Suppressive effects of boron application and temperature on the development of *Plasmodiophora brassicae* in canola and pak choy.

Mary Ruth McDonald, Abhinandan Deora, Kalpana Sharma and Bruce D. Gossen
Department of Plant Agriculture, University of Guelph, Guelph, ON NIG 2W1 Canada (MRM, AD, KS)
Saskatoon Research Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 0X2 Canada (BDG)

Introduction

Plasmodiophora brassicae Woronin is a soil-borne protist that causes clubroot of Brassica crops, including many vegetable crops and canola. Clubroot has become an important threat to on canola (*Brassica napus* L.) production in western Canada since it was first recognized there in 2003 (Tewari *et al*., 2004). Two disease management practices that are of particular interest for inclusion in IPM programs to manage clubroot are early seeding to avoid temperatures that are favourable for clubroot development, and application of boron to inhibit the initial stages of pathogen development in susceptible plants. A recent study demonstrated that the impact of temperature on primary infection and development of the pathogen under controlled conditions were similar in canola and Shanghai pak choy (*B. rapa* L. var. *communis* Tsen and Lee), a fast growing Asian Brassica crop (Gossen *et al*., 2011). Field application of B reduces clubroot in vegetable Brassicas (Dixon, 1996). Therefore, in the present study a trial was conducted on pak choy to identify the effect(s) of temperature on the incidence of root hair infection, the incidence of important developmental stages of *P. brassicae* in the root hairs, symptom development, and final severity of clubroot. A second trial was conducted on canola and pak choy under controlled to assess the impact of various commercial formulations B on clubroot development across a range of concentrations, and on canola under field conditions. Each trial was repeated.

Materials and methods

Temperature versus root hair infection and clubroot severity: Seedlings of Shanghai pak choy cv. Mei Quing Choy (Stokes Seeds Ltd., ON, Canada) were grown in a modified sand-liquid culture (Donald and Porter 2004). The study was arranged in a completely randomized design within each growth cabinet, with four replicates per sampling date and three seedlings per experimental unit. Each seedling was grown in a 5-mL pipette tips of noncalcareous sand, inoculated at 10 days after seeding (DAI) with a spore suspension (300 µL of 10^8 spores mL^{-1}) at the base, and transferred to growth cabinets at 10°, 15°, 20°, 25° and 30° C with 14-h photoperiod and 65% RH. For assessment of RHI, seedlings were harvested at 2-day intervals starting 2 days DAI to a maximum of 28 DAI. Samples were fixed in acetic acid: ethanol (1:1). Each root was stained with 125 ppm aniline-blue solution and 100 root hairs at 0–2 cm below the hypocotyl were assessed for RHI (%) and developmental stage of *P. brassicae* using a compound microscope.

For assessment of clubroot severity, seedlings were grown in tall plastic pots (19-cm-high Conetainers, Stuewe and Sons, Inc, Corvallis, OR). Each plant was inoculated with 5 mL of resting spore suspension of 1×10^6 resting spores mL^{-1} and the experimental layout was as described previously. Clubroot incidence and severity was assessed 6 weeks after inoculation. The roots were washed and the severity of clubbing/gall development was rated, and plants were separated into classes based on 0–3 scale, where 0 = no clubbing and 3 = > 2/3 of roots clubbed, and a disease severity index (DSI, range 0-100) was calculated.

Impact of Boron (B) – controlled environment: Pak choy and canola cv. 46H76 (Pioneer Hi-Bred, Caledon, ON) were assessed under controlled conditions. Plants were grown in liquid-sand culture at 24/20 ºC (day/night) in a growth room and inoculated with 1 mL of 1×10^6 resting spores mL^{-1}. Three commercial boron fertilizers: Boron (10% B as H_3BO_3, liquid, Alpine Plant Foods Corporation, New Hamburg, ON), BoronMax (8.1% B complexed with plant carbohydrates, liquid, NutriAg, Toronto, ON) and Solubor (20.5% B as $Na_2B_8O_{13}4H_20$, powder, Borax Inc., Valencia, CA), were assessed at 0, 0.25, 0.5, 1, 2, 4, 8, 16 and 32 kg ha^{-1} of B by drenching the sand at the time of sowing. The study was laid out in a factorial randomized complete block design (RCBD) with four replicates and assessed as described previously.

To assess clubroot severity, seedlings were grown, inoculated, and assessed as described previously. The B treatments were applied either immediately after sowing (early) or 10 days after inoculation (late). The study was arranged as a factorial RCBD with four replicate. There were three plants per experimental unit in the first repetition and six plants in the second.

Impact of Boron (B) – field trials: A range of B concentrations (0, 1, 2, 4, 8 and 16 kg ha^{-1}) were assessed in muck soil (pH ~ 6.3, organic matter ~ 70%) naturally infested with *P. brassicae* at the Muck Crop Research Station, ON. The study was arranged as a factorial RCBD with four replicates. Each plot consisted of two 5-m rows with 40 cm between rows, seeded at 25 seeds m^{-1}. Clubroot incidence and severity were assessed on 50 plants per plot at 6 weeks after seeding as described previously.

Statistical analysis: Data were assessed using analysis of variance and means were separated using Tukey's Mean Comparison test. There were no biologically important interactions with repetition. The impact of rates of B studies was assessed using regression in ANOVA. Differences are significant at $P \leq 0.05$ unless otherwise noted.

Results and Discussion

Root hair infection (RHI) occurred at each temperature, but development in root hairs (Fig. 1) and severity (Fig. 2) occurred more quickly at higher temperatures up to 25° C. Distinct clubbing developed only in plants grown at 20°–30° C. Swelling of the tap root was visible at 10 DAI in plants at 25° C, 14 DAI at 20° and 30° C, and 28 DAI at 25° C, but no symptoms were observed at 28 DAI at 10° C. RHI and severity were highest at 25°C, intermediate at 20° and 30°C, and lowest at 10° and 15° C and there was a positive correlation ($R^2 = 0.82$, $P<0.01$) between root hair infection at 20°–30° C and clubroot severity. This is the first report on the impact of temperature on development of *P. brassicae* within root hairs.

The impact of B formulation and rate were similar for each formulation and crop species, so only the data for Solubor on canola are presented. Also, differences between early and late treatments were very small, so these treatments were combined for presentation. Inhibition of RHI and pathogen development increased with increasing B concentration (Fig. 3). This supports reports from previous studies that B inhibits pathogen development (Webster and Dixon, 1991). Under controlled conditions, rates >2 kg ha^{-1} were phytotoxic to the crop; early treatment reduced germination and late treatment resulted in leaf burning and stunted growth (data not shown). In contrast, phytotoxicity in field conditions was observed only at >4 kg ha^{-1} (Fig. 5).

These studies demonstrate that low temperatures and B application can slow root hair infection, pathogen development, and reduce clubroot symptom. Field trials are underway to assess the impact of early sowing (when the temperature is low) and application of B at sowing time as components of clubroot management in canola under field conditions in Canada.

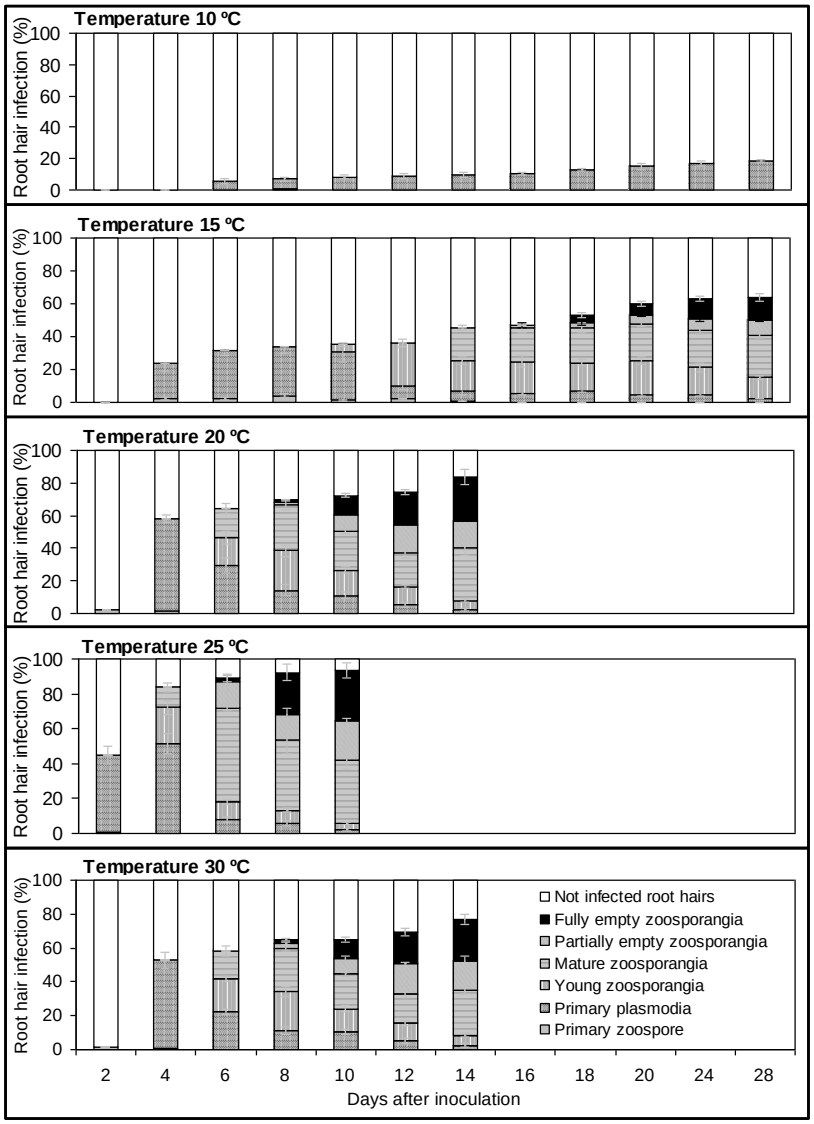

Fig. 1*. The stages of primary infection of* Plasmodiophora brassicae *over time from 10–30° C. Assessment was discontinued when swelling of the tap root was observed. Bars represent* ± *SE.*

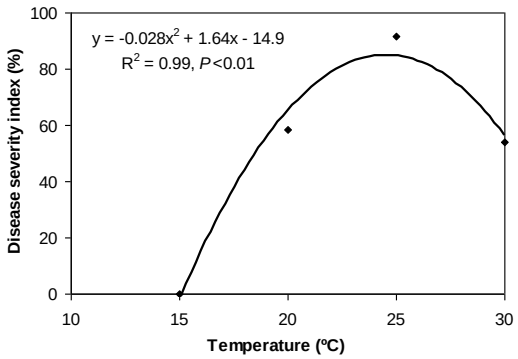

***Fig. 2**. Effect of temperature on clubroot severity (disease severity index) on pak choy at 24 DAI.*

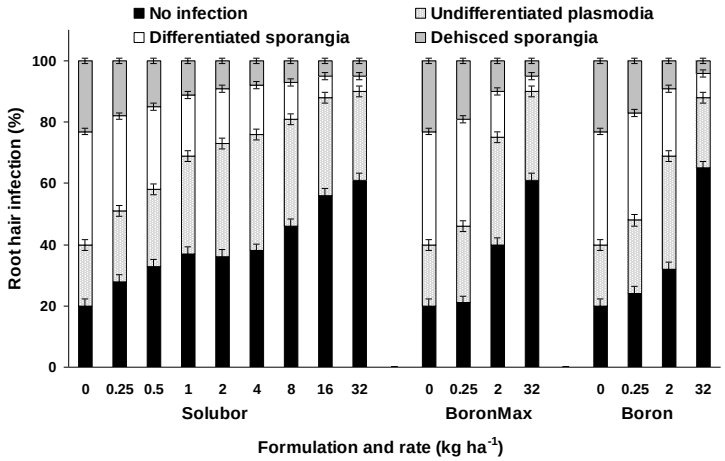

Fig. 3. Effect of rate (kg ha^{-1}) and formulation of boron on developmental stages of *Plasmodiophora brassicae* in infected root hairs of canola, combined across two repetitions.

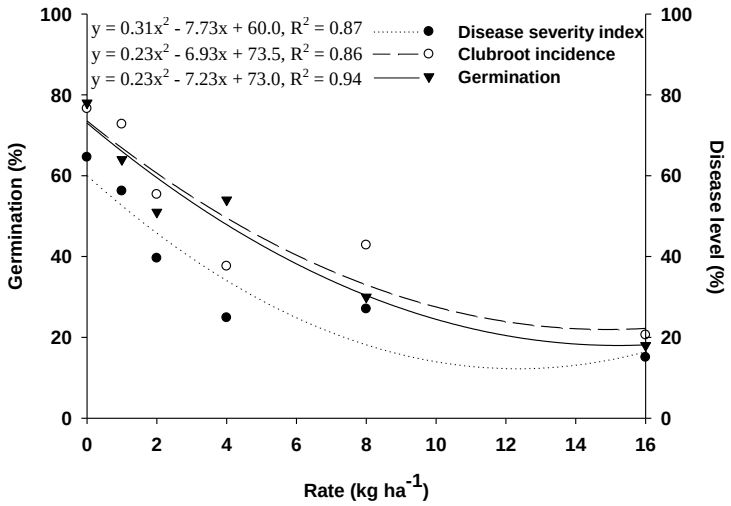

Fig. 4. Effect of rate of boron (kg ha^{-1}) on seedling germination (%) and clubroot incidence (%) and severity (disease severity index) in canola grown in muck soil near Bradford ON in 2010.

References

DIXON, G.R. (1996). Repression of the morphogenesis of *Plasmodiophora brassicae* Wor. by boron – A review. *Acta Hort*., *407*, 393–401.

DONALD, E.C & PORTER, I.J., (2004). A sand-solution culture technique used to observe the effect of calcium and pH on root hair and cortical stages of infection by *Plasmodiophora brassicae*. *Australasian Plant Pathol*. *33*, 585–589.

GOSSEN, B.D., KALPANA, K.C., & MCDONALD, M.R. (2011) Temperature influences development of clubroot under controlled conditions. *Plant Pathol*. (submitted)

HOWARD, R.J., STRELKOV, S.E., & HARDING, M.W. (2010). Clubroot of cruciferous crops- new perspectives on an old disease. *Can*. *J*. *Plant Pathol*., *32*, 43–57.

TEWARI, J.P., ORCHARD, D., HARTMAN, M., LANGE, R., TURKINGTON, T. K., & STRELKOV, S. (2004). First report of clubroot of canola caused by *Plasmodiophora brassicae* in the Canadian prairies. *Can*. *J*. *Plant Pathol*., *26*, 228–229.

WEBSTER, M.A., & DIXON, G.R. (1991). Boron, pH and inoculum concentration influencing colonization by *Plasmodiophora brassicae*. *Mycol*. *Res*., *95*, 74–79.