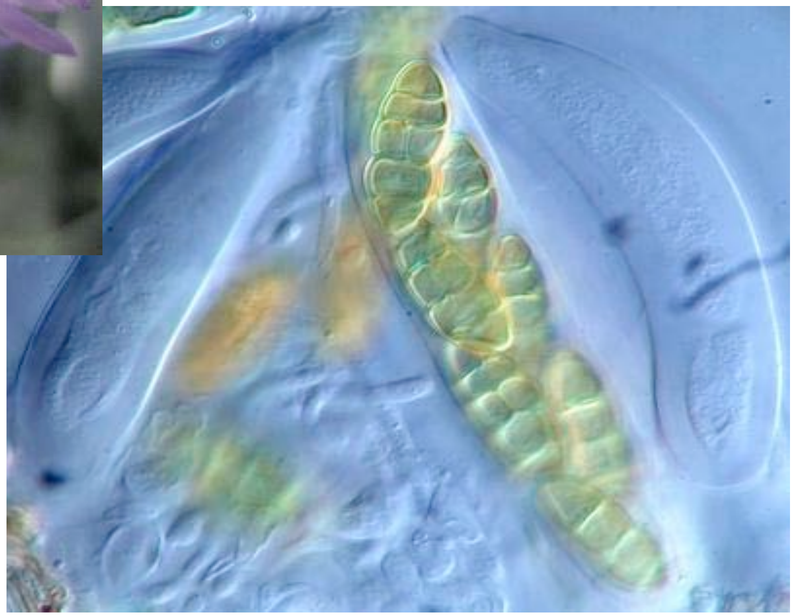
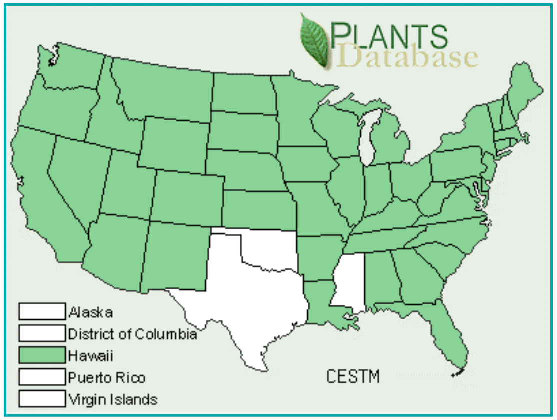
Roles of endophytic fungi in the invasive spotted knapweed (*Centaurea stoebe* L.)

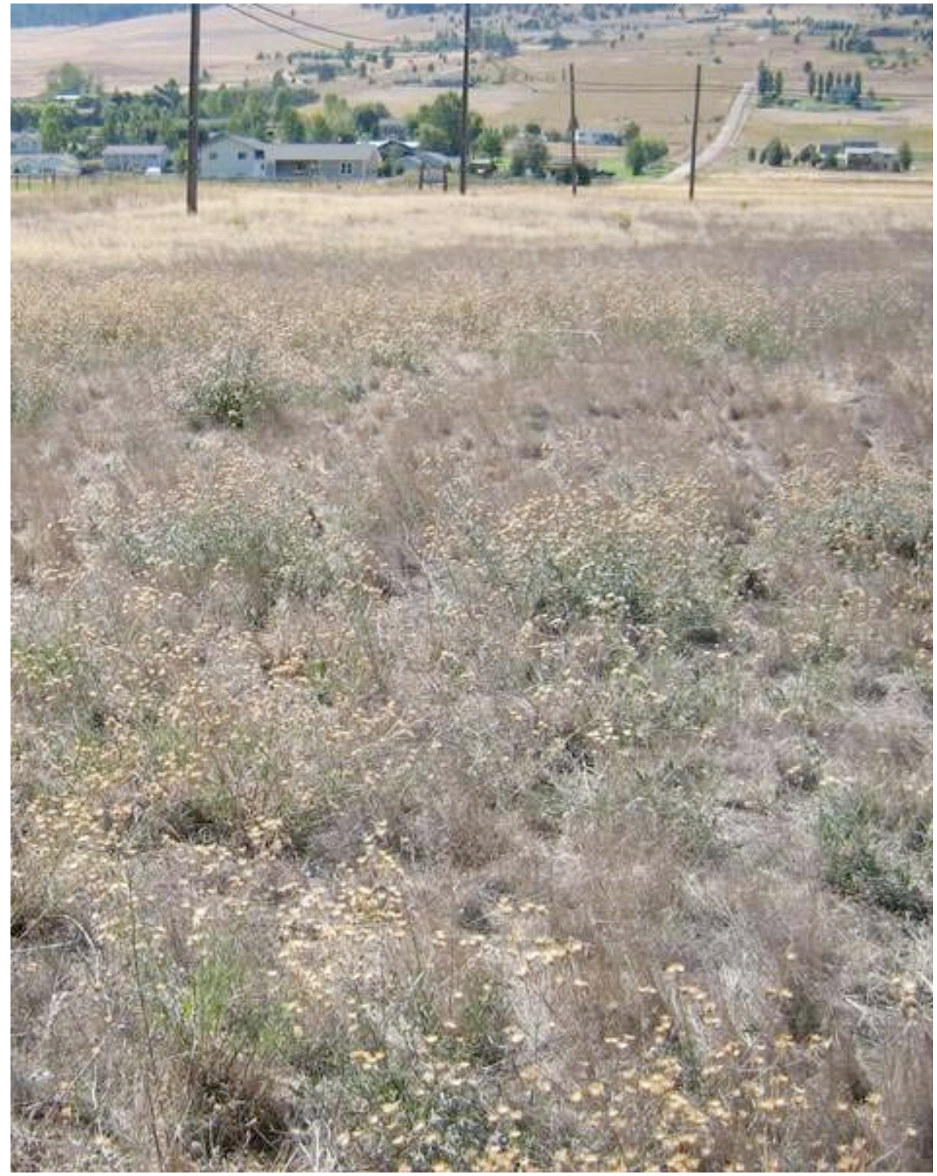
Alexey Shipunov,
Anil Kumar Raghavendra,
George Newcombe



Department of Forest Resources, University of Idaho

Spotted knapweed



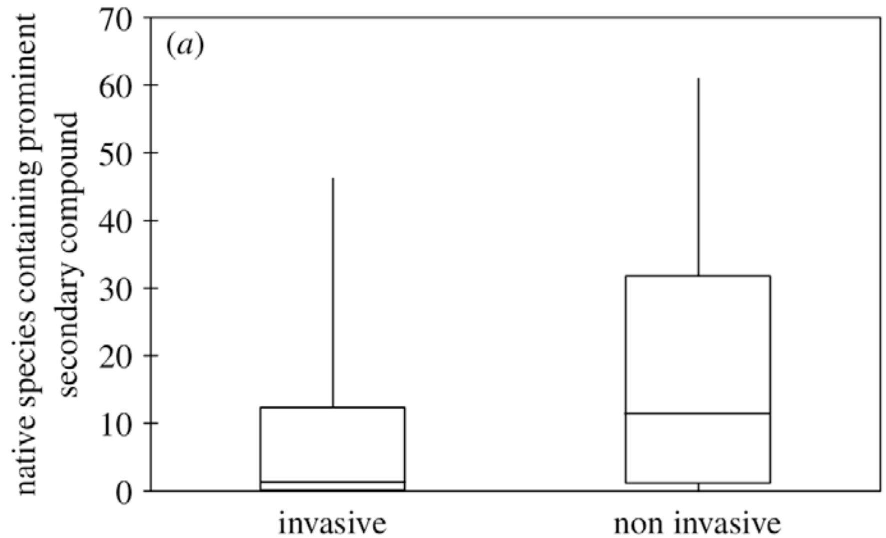


Spotted knapweed (*Centaurea stoebe* L.) is a noxious, invasive plant which was introduced into North America from Eurasia. First reported in North America in 1893, knapweed now infests millions hectares of rangelands and pastures.

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

“Novel weapons”

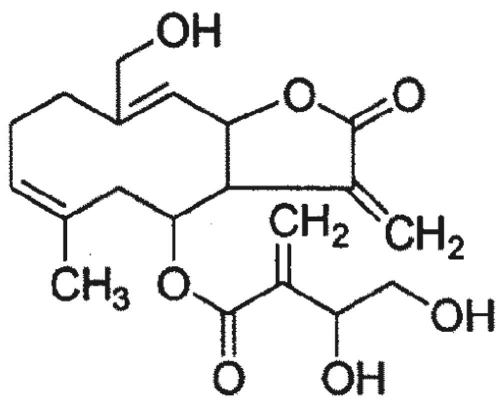
Many invasive North American plants have been reported to have antimicrobial, antiherbivore and allelopathic effects, which are most probably the consequences of unique (for American flora) secondary chemical compounds.



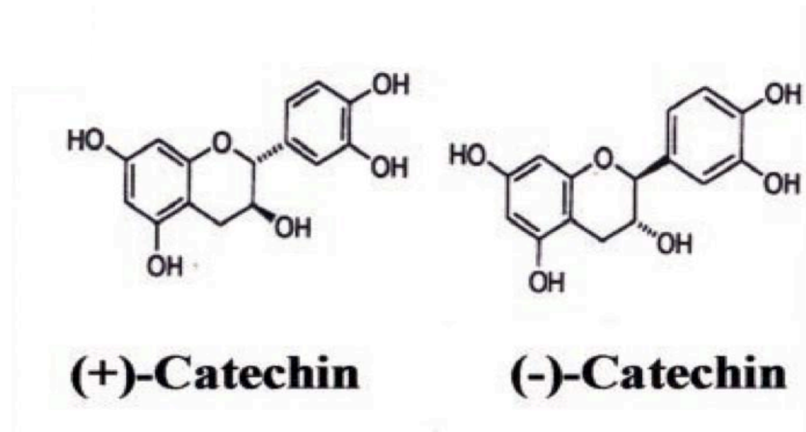
(From Cappucino & Arnason, 2006): **invasive** plants share their prominent secondary compounds with less native North American plants than **non-invasive** plants

Allelopathy

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

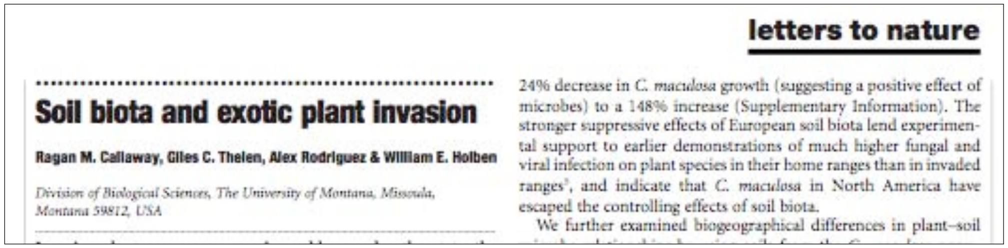


Cnicin (sesquiterpene lactone) was extracted from aerial parts of knapweed in 1967 and has been thought as main inhibitor of neighbor plants growth (Kelsey & Locken, 1987). However, some reporters told about little inhibitor effect of cnicin (Muir & Majak, 1983)



Catechins

Catechin or not catechin


letters to nature

Soil biota and exotic plant invasion

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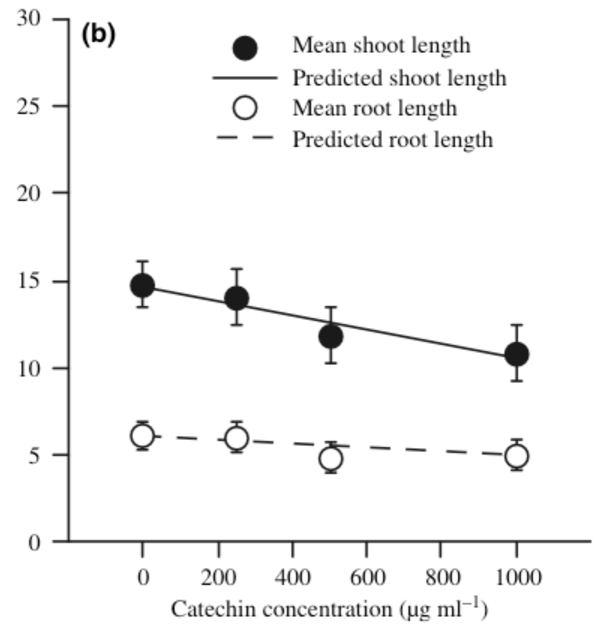
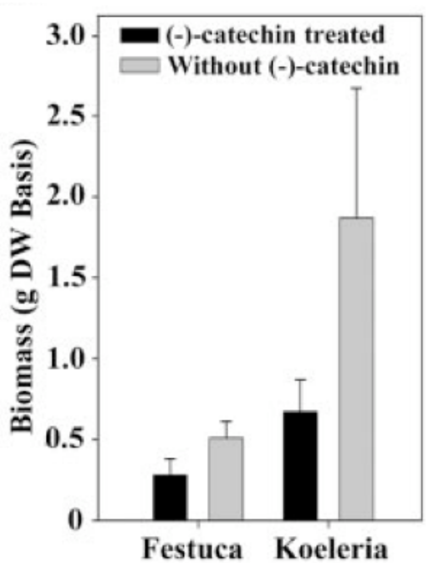
24% decrease in *C. maculosa* growth (suggesting a positive effect of microbes) to a 148% increase (Supplementary Information). The stronger suppressive effects of European soil biota lend experimental support to earlier demonstrations of much higher fungal and viral infection on plant species in their home ranges than in invaded ranges[5], and indicate that *C. maculosa* in North America have escaped the controlling effects of soil biota.

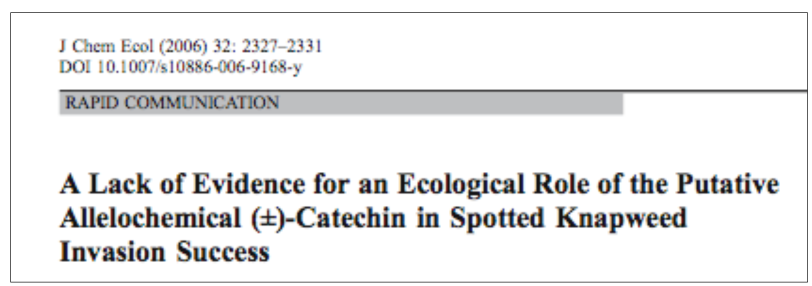
We further examined biogeographical differences in plant–soil

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, 2006) show the absence of catechin effect.

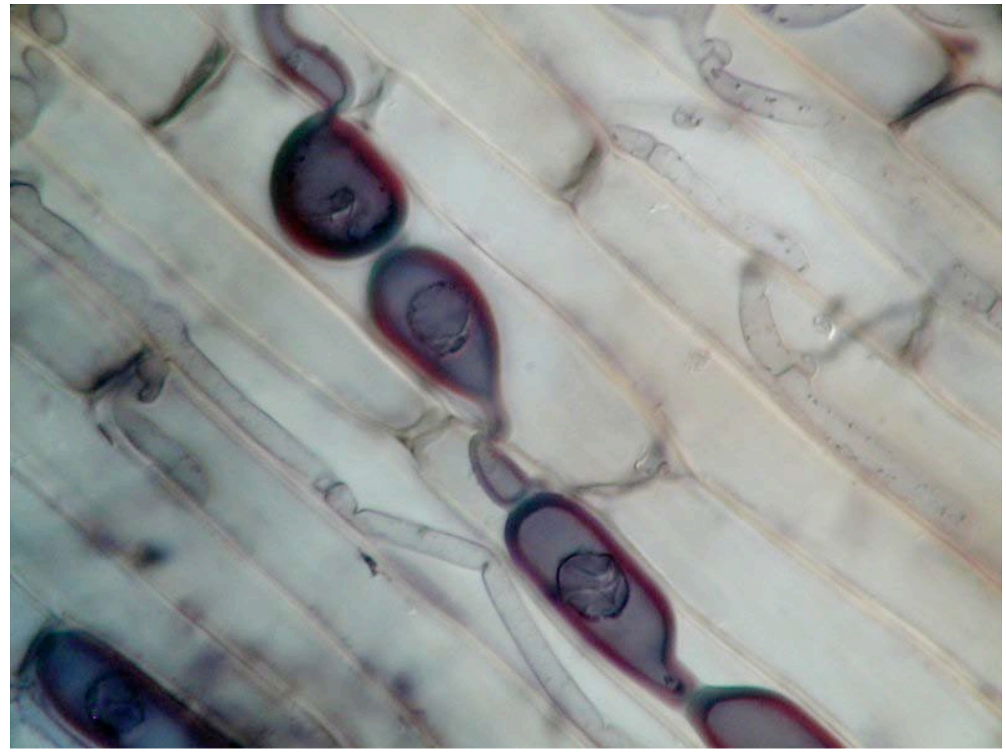

J Chem Ecol (2006) 32: 2327–2331
DOI 10.1007/s10886-006-9168-y

RAPID COMMUNICATION

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

Fungal endophytes

- Inhabit every plant
- Some endophytes are known to produce secondary metabolites which are beneficial to the host plant, e.g., taxol from *Taxus* trees
- Have full spectrum from parasitism to commensalism



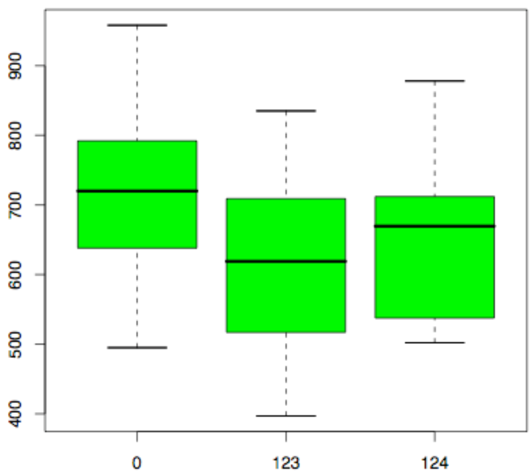
Therefore, the controversy could be explained if **investigated plants have different endophyte communities** and, as a consequence, different secondary compounds

Endophytes have different effects on knapweed

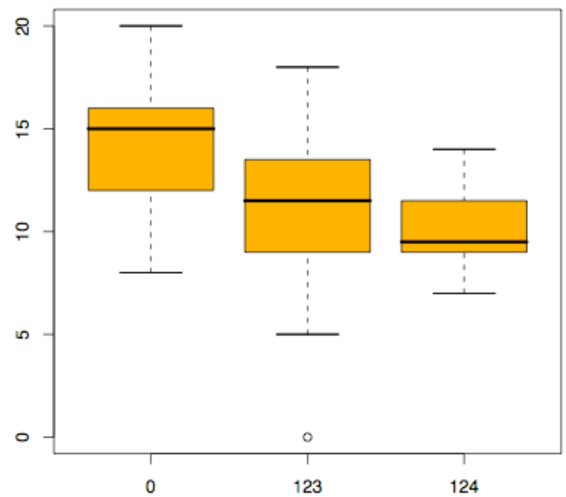
Endophyte strain 124 (*Fusarium* sp.) suppresses the flowering of knapweed

Different effects

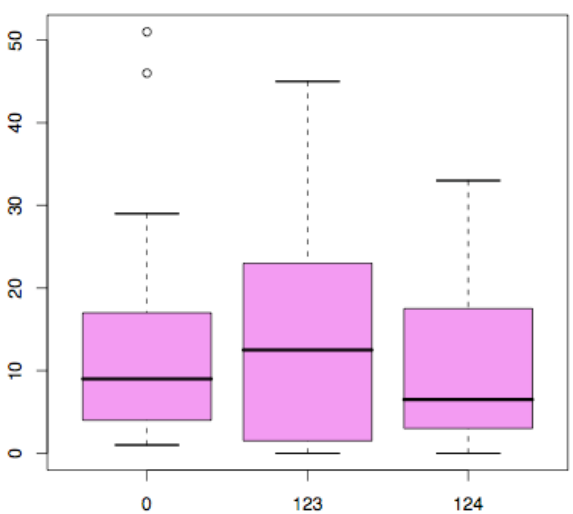
Height



Branching



Number of flowers



Some endophytes have strong negative effect on knapweed (i.e., they are close to pathogens)

Trays with inoculated seedlings

Trays with control seedlings

Even close species can be different

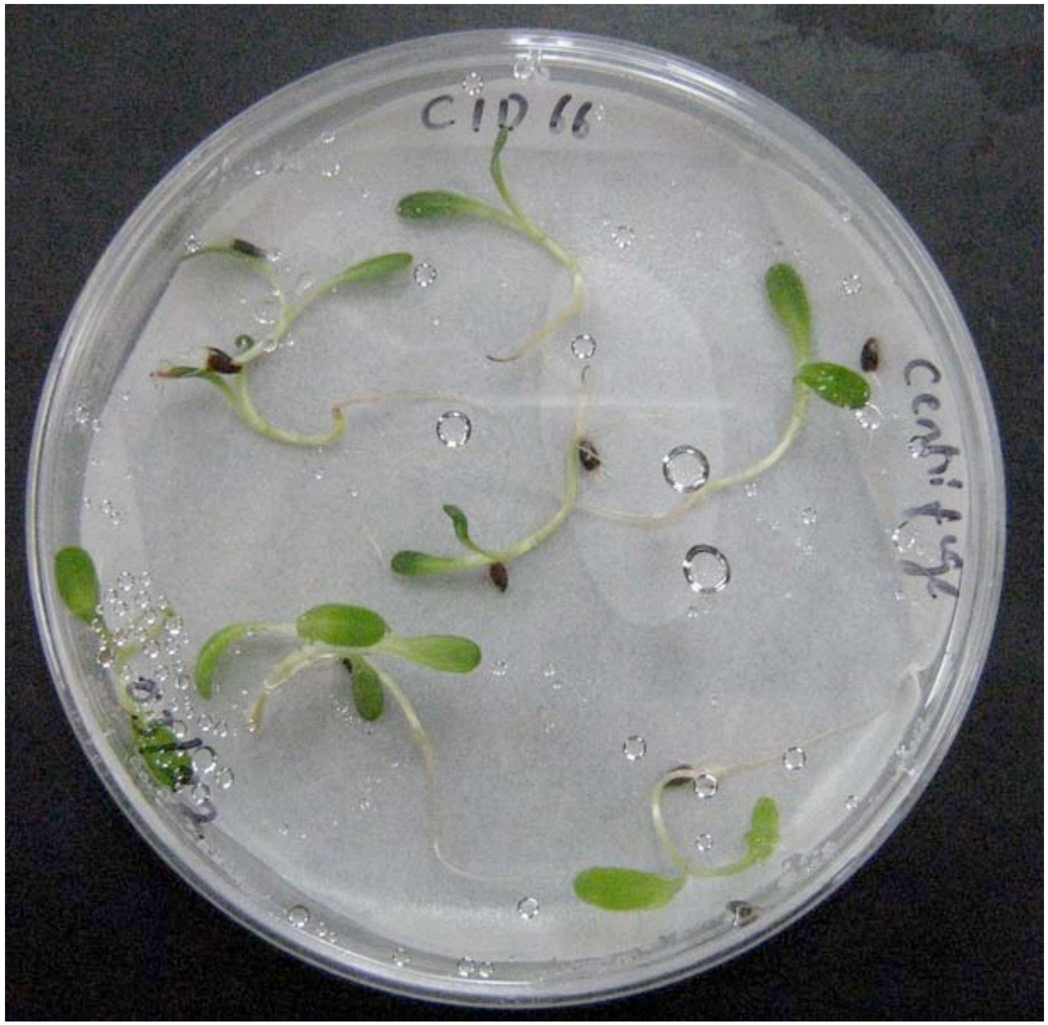
Effect of CID107 (*Fusarium* sp. 1)

Effect of CID44 (*Fusarium* sp. 2)

Endophytes and seed germination

Experiment with knapweed seeds

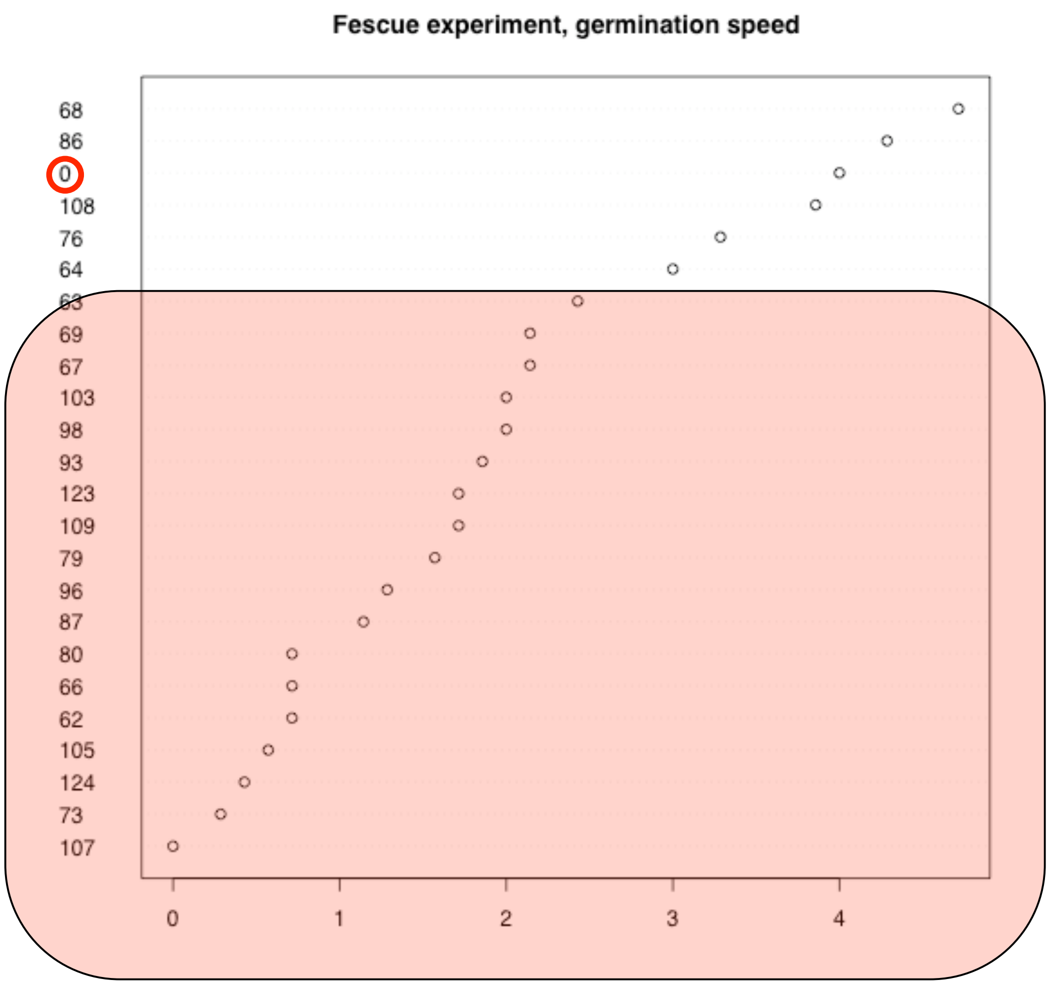
(fungal cultures were used)



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 (*Fusarium* sp.) can **kill** fescue seeds.

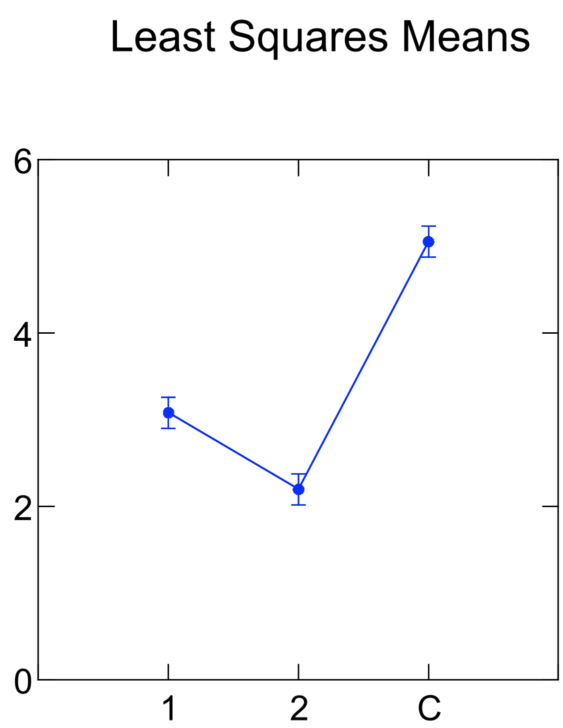
Competition experiment

E+
knapweed
and fescue

Fescue
alone:
control

Endophyte-free (E–)
knapweed and fescue

The differences in fescue biomass are statistically significant

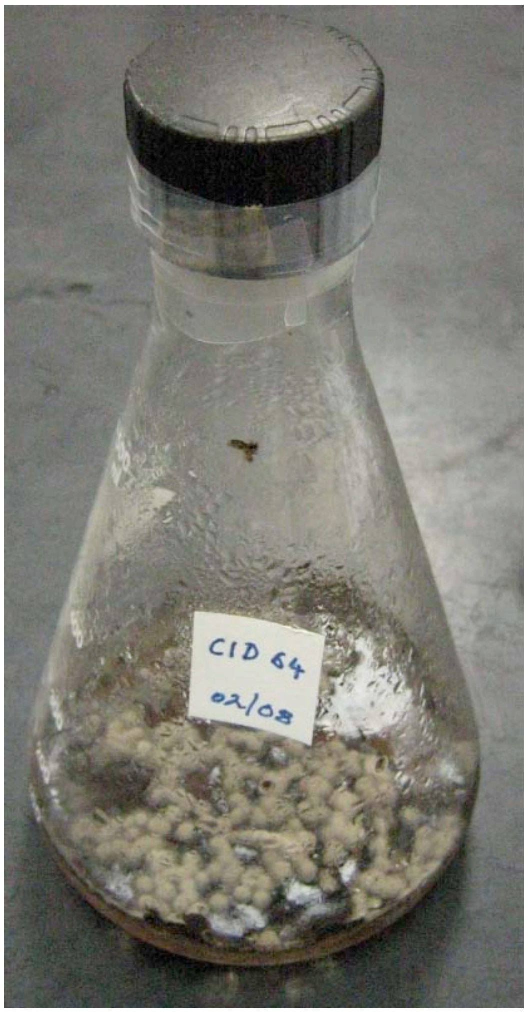


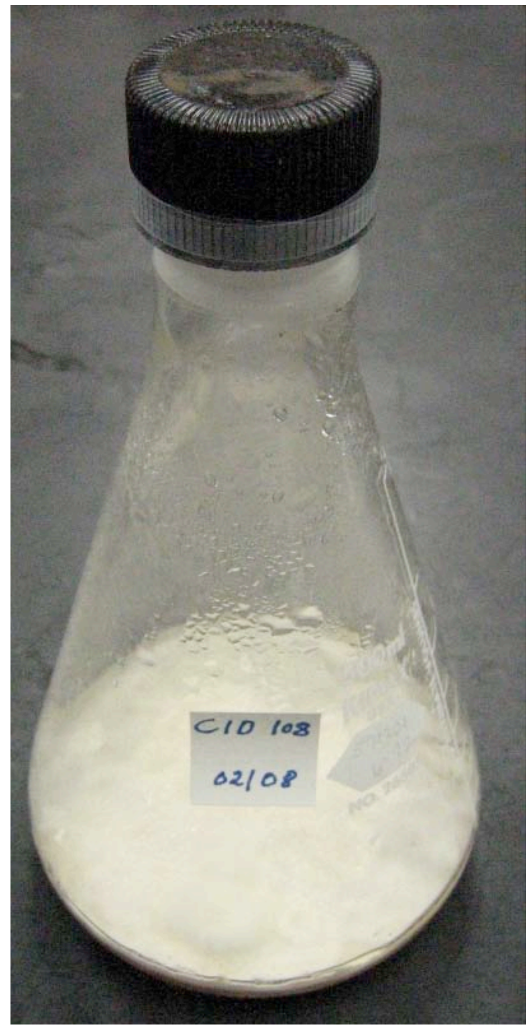
1 E– plants

2 E+ plants

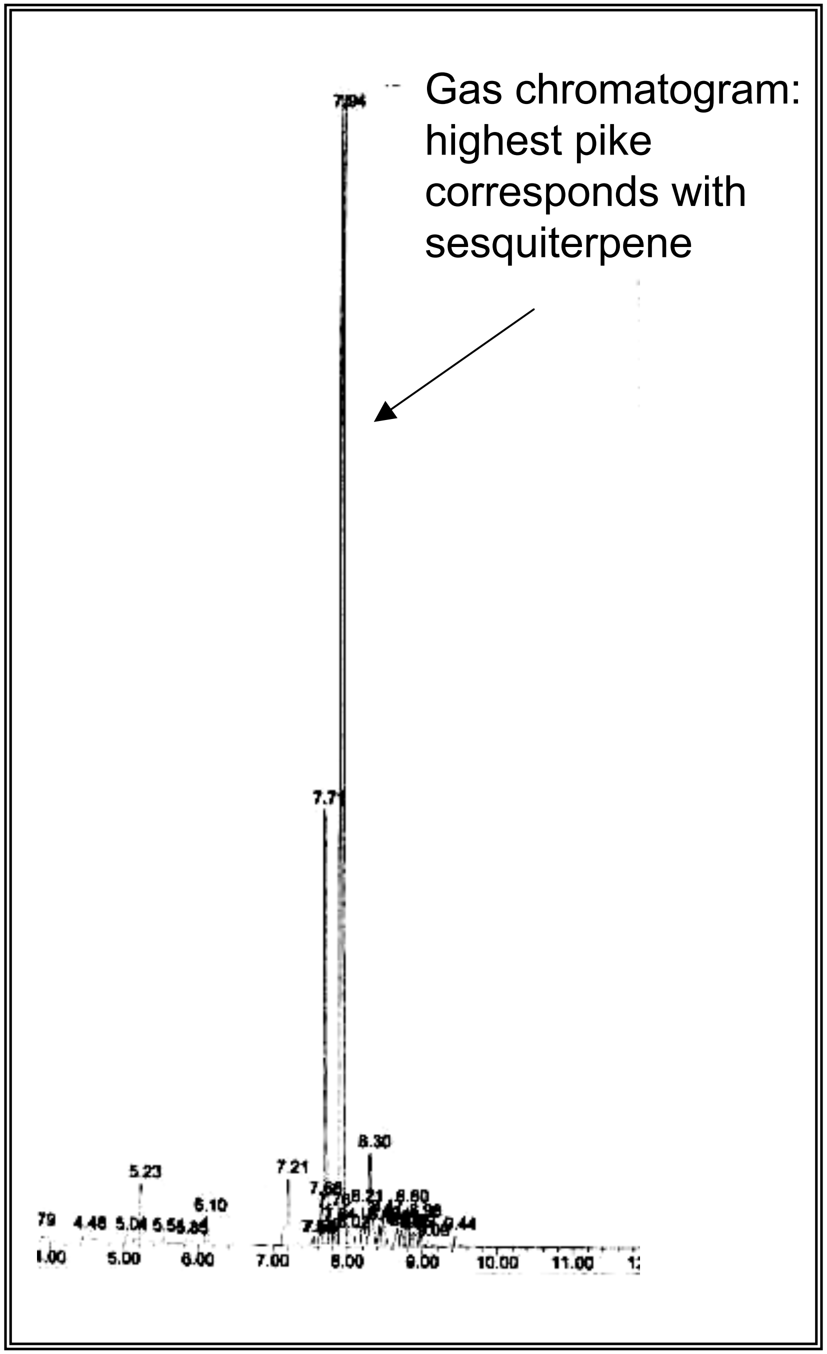
C Control (*Festuca idahoensis* alone)

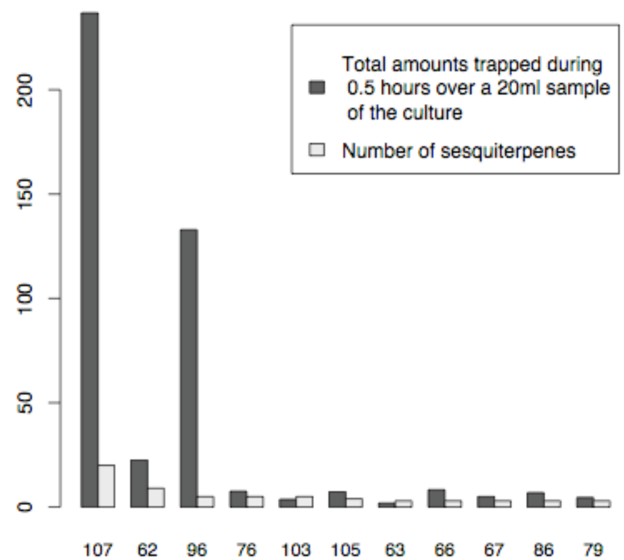
Liquid cultures and volatile compounds



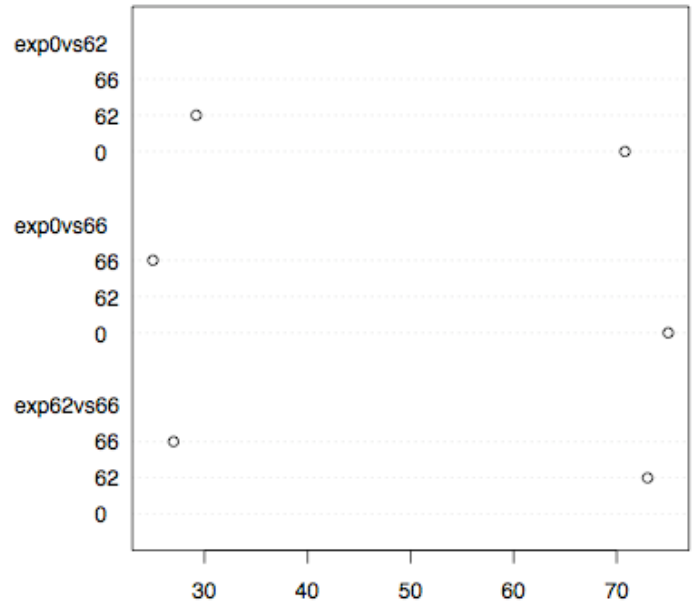


At least some endophytes can produce sesquiterpenes



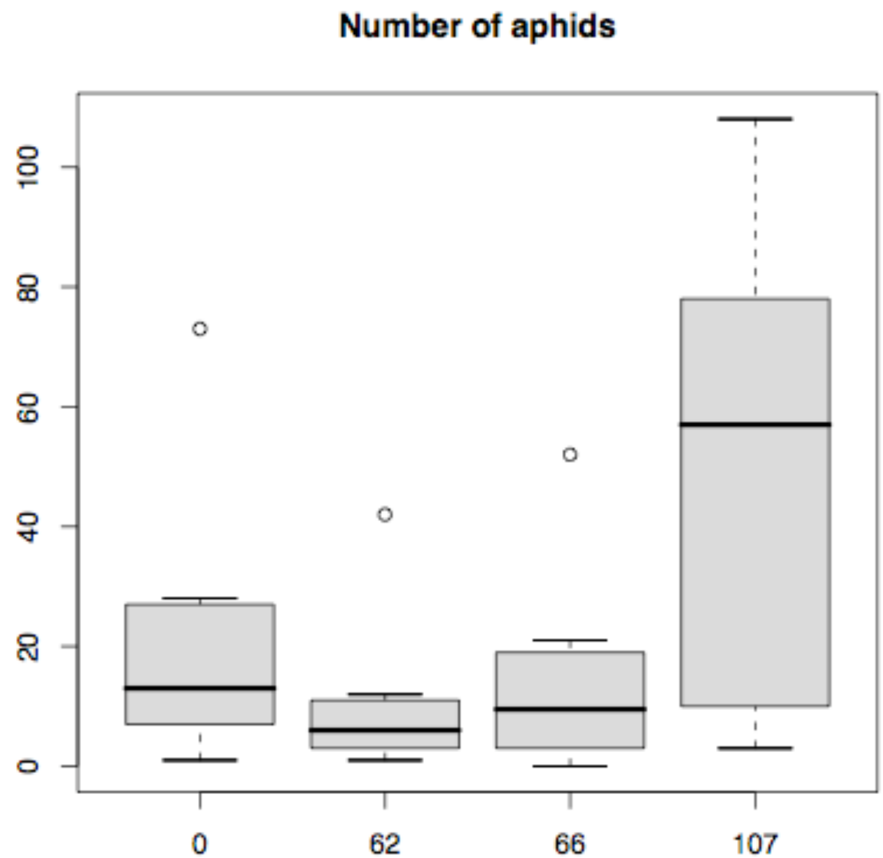


Insecticide effect



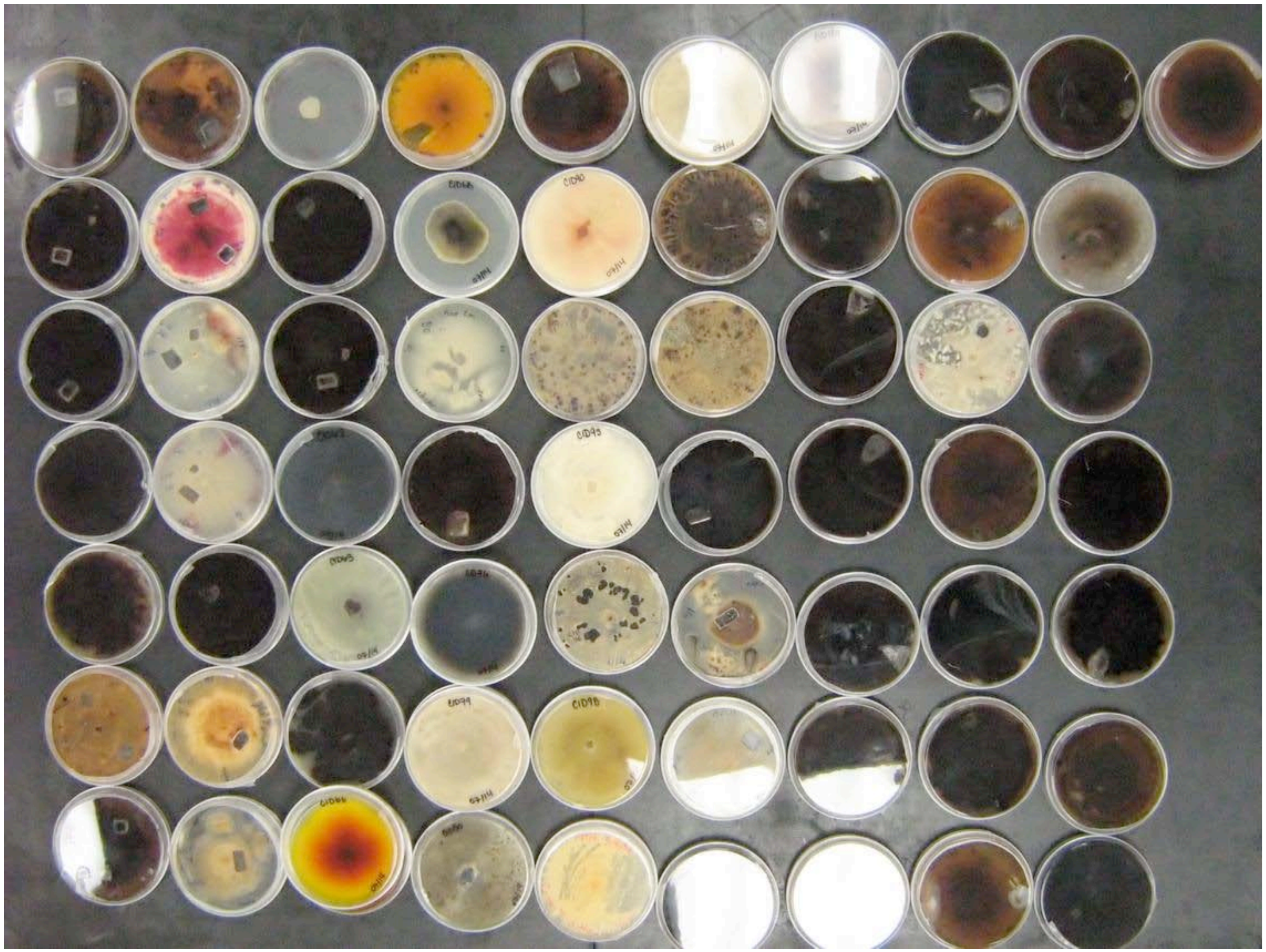
In a choice experiment, biocontrol weevils *Larinus minutus* demonstrated strong preference to non-inoculated flowers

Attraction of aphids



Some endophytes can attract other knapweed-eaters -- aphids

Did fungi come with their hosts?



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

Knapweed can bring “mal secco” disease?

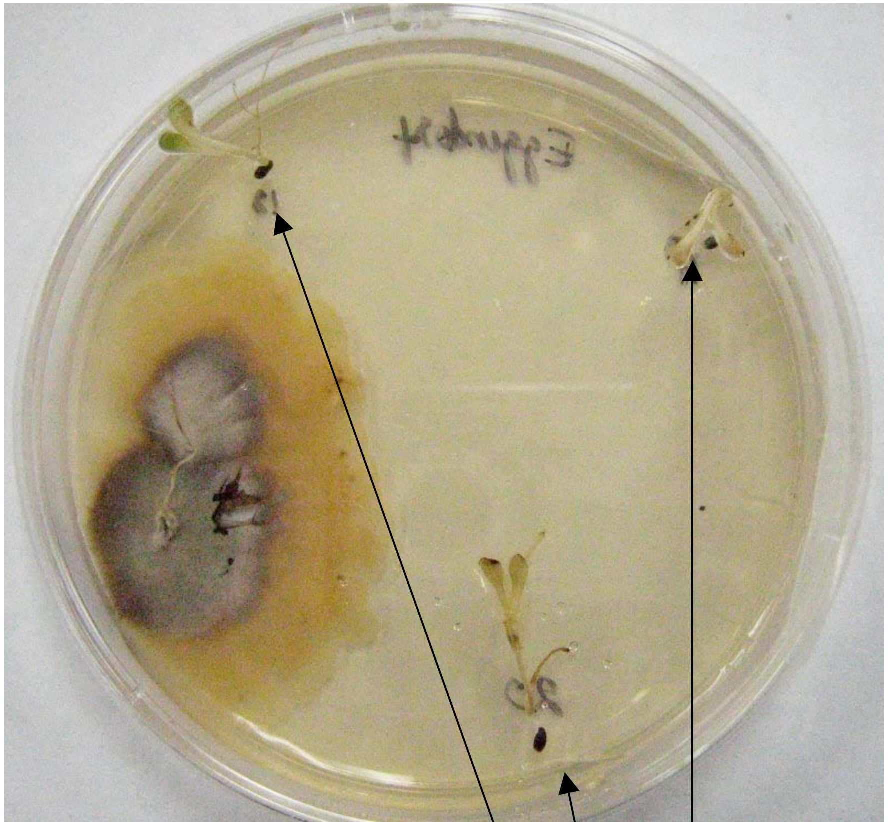
This endophyte (CID250, from Germany) have 99% identity with GenBank sequences of *Phoma tracheiphila*, very dangerous pathogen of *Citrus* trees

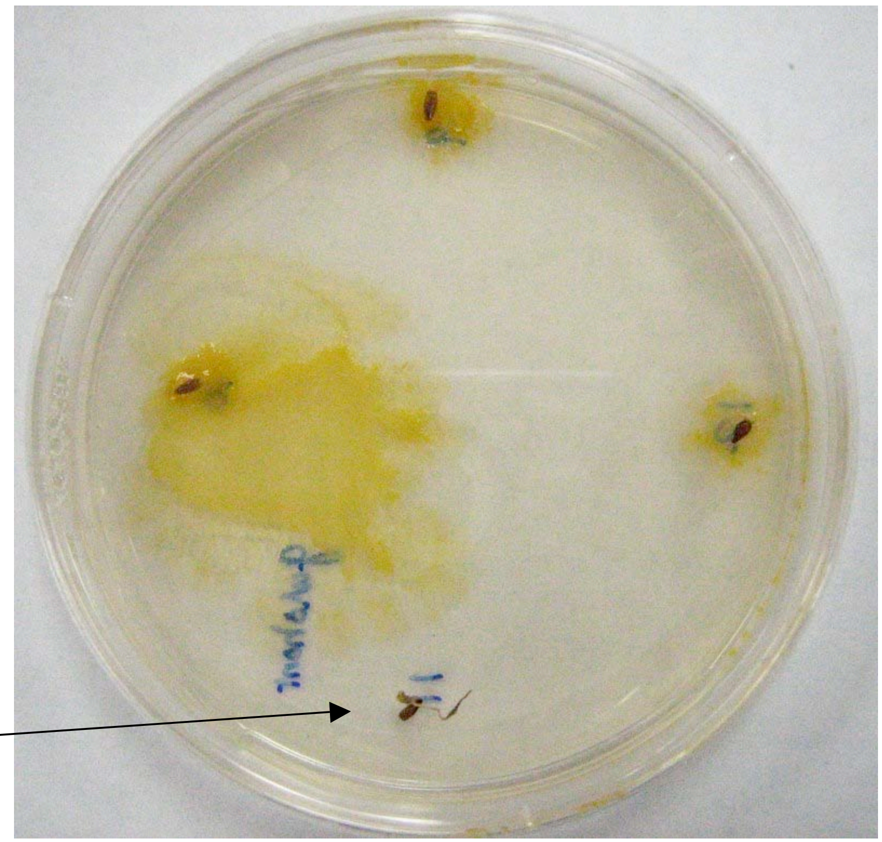
TWIG OF LEMON INFECTED BY *PHOMA TRACHEIPHILA*, THE CAUSAL AGENT OF MAL SECCO.

SUDDEN DIEBACK CAUSED BY MAL SECCO.

Isolation

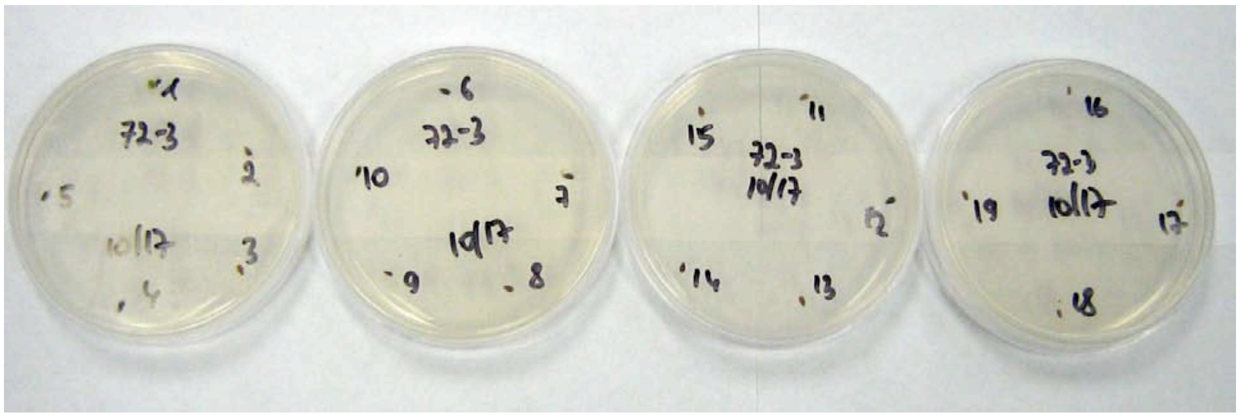
Endophytes are usually isolated from the achenes of knapweed





endophyte-free achenes

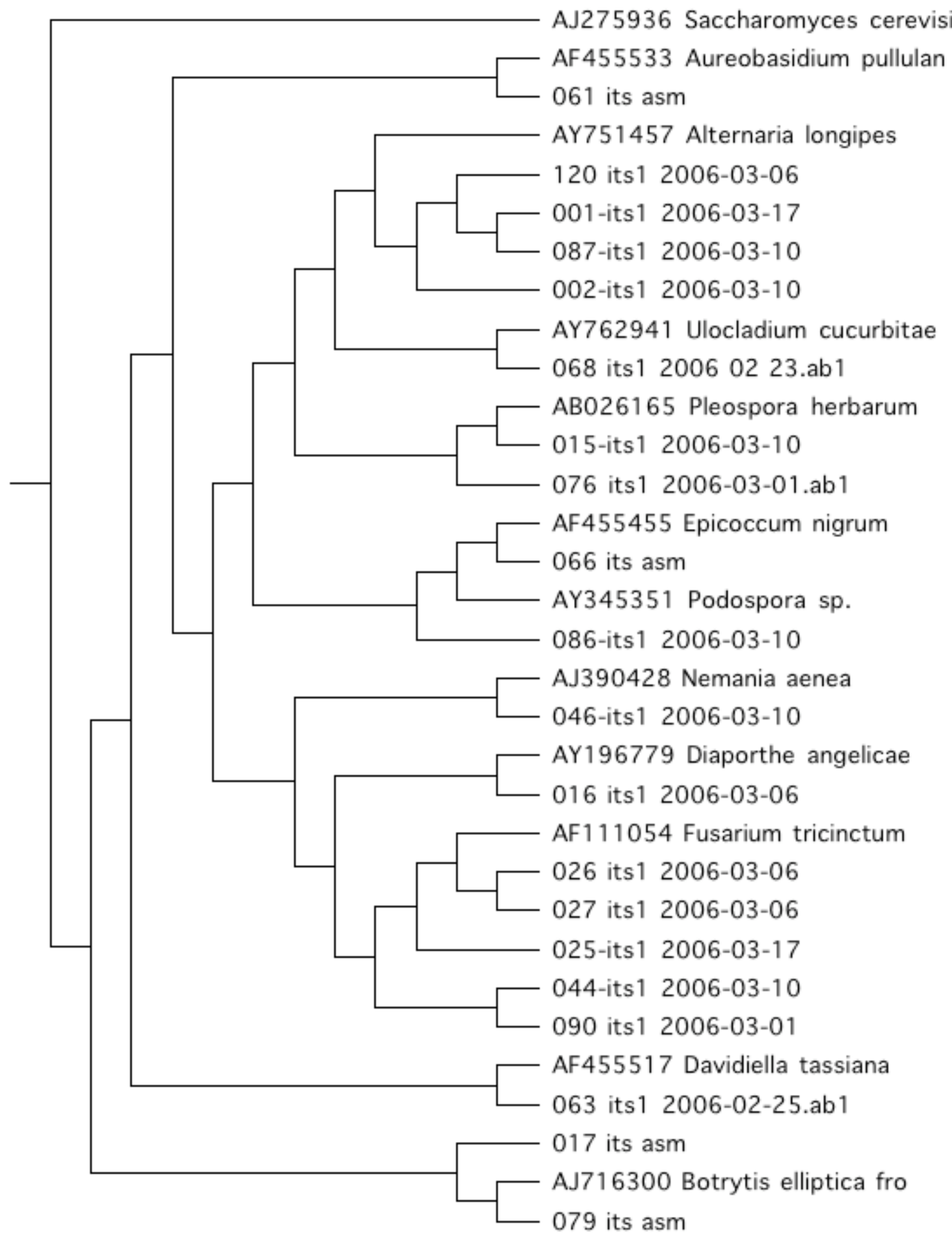
Isolation frequency varies from 0% to ~100%



Samples from Kamiah, ID

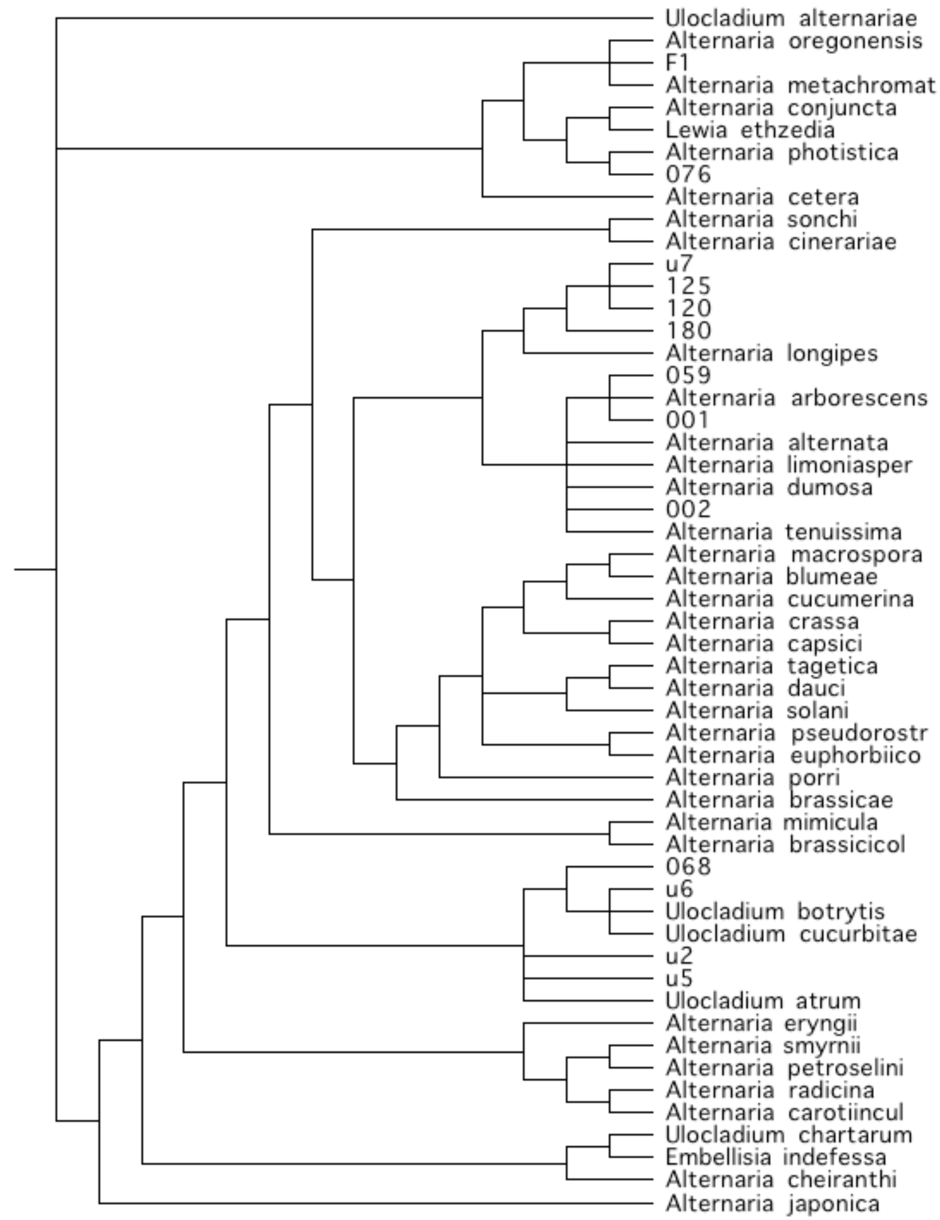
Samples from Grishneim (Germany)

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.

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

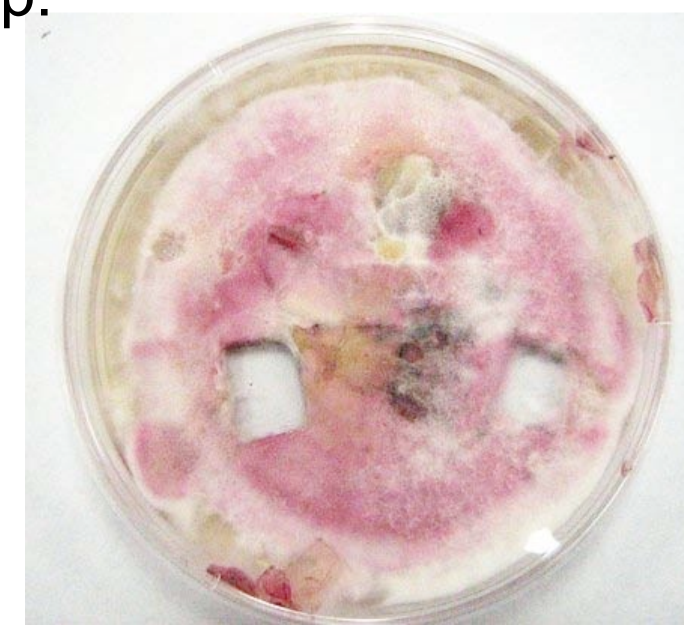


Majority rule consensus tree from MP analysis of “Alt a 1” gene sequences

Most frequent endophytes

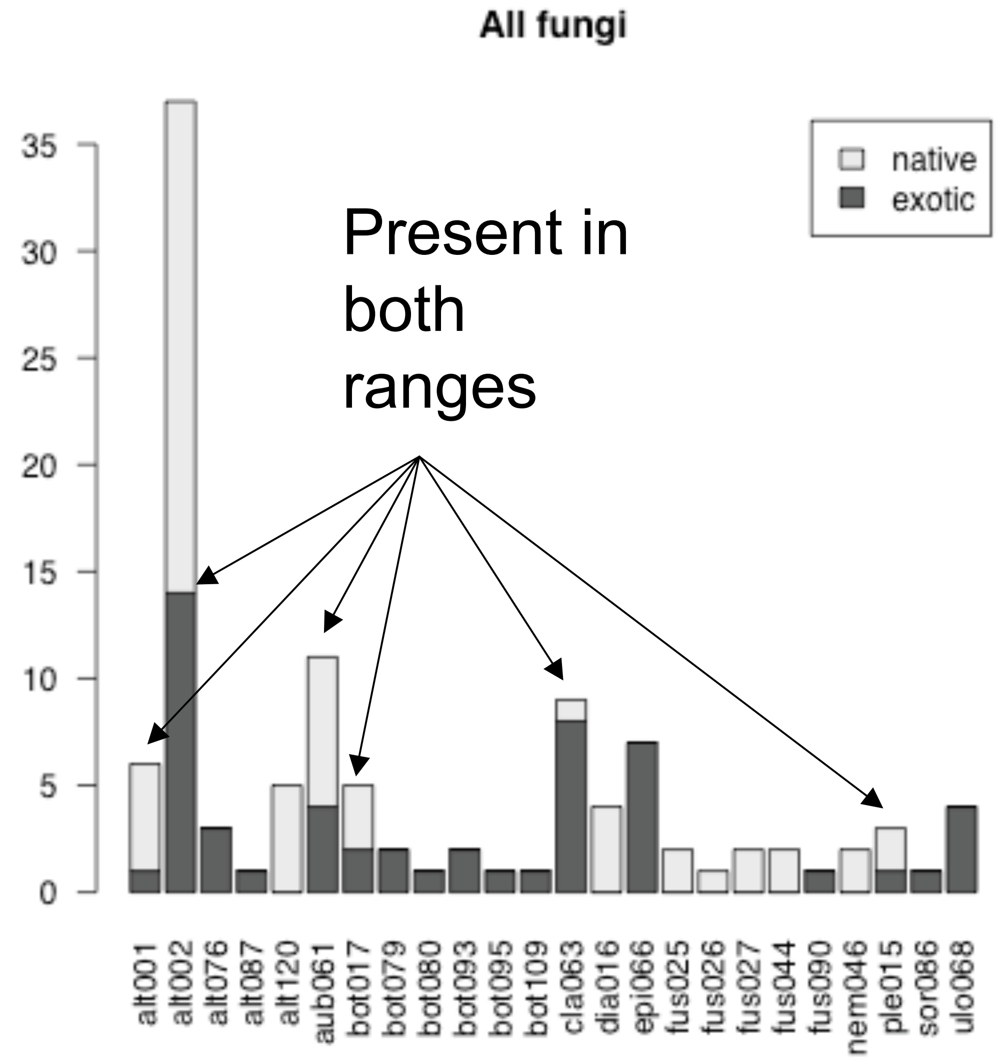
Botrytis spp.

Fusarium spp.

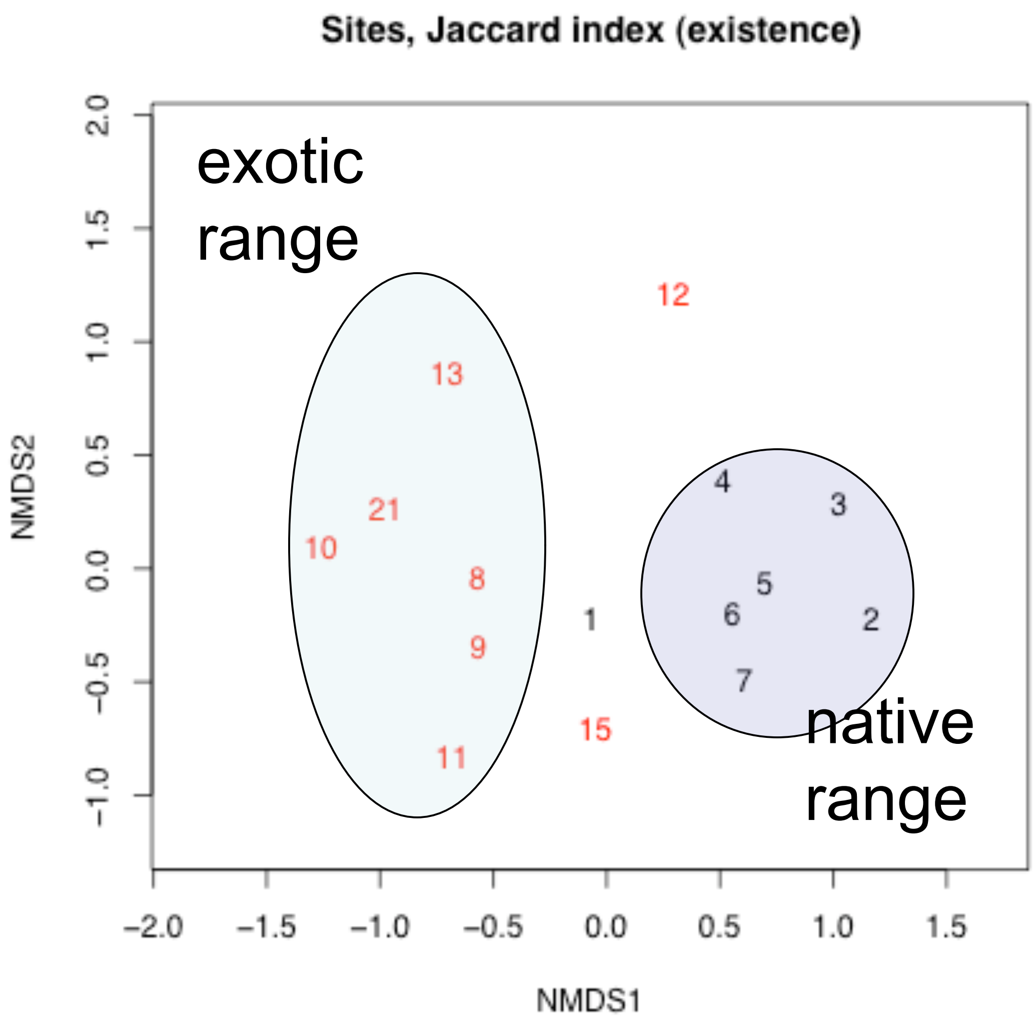


Alternaria spp.

Distribution among native and exotic ranges



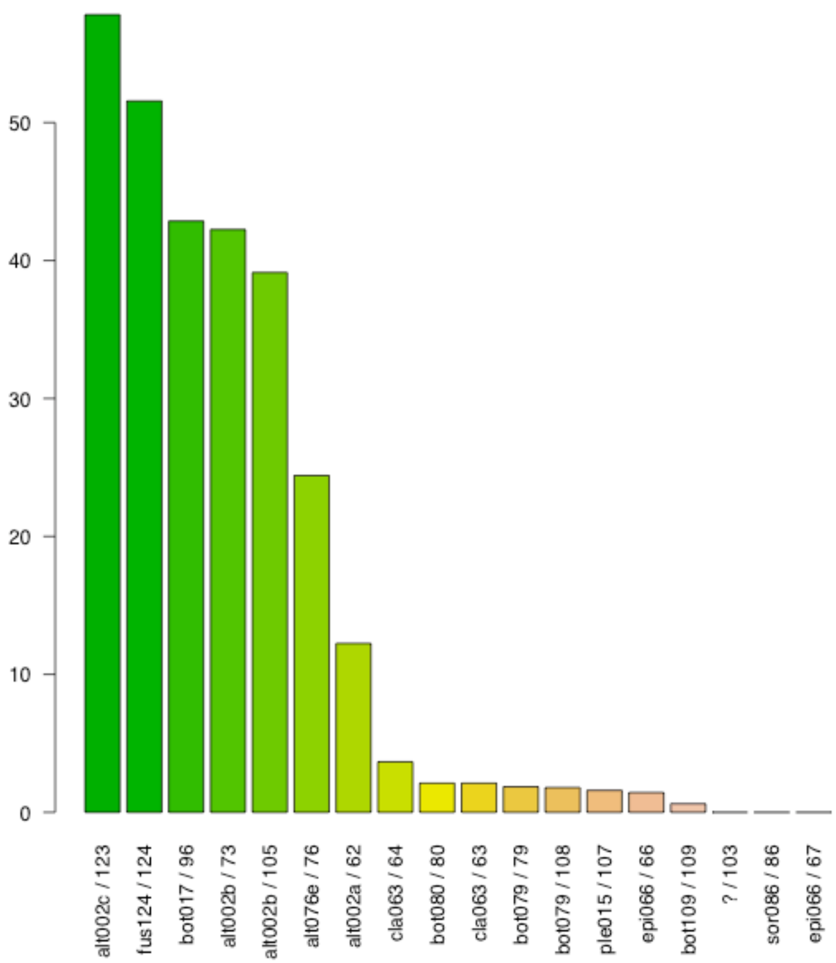
Are endophyte communities different?



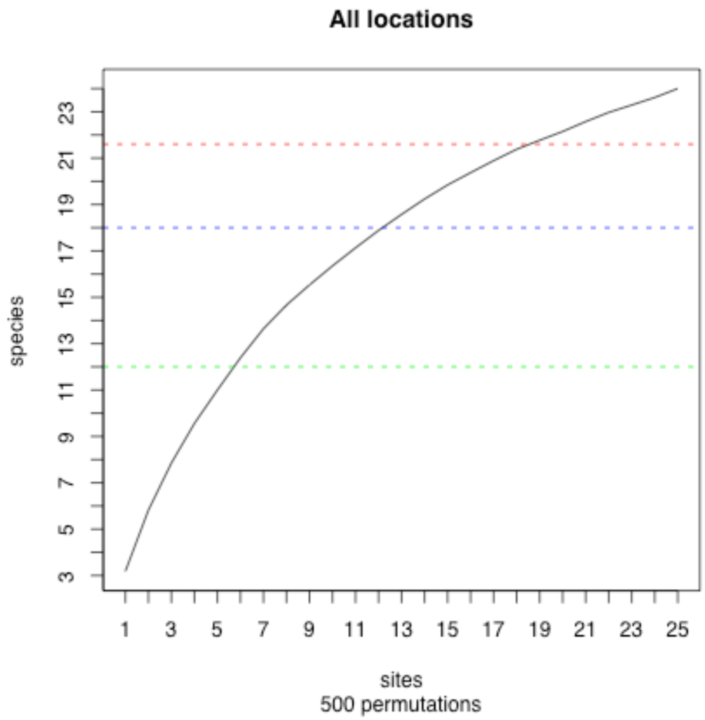
Endophyte-free plants

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

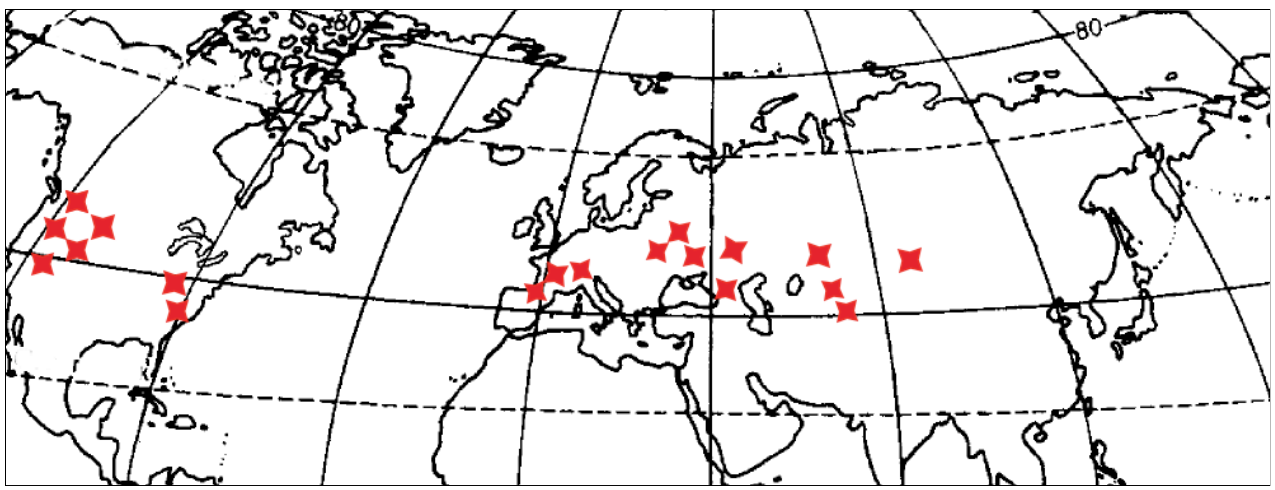


Then re-isolation were done. From all plants, we obtained only endophytes which were used for inoculation. *Alternaria* species have the best re-isolation frequency. **No endophytes were isolated from the control.** Thus, we have found the way to produce endophyte-free plans.



Sampling-2006

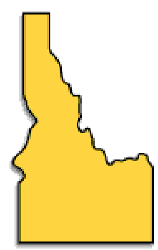
Accumulation curve for 2004/2005 (most of samples were collected in Idaho state or in southwestern Europe)



This year we have much wider sampling

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