Urban Forestry & Urban Greening 20 (2016) 97–102

Contents lists available at ScienceDirect

Urban Forestry & Urban Greening

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

The influence of systemic inducing agents on horse chestnut leaf miner (*Cameraria ohridella*) severity in white flowering horse chestnut (*Aesculus hoppicastanum* L.)

Glynn C. Percival[a,*], Simon P. Holmes[b]

[a] Bartlett Tree Research Laboratory, University of Reading, Cutbush Lane, Shinfield Field Site, Shinfield, Reading, Berkshire RG6 6AS, United Kingdom
[b] Tree Surveys, Unit H, Bridge Farm, Reading Road, Arborfield, Berkshire RG2 9HT, United Kingdom, United Kingdom

ARTICLE INFO

Article history:
Received 5 November 2015
Received in revised form 22 August 2016
Accepted 25 August 2016
Available online 26 August 2016

Keywords:
Insecticides
Integrated pest management
Leaf chewing insects
Plant health care
Secondary metabolites

ABSTRACT

Two separate trials during 2010 and 2011 were conducted using field grown white flowering horse chestnut trees (*Aesculus hippocastanum* L.) to asssess the insecticidal efficacy of three systemic inducing agents (SIR), namely (Messanger (active substance (a.s.) Harpin protein), Phoenix (a.s. Potassium phosphite) and Rigel (a.s. Salicylic acid derivative) applied to coincide with the appearance of each adult generation of the insect pest, horse chestnut leaf miner (*Cameraria ohridella* Deschka and Dimic; HCLM). A conventional synthetic insecticide (deltamethrin) used within the UK for HCLM control was included for comparison. A marked impact of SIR inducing agent and frequency of application on HCLM severity was recorded. The effectiveness of each SIR inducing agent on mean number of HCLM mines per leaf and percent HCLM larvae/pupae mortality increased when applied at increasing frequencies i.e. three sprays provided greater control than two sprays while two sprays provided greater control than one spray. The synthetic insecticide deltamethrin provided the greatest degree of HCLM control with two foliar sprays providing 100% HCLM control. Efficacy of SIR inducing agents based on reduction of HCLM mines per leaf after three sprays averaged across both the 2010 and 2011 growing seasons was in the order potassium phosphite > salicylic acid derivative > harpin protein > water control where leaf mining activity was reduced by 35–64%. Enhancement of leaf phenolic content was indicated as a means by which SIR agents reduced leaf mining activity. Results show commercially available SIR inducing agents exist that provide potentially acceptable degrees of HCLM control provided at least two sprays are applied during a growing season.

1. Introduction

Cameraria ohridella Deschka and Dimic, the horse chestnut leaf miner (HCLM) is now widely recognised as a destructive insect pest of the white flowering horse chestnut tree (*Aesculus hippocastanum* L.) throughout Europe (Kehrli and Bacher, 2003; Raimondo et al., 2003). Larva tunnel into the leaves of the tree feeding in the upper palisade parenchyma tissue in turn reducing photosynthetic activity and eventually causing physical destruction of leaf tissue (Percival et al., 2012; Straw and Bellet-Travis, 2004). Initially infested leaves become covered in small brown patches which rapidly spread throughout the canopy, giving the tree an autumnal appearance. Eventually infested leaves die and fall prematurely. If new leaves are formed they can be re-infected (Salleo et al., 2003; Thalmann et al., 2003).

Due to stringent UK government regulations regarding the registration and use of insecticides within urban landscapes few insecticides exist for HCLM control. Of those available diflubenzuron, an insect growth regulator has been shown to provide 80–100% control when applied as a foliar spray as the first generation of adult moths emerge (Tomiczek and Krehan, 1998; Percival et al., 2012). However, diflubenzuron is non-systemic and does not penetrate plant tissues, consequently total foliar spray coverage has to be achieved for successful control (Blümel and Hausdorf, 1997). Deltamethrin is a synthetic pyrethroid insecticide that possesses broad spectrum insect activity and persistence of 6–8 weeks on foliar tissue (Anon., 2015). Consequently, significant collateral damage to beneficial insecticides can occur (Ray and Fry, 2006). Organic pyrethroids while providing a rapid knock down of HCLM populations are an unfeasible option due to their rapid degradation in ultra violet light (Percival et al., 2012). Lack of persistence

* Corresponding author.
E-mail address: gpercival@bartlettuk.com (G.C. Percival).

http://dx.doi.org/10.1016/j.ufug.2016.08.009
1618-8667/

following application similarly occurs with insecticidal soaps and oils (Percival et al., 2012). Consequently HCLM control using these types of insecticides would rely on frequent spray applications which are not generally considered a long term cost effective management option (Percival et al., 2012; Straw and Bellet-Travis, 2004).

Tree resistance against pests and diseases can be enhanced by exposing plants to a range of natural and/or synthetic compounds such as inorganic potassium and phosphate salts, compost water extracts, low molecular weight proteins, oxalate, and unsaturated fatty acids (Bécot et al., 2000; Fobert and Després, 2005; Lattanzio et al., 2006; Percival, 2001). After exposure to these compounds a suite of physiological and biochemical changes occur within leaf, wood and root tissue to include synthesis of low-molecular-weight (LMW) compounds (phenolics, terpenoids, alkaloids) that possess toxic, antimicrobial, anti-nutritive and anti-digestive activity, synthesis of protein-based oxidative and hydrolytic enzymes, proteinase inhibitors, increased leaf lignification i.e. leaves become thicker, enhanced resin production and initiation of wound periderm (Bernards and Bastrup-Spohr, 2008; Keeling and Bohlmann, 2006; Facchini, 2001). The level of phenolic compounds in *A. hippocastanum* leaves has been shown to be of particular importance in distinguishing resilience to HCLM between the white and red horse chestnut (Oszmiański et al., 2014). Inducement of these physiological, biochemical and anatomical changes within plant tissue is termed systemic acquired (SAR) or systemic induced resistance (SIR). In essence, SIR is a form of pest and disease resistance caused by activation of the host plant's own genetically programmed defence pathways, resulting in changes that diminish the effects of subsequent insect pest and disease attack (Agrawal et al., 1999; Hammerschmidt, 2007). SIR potentially could prove to be a valuable tool in sustainable pest management as SIR induces multiple defence mechanisms which in turn reduces selection pressure against an insect pest or disease population making it less likely that genetic resistance to a particular insecticide can develop (Walters, 2009). SIR has been actively studied in herbaceous plant species, and, in recent years, woody plants, and is fast emerging as a potential, eco-friendly concept for enhancing tree resistance to pest and disease attack (Lattanzio et al., 2006; Percival, 2001). SIR elicitation in plants to protect against pathogenic bacteria and fungi of ecomomically important crops such as wheat, rice and potato has been recognized for over 100 years (Chester, 1933). By contrast, enhancing tree resistance against insect pests has received little investigation (Green and Ryan, 1972). Developments in plant protection technology have led to the formulation and commercialization of a range of SIR agents for use within the horticultural industry although their availability differs between countries (Percival and Haynes, 2008; Percival et al., 2009). Commercially available SIR agents include harpin protein (Trade name Messenger), benzothiadiazole, (Trade name Bion), potassium phosphite (Trade name Phoenix), salicyclic acid derivative (Trade name Rigel) and probanazole (Trade name Oryzemate®). Importantly, because of their limited non-direct chemical mode of action, SIR agents are less subject to the strict government legislative restrictions that relate to conventional synthetic pesticides used for insect control (Anon., 2015). The use of SIR agents as potential tree protectants against HCLM, has not been investigated.

Aims of this study were to i) evaluate the efficacy of foliar sprays of three commercially available SIR agents against HCLM, ii) compare their efficacy against an conventional synthetic insecticide registered for HCLM management, iii) determine the most effective dose rates and frequencies of application to achieve maximal control of HCLM over a growing season iv) elucidate the influence of each SIR agent on total leaf phenolic content and how this in turn influences resilience to HCLM.

2. Materials and methods

2.1. Field site and experimental trees

Planting distances were based on 2.5×2.5 m spacing. Bare rooted horse chestnut (*Aesculus hippocastanum* L.) were planted in November 2004 and trained under a central-leader system to an average height of $3.5\,\text{m} \pm 0.40\,\text{m}$ with mean trunk diameters of $10.0\,\text{cm} \pm 1.4\,\text{cm}$ at 60 cm above the soil level. The trial site was located at the University of Reading Shinfield Experimental Site, University of Reading, Berkshire (51°43/ N, −1°08/ W). The soil was a sandy loam containing 4–6% organic matter, pH of 6.4, available P, K, Mg, Na and Ca were 58.9, 619.3, 188.2, 54.5 and 2235.0 mg/L respectively. Weeds were controlled by hand prior to and during the study. No watering or fertilisation was applied during the two year trial. Historically the horse chestnut trees were attacked and infested with HCLM infestation on an annual basis. Prior to the trial commencing in 2010 trees were inspected in September 2009 and only trees with >70% of leaves affected with HCLM infestation with severe defoliation were included in the trial. A fungicide based on the triazole penconazole (Product name Topas, Headland Agrochemicals Ltd, Saffron Walden, Essex, UK) was applied every six weeks during each growing season commencing in early May and concluding in late September 2010 and 2011. All sprays were applied using a Tom Wanner Spray Rig sprayer at 50 ml penconazole per 100/L of water. Trees were sprayed until runoff, generally 0.50 L fungicide per tree.

2.2. Spray treatments and timing

All treatments (Table 1) were applied to coincide with the appearance of the adult moth at each generation namely: April 20, June 3 and August 17, 2010 and April 26, June 11, August 18, 2011. All SIR agents and deltamethrin were applied at the manufacturers recommended rate and double the manufacturers recommended rate (Table 1). To determine the influence of one vs two vs three sprays, in the case of the 2010 trial for example all 15 trees were sprayed on April 20, two thirds (10) of the trees were sprayed on June 3 and one third (5) of all trees sprayed on August 17. Foliar sprays of all products were applied until run-off using a 10 L Cooper Peglar electric back pack sprayer. During spray treatments polythene screens 3.5 m high were erected around each tree to prevent dispersal of sprays and possible cross contact of other trees. In addition two guard trees were used between each spray treated trees. Fifteen trees per product, 3 SIR agents, 1 synthetic insecticide $\times$ 2 doses $\times$ 1 water control were used; 135 trees in total. Trees were allocated into one of 3 blocks i.e. 3 replicates of 45 trees and randomised within each block. This experimental design was adopted in line with Official Recognition of Efficacy Testing Organisations in the United Kingdom guidelines for product efficacy testing and analysed as a three randomized complete block design.

2.3. Analysis of total leaf phenolic content

Analysis of total leaf phenolic content was conducted at week five after the final spray treatment by collecting six fully expanded leaves at random according to the height of the tree (2 leaves in the lower canopy, 2 in the middle canopy, 2 in the upper canopy) i.e. 6 leaves per treatment. One gram of green photosynthetic leaf tissue i.e. non-mined tissue previously dried for 24 h was placed into a 30 ml test tube and 10 ml of 80% aqueous methanol added. The suspension was stirred slightly and then tubes sonicated twice for 15 min. Tubes were then stored at 20 °C for 24 h. The extract was then centrifuged for at 1500*g* at 4 °C and the supernatant collected. Quantifiaction of total phenolic content was measured using the Folin–Ciocalteu colorimetric method (Gao et al., 2000). Plant