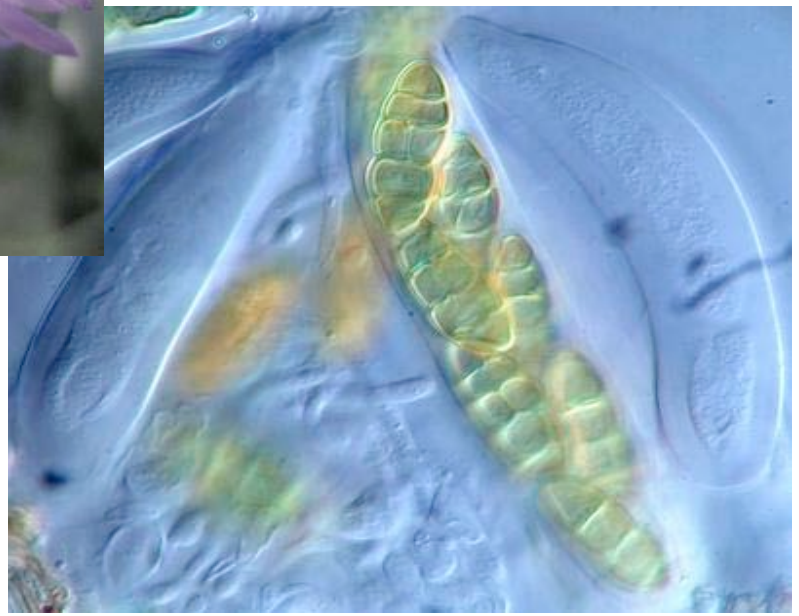
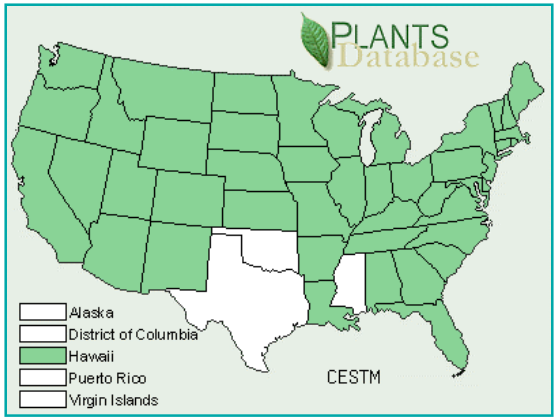
Endophytic fungi in the invasive spotted knapweed (*Centaurea stoebe* L.)

Alexey Shipunov,
Anil Kumar Raghavendra,
George Newcombe



Department of Forest Resources, University of Idaho

Spotted knapweed

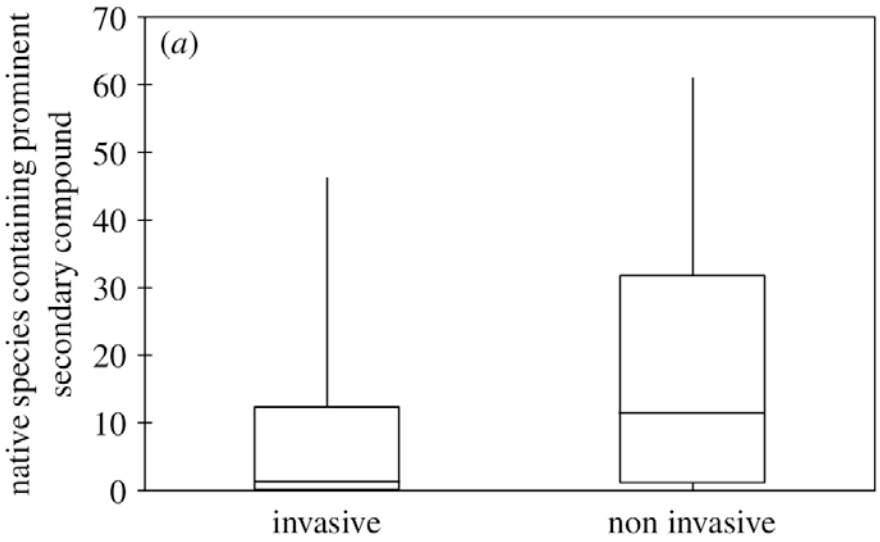


Spotted knapweed (*Centaurea stoebe* L.) is a noxious, invasive plant which was introduced into North America from Eurasia. First reported in North America in 1893, knapweed now infests millions hectares of rangelands and pastures.

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

“Novel weapons”

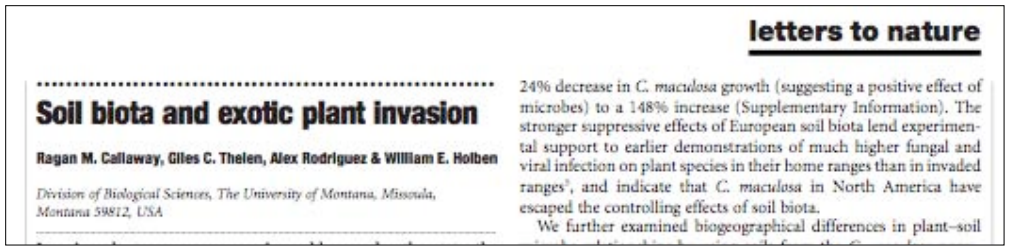
Many invasive North American plants have been reported to have antimicrobial, antiherbivore and allelopathic effects, which are most probably the consequences of unique (for American flora) secondary chemical compounds.



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

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



letters to nature

Soil biota and exotic plant invasion

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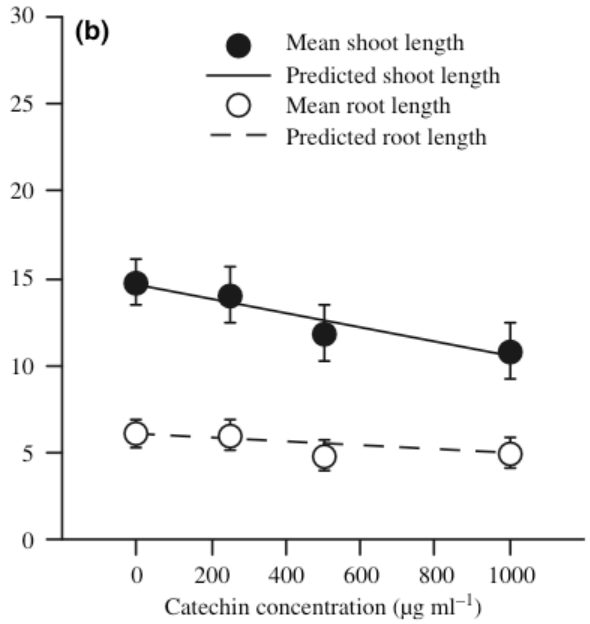
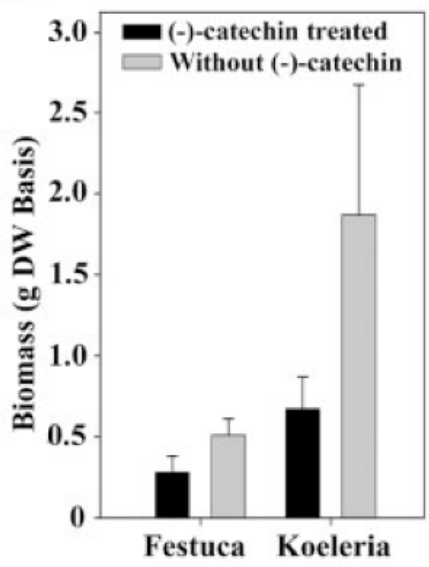
24% decrease in *C. maculosa* growth (suggesting a positive effect of microbes) to a 148% increase (Supplementary Information). The stronger suppressive effects of European soil biota lend experimental support to earlier demonstrations of much higher fungal and viral infection on plant species in their home ranges than in invaded ranges[3], 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.

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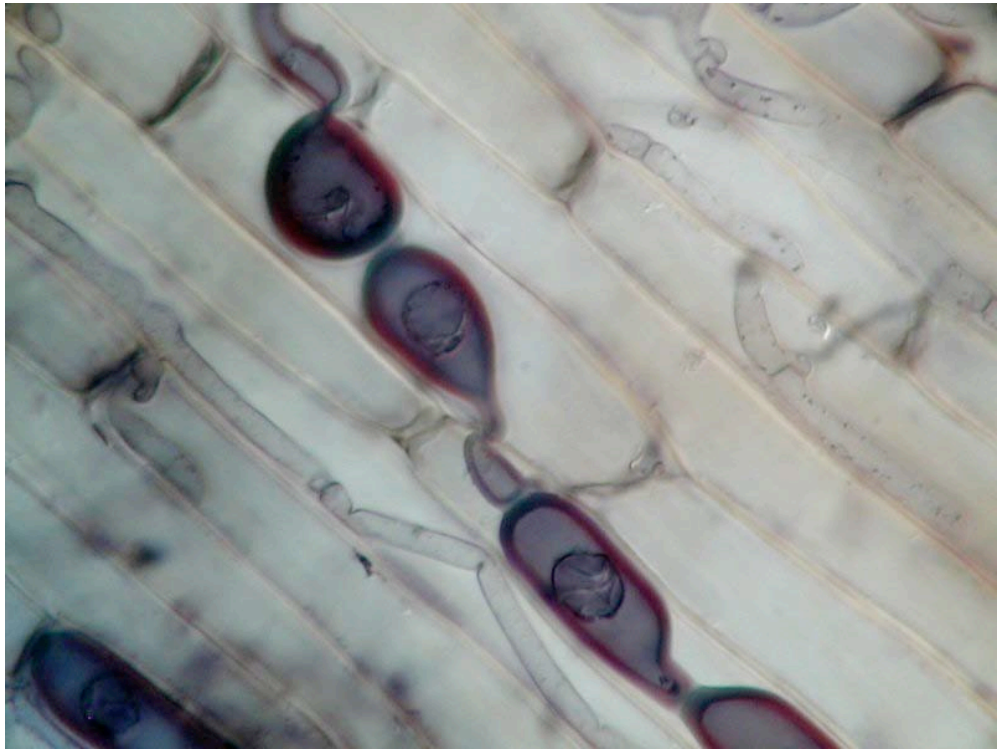
RAPID COMMUNICATION

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

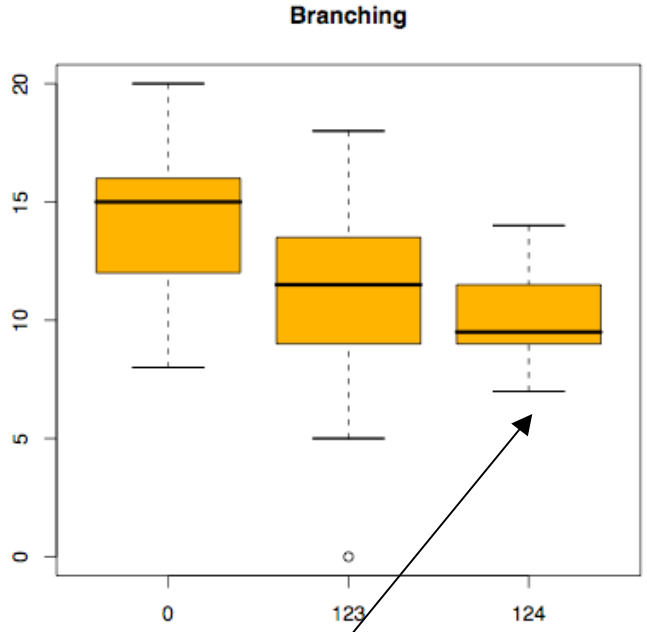
Fungal endophytes

- 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



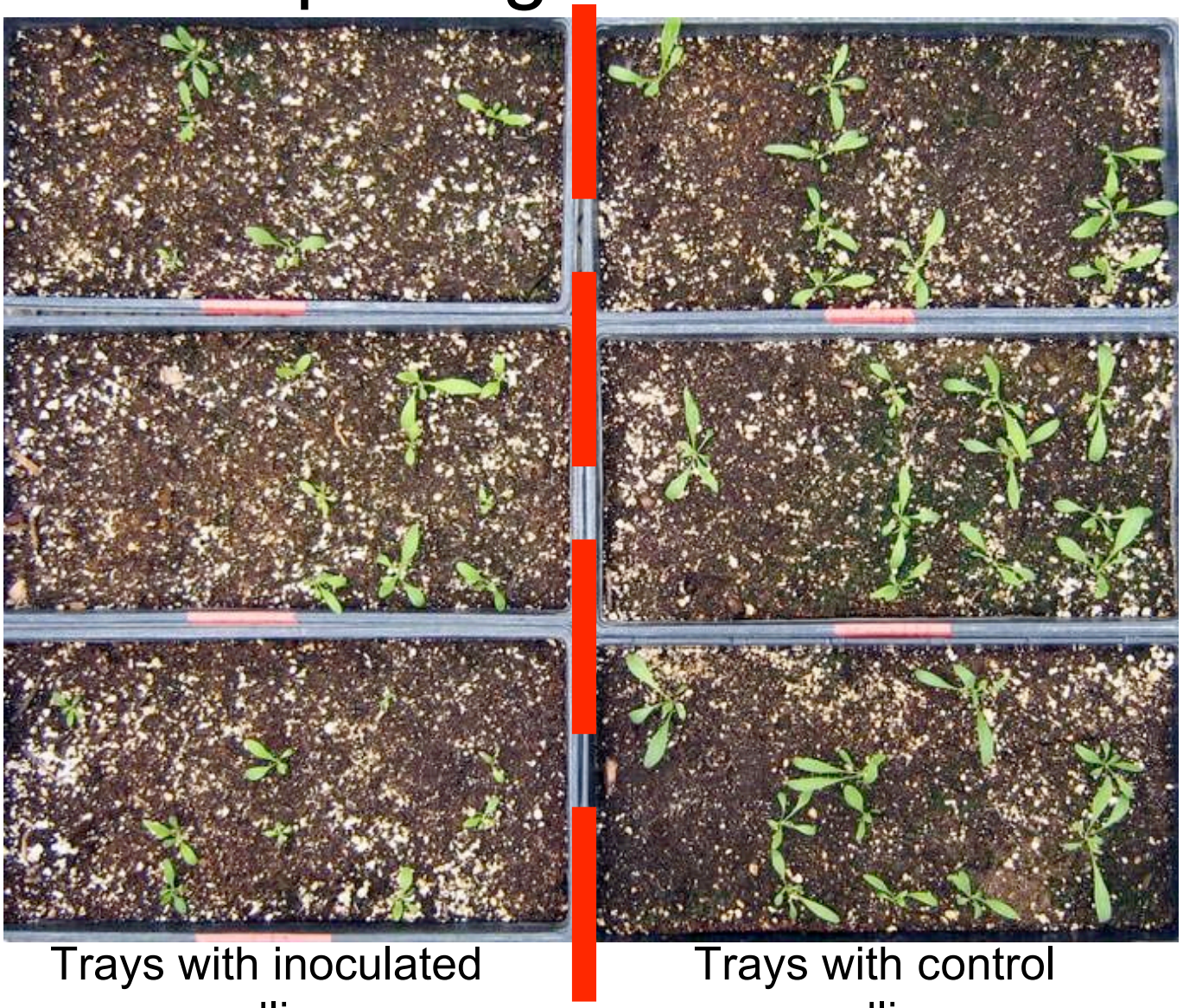
We are hypothesizing that fungal endophytes are influencing plant competition with knapweed

Negative effects



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

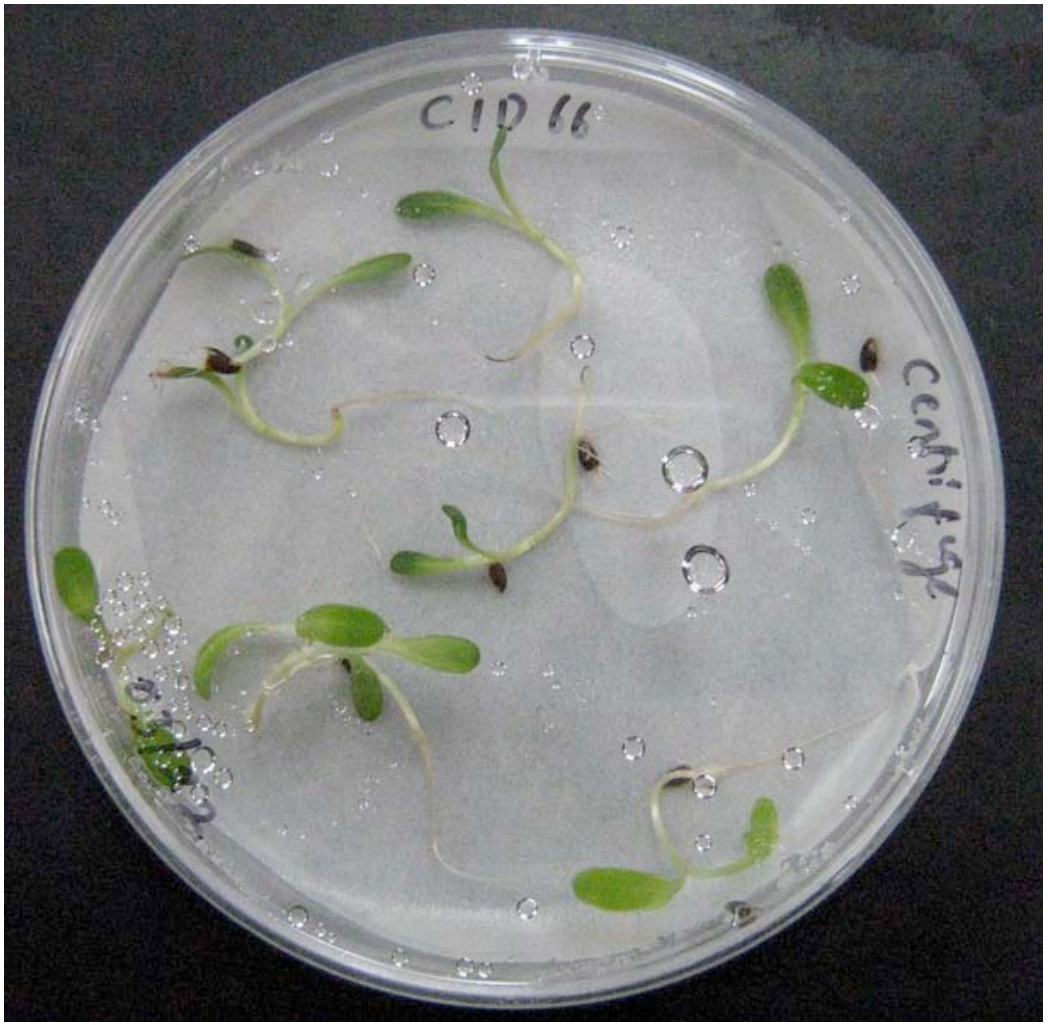
Some endophytes have even pathogenic effect



Trays with inoculated seedlings

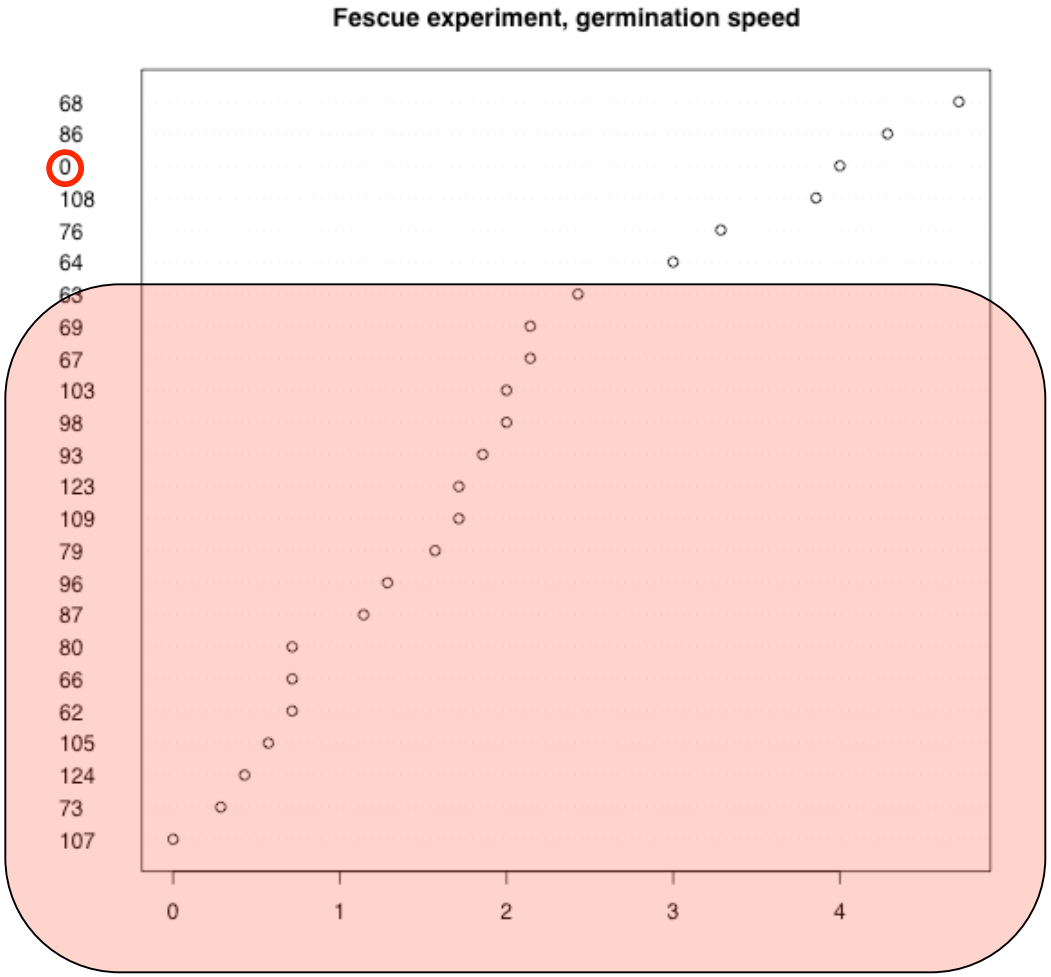
Trays with control seedlings

Endophytes can benefit knapweed seedlings



Experiments with knapweed and *Festuca idahoensis* seeds

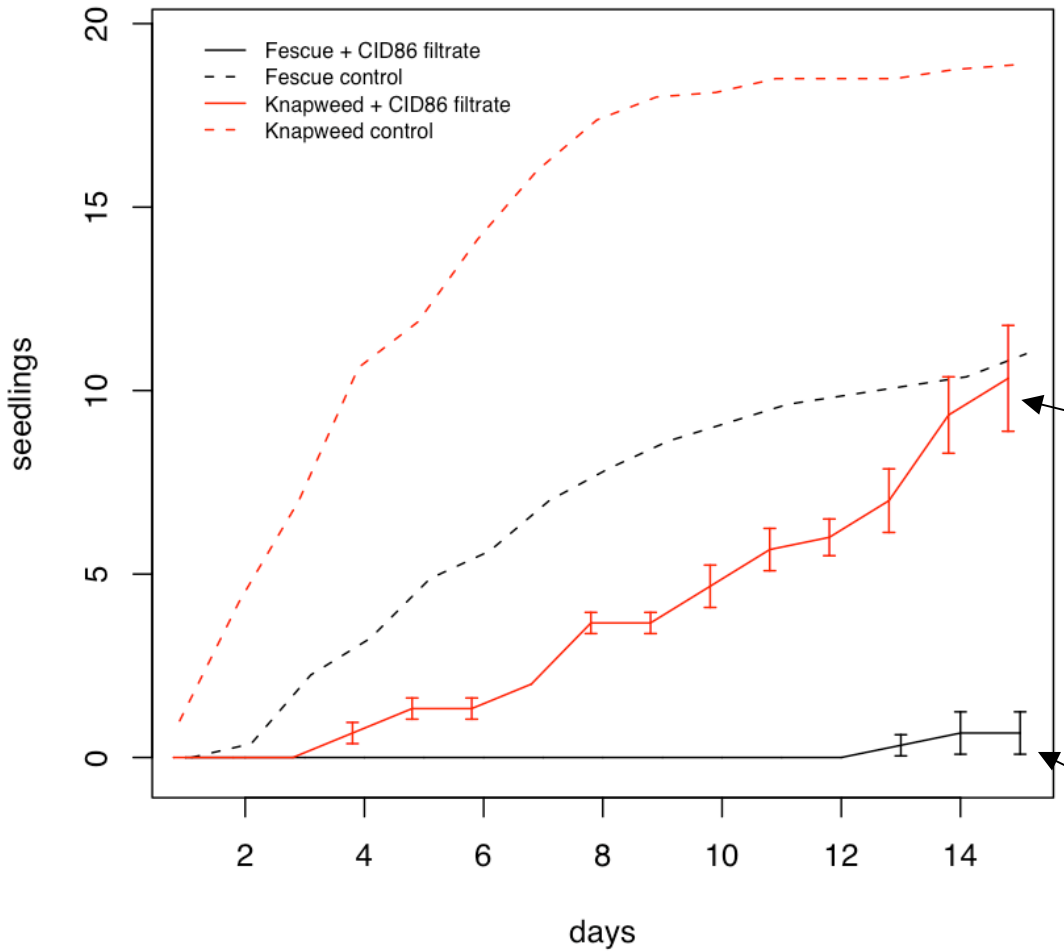
Endophytes are capable to suppress fescue seed growth

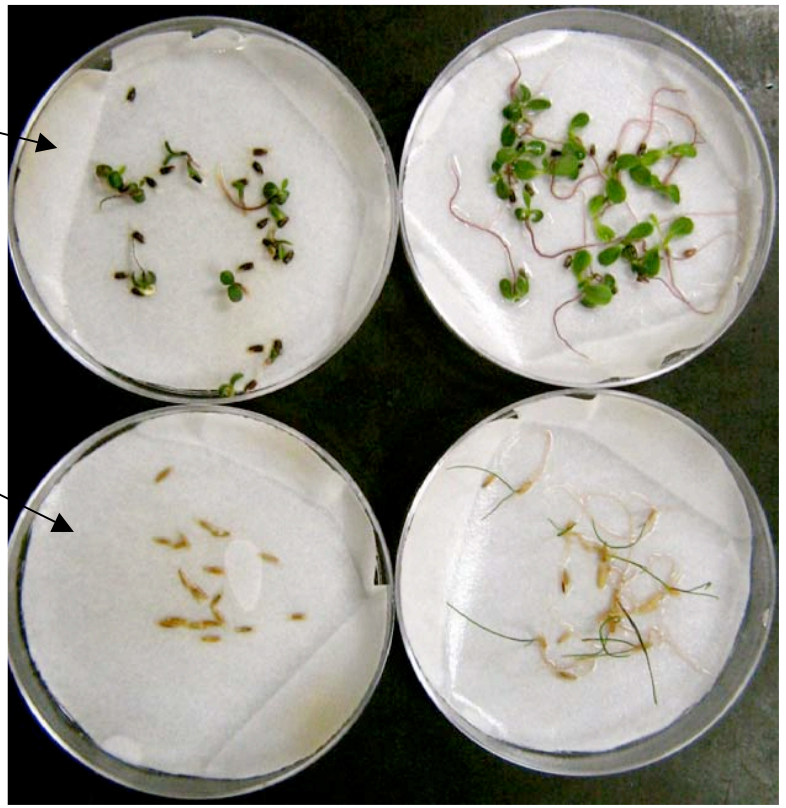


More than 2/3 endophyte strains have **statistically significant termination effect** on *Festuca idahoensis* seeds, whereas only 1/4 of them have similar effect on knapweed seeds. Moreover, some endophytes (*Fusarium* sp.) can **kill** fescue seeds.

Comparison of influence

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





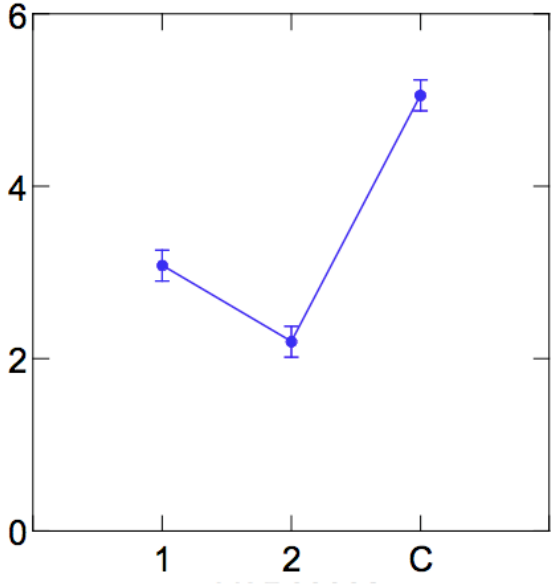
Competition experiment

E+
knapweed
and fescue

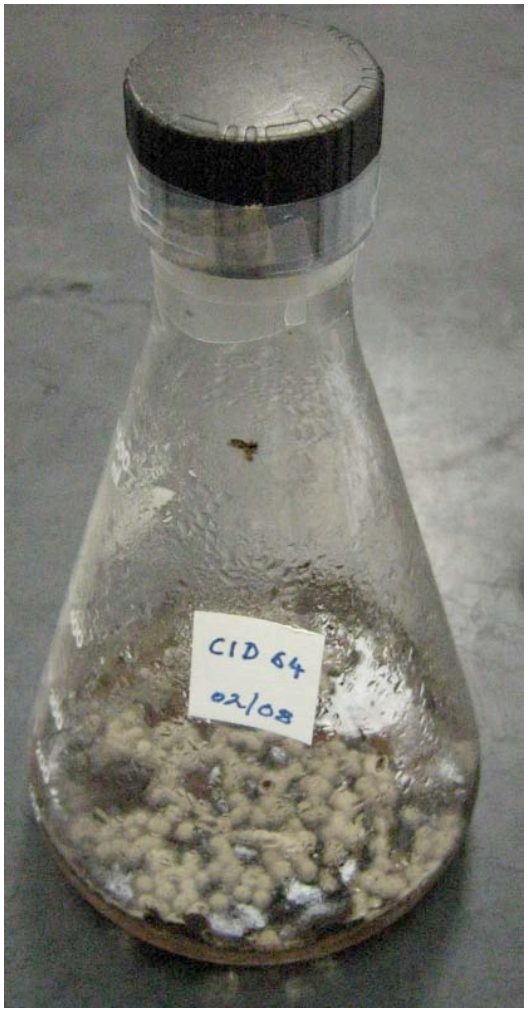
E–
knapweed
and fescue

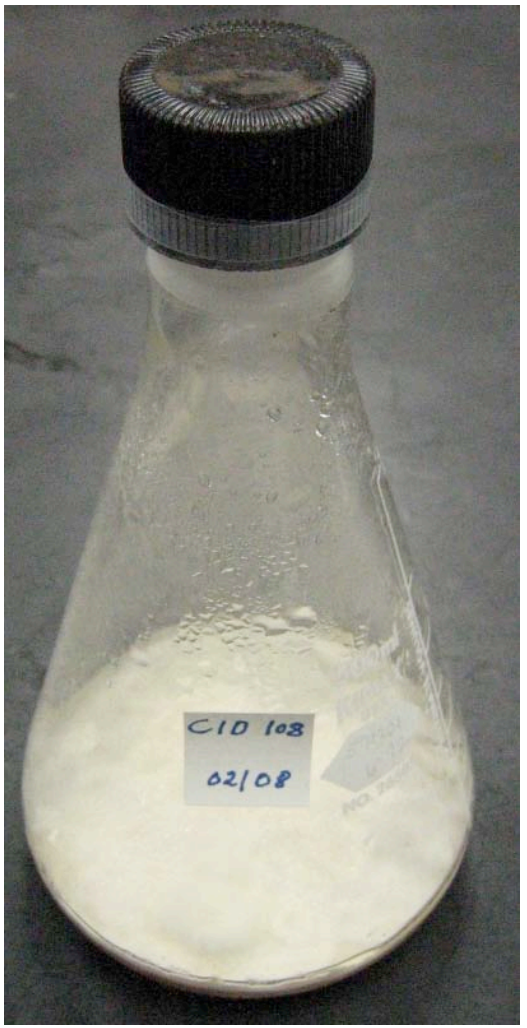
Fescue
alone:
control

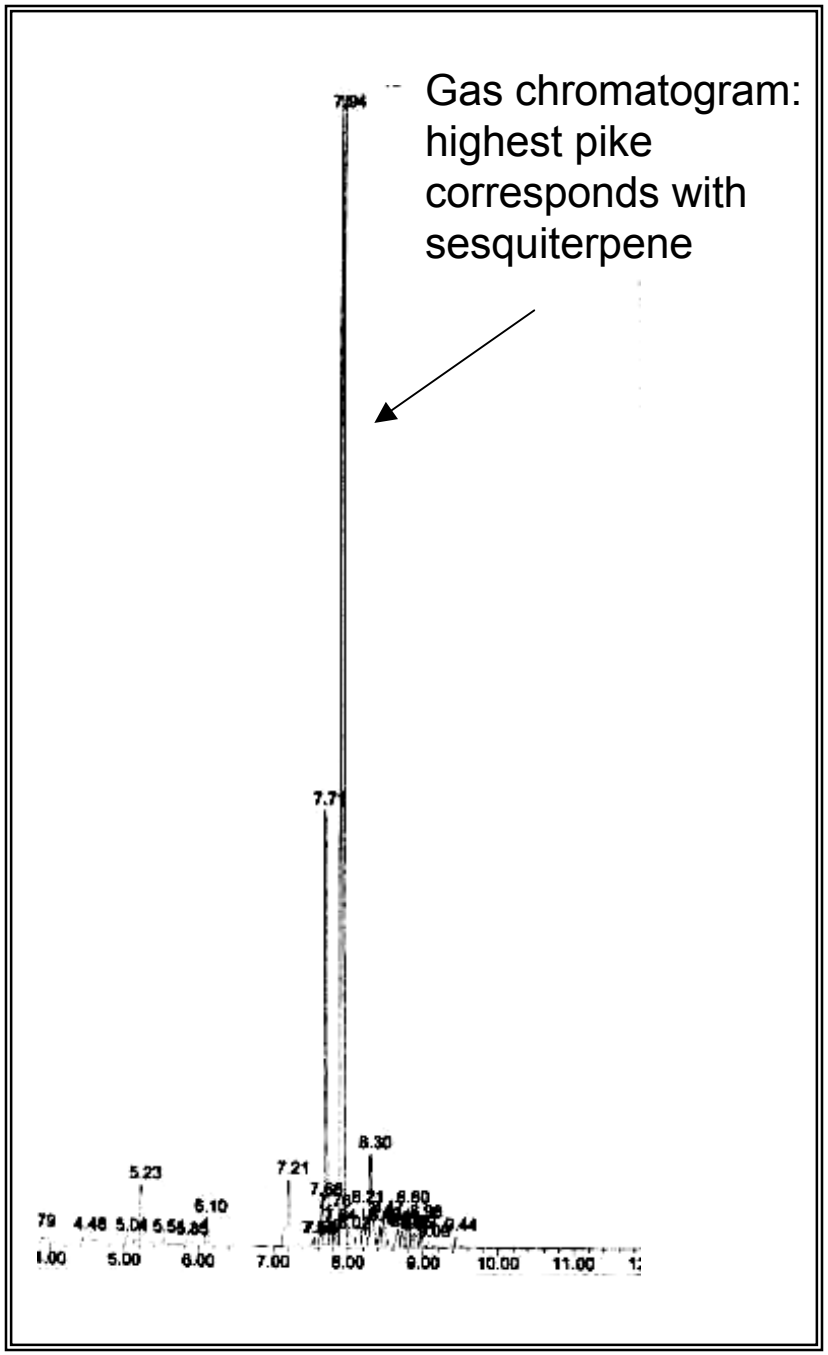
Differences in biomass are significant



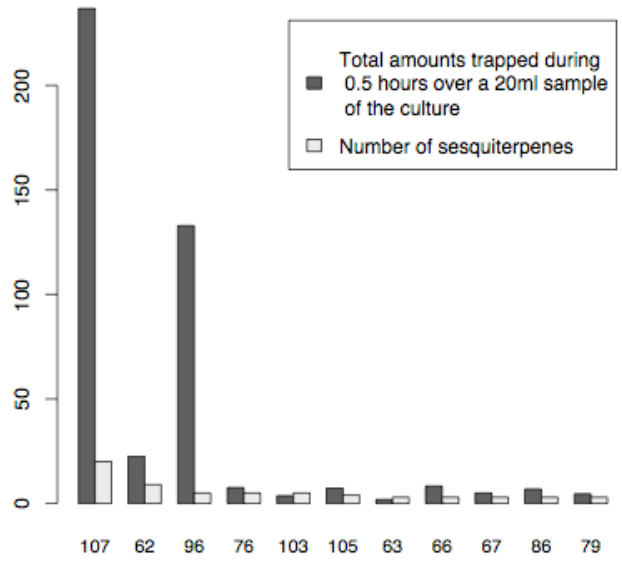
Liquid cultures and volatile compounds



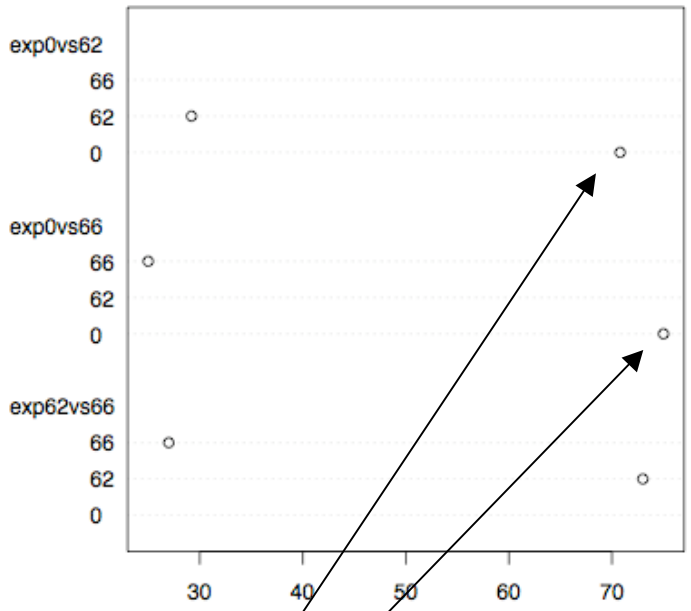




At least some endophytes can produce sesquiterpenes

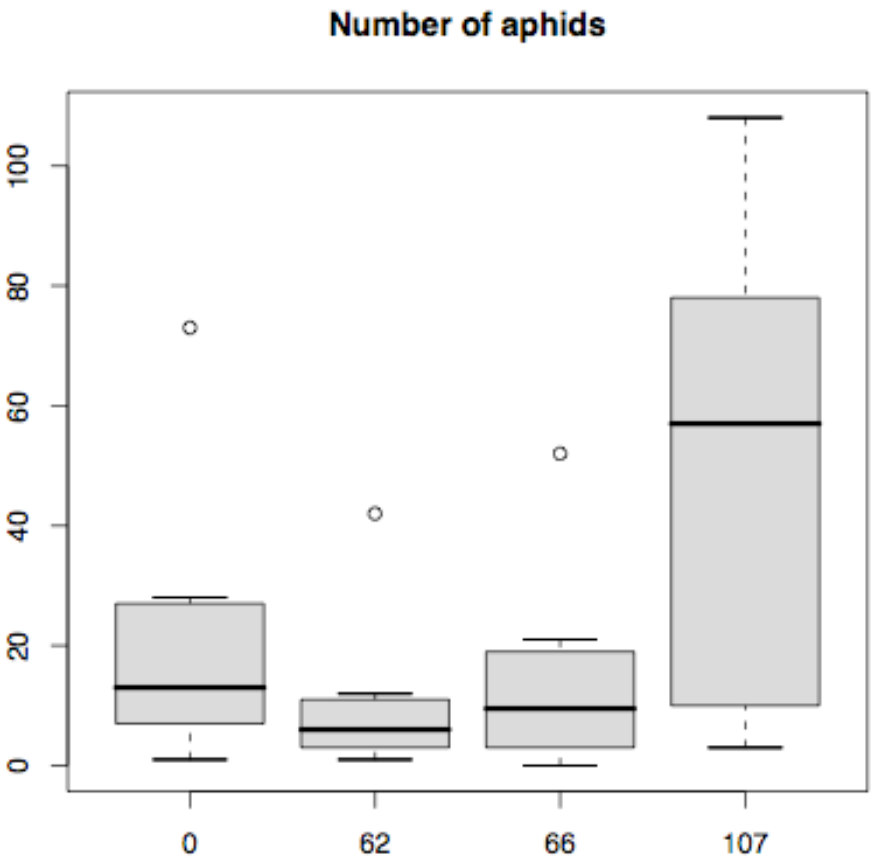


Insecticide effect



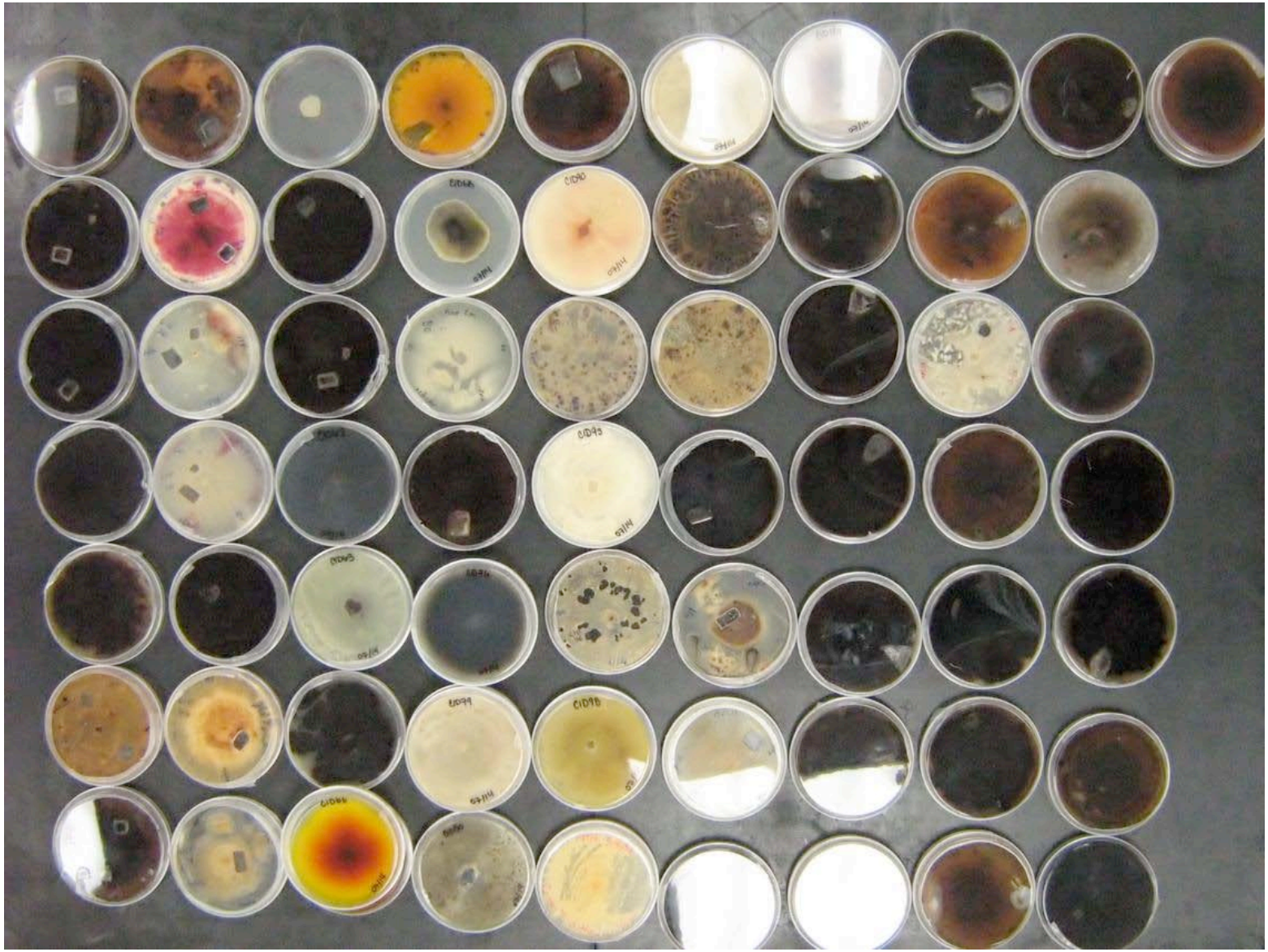
In a choice experiment, biocontrol weevils *Larinus minutus* demonstrated strong preference to non-inoculated flowers

Attraction of aphids

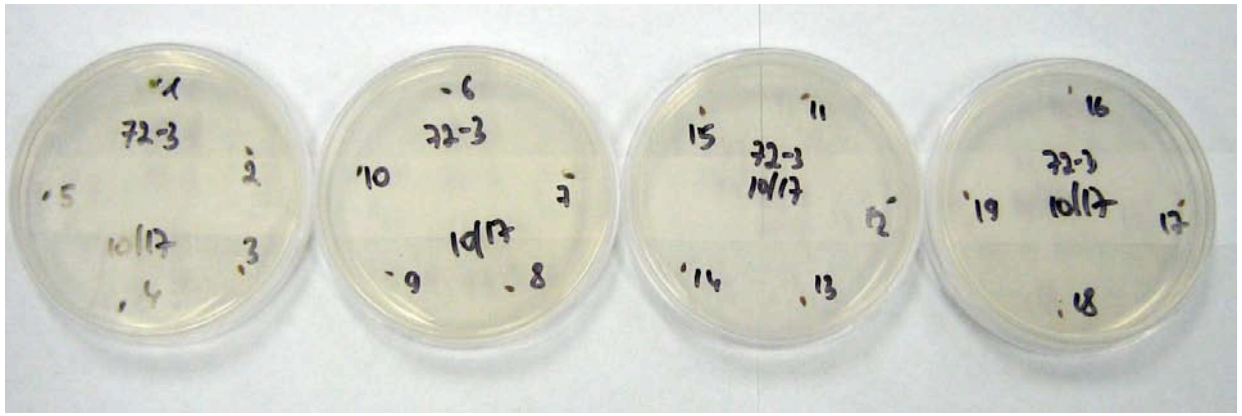


Some endophytes can attract other knapweed-eaters -- aphids

Knapweed endophyte diversity



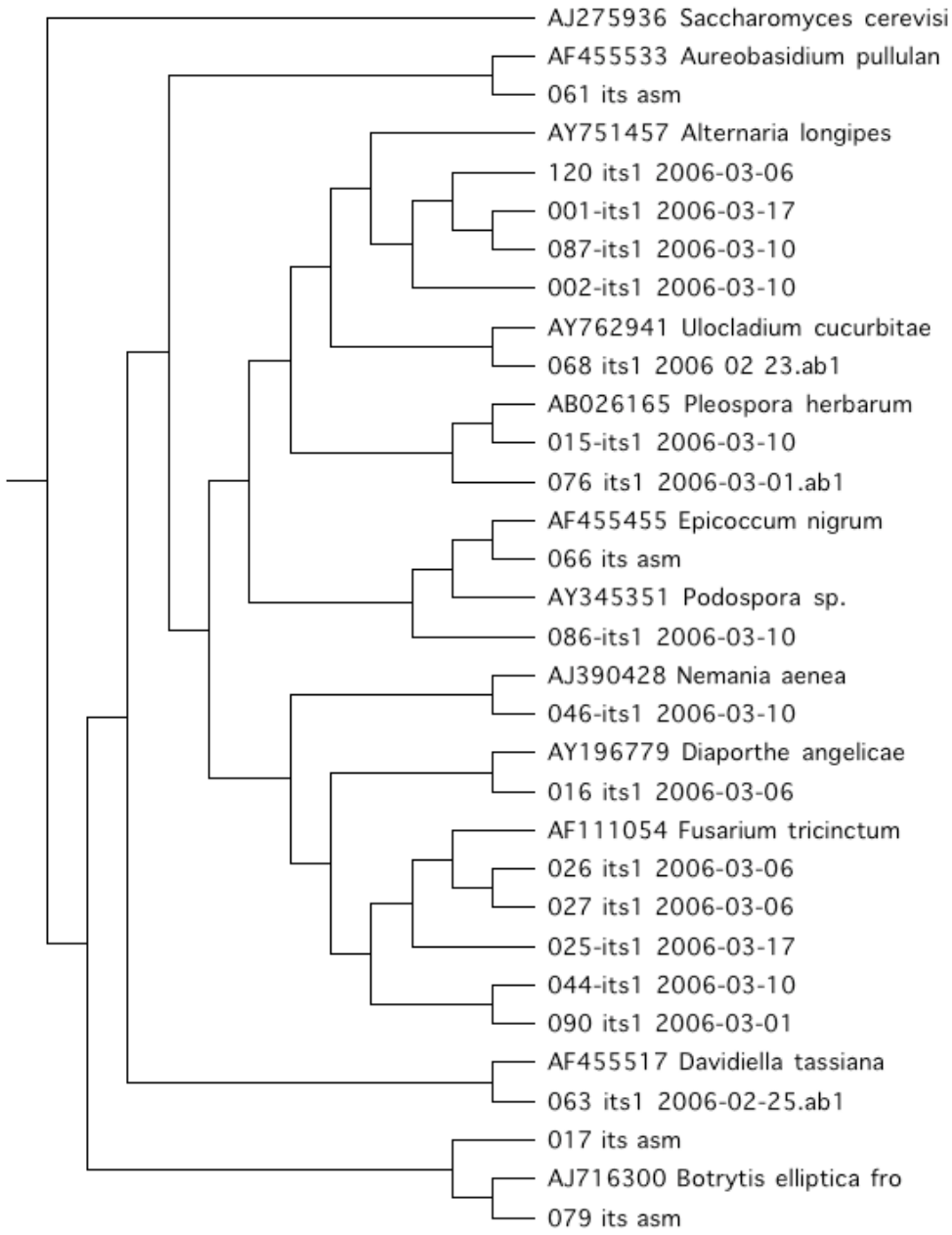
Isolation frequency varies from 0% to ~100%



Samples from Kamiah, ID

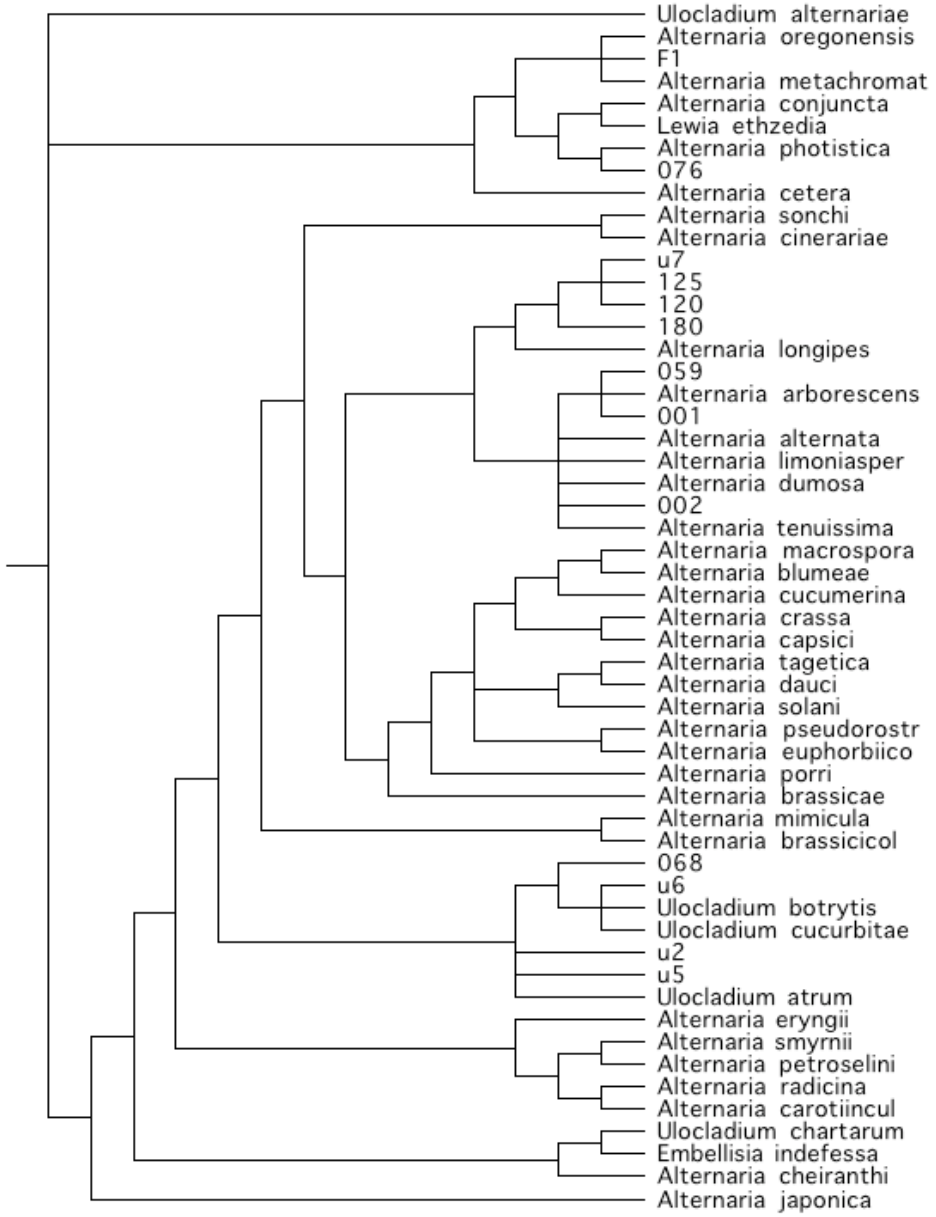
Samples from Grishneim (Germany)

Most endophytes belong to *anamorphic* fungi: it is nearly impossible to identify them without using molecular tools



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.

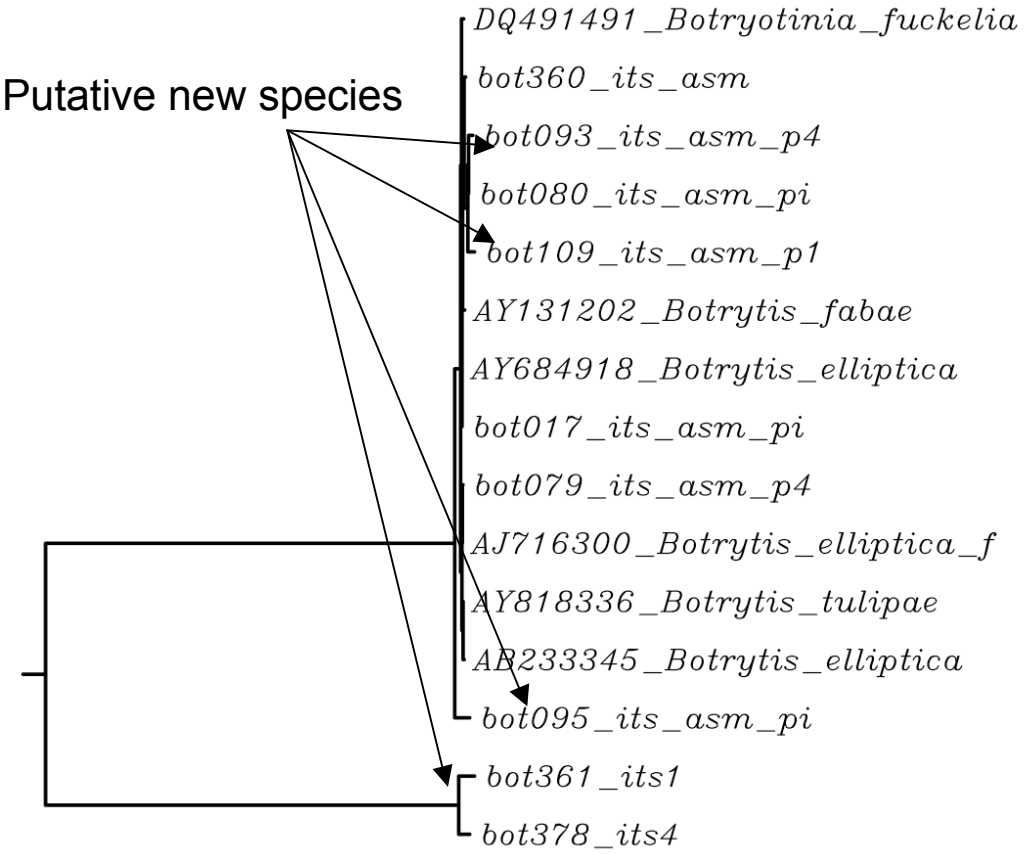
Recently (2005) founded single-copy *Alternaria* allergene gene "Alt a 1" was used to identify *Alternaria* and *Ulocladium* species



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

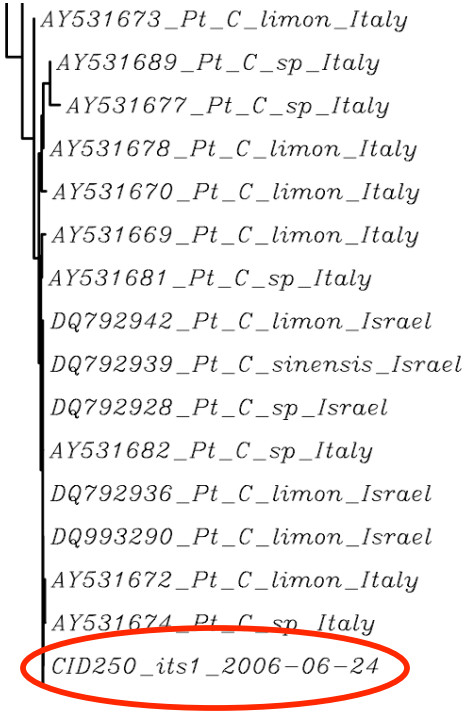
Some endophytes may represent novel lineages

Botrytis (anamorph of *Botryitinia*, Sclerotiniaceae)



“Host-jumping” vs. co-introduction

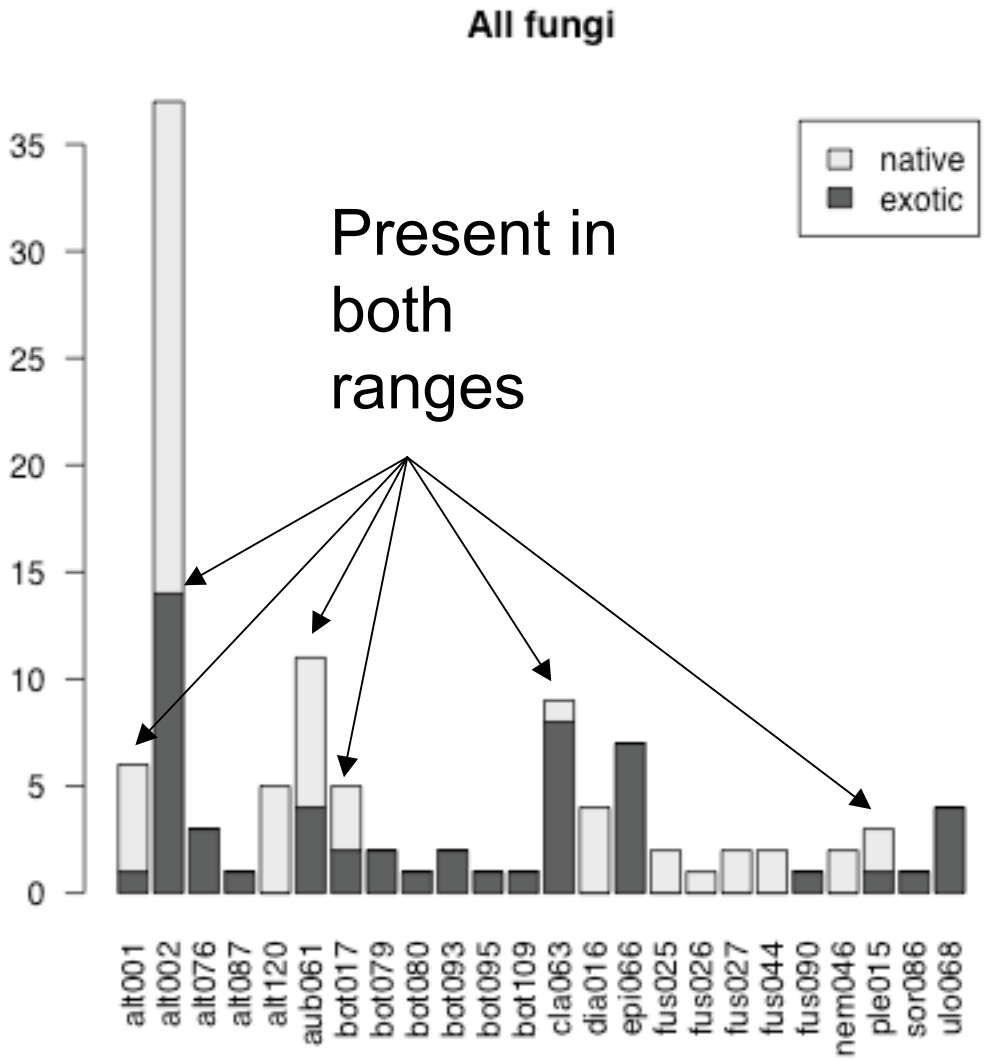
Endophytes in introduced plants like knapweeds must either have been **co-introduced** in seeds of their host, or have ‘**jumped**’ from other plants in the invaded range of their hosts



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

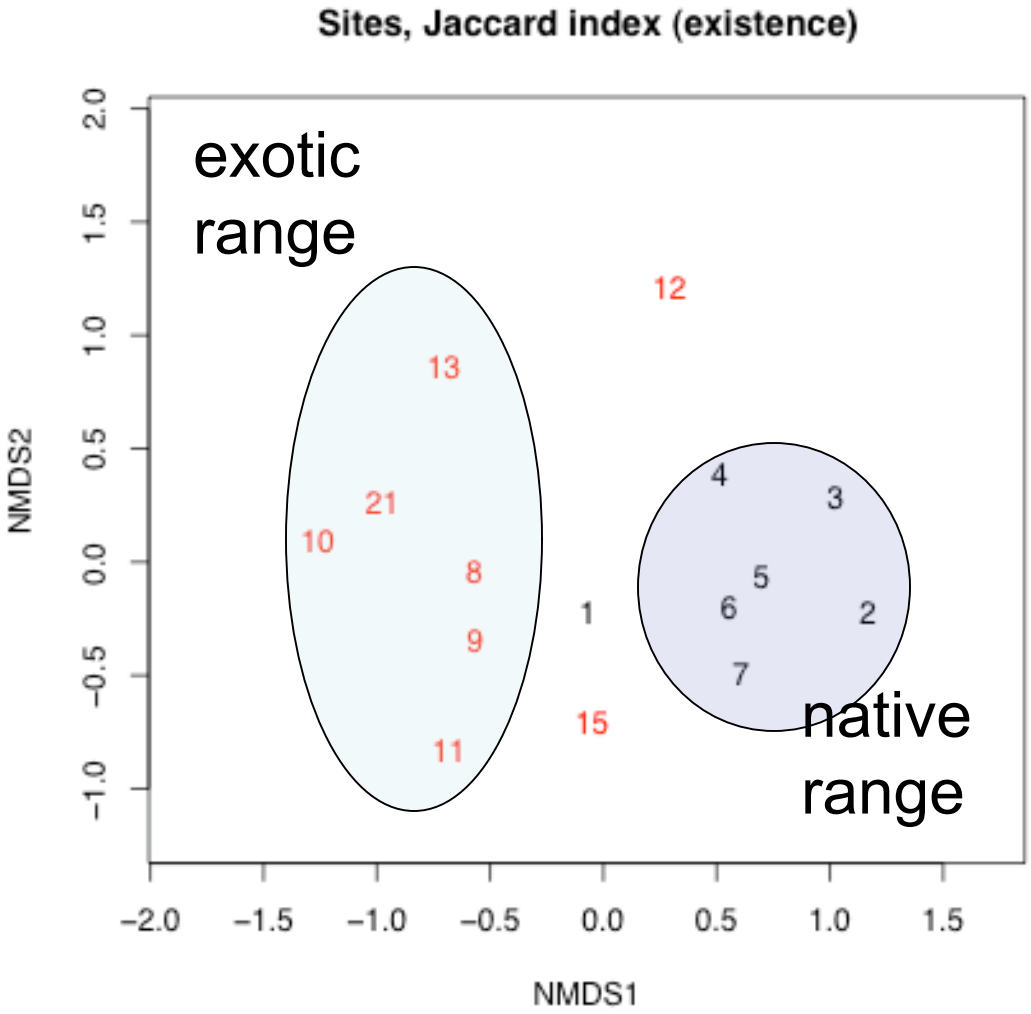
SUDDEN DIEBACK CAUSED BY MAL SECCO.

Distribution among native and exotic ranges



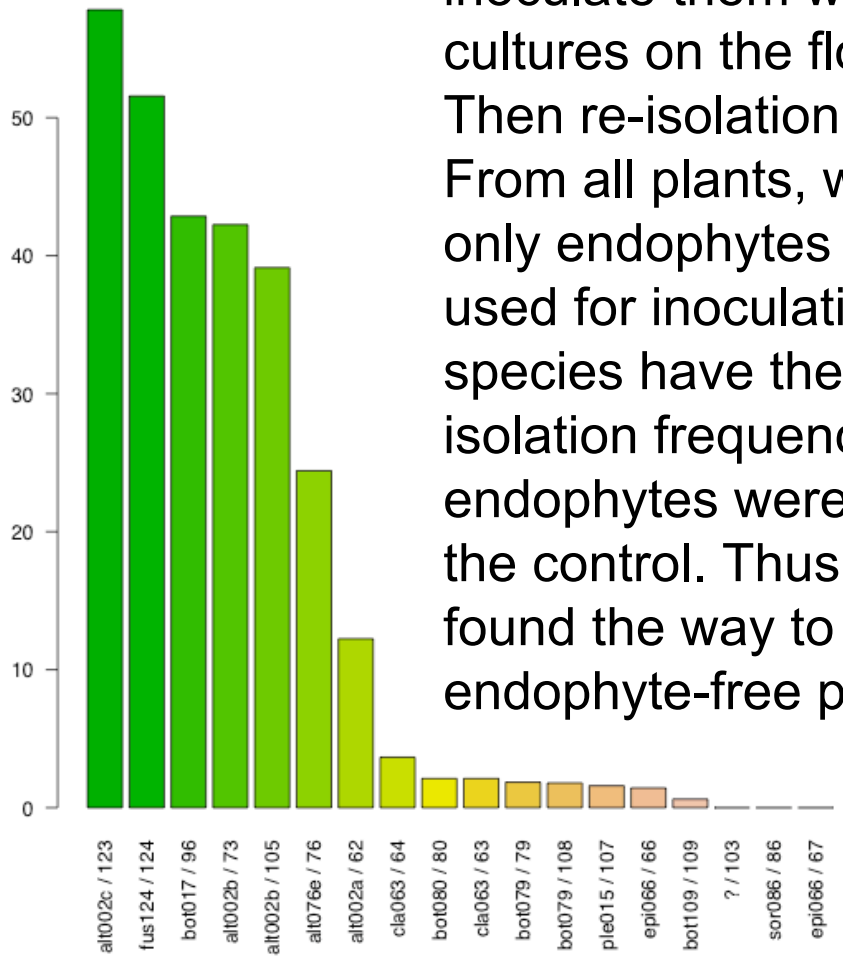
Data from 2004/2005 sampling

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.

In progress

1) New samples: from California to Canada and from Germany to Russian Urals
2) Geographical distribution of endophytes, isolation frequency, germination rates and climate data
3) Repeated samples: succession in endophyte world
4) Hidden fungi: cultivation-free methods
5) Fungal coexistence and/or hyperparasitism
6) Plant morphology and fungi: age and flowering stalk development
7) Extended experiments with aphids, with fescue and with volatiles

Acknowledgements

- Cort Anderson
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- 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