Cláudia da Silva Furtado

Lethal and sublethal effects of essential oils from *Pittosporum undulatum* and *Hedychium gardnerianum* on the egg parasitoid *Trichogramma cordubensis* (Hymenoptera: Trichogrammatidae)

UNIVERSIDADE DOS AÇORES
Ponta Delgada
Outubro de 2012

Cláudia da Silva Furtado

Mestrado em Biotecnologia em Controlo Biológico

Lethal and sublethal effects of essential oils from *Pittosporum undulatum* and *Hedychium gardnerianum* on the egg parasitoid *Trichogramma cordubensis* (Hymenoptera: Trichogrammatidae)

Orientadores:
Professora Doutora Patrícia Garcia
Professor Doutor José Silvino Rosa
Doutora Susana Cabral

UNIVERSIDADE DOS AÇORES
Ponta Delgada
Outubro de 2012

Agradecimentos

Em primeiro lugar, agradeço aos meus orientadores Professora Doutora Patrícia Garcia, Professor Doutor José Silvino Rosa e Doutora Susana Cabral por terem acedido orientar-me neste projecto final de mestrado. Sem as suas valiosas indiações e o seu conhecimento os meus passos não teriam sido, por certo, tão seguros. Agradeço, acima de tudo, a paciência e a disponibilidade inquestionável que sempre me dispensaram ao longo deste trabalho.

Agradeço ao Técnico Manuel Fernando pela recolha e irradiação dos ovos de *E. kuehniella*.

Agradeço aos meus amigos, açorianos e eborenses, que tanto me apoiram e incentivaram ao longo deste trabalho.

Aos meus Pais e Irmã, um agradecimento especial, pelo apoio incondicional que sempre me dedicaram ao longo desta e tantas outras jornadas.

Por fim, agradeço (mais uma vez) ao meu amigo e companheiro Marco Machado por tudo e mais alguma coisa.

Dedico este trabalho à minha família.

Resumo

Ensaios de laboratório e de campo têm demonstrado que os parasitóides do género *Trichogramma* são muito suscetíveis à maioria dos inseticidas de largo espectro, reduzindo a sua eficácia como agentes de controlo biológico. A integração deste agente de controlo biológico com outros métodos requer o conhecimento dos efeitos letais e subletais que os insecticidas podem ter sobre estes inimigos naturais. De modo a promover métodos ambientalmente seguros de controlo de pragas nos Açores, este trabalho teve como objetivo avaliar a segurança de óleos essenciais de *Pittosporum*m *undulatum* e *Hedychium gardnerianum* em diversos parâmetros biológicos do parasitóide oófago *Trichogramma cordubensis*, quando aplicado sobre ovos do hospedeiro, antes e depois do parasitismo. Para tal, avaliou-se os efeitos letais e sub-letais destes óleos às concentrações de 0.5% e 2.5%, por contacto direto e residual, na longevidade e fecundidade de fêmeas *T. cordubensis*, e na percentagem de emergência e tempo de desenvolvimento preimaginal da descendência. O óleo essencial que mais afetou os parâmetros biológicos do parasitóide, tanto por contato residual ou direto, foi *H. gardnerianum* à concentração de 2.5%. O número de ovos parasitados e a longevidade de *T. cordubensis* foram os parâmetros mais negativamente afetados. *H. gardnerianum* à concentração de 2.5%, teve um efeito residual elevado enquanto que nos outros tratamentos verificou-se o enfraquecimento do efeito ao longo do tempo. Quando aplicado sobre os ovos parasitados, as fases de desenvolvimento embrionário e larval foram as mais afectadas por *H. gardnerianum* a 2.5%. Os óleos essenciais de *P. undulatum*, às concentrações de 2.5% e 0.5%, e *H. gardnerianum* à concentração de 0.5% são compatíveis com libertações de *T. cordubensis*, visto que a estas concentrações têm um efeito residual reduzido e, quando aplicados após a fase larvar do parasitóide, os seus efeitos são neglígiveis. *P. undulatum*, à concentração de 0.5%, apresentou resultados promissores, indicando que pode ser usado com segurança em qualquer altura de exposição e fase de desenvolvimento de *T. cordubensis*. No entanto, mais estudos devem ser realizados de modo a determinar o efeito destes óleos essenciais no comportamento biológico de *T. cordubensis*, em condições de campo.

Palavras-chave: *Trichogramma*, efeitos secundários, contato direto e residual, extratos de plantas, *Pittosporum undulatum*, *Hedychium gardnerianum.*

Abstract

Laboratory and field studies have shown that *Trichogramma* wasps are highly susceptible to most broadspectrum insecticides, reducing their efficacy as biological control agents. Thus, the integration of this biological control agent with other methods requires knowledge of the lethal and sublethal effects they may have on these natural enemies. To promote environmentally friendly pest control methods in Azorean crops, this research aimed at assessing the safety of essential oils of *Pittosporum undulatum* and *Hedychium gardnerianum* on several biological parameters of the wasp *Trichogramma cordubensis*, when applied on host eggs before and after parasitism. We evaluated the lethal and sublethal effects of these essential oils at the concentrations of 0.5% and 2.5%, by residual and direct contact, on the longevity and fecundity of *T. cordubensis* and, on the offspring emergence rate and preimaginal development time. The essential oil that most affected the biological parameters of *T. cordubensis*, either by residual or direct contact, was *H. gardnerianum* at the concentration of 2.5%. The number of parasitized eggs and the longevity of *T. cordubensis* were the parameters most negatively affected. At the concentration of 2.5%, *H. gardnerianum* showed to have a high residual effect while, for the other treatments we observed a reduction of this effect over time. When applied on parasitized host eggs, the embryo and larval developmental stages of *T. cordubensis* were the most negatively affected by the treatment with *H. gardnerianum* at 2.5%. Our results suggest that the essential oils from *H. gardnerianum* at the concentration of 0.5% and, from *P. undulatum* at the concentrations of 0.5% and 2.5%, are compatible with *T. cordubensis* releases, since at such concentrations these extracts have a low residual effect and, when applied after completion of the larval developmental stage, their side effects are negligible. Furthermore, *P. undulatum* at the concentration of 0.5% showed promising results, indicating that it can be safely used at any time of exposure and preimaginal developmental stage of the *T. cordubensis.* However, more attention should be devoted to field experiments to more clearly determine the influence of these essential oils to *T. cordubensis* under agricultural conditions.

Keywords: *Trichogramma*, side-effects, residual and direct contact, plant extracts, *Pittosporum undulatum*, *Hedychium gardnerianum*

Índice

Chapter 1.

General Overview

The search for biologically active compounds from natural resources has always been of great interest to scientists looking for new sources and models for development of ecologically and environmentally friendly insect control agents (Isman, 2000; Lahlou, 2004; Isman *et al*., 2007; Rosa *et al*., 2009). The extensive use of pesticides is known to cause several problems in the environment, such as target pest resurgence and secondary pest outbreaks throughout the world's agroecosystems (Luck *et al*., 1977; Metcalf, 1986). Moreover, the reduction of the efficiency of biological control agents (BCA), such as parasitoids, in pesticide treated crop systems has been highlighted by several authors (Brunner *et al*., 2001; Hewa-Kapuge *et al*., 2003). Since insecticides may cause the death of the BCA (lethal effects) or change several other traits of their biology (either physiological or behavioural) without killing the individuals (sublethal effects), the success of Integrated Pest Management (IPM) programs depends, in part, on the optimal use of selective insecticides that are less harmful to natural enemies. Thus, in addition to direct mortality induced by insecticides, we should also have knowledge of the effects on physiological and behavioural traits of the natural enemies for a complete analysis of their impacts. (Desneux *et al*., 2007; Stark *et al*., 2007; Garcia, 2011).

An important way of avoiding this problem is the use of pesticides whose action spares natural enemies through either physiological or ecological selectivity (Saber *et al*., 2005). Essential oils, found in a variety of plants, have been suggested as an alternative source for insect control. These are volatile, natural, complex compounds characterized by a string odour, formed by aromatic plants belonging to a number of botanical families. Plants, as long-lived stationary organisms, must resist attackers over their lifetime, so they produce and exude constituents of the secondary metabolism, that play an important role in their defence mechanisms (Ntalli *et al*., 2011). Therefore, these chemical volatiles have functions in chemical defence, acting as insecticides (Bakkali *et al*., 2008), acaricides (Flamini, 2003), avoiding bacterial or fungi phytopathogen colonization (Karamanoli, 2002; Karamanoli *et al*., 2005), and/or attracting natural enemies of herbivores (Ntalli *et al*., 2011). Usually they are obtained by hydro-distillation and they comprise monoterpenes, sequiterpene lactones and triterpenes, which are examples of volatile plant compounds that may have commercial applications (Barney *et al*., 2005; Al-mazra'awi *et al*., 2009). Many of them are selective, biodegrade do nontoxic products and have few effects on the environment and non-target organisms (Isman, 2000). They can also provide an alternative for resistance

management because some plant extracts can be highly effective against insecticide-resistant insect pests (Isman, 2006; Ntalli *et al*., 2011). Thus, these plant compounds usually can be safely used in IPM (Schmutterer, 1992; Ahn *et al*., 1997; Yi *et al*., 2007).

The use of egg parasitoids for the control of pests has long been an essential part of pest management strategies in crop protection. Numerous egg parasitoids are effective natural enemies of important agricultural and forestry pests, killing the host before it causes any damage to the crops (Bastos *et al*., 2006). Among these, the genus *Trichogramma* Westwood has been considered for the control of pests for over than 100 years (Li-Ying, 1994). The genus *Trichogramma* is worldwide distributed and comprises about 145 described species. These minute wasps (0.2-1.5 mm) are solitary or gregarious endoparasitoids that can be easily produced in large scale using factitious hosts (Garcia, 1995). Moreover, the exploitation of their developmental pauses (diapause or quiescence) allows their storage at low temperatures for long periods, reducing the costs of mass rearing these insects (Garcia *et al*., 2002; Garcia *et al*., 2009). These egg parasitoids have a preference for Lepidoptera, but there are also records of parasitism on eggs of Coleoptera, Diptera, Hemiptera, Homoptera, Hymenoptera (Symphyta) and Neuroptera (Pinto *et al*., 1994).

Trichogramma cordubensis Vargas and Cabello (Hymenoptera: Trichogrammatidae) (Figure 1), a native thelytokous species of São Miguel Island (Azores, Portugal) is one of the most important species of egg parasitoids found on the island (Garcia *et al.*, 2006). According to Garcia (2000), this species is well adapted to the mild temperatures and high relative humidity that characterize the climate of the Azores, as well as to its natural hosts, that belong mostly to the family Noctuidae.

Figure 1. Female adult of *Trichogramma cordubensis* Vargas & Cabello (Hymenoptera: Trichogrammatidae), (30x).

Many studies have compared the relative toxicity of pesticides, including insecticides, fungicides and herbicides, to *Trichogramma* in screening trials. Laboratory and field studies have shown that these wasps are highly susceptible to most broad-spectrum insecticides, reducing their efficacy as BCA, by adversely affecting emergence rates (Hohmann, 1991, 1993; Suh *et al*., 2000; Takada *et al*., 2001; Vieira *et al*., 2001), adult mortality (Jacobs *et al*., 1984; Suh *et al*., 2000; Brunner *et al*., 2001; Vieira *et al*., 2001; Hewa-Kapuge *et al*., 2003) and parasitism of host eggs (Jacobs *et al*., 1984; Hagley *et al*., 1989; Hohmann, 1993; Hewa-Kapuge *et al*., 2003; Garcia *et al*., 2006). Thus, the integration of *Trichogramma* with chemical methods for pest management requires knowledge of the lethal and sublethal effects that the chemicals may have on the natural enemies, taking into particular consideration the developmental stages of the organism which will have the highest incidence of exposure (Yi *et al*., 2007).

To promote environmentally friendly pest control methods in Azorean crops, this research aimed at assessing the safety of the essential oils of *Pittosporum undulatum* Vent. (Pittosporaceae) and *Hedychium gardnerianum* Sheppard ex Ker-Gawler (Zingiberaceae), two invasive plant species of the Azores, to *T. cordubensis.* *Hedychium* is commonly known as ginger lilies and is a genus of herbs with thick, fleshy and branched rhizomes. Native to South Asia, the genus *Hedychium* has over 80 species, widely cultivated for ornamental purposes especially for their sweet scented flowers (Sabulal *et al*., 2007). *Heychium gardnerianum* was introduced in the Azores in the middle of the 19th century, spreading rapidly over the island of São Miguel, wherever the native forest becomes degraded, as well as being scattered in the dense laurel forest (Medeiros *et al*., 2003). The genus *Pittosporum* comprises about 150 species of tropical and subtropical Africa, Asia, Australia, New Zealand, and some Pacific Islands (Wagner *et al*., 1999). *Pittosporum undulatum* is an evergreen, 4-13m tree, with a fast growing leaf canopy, that is often used as an ornamental plant, due to its attractive fragrant flowers (Medeiros *et al*., 2003). It is native to south eastern Australia but has now spread to a great number of islands including the Azores. Its spontaneous spreading, has significantly transformed the islands' landscape, invading moist disturbed forests from low to middle elevations and threatening the survival of the native forest (Binggeli, 1998).

According to Rosa *et al*. (2009), the essential oils of these two plants are toxic and have ovicidal, growth inhibition and antifeedant activity against *Pseudaletia*

unipuncta (Lepidoptera: Noctuidae). This species, known as the armyworm, is a polyphagous insect and one of the most important pests of graminaceous crops, including pasture, in the Azores islands (Vieira *et al*., 2003). Other studies demonstrated that limolene and α-pinene, the main components present in the essential oils of these two plants, have been tested successfully against Coleopteran (Bekele *et al*., 2001; Ngamo *et al*., 2007) and other Lepidopteran pests (Miyazawa *et al*., 1998; Hummelbrunner *et al*., 2001). Furthermore, other recent studies have shown that the essential oils of *H. gardnerianum* display good molluscicidal (Teixeira *et al*., 2011; 2012) and antimicrobial activity against several *Staphylococcus* strains (Medeiros *et al*., 2003). Jadhav *et al*. (2007), reported *in vitro* insecticidal activity against human head lice by the essential oil of *Hedychium spicatum*, which is a species closely related to *H. gardnerianum*.

Therefore, and as suggested by Rosa *et al*. (2009), the essential oils of *H. gardnerianum* and *P. undulatum* can be exploited for the development of bioactive compounds as a new source of agrochemicals. Thus, in the present study we will evaluate the effects of these essential oils, by residual and direct contact, tested at two different concentrations, on the longevity and fecundity of the wasp *T. cordubensis* and, on the offspring emergence rates and preimaginal development times, to assess their possible integration in insect pest management programs.

References

Ahn J, Kwon M, Park H, Han C (1997) Potent insecticidal activity of *Ginkgo biloba* derived trilactone terpenes against *Nilaparvata lygens*. In: Hedin P A, Hollinworth R, Miyamoto J, Masler E, Thompson D (Eds.) Phytochemicals for pest control. American Chemical Society, pp 90-105

Al-mazra'awi MS, Ateyyat M (2009) Insecticidal and repellent activities of medicinalplant extracts against the sweet potato whitefly, *Bemisia tabaci* (Hom.: Aleyrodidae) and its parasitoid *Eretmocerus mundus* (Hym.: Aphelinidae). Journal of Pest Science 82:149-154

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils - A review. Food and Chemical Toxicology. 46:446-475

Barney JN, Hay AG, Weston LA (2005) Isolation and characterization of allelopathic volatiles from mugwort (*Artemisia vulgaris*). Journal of Chemical Ecolology 31:247-65

Bastos CS, Almeira RP, Suinaga F (2006) Selectivity of pesticides used on cotton (*Gossypium hirsutum*) to *Trichogramma pretiosum* reared on two laboratory-reared hosts. Pest Management Science 62:91-98

Bekele J, Hassanali A (2001) Blend effect in the oxicity of the essentials oils constituent of *Ocimum kikimandssharicum* and *Ocimum kenyense* (Labiateae) on two post-harvest insect pest. Phytochemistry 57:385–391

Binggeli P (1998) An overview of invasive woody plants in the tropics. School of Agricultural and Forest Sciences Publication Number 13, University of Wales, Bangor, UK.

Brunner J, Dunley JE, Doerr MD, Beers EH (2001) Effect of pesticides on *Colpoclypeus florus* (Hymenoptera: Eulophidae) and *Trichogramma platneri* (Hymenoptera: Trichogrammatidae), parasitoids of leafrollers in Washington. Journal of Economic Entomology 94(5):1073-1083

Desneux N, Decourtye A, Delpuech JM (2007) The Sublethal Effects of Pesticides on Beneficial Arthropods. Annual Review of Entomology 52:81-106

Flamini G (2003) Acaricides of natural origin, personal experiences and review of literature (1900-2001). Studies in Natural Products Chemistry 28(9): 381-451

Garcia P, Tavares J (1995) Parasitic capacity, longevity and development of *Trichogramma cordubensis* (Hym. Trichogrammatidae) at three temperature regimes. Les Colloques de l'INRA 73:71-74

Garcia P (2000). Biologia de *Trichogramma cordubensis* Vargas & Cabello (Hym., Trichogrammatidae) numa perspectiva de controlo biológico. PhD Thesis, Departamento de Biologia, Universidade dos Açores, pp 238

Garcia P, Wajnberg E, Pizzol J, Oliveira L (2002) Diapause in the egg parasitoid *Trichogramma cordubensis*: role of temperature. Journal of Insect Physiology 48:349-355

Garcia P, Cabral S, Oliveira L, Rodrigues A (2006) Effects of deltamethrin on the reproduction of *Trichogramma cordubensis* (Hymnoptera: Trichogrammatidae). Biocontrol Science and Technology 16(7):699-708

Garcia P, Pereira N, Oliveira L (2009) Side-effects of organic and synthetic pesticides on cold-stored diapausing prepupae of *Trichogramma cordubensis*. BioControl 54:451–458

Garcia P (2011) Sublethal Effects of Pyrethroids on Insect Parasitoids: What We Need to Further Know. Pp. 477-494. In: Margarita Stoytcheva (Ed.) Pesticides-Formulations, Effects, Fate., InTech, Croatia, pp 808

Hagley EAC, Laing JE, (1989) Effect of pesticides on parasitism of artificially distributed eggs of the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) by *Trichogramma spp.* (Hymenoptera: Trichogrammatidae). Proceedings of the Entomological Society of Ontario 120:25-33

Hewa-Kapuge S, McDougall S, Hoffmann A (2003) Effects of methoxyfenozide, indoxacarb, and other insecticides on the beneficial egg parasitoid *Trichogramma* nr. *brassicae* (Hymenoptera: Trichogrammatidae) under laboratory and field conditions. Journal of Economic Entomology 96(4):1083-1090

Hohmann C (1991) Efeito de diferentes insecticidas sobre a emergência de *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). Anais da Sociedade Entomológica do Brasil 20(1):59-65

Hohmann C (1993) Efeito de alguns insecticidas sobre adultos de *Trichogramma pretiosum* Riley. Anais da Sociedade Entomológica do Brasil 22(3):563-568

Hummelbrunner LA, Isman MB (2001) Acute, sublethal, antifeedant, and synergistic effects of monoterpenoid essential oil compound on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). Journal of Agricultural and Food Chemistry 49:715–720

Isman MB (2000) Plant essential oils for pest and disease management. Crop Protection 19: 603–608.

Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology 51:45-66

Isman MB, Akhtar Y (2007) Plant natural products as a source for developing environmentally acceptable insecticides. In: Ishaaya I, Nauen R, Horowitz AR (Eds.) Insecticidal design using advanced technologies. Springer-Verlag, The Netherlands, pp 235–245

Jacobs RJ, Kouskolekas CA, Gross HR (1984) Responses of *Trichogramma pretiosum* (Hymenoptera: Tricogrammatidae) to residues of permethrin and endosulfan. Environmental Entomology 13(2):355-358

Jadhav V, Kore A, Kadam VJ (2007) In-vitro pediculicidal activity of *Hedychium spicatum* essential oil. Fitoterapia 78:470–473

Karamanoli K (2002). Secondary metabolites as allelochemicals in plant defence against microorganisms of the phyllosphere. In: Reigosa M, Pedrol N (Eds). Allelopathy: from molecules to ecosystems. USA-Enfield (NH) Science Publishers Inc, pp 277–288

Karamanoli K, Menkissoglu-Spiroudi U, Bosabalidis AM, Vokou D, Constantinidou A (2005) Bacterial colonization of the phyllosphere of nineteen plant species and antimicrobial activity of their leaf secondary metabolites against leaf associated bacteria. Chemoecology 15:59–67

Lahlou M (2004) Methods to study the phytochemistry and bioactivity of essential oils. Phytotherapy Research 18:435–448

Li-Ying L (1994) Worldwide use of *Trichogramma* for biological control on different crops: a survey biological control with egg parasitoids. In: Wajnberg E, Hassen S (Eds.) CAB International, UK, pp 37–53

Luck F, Bosch R, Garcia R (1977) Chemical insect control – a trouble pest management strategy. Bio-science 27:27-34

Metcalf L (1986) The ecology of insecticides and chemical control of insects. In: Kogan M. (Ed.) Ecological theory and integrated pest management practices. John Wiley and Sons, New York, pp 251-257

Medeiros JR, Campos LB, Mendonça SC, Davin LB, Lewis NG (2003) Composition and antimicrobial activity of the essential oils from invasive species of the Azores, *Hedychium gardnerianum and Pittosporum undulatum*. Phytochemistry 64:561-565.

Miyazawa M, Wada T, Kameoka H (1998) Biotransformation of (+)- and (–)-limonene by the larvae of common cutworm (*Spodoptera litura*). Journal of Agricultural and Food Chemistry 46: 300–303

Ngamo TSL, Ngatanko I, Ngassou MB, Mapongmestsem PM, Hance T (2007) Insecticidal efficiency of essential oils of 5 aromatic plants tested both alone and in combination towards *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Research Journal of Biological Sciences 2:75–80

Ntalli N, Menkissoglu-Spiroudi U (2011) Pesticides of botanical origin: A promising tool in plant protection. Pp 3-24 In: Margarita Stoytcheva (Ed.) Pesticides-Formulations, Effects, Fate., InTech, Croatia, pp 808

Pinto J, Stouthamer R (1994) Systematics of the Tricogrammatidae, Biological Control with egg parasitoids. Wanjnberg E.; Hassan S. (Eds). CAB International, UK, pp 5-28

Rosa JS, Mascarenhas C, Oliveira L, Teixeira T, Barreto MC, Medeiros J (2009) Biological activity of essential oils from seven azorean plants against *Pseudaletia unipuncta* (Lepidoptera: Noctuidae). Journal of Applied Entomology 134:346-354

Saber M, Hejazi M, Kammal K, Moharramipour S (2005) Lethal and sublethal effects of fenitrothion and deltamethrin residues on the egg parasitoid *Trissolcus grandis* (Hymenoptera: Scelionidae). Biological and Microbial Control 98(1):35-40

Sabulal B, George V (2007) Chemical composition and antimicrobial activities of the essential oils from the rhizomes of four *Hedychium* Species from South India. Journal of Essential Oil Research 19:93–97

Schmutterer H (1992). Control of diamondback moth by application of neem extracts. In: Talekar S. and Griggs T. (Eds) Diamondback moth management and other crucifer pests. Proceedings of the 2nd International Workshop, Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp 325-332

Stark JD, Sugayama RL, Kovaleski A (2007) Why demographic and modelling approaches should be adopted for estimating the effects of pesticides on biocontrol agents. BioControl 52:365-374

Suh C, Orr D, Duyn J. (2000) Effect of insecticides on *Trichogramma exiguum* (Trichogrammatidae: Hymenoptera) preimaginal development and adult survival. Journal of Economic Entomology 93(3):577-583

Takada Y, Kawamura S, Tanaka T (2001) Effects of various insecticides on the development of the egg parasitoid *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae). Journal of Economic Entomology 94(6):1340-1343

Teixeira T, Rosa JS, Rainha N, Baptista J, Rodrigues A (2011) Assessment of molluscicidal activity of essential oils from five Azorean plants against *Radix peregra* (Müller, 1774). Chemosphere 87(1):1-6

Teixeira T, Rainha N, Rosa J, Lima E, Batista J (2012) Molluscicidal activity of crude water and hexane extracts of *Hypericum* species to snails (*Radiz peregra*). Environmental Toxicology and Chemistry 31(4):748–753

Vieira A, Oliveira L, Garcia P (2001) Effects of Conventional pesticides on the preimaginal development stages and on adults of *Trichogramma cordubensis* (Hymnoptera: Trichogrammatidae). Biocontrol Science and Technology 11:527-534.

Vieira V, Pintureau B, Tavares J, McNeil JN, (2003) Differentiation and gene flow among island and mainland populations of the true armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae). Canadian Journal of Zoology 81:1367–1377

Wagner WL, Herbst D, Sohmer S (1999) Manual of the Flowering Plants of Hawai'i. 2 vols. Bishop Museum Special Publication 83, University of Hawai'i and Bishop Museum Press, Honolulu, HI.

Yi C, Kwon M, Hieu T, Jang Y, Ahn Y (2007). Fumigant toxicity of plant essential oils to *Pluttela xylostella* (Lepidoptera: Yponomeutidae) and *Cotesia glomerata* (Hymenoptera: Braconidae). Journal of Asia-Pacific Entomology 10(2):157-163