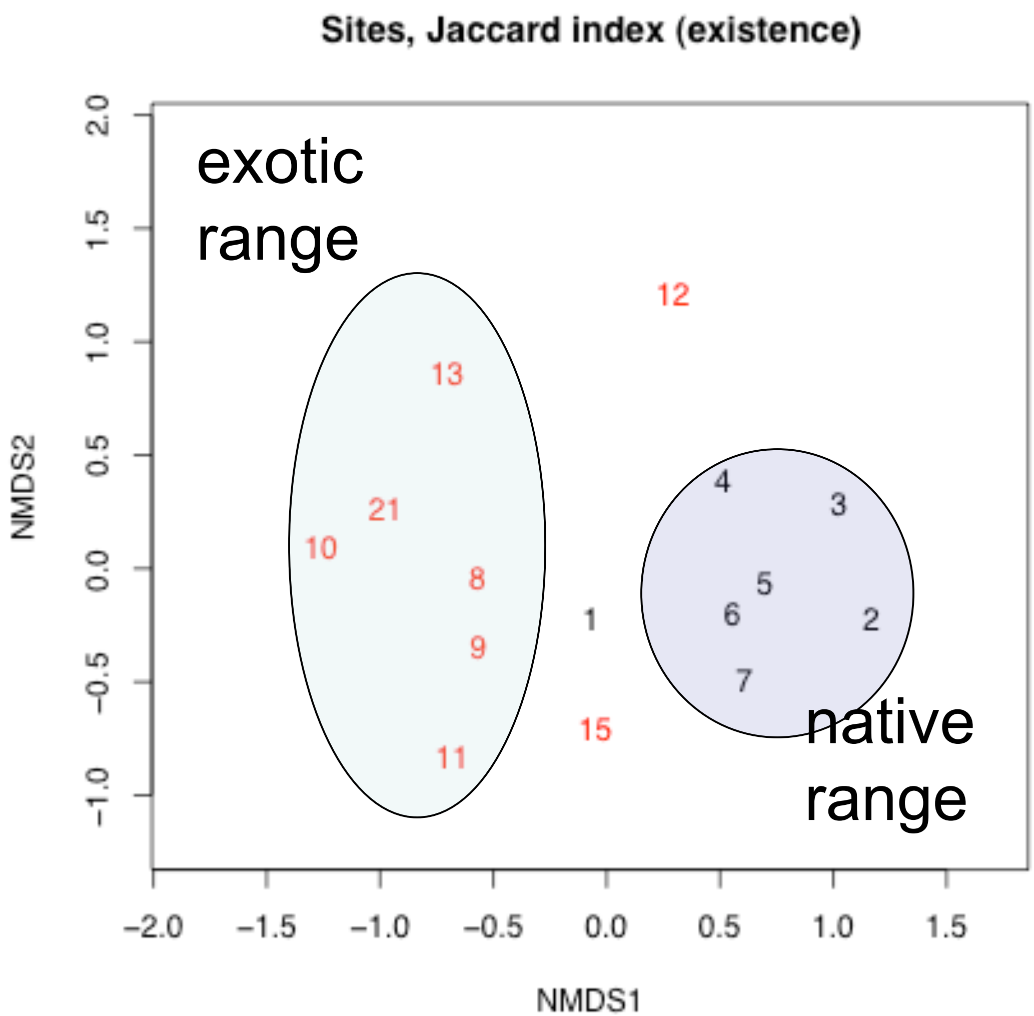


Majority rule consensus tree from MP analysis of “Alt a 1” gene sequences

Distribution among native and exotic ranges



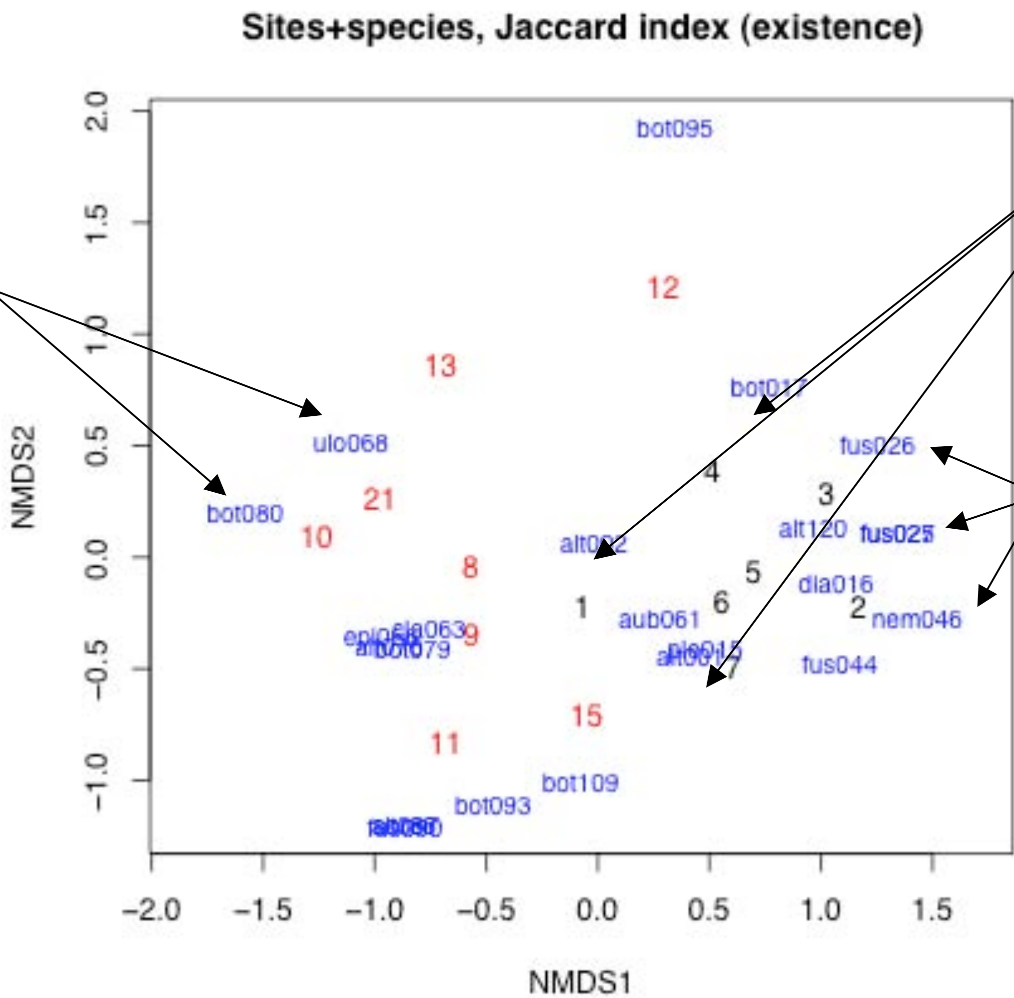
Are endophyte communities different?



Patterns of co-occurrence

ITS haplotypes common to exotic range

To both



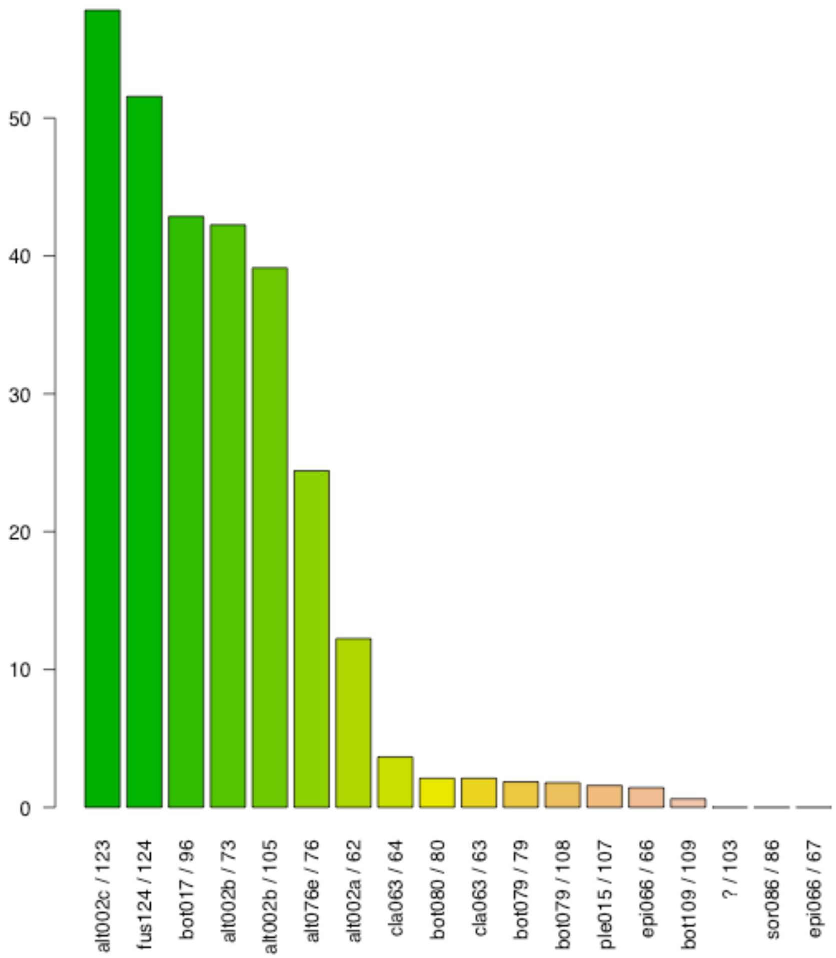
Haplotypes common to native range

At the time, we have support for both co-introduction and "host-jumping" hypotheses.

Endophyte-free plants

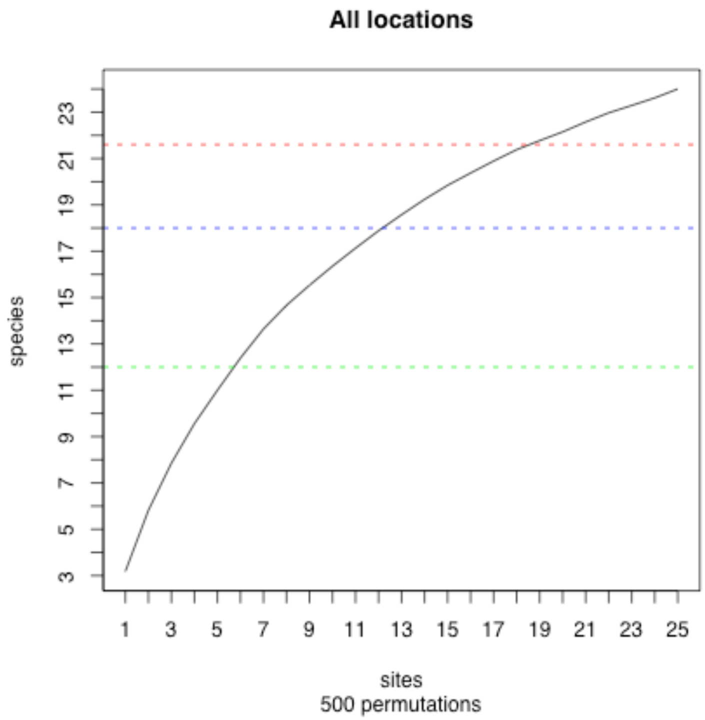
Plants from natural habitats are usually rich of endophytes (70%–90% of seeds). However, some of our samples contain no endophytes. We cultivated the 2nd generation of knapweed and inoculate them with liquid fungal cultures on the flowering stage.

Re-isolation

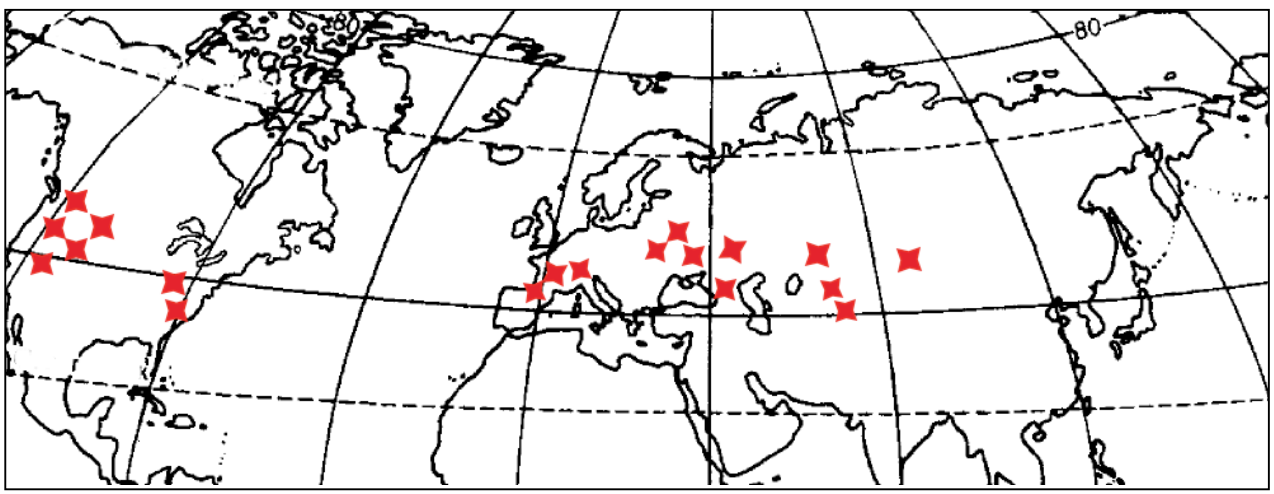


Then re-isolation were done. From all plants, we obtained only endophytes which were used for inoculation. *Alternaria* species have the best re-isolation frequency. **No endophytes were isolated from the control.** Thus, we have found the way to produce endophyte-free plans.

Sampling-2006



Accumulation curve for 2004/2005 (most of samples were collected in Idaho state or in southwestern Europe)

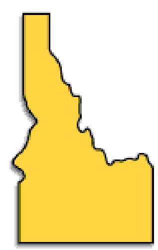


Places of interest

We are trying now to organize much wider sampling and **need your help**!

Acknowledgements

- Cort Anderson
- Rebecca Ganley
- Sanford Eigenbrode
- Hongjian Ding
- Maryse Crawford
- The team of R project for statistical computing
- Jari Oksanen, author of “vegan” R package for vegetation ecologists
- Idaho State Government



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