Biological Control 64 (2013) 138–142

Contents lists available at SciVerse ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Weevil borne microbes contribute as much to the reduction of photosynthesis in water hyacinth as does herbivory

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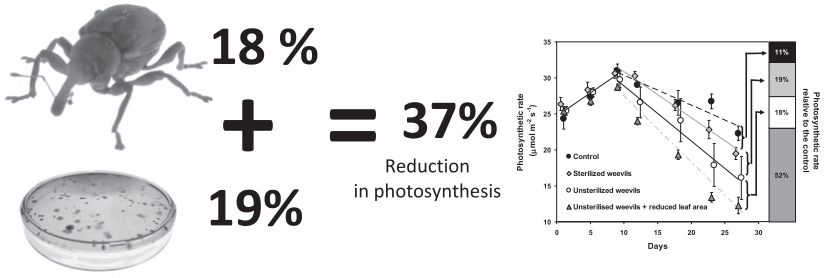
HIGHLIGHTS

- Water hyacinth weevils were demonstrated to be vectors for phyopathogens.
- Weevils carried both fungi and bacteria and transferred these to leaves on which they fed.
- These pathogens contributed as much to the decrease in photosynthetic productivity as did biomass removal.
- Hence, the selection and use of biocontrol agents needs to include their role as pathogen vectors to maximise efficiency.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 June 2012
Accepted 26 October 2012
Available online 5 November 2012

Keywords:
Photosynthesis
Microbes
Hosts
Water hyacinth
Weevil
Herbivory

ABSTRACT

Arthropods released for weed biocontrol can have effects other than simply removing biomass and frequently decrease photosynthetic rate more than can be attributed to the mere loss of photosynthetic surface area. Some of this effect may result because biological control agents facilitate the transfer and ingress of deleterious microbes into plant tissues on which they feed. We evaluated this facilitation effect using water hyacinth (*Eichhornia crassipes*) and a weevil (*Neochetina eichhorniae*) and compared the reductions in photosynthetic rates between leaves subject to herbivory by adult weevils sterilized with 3.5% chlorine bleach, to those that were unsterilized. The results showed that weevils carried both fungi and bacteria, transferred these to leaves on which they fed, and that microbes and biomass removal contributed almost equally to the 37% decrease in photosynthetic productivity. Hence, maximising the effectiveness of using arthropods that damage leaf surfaces for biocontrol requires the presence of microorganisms that are deleterious to plants.

1. Introduction

Water hyacinth (*Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae), is native to South America (Bechara, 1996), but has become an invasive in many parts of the tropics and subtropics forming dense mats on waterways that impact biodiversity, fisheries, transport and hydroelectric production (Mailu, 2001; Midgley et al., 2006). The only effective long-term control of this weed has been with biological control, particularly using the weevils *Neochetina eichhorniae* (Warner) and *N. bruchi* (Hustache) (Coleoptera: Curculionidae) (Hill and Olckers, 2001).

These weevil species reduce water hyacinth vigour by decreasing plant size, vegetative reproduction, and flower and seed production (Del Fosse, 1978; Coetzee et al., 2005). The weevil larvae tunnel in the petioles and crown of water hyacinth (Bashir et al., 1984), while adults chew small rectangular patches from the photosynthetic surfaces. This combined damage decreases the

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http://dx.doi.org/10.1016/j.biocontrol.2012.10.011

photosynthetic rate of leaves. However the effect is larger than can be attributed to the loss of photosynthetic surface area alone (Ripley et al., 2008), and suggests that weevils could facilitate the transfer and ingress of saprophytic and other deleterious microorganisms to plant leaves.

These introduced microbes may be airborne and enter via herbivory feeding scars, or may be carried on insect cuticles and in frass and therefore enter directly into the feeding scars (Paine et al., 1997; Moran, 2005). Introductions resulting from herbivory have been demonstrated for *N. eichhorniae* feeding on water hyacinth and for Scolytidae beetles feeding on conifers, both of which introduce pathogenic fungi into plants on which they feed (Charudattan et al., 1978; Paine et al., 1997; De Nooij, 1988). Microorganism infections elicit general symptoms that include increased respiration rates, increased permeability of plasma membranes, decreased photosynthetic rates, water and nutrient deficiencies (de Nooij et al., 1992; Lambers et al., 2008), all of which can contribute to decreased growth and cause necrosis of plant tissue (Del Fosse, 1978; Charudattan et al., 1978; Agrios, 2005; Hatcher, 1997).

This study aimed to determine the contribution of weevil-borne microbes to the decline in photosynthetic rate of water hyacinth and compared the effect of herbivory by unsterilized weevils with that of weevils that were externally sterilized of bacteria and fungi. This tests the hypothesis that these biocontrol agents decrease plant vigour, not only by removing biomass, but also by facilitating the transfer and entry of deleterious microbes to plant tissues.

2. Materials and methods

2.1. Plant and insect material

Water hyacinth plants were sourced from a wild population at New Years Dam, Eastern Cape Province, South Africa (33°17′35, 89″ S 26°07′18, 85″ E) and were maintained insect free in 70 L containers in a clear polythene tunnel. During the experimental period mean day/night tunnel temperatures were 40/21.5 °C, and humidity ranged between 34% and 90%. Containers were filled with tap water and supplied with 0.68 g L^{-1} of controlled release NPK fertiliser (Osmocote®) and 11.2 g L^{-1} of iron chelate to ensure healthy growth and vegetative production of daughter plants. Similar sized daughter plants, were selected from stock plants and four or five individuals were maintained in 25 L tubs under the same growth conditions. Weevils (*N. eichhorniae*) used for herbivory treatments were sourced from colonies maintained in a polythene tunnel at Rhodes University, which had originated from individuals collected from *E. crassipes* plants growing naturally on New Years Dam (33°17′35,89″ S 26°07′18,85″ E).

2.2. Weevil sterilization

Weevils were externally sterilized by placing four individuals in a plastic centrifuge tube with 15 ml of 3.5% sodium hypochlorite and vortex mixed for one minute under sterile conditions, followed by another two vortex mixes with sterilized distilled water. Weevils that were not subject to this procedure but sourced directly from the stock culture are referred to as unsterilized weevils.

The effectiveness of this sterilization procedure was determined by vortex mixing individual sterilized or unsterilized weevils in 1 ml of sterile deionised water. 100 µl aliquots of this aqueous extract were then spread onto potato dextrose agar (PDA) and nutrient agar (NA) plates under sterile conditions. Both types of plates were used in order to allow a broad spectrum of microorganisms to be cultured. Plates were incubated for 72 h at 25 and 32 °C, respectively, and the number of bacterial and fungal colonies that developed was counted. This was replicated three times. A similar procedure was used to determine the microorganisms associated with control leaves and leaves that were exposed to herbivory by unsterilized and sterilised weevils. Herbivory treatments were imposed by confining four weevils to an individual leaf, on five separate plants, for 4 days. Weevils were then removed from the leaves and these, and control leaves, were excised from plants and vortex mixed in 10 ml of sterile deionised water. 100 µl aliquots of these aqueous extract were then plated and analysed as above.

2.3. Herbivory treatments

The effect of herbivory by sterilized and unsterilized weevils on water hyacinth photosynthetic rates and gas exchange was compared to control leaves that were not subject to herbivory. Herbivory treatments were imposed by enclosing a single fully expanded leaf in a fine mesh bag with four weevils and this was replicated on five plants. Control leaves were enclosed in mesh bags without any weevils. Treatments were maintained on the same leaves for a period of 27 days and at intervals of between 3 and 5 days, leaf photosynthetic and gas exchange parameters were measured. On each of these occasions the weevils were removed from the leaves, and after gas exchange measurements, were replaced with four

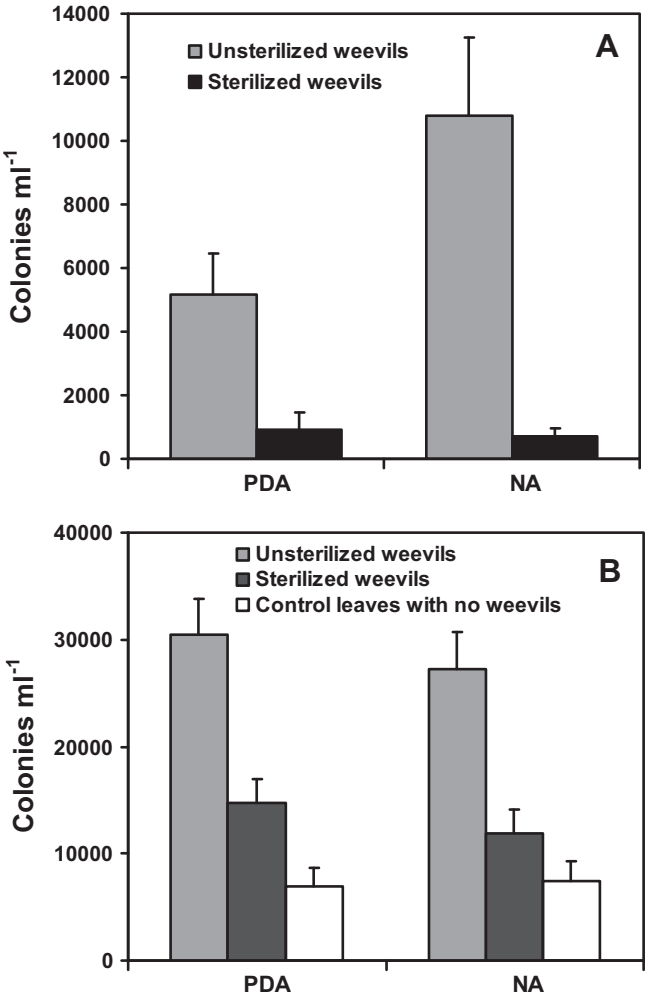

Fig. 1. Mean (SE) number of bacterial and fungal colonies washed from unsterilized or sterilized weevils (A; $n = 3$) or from control leaves and leaves subject to herbivory by sterilized, or unsterilised weevils (B; $n = 5$), and cultured on potato dextrose agar (PDA) or nutrient agar (NA).