Biological Control 121 (2018) 119–128

Contents lists available at ScienceDirect

Biological Control

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

The impact and production of the Brazilian peppertree biological control agent *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae) is affected by the level of host-plant fertilization

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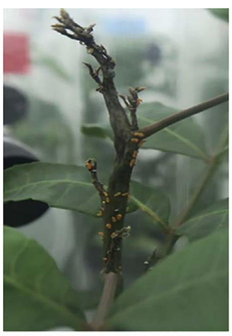
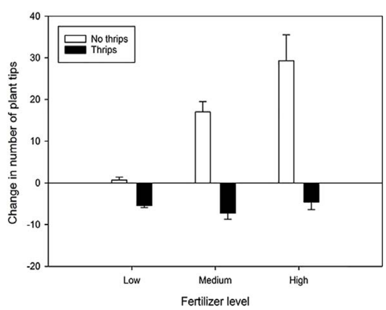
GRAPHICAL ABSTRACT





ARTICLE INFO

Keywords:
Schinus terebinthifolia
Thrips
Pre-release impact assessment
Mass production
Nitrogen reallocation

ABSTRACT

Brazilian peppertree, *Schinus terebinthifolia* Raddi (Anacardiaceae) is an invasive weed of natural and agricultural areas of Florida, Hawaii, and Texas (USA). A thrips, *Pseudophilothrips ichini* (Hood) is being developed as a potential agent for biological control of this invasive weed. As field releases are planned, methods are needed for the mass production and to predict the impact of the thrips. Brazilian peppertree seedlings were grown in potting media amended with three fertilizer (24N-8P-16K) levels: low (0 g/l water), medium (1.8 g/l water) or high (3.6 g/l water). The greatest number of *P. ichini* thrips developed on the medium fertilized plants. To measure *P. ichini* thrips impact, we compared growth of these fertilized seedlings with or without thrips. While maintaining the number of *P. ichini* thrips adults constant (20 adults), we measured the impact of one or two generations of feeding. Fertilizer level had a significant effect on all plant responses. Both one and two generations of thrips significantly decreased plant height, number of branches, leaflets, leaves, and tips. Two generations of thrips feeding caused significant reductions in branch, leaf, and total biomass. Thrips feeding increased percent nitrogen in roots and decreased percent nitrogen in branches at the medium and high fertilizer levels. Percent nitrogen of field collected stems and leaves matched that of the low fertilizer level. These results suggest a medium fertilizer application is best for mass production of *P. ichini* thrips. Moreover, sustained damage for more than one generation of thrips feeding will have a significant impact on total biomass of *S. terebinthifolia* seedlings. The use of fertilized nursery sites may help in initial establishment and field mass production of *P. ichini* thrips.

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https://doi.org/10.1016/j.biocontrol.2018.02.014
Received 15 November 2017; Received in revised form 9 February 2018; Accepted 15 February 2018
Available online 16 February 2018
1049-9644/ Published by Elsevier Inc.

1. Introduction

Introduced biological control agents are subjected to rigorous host range testing to assure they pose minimal direct threats to non-target species. However, once released, not all agents successfully exert population-level control on the target weed (Winston et al., 2014). Agents may establish and attain increased densities but exert weak or no population-level benefits (McFadyen, 1998). Not only is the development and release of ineffective agents a poor use of resources, but these agent populations can become superabundant, providing food web subsidies that might have far reaching unintended consequences (Callaway et al., 1999; Pearson and Callaway, 2003; Pearson et al., 2000). The selection of not only specific, but effective agents needs to be emphasized when deciding to release biological control agents (McClay and Balciunas, 2005). Pre-release studies that examine impacts of a biological control agent on target weeds can assist in predicting the benefits of potential releases (McEvoy and Coombs, 2000).

Brazilian peppertree, *Schinus terebinthifolia* Raddi (Anacardiaceae), is one of the worst weeds of agricultural, pastural and natural areas in South Florida, Hawaii, and South Texas (Schmitz et al., 1997; Yoshioka and Markin, 1991). Research on the biological control of this weed discovered a safe and apparently damaging thrips species, *Pseudophilothrips ichini* (Hood) (Thysanoptera: Phlaeothripidae) (Wheeler et al., 2017; Wheeler et al., 2016). To prepare for the implementation of this biological control, optimal rearing methods are needed to improve the likelihood of establishment. Typically, phloem feeding insects like thrips thrive when fed high nutrient plants (Butler et al., 2012). For example, damage from pest thrips is greater when they attack well fertilized plants and thrips populations can be reduced by growing plants at lower fertilizer levels (Chau and Heinz, 2006; Chau et al., 2005). However, little is known about the production of beneficial thrips species, such as biological control agents, at different fertilizer levels. Additionally, to predict the value of this biological control agent, the impact thrips might have on the target weed needs to be examined. Previous field observations in the native range indicated that *P. ichini* thrips adults and larvae attacked new leaves and stems at the actively growing tips, which were eventually killed, preventing the plant from flowering (Wheeler et al., 2016). Additionally, preliminary laboratory studies indicated that *P. ichini* thrips would decrease the growth of Brazilian peppertree seedlings (Manrique et al., 2014). The research presented here seeks to build on these results and determine the effect of fertilizer rate on thrips production. Also, we examined the effect of one and two generations of *P. ichini* thrips feeding on Brazilian peppertree plants grown at different fertilizer levels.

Pre-release assessments of an agent's potential efficacy to impact the target weed can be conducted with different approaches, all of which have limitations in interpretation (Blossey, 1995; McFadyen, 1998; Sheppard, 2003). Approaches to predict potential impact generally include manipulation of agent populations and measurement of plant responses (Briese, 1996; Ding et al., 2006; Huang et al., 2011; Manrique et al., 2014; Wang et al., 2012). While studies conducted in the native range with manipulated populations can be useful to predict herbivore impacts on weeds (Goolsby et al., 2006, 2004; Huang et al., 2011; Wang et al., 2012), interpreting the implications of these results for the invaded range may be challenging in that population-level impacts on the weed are difficult to determine (McClay and Balciunas, 2005). Although there has been keen interest in selecting the most effective agent prior to release (e.g., Blossey, 1995; Goeden, 1983; Harris, 1973), biological control research has mostly avoided the topic of potential impact, possibly due to a reluctance to commit scarce resources that must be spent on obligatory host specificity tests. Furthermore, results from these pre-release impact studies may lead to the rejection of otherwise effective agents (McClay and Balciunas, 2005, but see Goolsby et al., 2009, 2004). Despite these concerns, predictions of the impacts of potential agents are still requested by USDA testing protocols (TAG-BCAW-Manual, 2016). The results presented here include pre-release impact studies that predict the effectiveness of *P. ichini* thrips on Brazilian peppertree. The results predict the potential, rather than actual impact of the thrips feeding on Brazilian peppertree plants in the field.

2. Materials and methods

2.1. Plants – one thrips generation

In March 2016, seeds were planted at the USDA/ARS, Invasive Plant Research Laboratory, Fort Lauderdale, FL (26.085012° N, 80.240031° W). Seeds were collected locally from an on-site garden and planted in seedling trays containing Fafard Superfine Germinating Mix® (Sun Gro Horticulture, Agawam, MA) combined with minor nutrients (Southern Ag Essential Minor Elements®, GreenPro Solutions Inc., Jonestown, PA) (14 g/19 l germinating mix). When the first true leaves expanded, seedlings were transplanted into 3.8-l pots that contained Fafard 3B® potting media and a top dressing of the same minor plant nutrients (5 g/pot). The plants were placed outdoors in a common garden and two weeks after transplanting, one of three fertilizer treatments were assigned to each plant. Treatments included three levels of liquid Miracle Gro® All Purpose Plant Food (24N-8P-16K) applied at 0 g, 1.8 g, and 3.6 g/l water. Each fertilizer treatment was dispensed in 350 ml water/pot and was repeated every 2 weeks. These fertilizer levels were selected as they were expected to approximate a wide range of conditions from nutrient poor to rich sites in the invaded range. Furthermore, the highest fertilizer level was the recommended rate provided by the vendor. Plant placement in the common garden was rearranged randomly every week. Plants were kept free of pests with one insecticide application (acephate 9.4% active ingredient; Ortho Systemic Insect Killer®; Scotts Miracle Gro, Marysville, OH, USA; 29.6 ml per 3.8 l water) applied one month before the study began. Also, several applications of a Joy® dishwashing liquid solution (11 ml/3.8 l water) (Proctor and Gamble, Cincinnati, OH) were applied to prevent infestations of *Scirtothrips dorsalis* Hood (Chilli thrips) (Thysanoptera: Thripidae) prior to testing. However, no insecticides were applied within 4 weeks of testing. Once the plants were 4 weeks old (13–40 cm tall), they were introduced to the quarantine laboratory. After taking initial demography measurements (details below), all plants were placed inside vented acrylic cylindrical cages (45-cm tall × 15-cm diameter) and moved to a greenhouse 27 ± 2 °C; 60% ± 10% RH. Eight replicate plants of each fertilizer treatment were included.

2.2. Plants – two thrips generations

The methods used to produce experimental plants for the two thrips generation studies were similar to those described above with the following differences. In February 2017, locally collected seeds were planted with Fafard 4B® potting media including minor nutrients. Again, seedlings were transplanted into larger pots and the fertilizer treatments included three levels of liquid Miracle Gro® All Purpose Plant Food (24N-8P-16K) applied at 0, 1.8 g, and 3.6 g/l water. The same fertilizer treatments were repeated every two weeks. Weekly leaf and soil drenches of neem oil (Southern Ag Triple Action Neem Oil: Broad Spectrum Fungicide/Insecticide/Miticide, Southern Agricultural Pesticides, Inc., Palmetto, FL; 15 ml/946 ml warm water) for broad mites (*Polyphagotarsonemus latus* Banks (Acari: Tarsonemidae) was applied to all plants prior to *P. ichini* infestations. Ten replicate plants of each fertilizer treatment were included.

2.3. Insects – one thrips generation

Adult *P. ichini* thrips, of unknown age and sex, were collected from a quarantine colony maintained at the USDA/ARS, Invasive Plant Research Laboratory, Fort Lauderdale, FL, USA. Of the eight plants per fertilizer treatment, five plants were infested with 20 adult thrips and