Efficacy of organic sulfur compounds from garlic/onion on white rot Sclerotia germination

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White rot disease

Schlerotium cepivorum

Garlic with white rot disease

- Few sclerotia in a liter of field soil potentially can result in crop failure
- Long dormant period in the absence of allium hosts
- Once white rot is identified in a field, growers are not able to grow allium crops.

Hypothsis and approach

- If we can stimulate the germination without the presence of host plant, sclerotia will die

Objectives

- Develop a fast laboratory approach to stimulate sclerotia germination
- Use laboratory approach to screen bio-stimulants
- Provide bio-stimulant input for field trials

Previous Experiment-Sclerotia isolation (Dung Jeremiah)

- Sclerotia isolation
- Sclerotia preparation and activation
- Sclerotia viability

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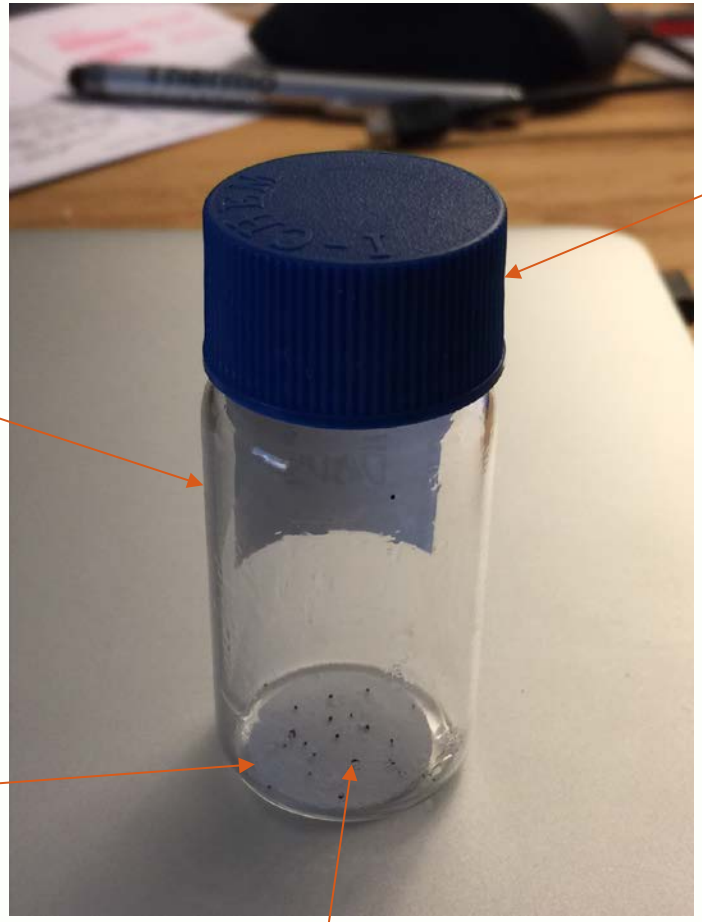
Previous experimental set-up

I-Chem Certified pre-cleaned vial, 20 mL



Teflone lined Silicone Septa

When add PDA agar to the vial, achieve germination

When just filter paper, no germination

Filter paper, maintain moisture

Sclerotia

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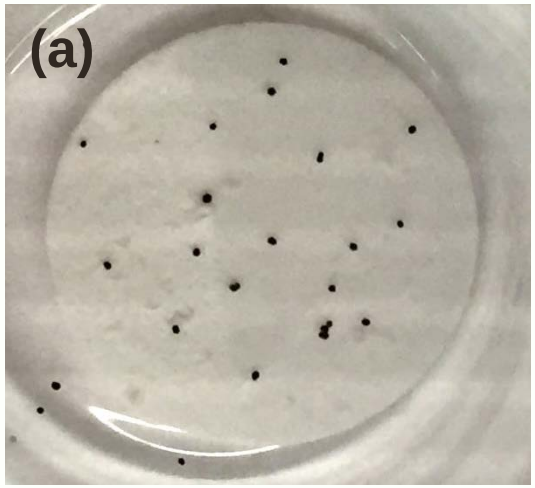
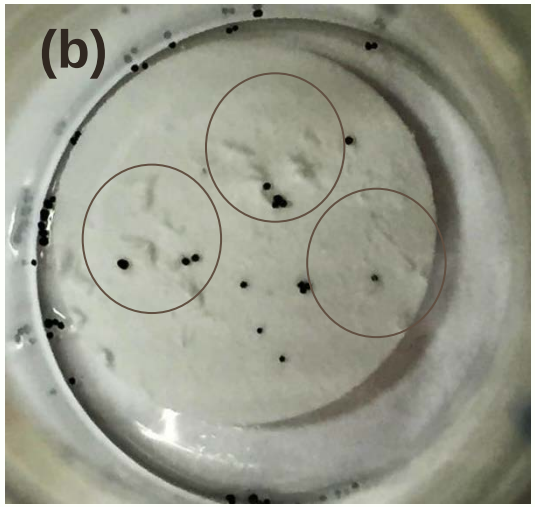
- **Germination Results -- *Set A***



(a) Control
Filter paper, 10 sclerotia, 100 µl H_2O,
10 µl methanol in 20 mL vial

No germination



(b) DADS stimulant (5 ppm in headspace)
Filter paper, 10 sclerotia, 100 µl H_2O
10 µl 1% (10000 ppm) DADS in 20 mL vial

Germination level: (+++)

Figure 4. Germination Result of Set A

- *S. cepivorum* were provided by Dr. Dung; +++ represent the germination level

However, we have difficulties to germinate the sclerotia consistently

- Sclerotia should not geminate without stimulants
- Keep stimulants in a defined container
  - Sulfur compounds are volatile
  - Escape
  - Cross-contamination
- Need best germination environment
  - Temperature, moisture, oxygen

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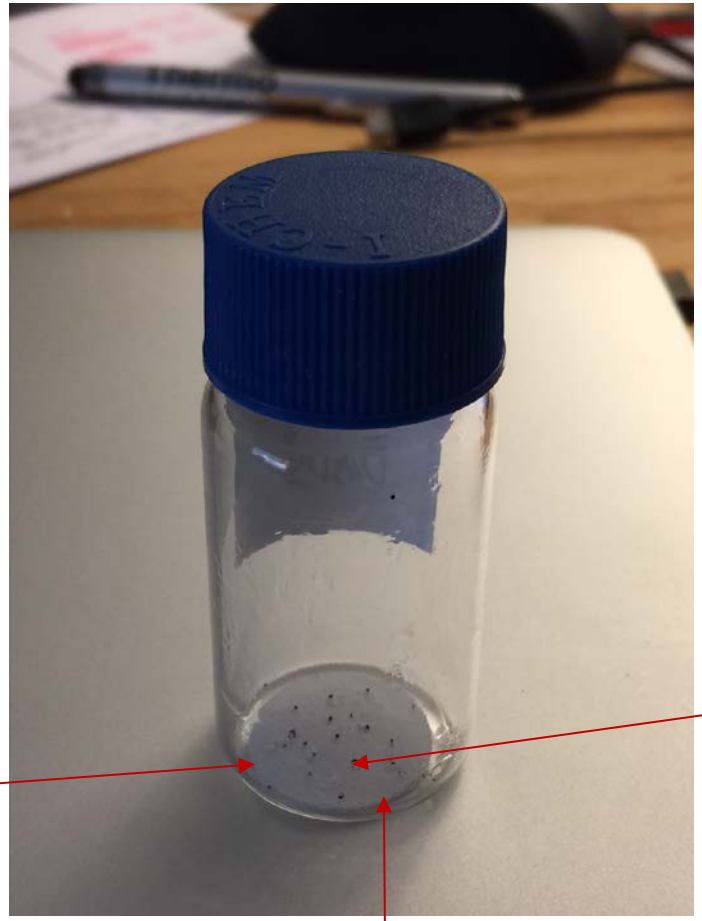
Modifications for experimental set-up



Filter paper, maintain moisture

Sclerotia

PDA agar could be added to the bottom

Many, Many trials…

- **Problems**

➢Molds grew easily on PDA agar

➢Germination was not reproducible due to uncontrollable temperature and moisture

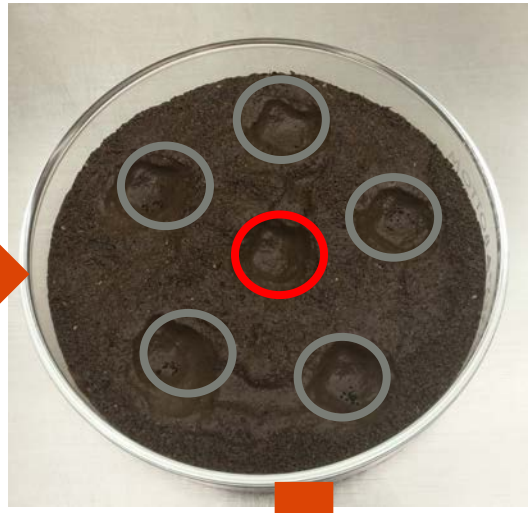
New Experimental Design

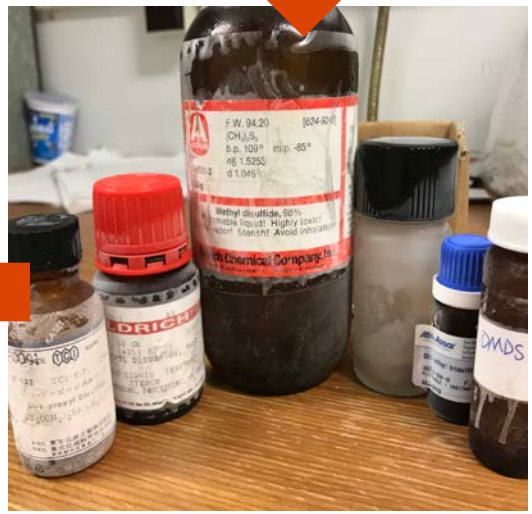
- Evaluate field soil as germination media
- Introduce incubation oven to achieve better environment control (temperature, moisture)

Sieve field soil through #10 and #20 sieves

30 cc soil and 13.5 ml water in petri dish

Sclerotia plating

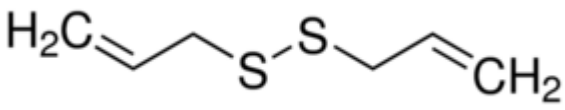




Apply sulfur standards

Seal with tape

Inspect sclerotia germination using a dissecting microscope

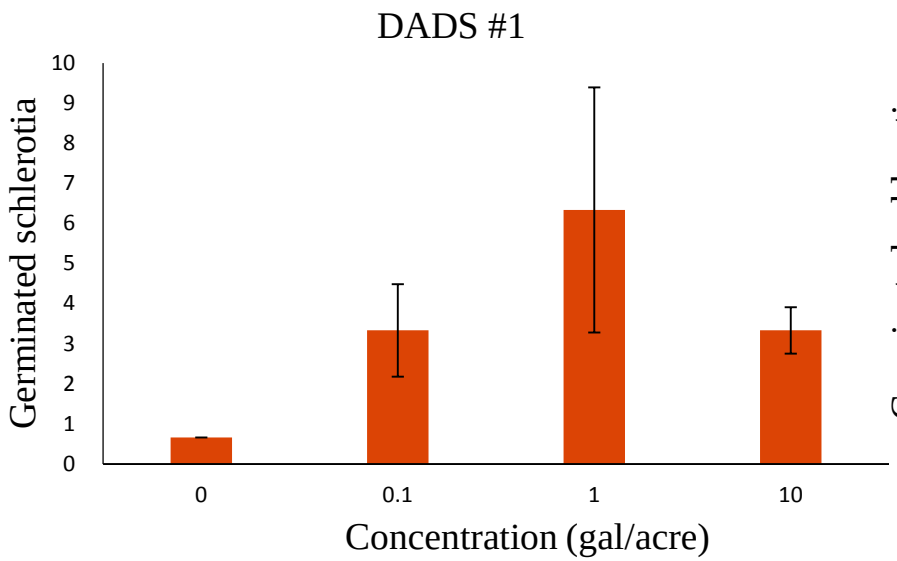


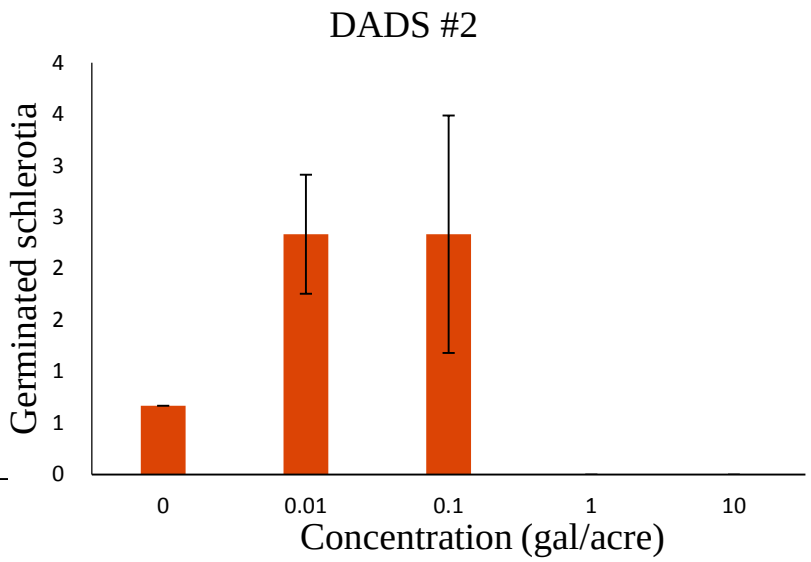
Concentrations of sulfur standards

Treatment	Concentration (gal/acre)	Applied amount
Dially disulfide，	0.1	7 µL 10 % solution
	1	7 µL pure solution
	10	70 µL pure solution

Triplicate for each treatment

Dose Response of DADA on Germination

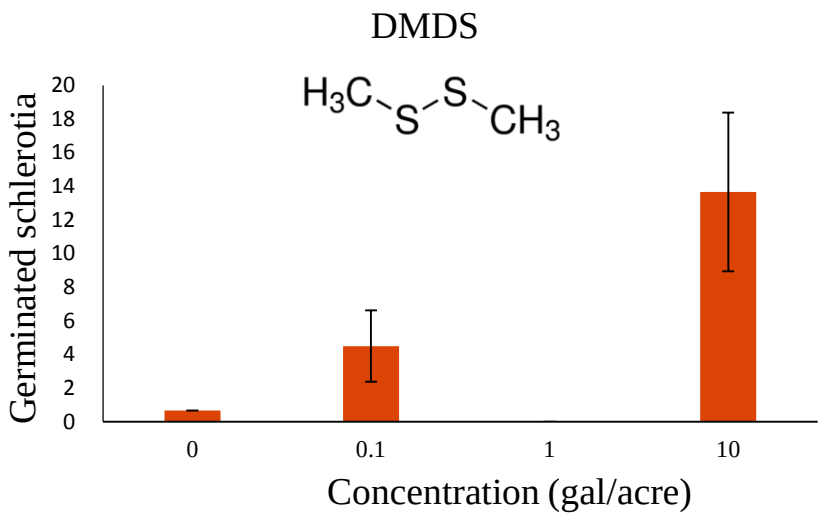


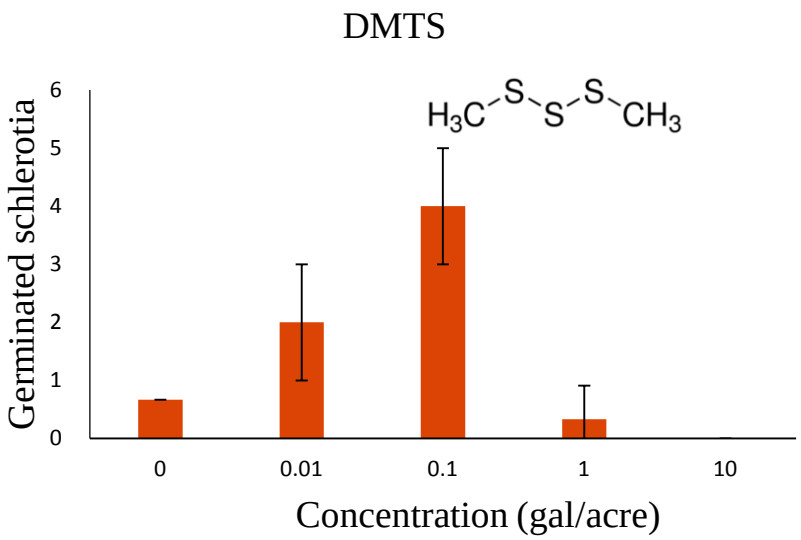


Warning: DADS was added on the top of soil, effective dosage will be different if added in the soil due to interaction with soil matrix

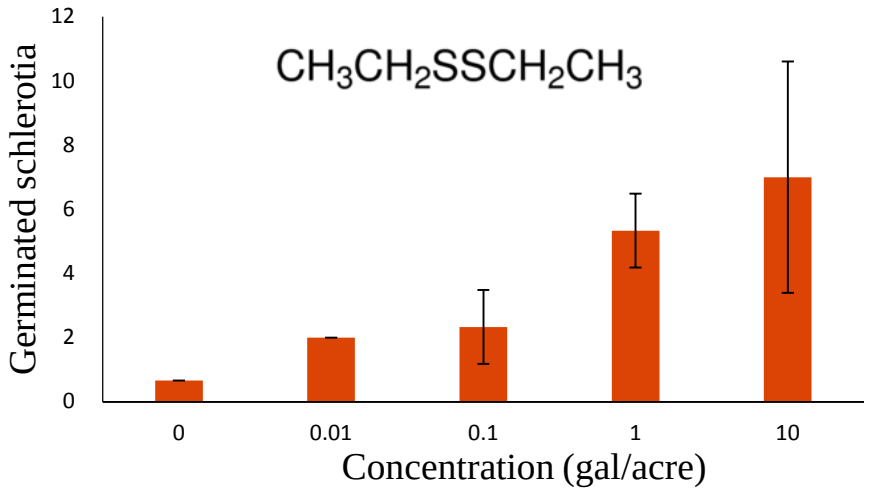
Treatment	Concentration (gal/acre)	Applied amount
Dimethyl disulfide (DMDS)	0.1	7 µL 10 % solution
	1	7 µL pure solution
	10	70 µL pure solution
Sulfur powder	0.01	10 mg sulfur powder
	0.1	100 mg sulfur powder
	1	1000 mg sulfur powder
Dimethyl trisulfife (DMTS) Diethyl disulfide (DEDS), diisopropyl disulfide (DIPDS) Dipropyl disulfide(DPDS)	0.01	7 µL 1 % solution
	0.1	7 µL 10% solution
	1	7 µL pure solution
	10	70 µL pure solution

Other Organic Sulfur on Germination

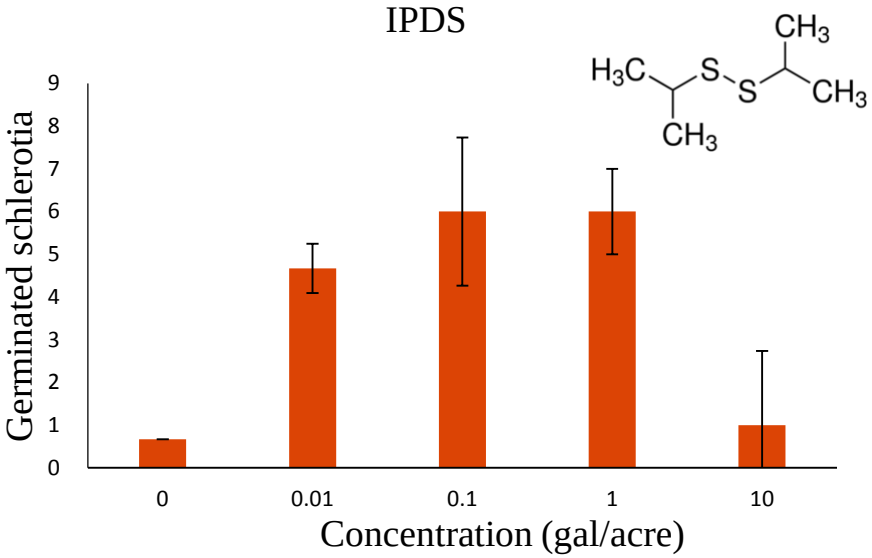




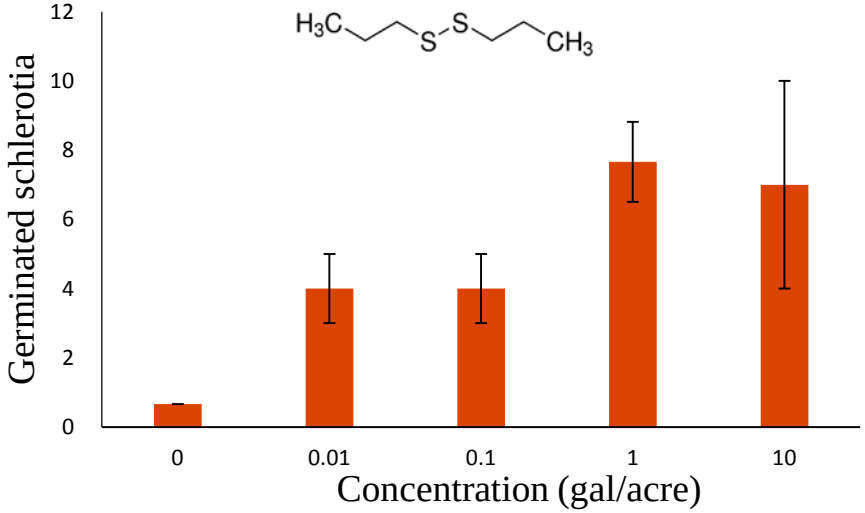
DEDS



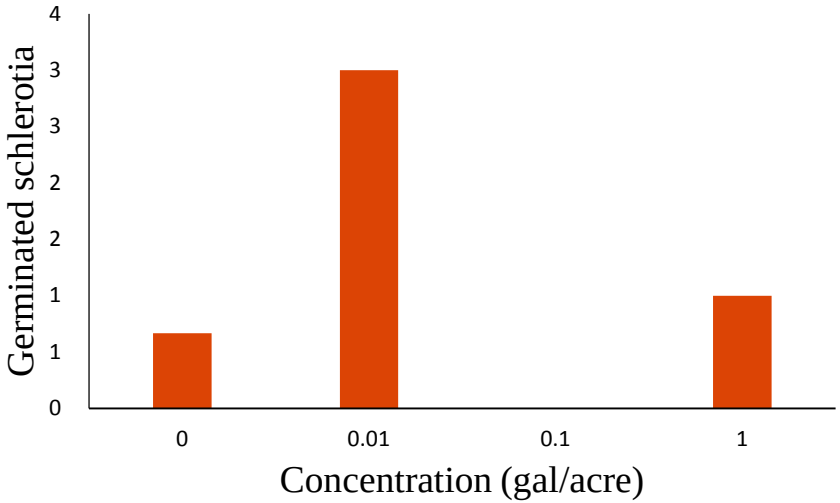
IPDS



DPDS



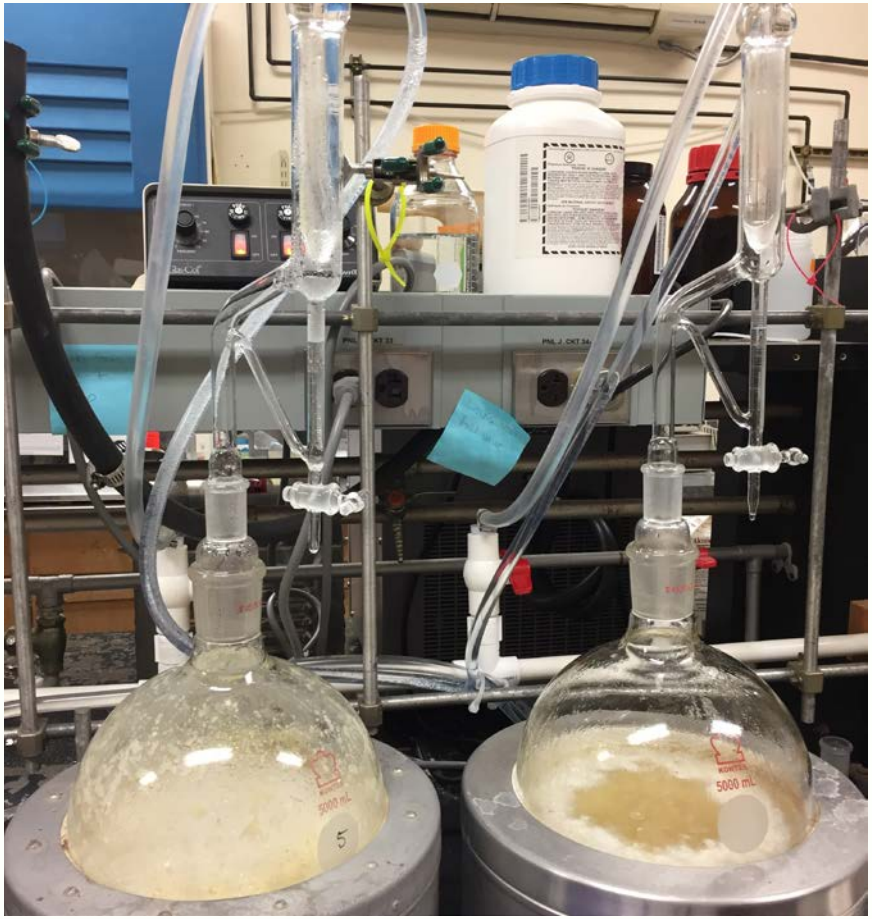
Sulphur powder

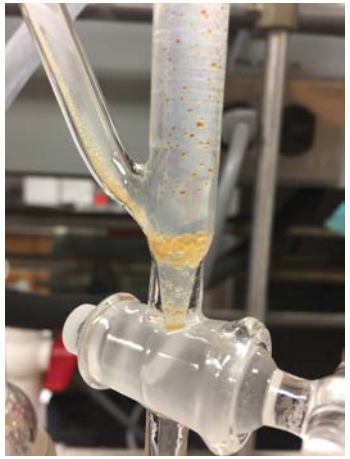


Short summary

1. In terms of DADS #1, DADS #2, DMTS, IPDS and sulfur powder treatments, lower concentrations (0.01, 0.1 gal/acre) had a better stimulant effect on schlerotia germination compared to higher concentrations (1, 10 gal/acre), leading to higher amount of germinated schletotia.
2. For DMDS, DMDS and DPDS, treatment with higher concentrations had increased amount of germinated schlerotia. 10 gal/acre of DMDS had the largest amount of germinated schlerotia among all treatments.
3. Absence of germinated schlerotia in control treatments indicated little cross-contamination during incubation.

Garlic oil distillation





- 400g garlic (California early/California late)
- 1.5 L Milli-Q water
- Blend garlic with water, and let it sit in the hood for 2 hrs
- Distillation and collect the oil phase

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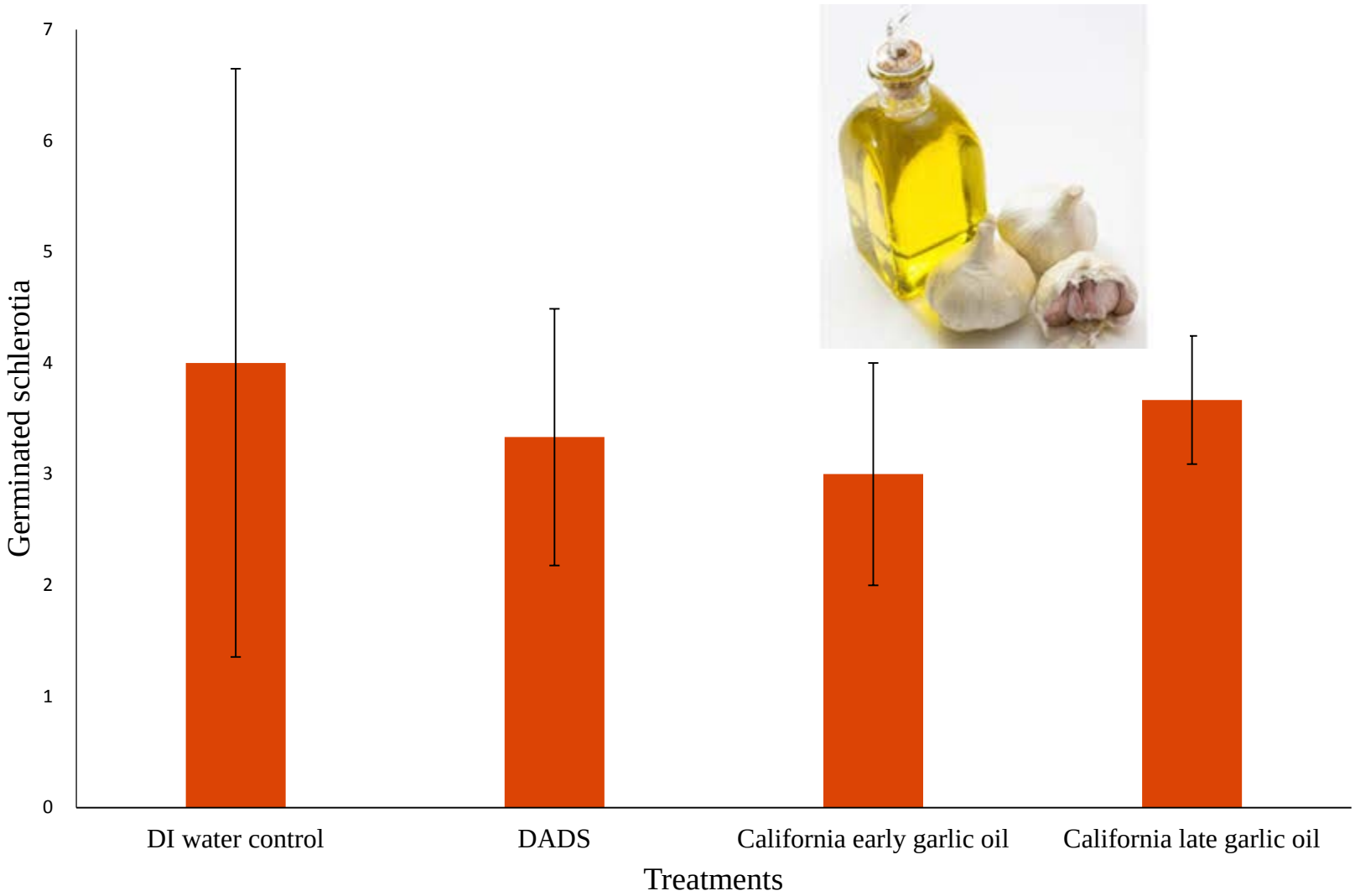
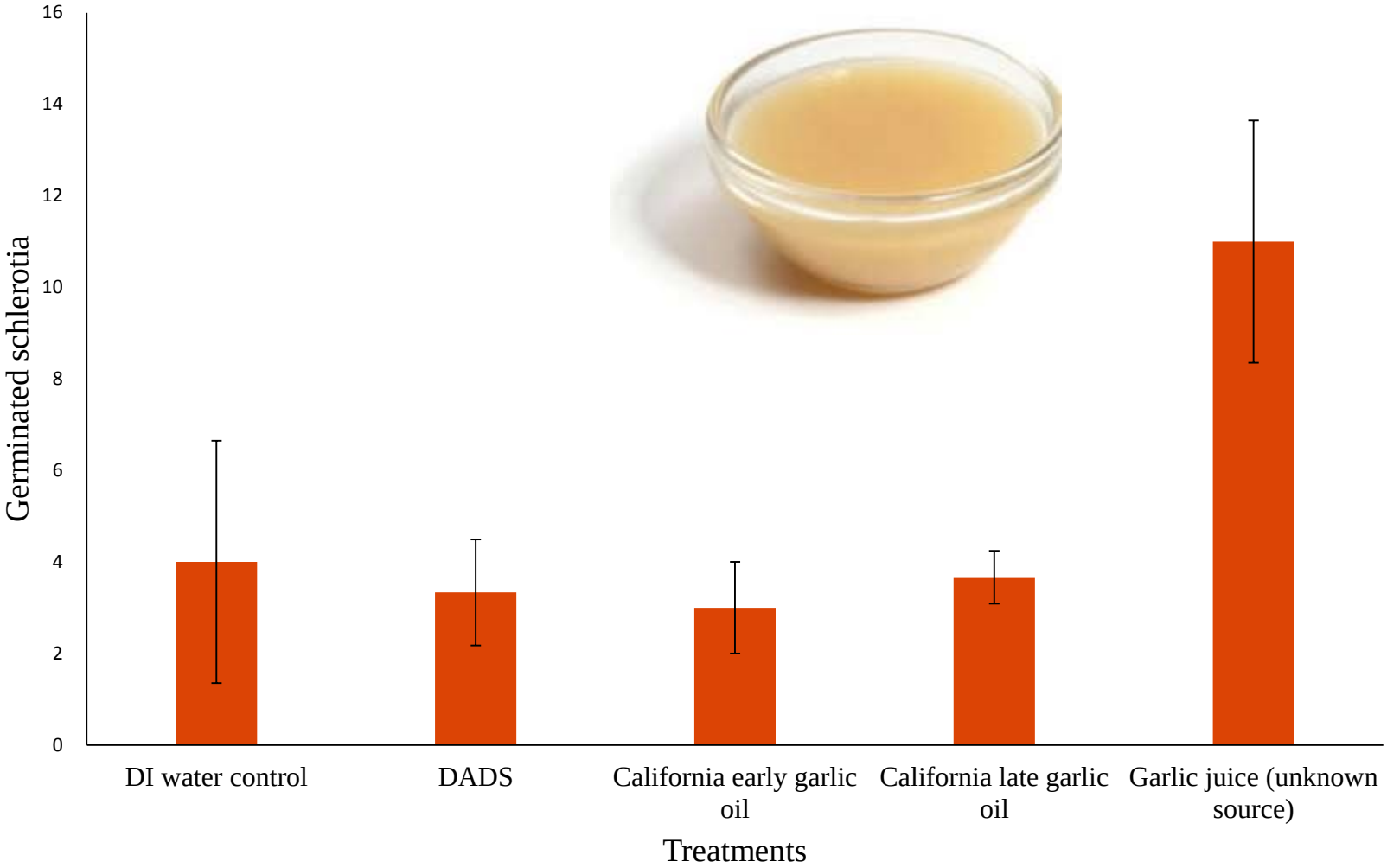
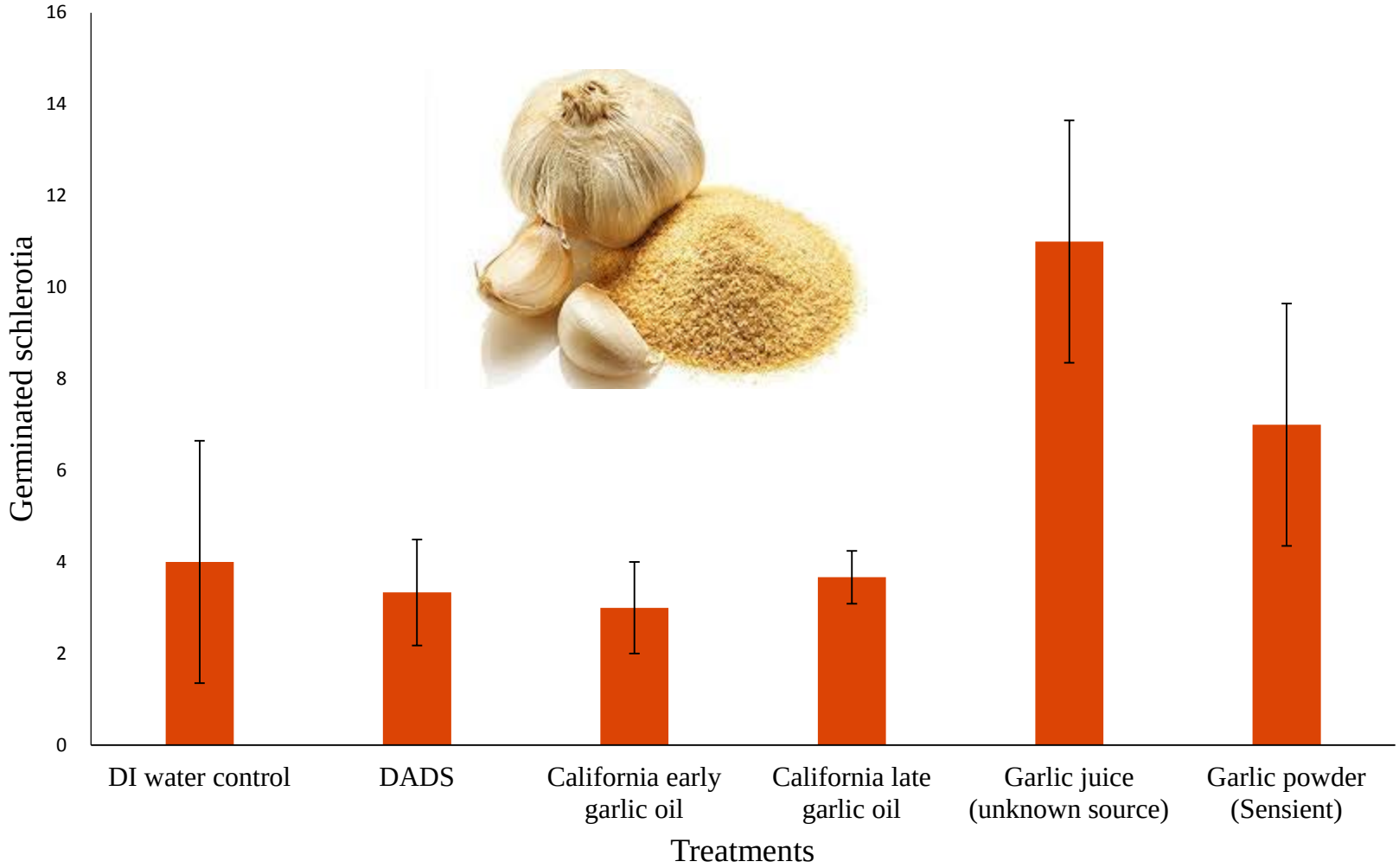
Garlic Oil (1 gal/acre) on Sclerotia Germination



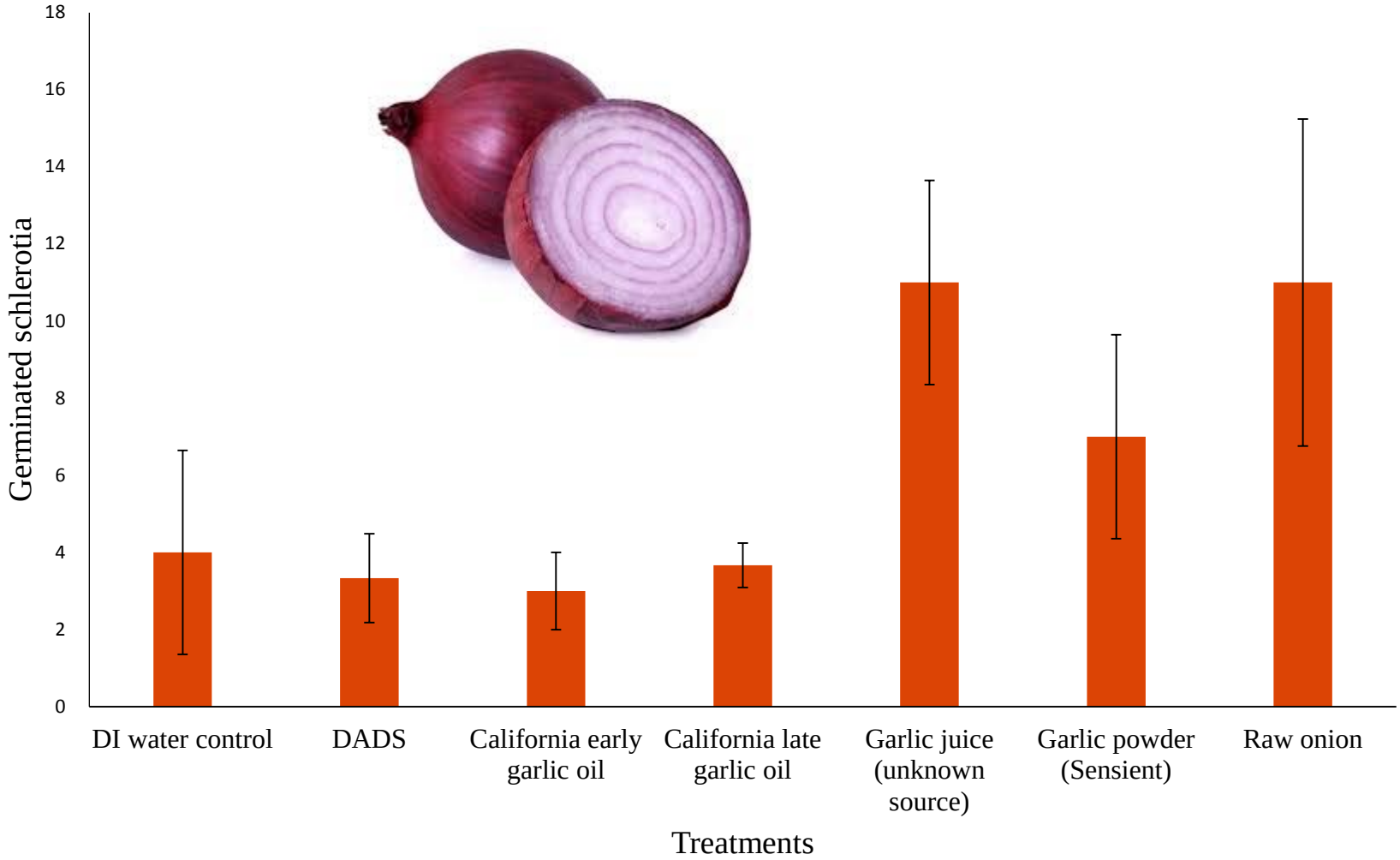
Garlic Juice On Sclerotia Germination



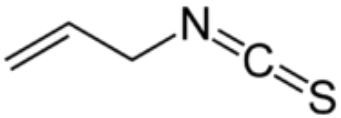
Garlic Powder on Sclerotia Germination



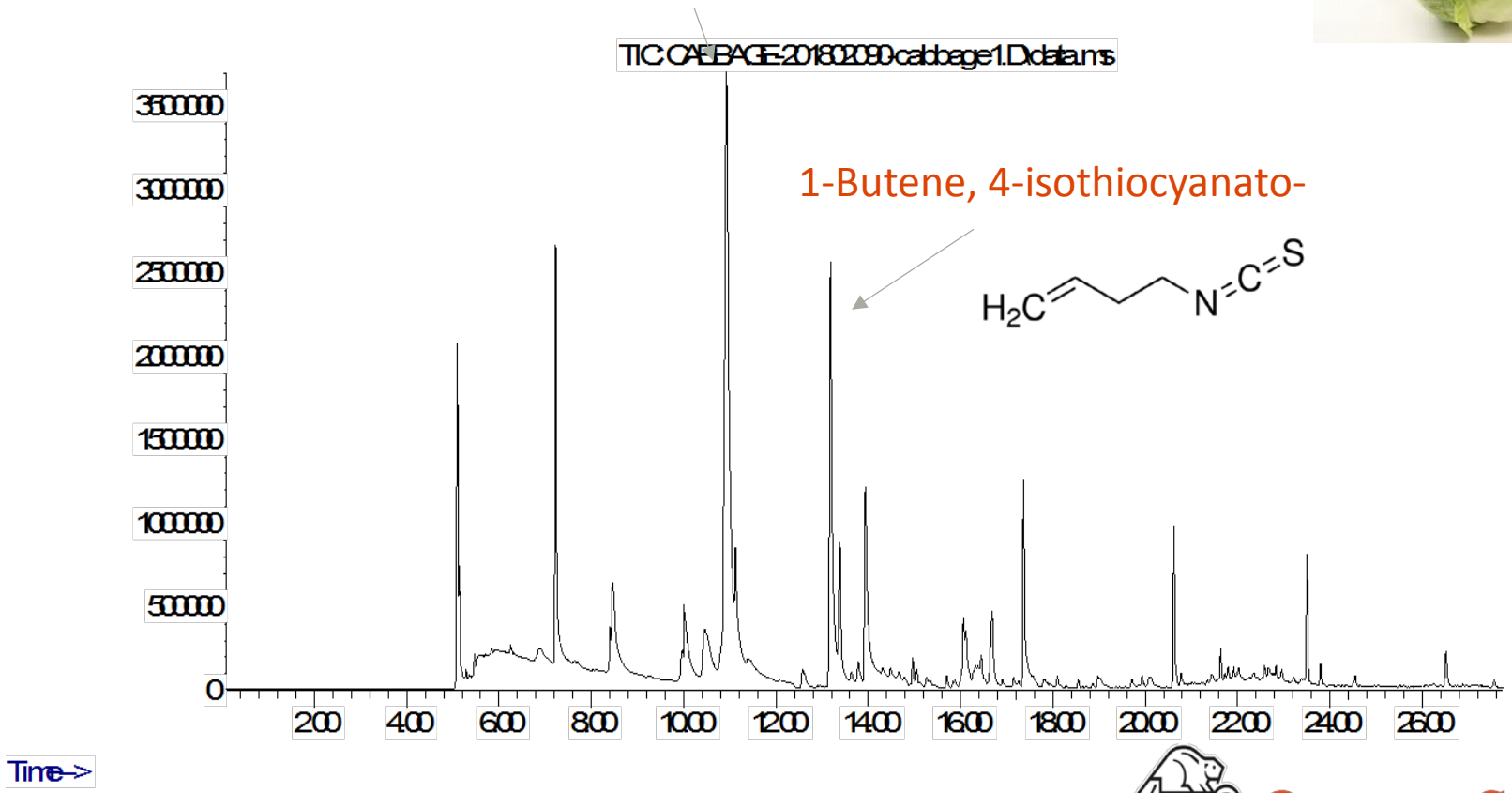
Raw Onion on Sclerotia Germination



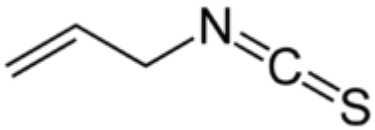
Volatile Compounds in Cabbage

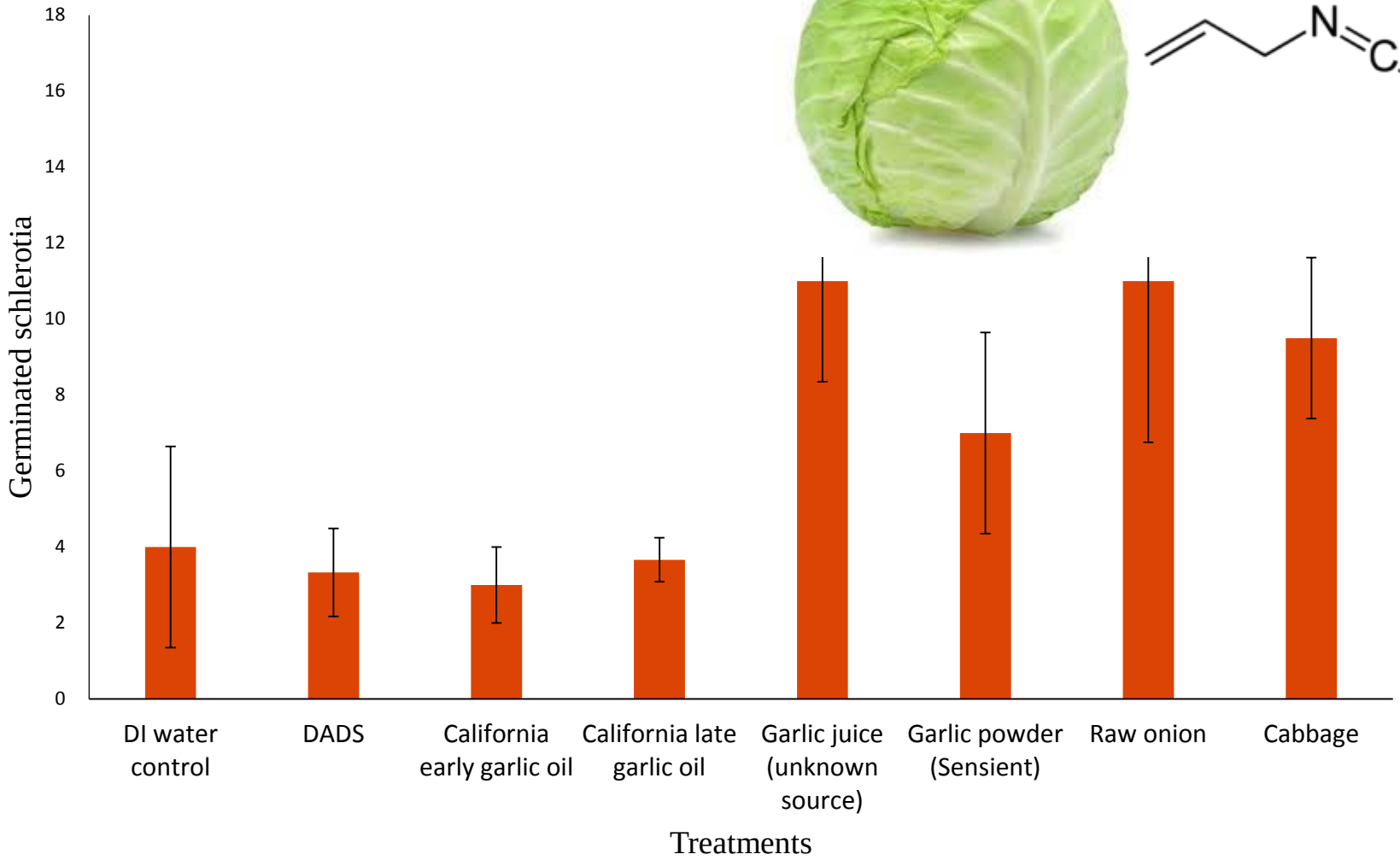


Allyl isothiocyanate



Germination results





Germination results

Summary

- Soil-based germination seems more reliable
- Germination dose-response correlation was observed
- Even very low application rate of DADS can stimulate the germination, although types of soil may affect the effective dosage
- High dosage can inhibit sclerotia germination
- In addition to DADS, many other sulfur organic compounds can also stimulate germination
- Garlic oil, garlic juice, garlic powder all can stimulate germination
- Cabbage can also stimulate the sclerotia germination