



***Ustilago bullata*, a potential
biocontrol for *Bromus* species**

**Hilbig, Bridget E.* Michael F. Allen, Matthew O'Neill, Elizabeth Holmes,
and Edith B Allen.**

**University of California-Riverside Dept. of Botany and Plant Sciences,
Riverside, CA.**

***bhilb001@ucr.edu**

Bromus diandrus, *B. rubens*, *B. tectorum*

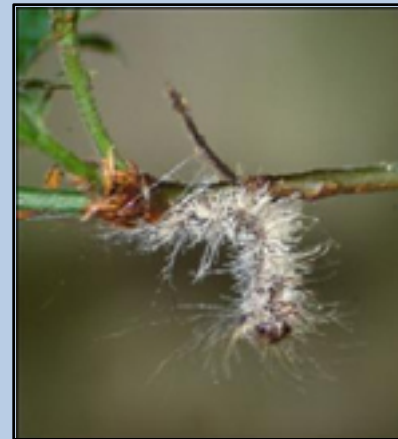
- Dominant winter annual grasses
- Dominance is associated with dramatic increases in fire
- Respond positively to disturbance by fire
- Little success in eradication of *Bromus* on large scale
- Current methods include prescribed burns, mowing or grazing, and herbicide use
 - each method having advantages and disadvantages



B. diandrus

Biological Control

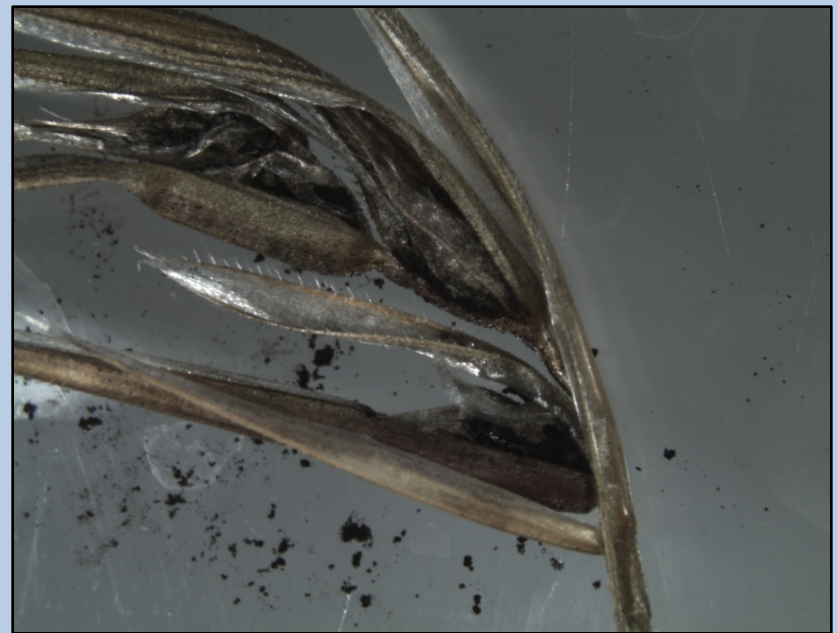
- Introduced biological agents are most effective when they exhibit:
 - Narrow host range
 - Climatic adaptability
 - Synchrony with host life cycle
 - High reproductive potential
 - Survival at low host density



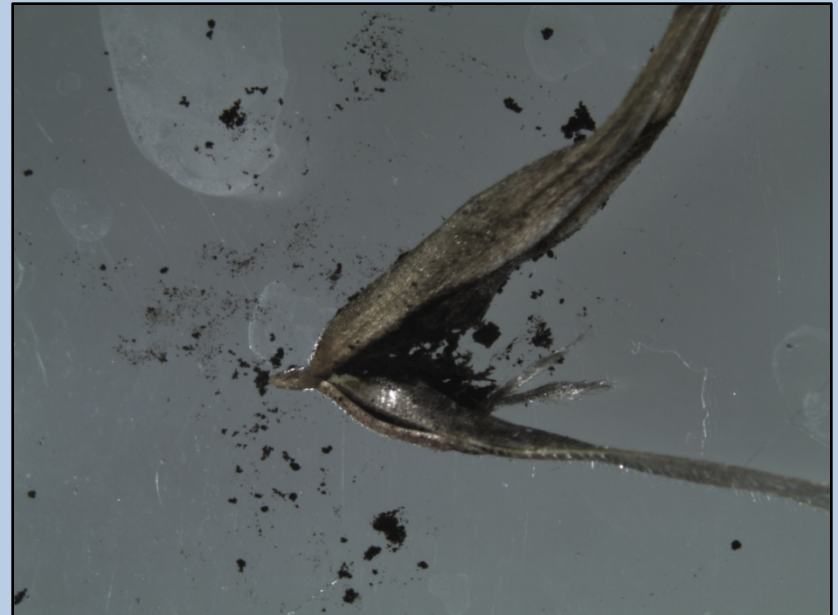
*Entomophaga
maimaiga*

Ustilago bullata

- Fungus
- Causal organism for head smut disease of grasses
- Seedling-infecting pathogen that grows systemically and then sporulates in the host inflorescence eliminating seed production
- Meyer's work investigated host-specificity of *U. bullata* and almost never found pathotypes that could infect native grasses



Ustilago bullata observed on *Bromus diandrus*



Ustilago bullata can sometimes reach epidemic proportions (Gossen & Turnbull 1995)

May 2013

90% of *B. rubens* and *B. tectorum*
Infected with *Ustilago bullata*

April 2014

Bromus << 1% cover



Photos by Edith Allen

Factors that determine disease incidence

1.) Host density

- Dense grass promotes high incidence of disease
- Low density of grass results in lower incidence of smut

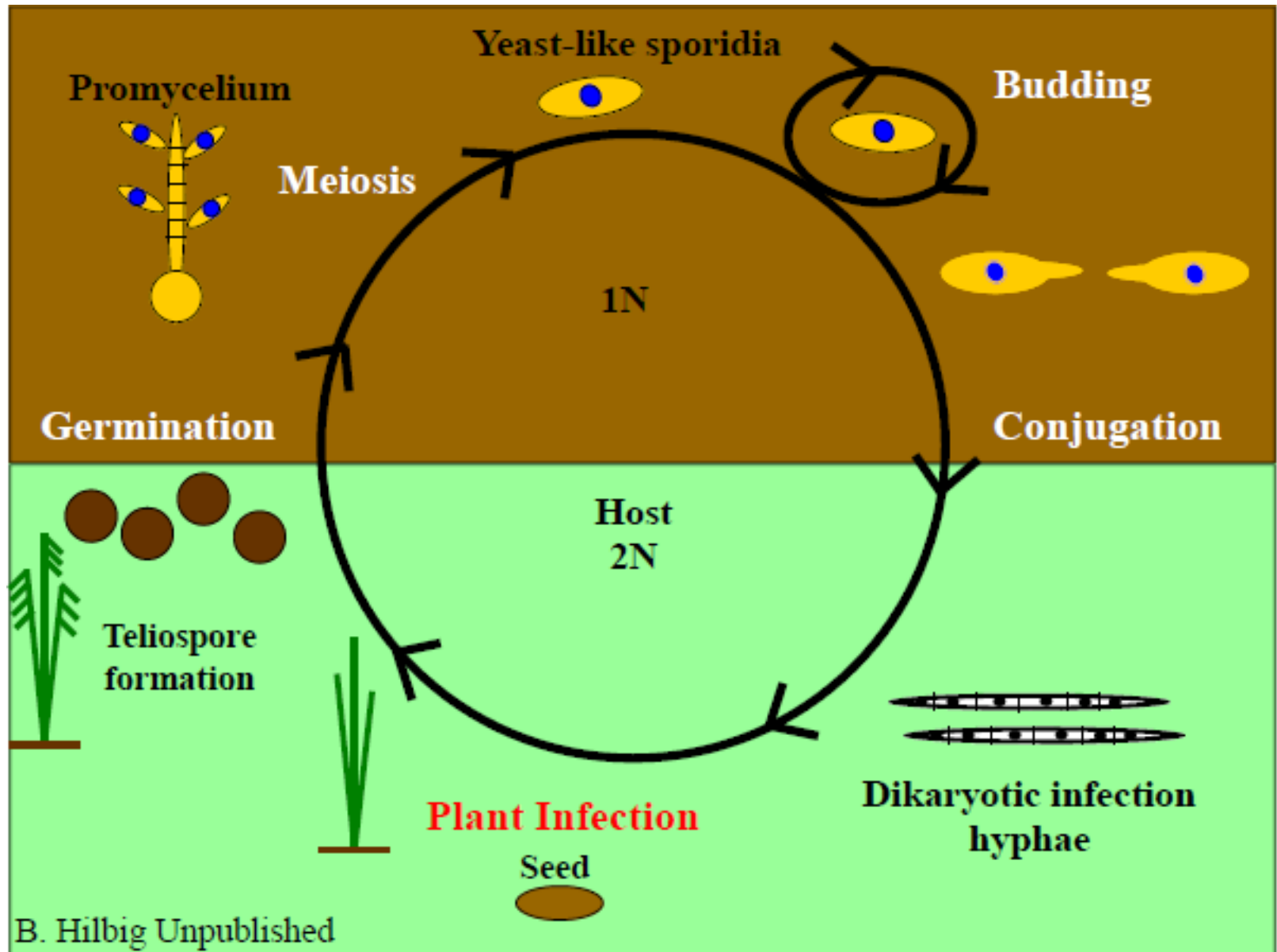
2.) Requires close synchronization with host seed germination

- Infections occur when the hosts coleoptile emerges
- Bromus seeds can germinate any time from early Fall through mid-spring

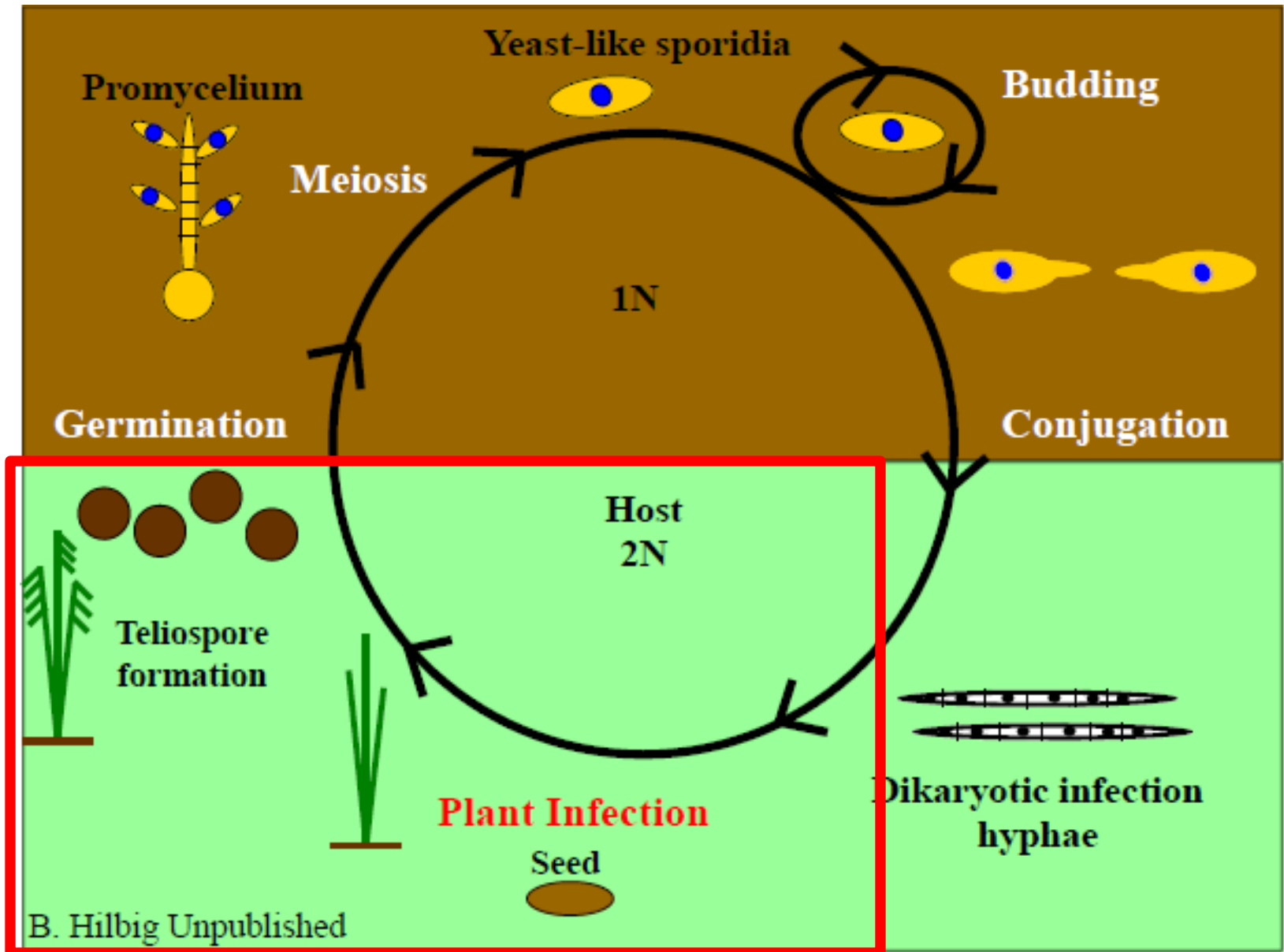
3.) Temperature

- Infection occurs between 12-25 °C, but disease is less frequent when temperature exceed 20 °C (Boguena *et al.* 2014)
- We have found that Mediterranean climate sites have lower incidence than high desert sites

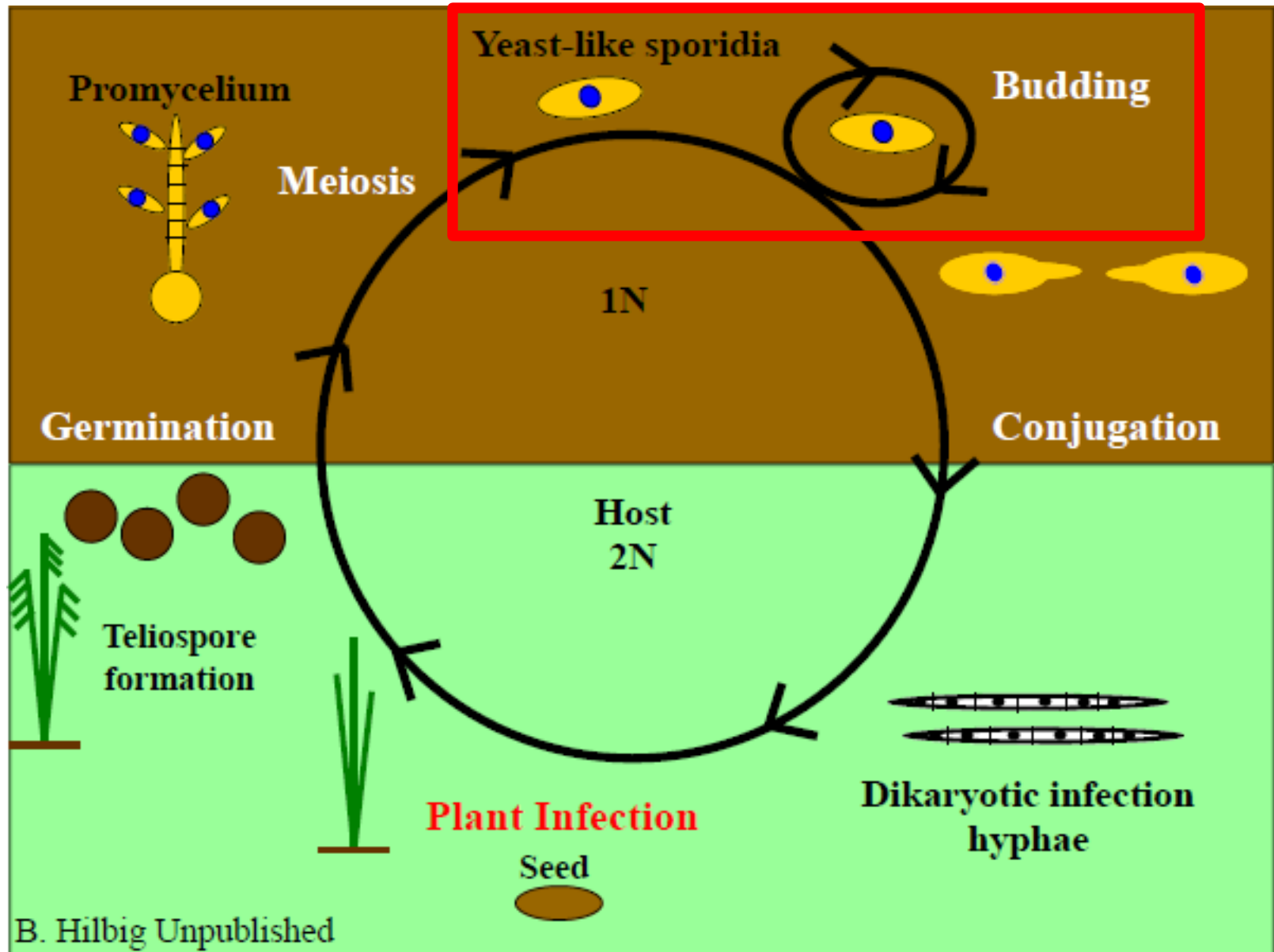
Complex Dimorphic Life Cycle



Complex Dimorphic Life Cycle



Complex Dimorphic Life Cycle

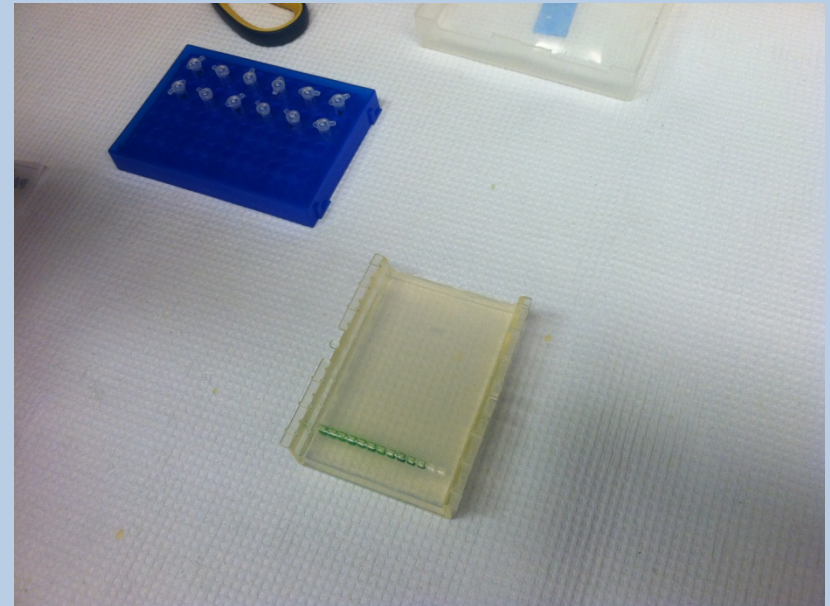


Goals

- 1.) Compared species of *Ustilago* infecting *Bromus rubens* in its native range (Spain) to species in its invaded range (California)
 - * Origin of *Ustilago bullata*?
- 2.) Isolate and germinate teliospores to test *Ustilago bullata* as a biocontrol agent
- 3.) Test on non-target species

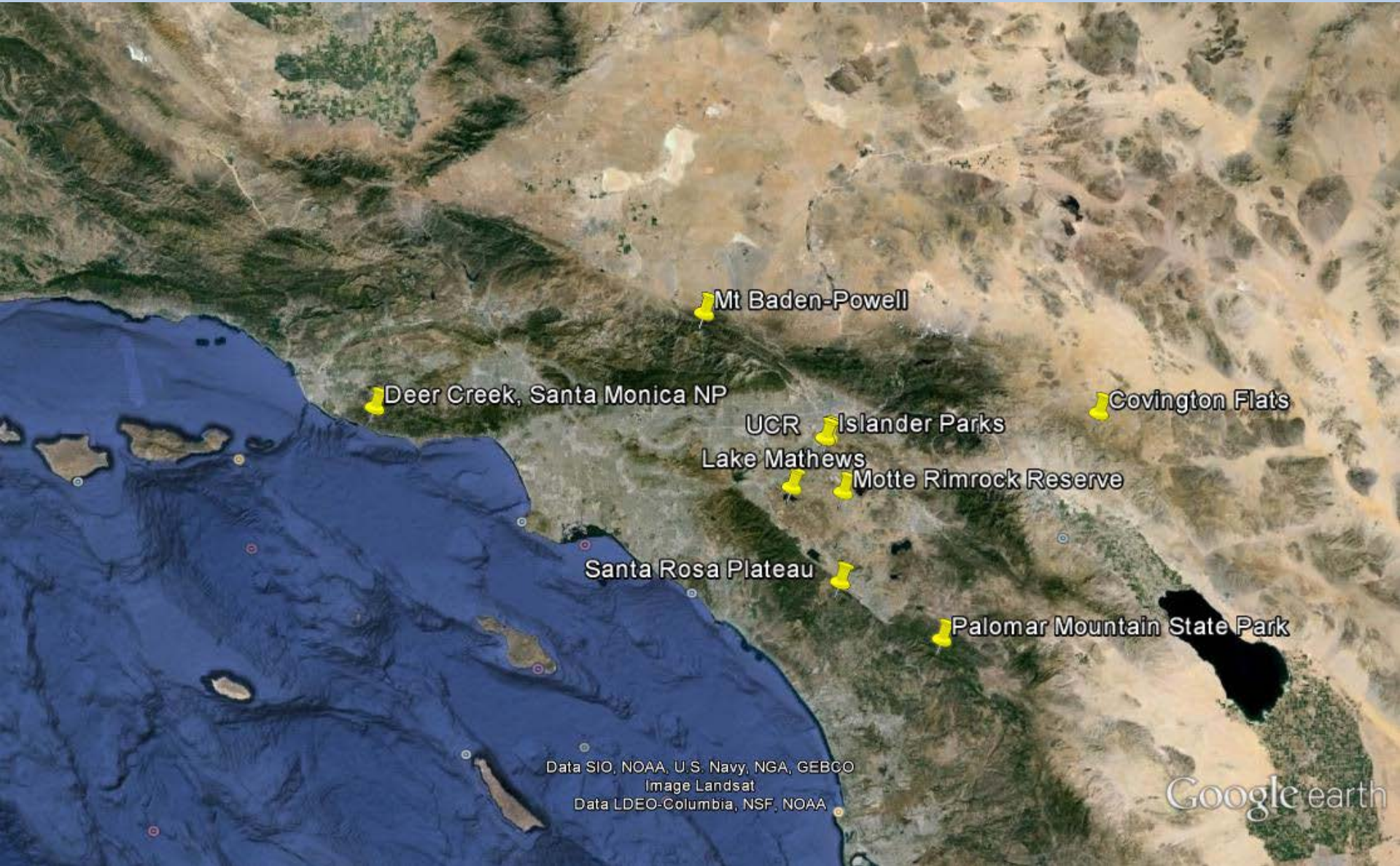
Methods

- Smutted host plants *Bromus diandrus*, and *B. rubens* were collected from different vegetation types in southern California and Spain and stored at room temperature.
- Fungal DNA was extracted from teliospores of infected seeds and sequenced for identification of fungal species



California Samples

Been found in many different ecosystems throughout southern California



Spanish Populations





Ustilago bullata
observed on
Bromus rubens,
B. tectorum,
and
B. diandrus

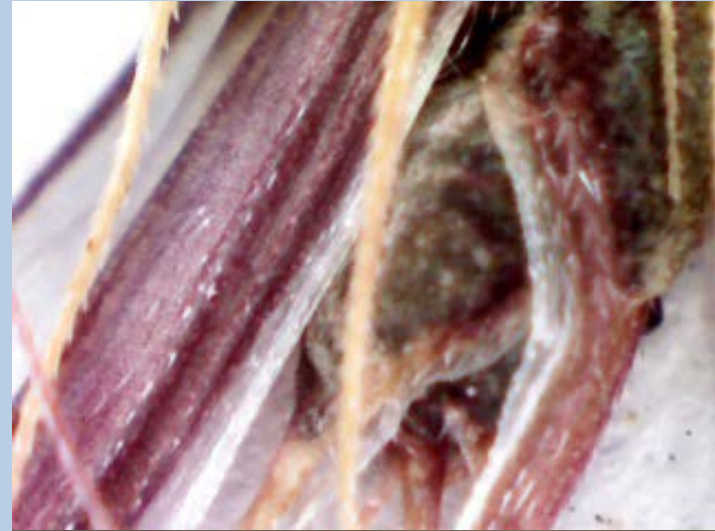


Photo: Edith Allen



Results

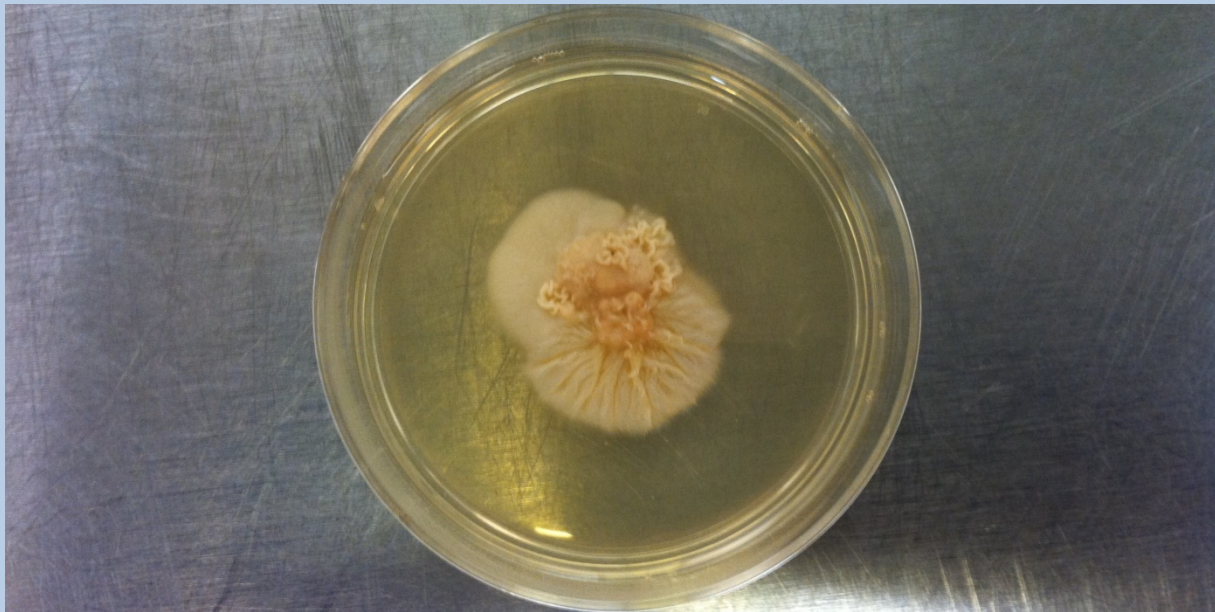
- Spanish populations revealed three species of *Ustilago* in *Bromus rubens* including *U. bullata*, *U. hordei*, and *U. avenae*
- *U. bullata* was the only species found infecting *Bromus* species in California populations
- Based on phylogenetic analyses we found similar strains of *U. bullata* in Spanish and California population
 - Other studies have demonstrated *U. bullata* races on *Bromus tectorum* traveled with it to the introduced range (Meyer *et al.* 2005)



Tree by Matt O'Neill

Culturing *Ustilago*

- Is difficult!
- Smutted seeds of *Bromus diandrus* from the abundant stand at UC Riverside, California were surface sterilized with 10% bleach, germinated on potato dextrose agar with antibiotics.
- Media were left to incubate at room temperature (21°C)



- Resulting yeast cultures were identified through DNA sequencing.

Ustilago bullata

- Introduced biological agents are most effective when they exhibit:
 - Narrow host range ?
 - Climatic adaptability ?
 - Synchrony with host life cycle YES
 - High reproductive potential YES
 - Survival at low host density ?

Future directions

- Inoculation of *Bromus* with shake cultures of yeast –like sporidia
- Test for host specificity
 - Does it infect native grasses
 - Cereal crops?
- Inoculate in field trials



Acknowledgements

Matthew O'Neill

Michael Allen

Edith Allen

Akif Eskalen

Elizabeth Holmes

Joey Mayorquin

Violet Khin

Amanda Haraksin

Members of the Allen and Eskalen
labs

