Evaluation of methods for sterilizing boysenberry leaves for downy mildew infection studies

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Introduction

Downy mildew of boysenberry (*Rubus* sp.), caused by the oomycete *Peronospora sparsa* (Fig. 1), is a major disease problem for New Zealand growers. Investigation of the biology and epidemiology of this biotrophic pathogen requires the production of sporangial inocula from leaf discs. This study assessed three sterilization methods to reduce surface contaminants on leaves used for sporulation.

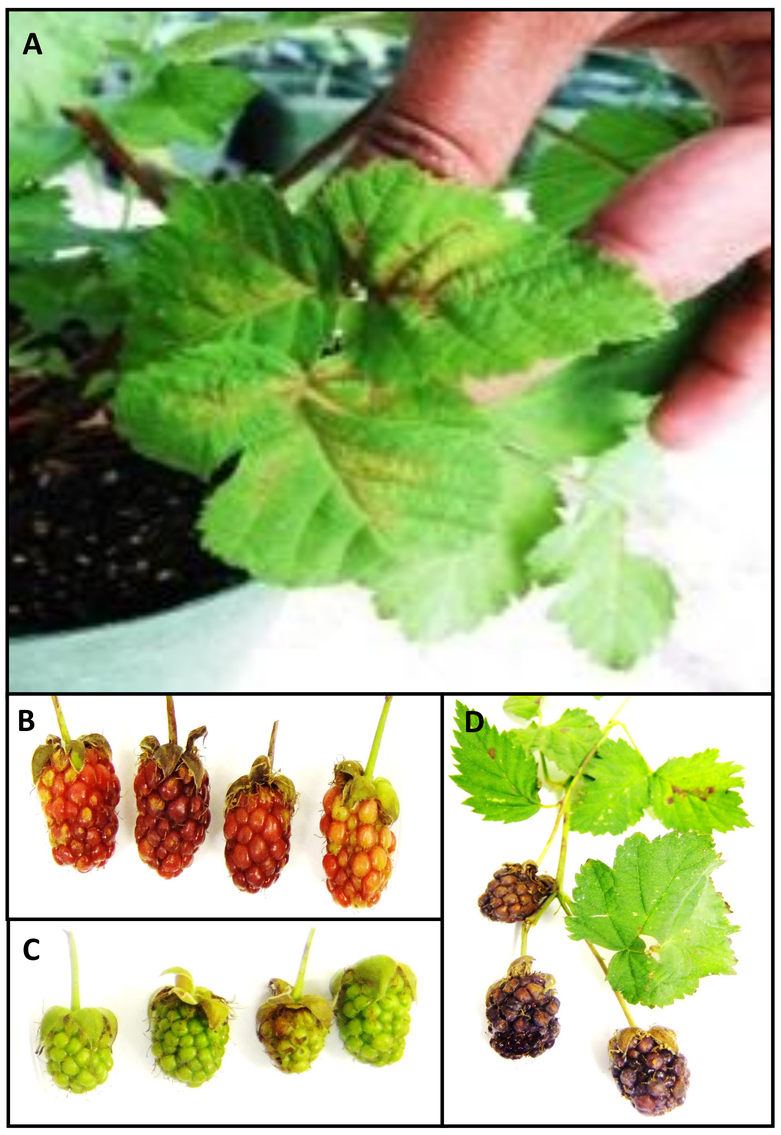

Figure 1. Downy mildew symptoms on boysenberry leaf (A), Healthy ripe (B) and unripe berries (C) and dryberries (D)

Methods

A. Methods of leaf sterilization

Detached boysenberry (*Mapua*) leaves (greenhouse)

- Treatments:
  - I. Control (no treatment)
  - II. Sterile water twice (5 min. each rinse)
  - III. 70% Ethanol (2 sec.) followed by sterile water (5 min.)
  - IV. 10% bleach (0.25% sodium hypochlorite) (30 sec.) followed by sterile water (5 min.)
- PDA and/or NA leaf imprints of upper and lower surfaces.
- Microbial enumeration after 7 days, 20°C (2 experiments).

B. Capability of infection on sterilized leaves

- Leaves inoculated on the lower surfaces, 3 x 20 µL drops of *P. sparsa* sporangia (2 x 10^4 /mL).
- Lesion incidence scored after 1 month on 1.5% WA (w/v) at 20°C.

Results

1. In Experiment 1 on PDA, all sterilization treatments had similar effects on numbers of fungal colonies ($P>0.05$; Fig. 2), with very few bacterial colonies.

2. In Experiment 2, numbers of fungi on PDA and NA , were greater for sterile water than ethanol or bleach ($P\leq0.05$; Fig. 2 & 3A).

Too few bacteria on PDA and NA to show differences between sterilization treatments, whereas numbers were greater on NA in the control treatment for upper surfaces ($P\leq0.05$,LSD 3.916).

In both experiments, leaf surfaces did not differ for bleach, ethanol or sterile water on PDA, but numbers of fungal colonies were greater on upper surfaces for sterile water and control treatments on NA ($P\leq0.05$; Fig. 2 & 3).

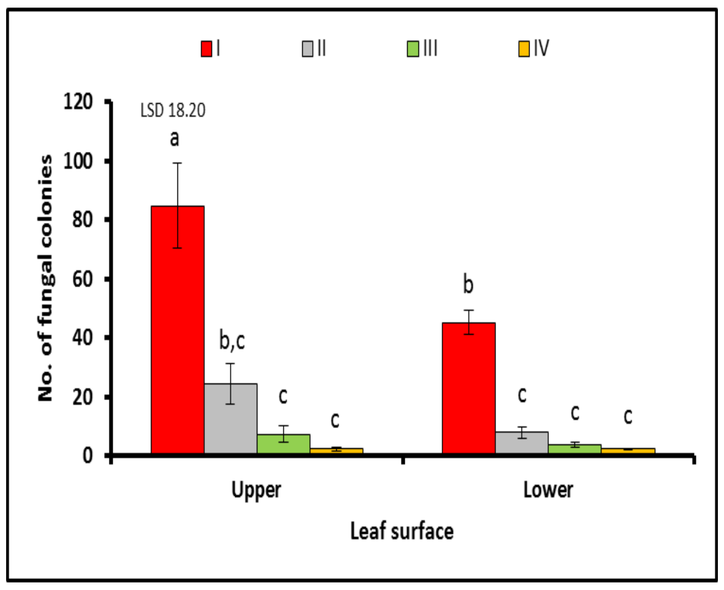

Figure 2. Number of fungal colonies on PDA from leaf surfaces after different sterilization treatments

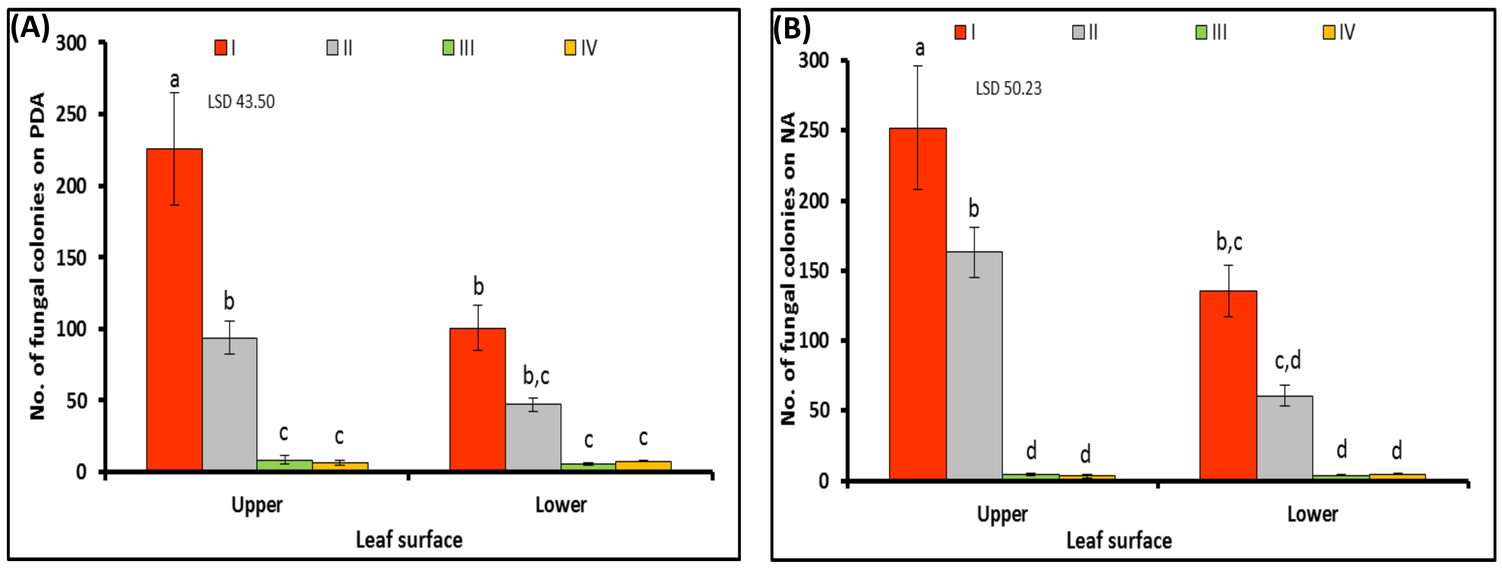

Figure 3. Number of fungal colonies on PDA (A) and NA (B), from leaf surfaces after different sterilization treatments

3. Leaves from the sterile water treatment had similar infection levels as the control. The ethanol treatment had the highest proportion of infection and the bleach had the lowest (Fig. 4 & 5).

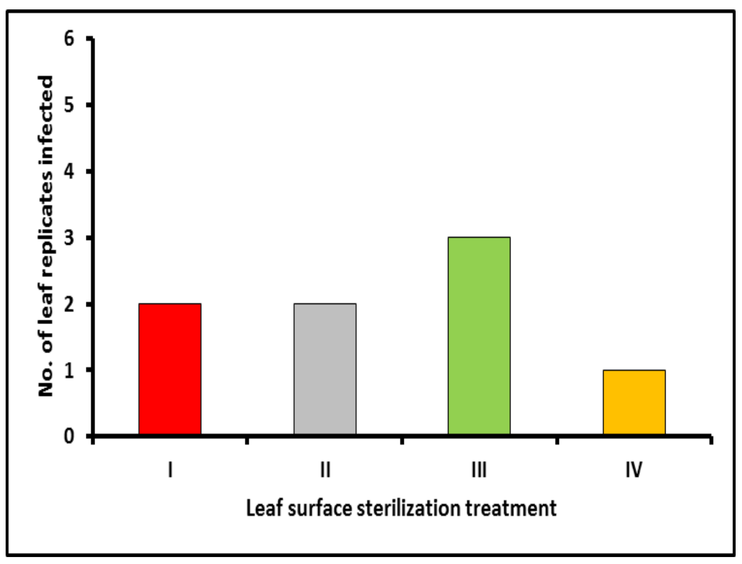

Figure 4. Effect of the sterilization for infection of leaves by *P. sparsa*

Figure 5. Susceptibility of leaf region (lower surface) to infection by *P. sparsa.*

Conclusions

1. Either PDA or NA can be used to recover fungi from leaf surfaces, but NA must be used for recovery of bacteria.
2. Although 70% EtOH and 10% bleach were better for surface sterilization, sterile water had least effect on infection success and so will be used in future experiments.

Acknowledgements

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