Effects of time, temperature, and host death on maturation of resting spores of *Plasmodiophora brassica*

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Introduction and Objectives

Plasmodiophora brassicae Woronin is an obligate, biotrophic pathogen that causes clubroot on canola (*Brassica napus* L.) and other species in the Brassicacae. Hypertrophy and hyperplasia in the roots of infected plants produce characteristic 'clubbed' root symptoms (Fig. 1). Following infection, young plasmodia colonize the root tissue, then develop over time into resting spores, which are released into the soil as the clubbed root deteriorates as the plants mature or die. Mature resting spores are long-lived, but immature spores are comparatively short-lived and produce little or no infection. The factors that affect maturation of resting spores are not well understood.

The objectives of the study were to determine:

1) whether maturation of resting spores of *P. brassicae* requires a living host,
2) the effect of temperature on maturation, and
3) whether the state of decay of clubs is associated with resting spore maturity.

Fig. 1. Clubroot on canola

Results and Discussion

- Freezing and thawing, or application of glyphosate, killed roots but did not kill resting spores or prevent their maturation (Fig. 2).
- Spore maturation continued under most conditions, except when clubs were stored at 5 ºC or frozen (Fig. 2).
- Clubs from plants harvested at 5 wk after seeding contained enough mature resting spores to cause 60% infection in the bioassay (Freezer treatment, Fig. 2).
- Freezing young clubs to kill plant cells, or killing plants with glyphosate, before storing clubs at 5º or 10 ºC resulted in more severe clubroot in bioassays, compared with fresh young clubs that were stored at 5 or 10 ºC (Fig. 2).
- Freezing clubs or killing plants with glyphosate before storing clubs at 5º or 10 ºC increased the number of mature spores.
- Spores reached maturity in clubs that were both more and less decayed (Fig. 2).

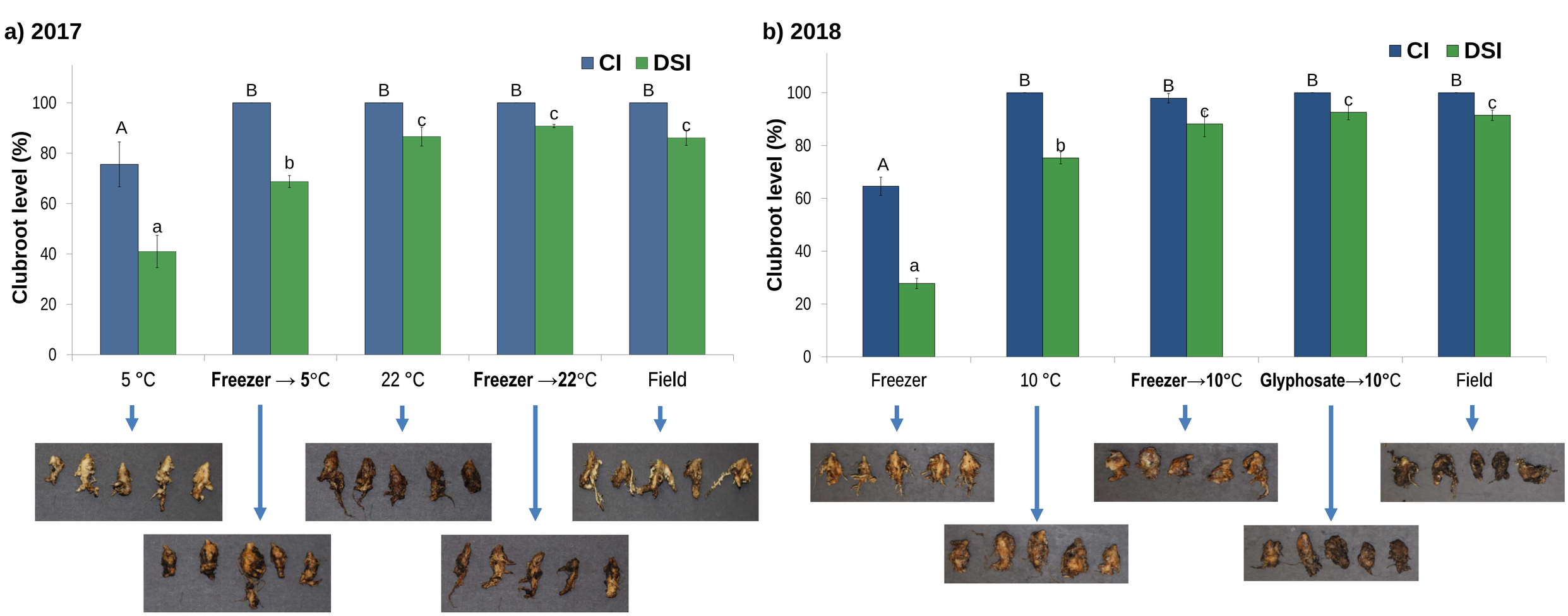

Fig. 2. Clubroot incidence (CI) and severity (disease severity index, DSI) in a bioassay on canola inoculated with resting spores from plants treated at 5 wk and spores collected at 9 wk after seeding in a) 2017 and b) 2018. Pictures of the state of decay of clubs from which spores were isolated are included for each treatment. Bars with the same letter and case did not differ at $P < 0.05$ based on Fisher's protected LSD test.

Materials and Methods

- Replicated field trials were conducted in 2017 and 2018.
- Five-week-old clubs were harvested, frozen (3 days @ -20 °C) or not frozen, and stored at 5 °C or 22 °C in 2017, and at 10 °C in 2018. Control plants were left to grow in the field for the duration of the study.
- In 2018, clubs were also stored at -20 °C (freezer) for the duration of the experiment as a negative control, and some plants were sprayed with glyphosate, dug up one week later and clubs stored at 10 °C (glyphosate to 10 °C).
- Spore maturity was assessed using a variation of qPCR that uses propidium monoazide (PMA-PCR) to quantify viable, mature spores (data not shown).
- Bioassays of spore viability were conducted, in which susceptible canola plants were inoculated with spores from each treatment at the end of each trial (9 weeks after seeding). Clubroot symptoms were assessed at 6 weeks after inoculation.

Conclusions

- Freezing and thawing, or application of glyphosate, did not kill resting spores.
- A living host was not required for spore maturation.
- Death of the host plant appeared to accelerate the maturation of resting spores.
- State of decay of clubs is not always associated with spore maturity.
- Infected plants must be killed very early to avoid adding more inoculum to the soil

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