Generating knowledge about the *Dracaena marginata* pest complex to improve management strategies for crops exported from Costa Rica

by

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ABSTRACT

Dracaena marginata, an important ornamental plant exported by Costa Rica, represents 4% of the country's Gross Domestic Product. Its market has grown from $70 million in 2005 to $85 million in 2008; one of the main markets being the United States (Arce et al. 2009). However, a plant size limitation of 18 inches for vegetative material of *D. marginata* by the US authorities due to pest infestation risks, has constrained production. Additionally, the repeated interception of shipments with quarantine pests, predominantly represented by leafhoppers, katydids, armored scales and snails, jeopardizes its future in the export market.

This study aimed to develop knowledge on the population dynamics of the pest complex found in *D. marginata* and the actual relationship between plant size and pest incidence. It looked at the role of weed cover on insect abundance and fluctuations of the populations through time to try and detect windows of opportunity to maximize the effectiveness of control measures. The relationship between pest abundance in the field and the interception rate of export shipments at the ports of entry in the United States was also studied. Specific studies were also carried out on the dynamics of the leafhopper *Empoasca* sp. and the efficacy of chemical insecticides for its control. This cicadellid species was considered particularly important at the interception level because its eggs are abundant on the plant leaves but undetectable during ordinary inspection.

Four groups of quarantine pests (leafhoppers, katydids, armored scales and snails) were present in *D. marginata* fields throughout the year. However, population fluctuations were not statistically significant after one year of monthly evaluations. Parasitism by micro hymenopteran wasps was important, but there were periods of high leafhopper egg populations where very low parasitism occurred. There were no statistical differences regarding plant size and pest incidence. However, there was a trend of finding higher pest populations in shoot tips with smaller cane heights, possibly due to proximity to pest populations found in the ground cover.

There were no statistical relationships between the level of infestation in the field and the number of interceptions at the ports of entry in the US. However, a significant correlation was found between Index of Units Shipped and the number of interceptions, indicating that the capacity of exporters to maintain quality control at the packing houses decreases as the size of the shipment increases.

An effective staining procedure (Neergaard 1997) was selected for detecting and quantifying *Empoasca* eggs on *D. marginata* leaves. This tool allowed for the determination of fluctuations on the abundance of *Empoasca* eggs under different weed cover compositions. The absence of weed cover induced a high oviposition of *Empoasca* on *Dracaena*, possible due to decreased host availability. *D. marginata* plants growing with the covercrop *Drymaria* had higher egg densities whereas plants growing with weed cover composed by Rubiaceae, grasses, and weedy cover showed lower pest incidence.

Imidacloprid based insecticides killed 100% of the hatching nymphs of Empoasca when applied as a curative or preventive treatment. Imidacloprid and Deltamethrin killed 100% of the Empoasca adults four weeks after application but dropped at week six. Muralla and Carbaryl killed 100% at week four and 93% at week six.

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DEDICATION

To Kathy, Dany and Pri: Source of strength and light in my life.

To my parents, for giving me the chance to start this long journey.

A Kathy, Dany y Pri: Fuentes de Fortaleza y luz en mi vida.

A mis padres, por darme la oportunidad de iniciar este largo viaje.

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CHAPTER 1

General Introduction

Dracaena marginata as crop bound for the export market

The genus *Dracaena* belongs to the botanical family Ruscaceae. Its center of origin is located in tropical and subtropical regions of Africa, Asia and Australia (Sanchez 2003, Poole et al. 2006). This genus comprises about 40 species from which six species *D. deremensis*, *D. fragrans*, *D. marginata*, *D. reflex*, *D. sanderiana*, and *D. surculosa* (godseffiana) are cultivated as foliage plants. These species are favored as interior ornamental plants because of their diverse shapes, colors and forms available in the market and because of their ability to survive under low-light conditions with minimum care (Chen et al. 2002).

The species *D. marginata* is native to Madagascar and has lanceolate, olive green leaves with reddish edges (Lötschert and Beese 1983). Three varieties of this plant that have been commercialized for export include: the green variety that matches the description above, the tricolor, with a yellow band separating the red and green stripes, and magenta variety in which the edges are dark highlighting deep purple appearance (Figure 1.1). The plants can reach a height of up to 3 m (Sánchez 2003).

Dracaena marginata is grown as ornamental crop in tropical regions of the world for export to temperate climates for use as indoor houseplants. In Costa Rica, different varieties are grown for the export market to the United States. The most commonly produced are bicolor, green and magenta. *D. marginata* is sold in a wide variety of forms including bare canes, unrooted tips, branches and rooted plants (Acuña et al. 1991). The plant is grown in Costa Rica at sites that are a few meters above sea level (m.a.s.l.) up to 1200. They are generally grown under full sun exposure (Acuña et al. 1991). Predominantly small to medium size stakeholders, ranging from one to five hectares, manage production of these plants as a family business. Dracaenas are part of the ornamental products, which represent 4% of the Gross Domestic Product in Costa Rica. The ornamental export market has grown from $70 million in 2005 to $85 million in 2008 (Arce et al. 2009) and *D. marginata* represents around 30% of this figure. However, the future of *D. marginata* exports has been jeopardized by the level of interceptions of quarantine pests in shipped plants to the United States market.

Figure 1.1. Varieties of *D. marginata:* green, magenta and Bicolo, from left to right.

Propagative material, destined for the US market is strictly controlled and cannot be larger than 45.72 cm (18 inches). This regulation is based on the assumption that taller plants are more prone to pest incidence due to their longer exposure under field conditions. As reported by Colpetzer (2005), leafhoppers, katydids, armored scales and snails are the most frequently reported pests on *Dracaena* shipments sent from Costa Rica to the United States. These invertebrate pests are commonly found associated with *D. marginata* plantations throughout the different growing areas in Costa Rica, however, very little is known about their population dynamics and the actual relationship between plant size and pest incidence.

Pests of quarantine importance for the United States market associated with *Dracaena marginata.*

Pest constraints of those plants bound for the export market are driven by the quarantine restrictions imposed by the United States and Europe to reduce risks to their agricultural industries. Principle pests of quarantine importance include a snail, *Succineacostarricana* von Martens (Gastropoda, Stylommatophora, Succineidae), and three families of insects: katydids (Orthoptera: Tettigoniidae), armored scales (Hemiptera: Diaspididae) and leafhoppers (Hemiptera: Cicadellidae) (Coltpetzer 2005). Prado et al. (2008)

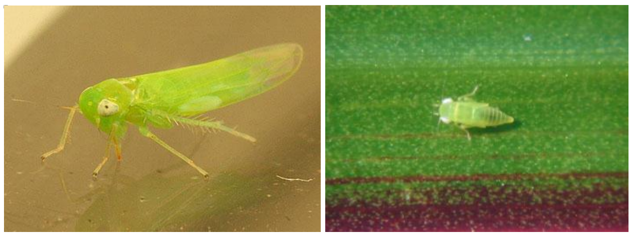
used a systematic sampling method to study the distribution, abundance and interactions of this group of pests, as a first step toward identifying opportunities to reduce the population of quarantine insects in *Dracaena* plantations. Their study determined that leafhopper abundance could be explained by differences in insecticide and herbicide use patterns in each of the production zones and patterns of precipitation. It also revealed that management practices, which targeted leafhoppers resulted in increased abundance of secondary pests such as armored scales and katydids because of impacts on natural enemy communities.

The pest complex of *D. marginata*

Dominant pest species identified during recent ecological studies in *D. marginata* production systems in Costa Rica include: *Oncometopia clarior, Caldwelliola reservata and Empoasca sp* (Hemiptera: Cicadellidae), *Microcentrum* sp. and *Conocephalus* sp. (Orthoptera: Tettigoniidae) (CSP 2008), *Aspidiotus* sp., *Chrysomphalus* sp. and, *Pinnaspis* sp. (Hemiptera: Diaspididae) (Prado 2006, CSP 2008), together with snails *Succineacostarricana* and *Ovachlamys fulgens*. These data are consistent with the taxonomic grouping of pests detected in exported shipments that have been intercepted at ports inspection in the United States (Coltpetzer 2005, PIN 2006). Each of these pests has been reported to be present wherever *Dracaena* is grown in Costa Rica.

According to the figures reported by PIN (2006) and the identification made by CSP (2008), three species *Oncometopia clarior, Caldwelliola reservata and Empoasca sp* (Hemiptera: Cicadellidae) are responsible for 41% of the interceptions of *D. marginata* in the port of Miami, Florida. *Oncometopia clarior* (Homoptera: Cicadellidae) (Figure 1.2a) is a xylem feeding leafhopper of the proconiini tribe (King and Saunders 1984) that has been identified as one of the main quarantine pests in *Dracaena marginata* for the US market (CSP 2008). This species has a wide range of plant hosts including citrus, and species in the Malvaceae, Amaranthaceae, Asclepidaceae and Poaceae. It is widely distributed and known for its capacity to transmit plant pathogenic bacteria *Xylella fastidiosa*, a causing agent of Pierce´s disease in citrus trees (Paiva et al. 2001) and grapes (Blua et al. 1999). Females insert their egg masses under the leaf cuticle using their ovipositor and then cover them with a layer of white powder called brochosome (Rakitov 2004; Hans et al. 2005). This protein-based powder protects the eggs against bacterial infections and parasitic wasps (Rakitov 2004). The eggs hatch purple nymphs after about 15 days. This insect passes through 5 nymphal stages (King and Saunders 1984). Adults and nymphs can feed on a wide variety of plants and studies suggest that they need to feed on more than one species. Studies carried out in *Dracaena marginata* in Costa Rica, show that although adults feed and lay eggs on the crop they spend more time on weeds like *Cyathula prostata*and *Lantana camara*, where they can also feed and lay their eggs (Pérez 2007). *Empoasca fabae* (Homoptera: Cicadellidae), also known as the potato leafhopper (Figure 1.2b), has a wide range of hosts (more than 200 plant species) including beans, sweet potatoes, potatoes, grasses (King and Saunders 1984), *Dracaena marginata* (CSP 2008), and other crops. They lay single eggs, about 0.5 mm long within the young leaf blades near the leaf nerves, taking between 12 to 15 days to hatch under tropical conditions. *Empoasca* also passes through 5 nymphal stages of white-green color, lasting about 15 days to become adults. The adults are a light green color, about 3mm long and can live up to 60 days. The females mate and then feed for a few days before starting to lay eggs. Both adults and nymphs feed and hide in the underside of the leaf (King and Saunders 1984). In potatoes, *E. fabae* can transmit a virus and also causes feeding damage known as hopper burn that significantly reduces plant photosynthetic capacity (Kaplan et al. 2008). In *Dracaena marginata*, nymphs remain hidden and feed on young leaves, whereas adults move contiuously between the crop and weed cover, especially into grasses (CSP 2008). Feeding damage on *D. marginata* appears as lines of white dots on the leaf blade as they feed on the phloem. Heavy infestations can cause cosmetic damage to the plant but no disease transmission has been reported for this plant. The small size of the eggs and its position within the leaf makes it very difficult to find and to control, since the waxy surface of the leaf offers a good protection against contact insecticides. The small nymphs coming out of the tips are a cause of interceptions when the plant is exported to the United States (CSP 2008). *Caldwelliola reservata* Fowler (Hemiptera: Cicadellidae) is a phloem feeder from the tribe cicadellini (Figure 1.2c) from which little information is available in the literature. King and Saunders (1984) describe the insect as beige in color, about 5-7 cm long and producing eggs of about 3 mm. This species is also a vector of the plant pathogenic bacteria *Xylella fastidiosa* (King and Saunders 1984, McKamey 2006).

a- *Oncometopia clarior*: adult, nymph and egg mass



b- *Empoasca* sp.: Adult and nymph

c- *Caldwelliola reservata*: Adult, nymph and eggs.

Figure 1.2. Principle cicadellid leafhoppers and their life cycle stages associated with *Dracaena marginata*: a) *Oncometopia clarior*, b) *Empoasca* sp, c) *Caldwelliola reservata*.

Armored scales (Figure 1.3a) are Heteropterans from the Diaspididae family. The females are sessile and develop a hard waxy cover, ranging from brown to black in color, under which the soft body is protected while males have wings and can fly (Powell and Lindquist 1994). The eggs are kept underneath the waxy cover and hatch when the female dies (Pape 1976). The first instar are called crawlers, are very mobile and are the spreading stage of the insect becoming sessile when they reach the second instar (Davis 1991). Armored scales are sucking insects and the main damage to the plant is cause by feeding activity. The injection of toxins produces yellow to reddish spots I the leaves, whereas plants may die under heavy infestations (Pape 1976)

Katydis (Figure 1.3b or bush crickets belong to the family Tettigoniidae, they are very variable in size, are commonly green and live in herbage or trees. Their wings often resemble leaves and have flatten, knifelike ovipositor and are generally phytophagous or omnivorous (Guillott 2005).

Succinea costaricana von Martens (Gastropoda, Stylommatophora, Succineidae) (Figure 1.3c) is one of the commonly found snail species on *D. marginata* (CSP 2008). It has been recorded from Costa Rica to Mexico (Monge-Nájera 1995). Some species of the genus *Succinea*are agricultural pests, and can be vectors of diseases or parasites (Godan 1983). Although *S. costaricana* does not damage the foliage of *D. marginata*, it is important since it is considered a quarantine pest (Villalobos and Monge-Najera 2004).

Figure 1.3. Armored scale (Florida red scale, *Chrysomphalus aonidum*)(A), Katydid (B) and Snail (*Succineacostaricana*)(C)

This study aimed at comparing pest abundance on commercial *D. marginata* plants of different sizes under a variety of field conditions, to determine if there is a relation between these two factors. The reduction of pest abundance solely, through improved timing of pesticide applications in the field, may not be sufficient for reducing the interception of quarantined pests. Studies of quarantine pests in red ginger flowers, *Alpinia purpurata* indicated that 100% control of the pest complex could only be achieved by applying foliar sprays of insecticides in the field just before harvest and by dipping and washing flowers after harvest (Hata et al. 1992). Thus, the second objective of this study was to determine if there was a relationship between the relative abundance of quarantine pests in *Dracaena marginata* production fields and the rates of their interception at the US Port of Miami over the course of the year. Presence or absence of these relationships is critical to identify control points that can reduce rates of interception of pests on *Dracaena* plants.

OBJECTIVES

General objective

The objective of this study was to generate information on the dynamics of four quarantine pests for *D. marginata* (leafhoppers, armored scales, katydids and snails) in order to understand the role of key factors such as seasonal abundance and the effects of weed cover composition and plant size on pest incidence.

Specific objectives

1. Determine the seasonal abundance of pest populations and their natural enemies, and the probable factors producing this behavior.
2. Determine the relation between seasonal pest abundance and the interception rate of exported *D. marginata* plants to the United States market.
3. Study the relationship between pest abundance and plant height.
4. Determine the impact of different weed covers on the incidence of cryptic cicadellid eggs and suggest a potential mechanism for the differences.
5. Use the generated knowledge on seasonal pest abundance, plant size and the influence of weed cover on cryptic egg incidence, and pesticide efficacy and longevity to propose a management strategy aimed at reducing pests in *D. marginata* for the export market to the United States.

HYPOTHESIS

1. The populations of pests of *D. marginata* fluctuate throughout the year, exhibiting peaks that require early control actions to avoid reaching undesirable pest levels.
2. The peaks of pest populations in the field can be correlated with an increase in interceptions of *D. marginata* shipments sent to the United States market.
3. Taller plants have a higher risk of being infested with pests due to the longer period of exposure to field conditions.

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CHAPTER 2

Seasonal abundance of quarantine pests on *Dracaena marginata* fields in two production zones of Costa Rica

INTRODUCTION

The foliage plant *Dracaena marginata* is commonly grown by Costa Rican nursery producers and is a critical component of the $85 million per year export market of ornamental products in this country (Arce et al. 2009). Domestically there are relatively few phytosanitary problems of economic concern, which are limited to minor cosmetic damage caused by fungal and bacterial infections (Griffith 1987, Infoagro 2010). In contrast, the sale of plants bound for export can be blocked when plants are found to contain pests of regulatory importance to the importing country. Quarantine restrictions against regulated pests are designed to reduce the risk of spreading exotic pests in the importing countries while facilitating continued trade. These levels of tolerance are based on pest risk assessments and backed up by efficient technical actions and measures for prevention, detection and eradication (Mumford 2002). Protocols for detection of pest and diseases at the ports of entry have been shown to be statistically efficient (Venette *et al.* 2002). However, the increasing volume of agricultural products in the international market, pose a growing risk for invasive species to pass through customs undetected.

Mumford (2002) states that, “the effectiveness of a quarantine measure should be judged by its ability to reduce the expected probability of successful invasion, rather than the certainty of preventing it.” From this stand point and in order to lower the risk from the country of origin, it is necessary to take preventive actions at the early stages in the production process. Technical knowledge is required for increasing the efficacy of pest management strategies in the production fields bound for the export market and must come from understanding the existing system. For *D. marginata*, this process starts by properly identifying the quarantine threats and understanding their ecology. Principle pests of quarantine importance for this crop include a snail, *Succinea costarricana* von Martens (Gastropoda, Stylommatophora, Succineidae), and three families of insects: katydids (Orthoptera: Tettigoniidae), armored scales (Hemiptera: Diaspididae) and leafhoppers

(Hemiptera: Cicadellidae) (Coltpetzer 2005). Within these groups, leafhoppers have been responsible for about 75% of these interceptions in the United States over the past 20 years.

Prado *et al.* (2008) used a systematic sampling method to study the distribution, abundance, and interactions of this group of pests, as a first step towards identifying opportunities to reduce the population of quarantine insects in *Dracaena* plantations. Their study determined that leafhopper abundance could be explained by differences in terms of insecticide and herbicide use in each of the production zones and patterns of precipitation. It also revealed that management practices which targeted leafhoppers resulted in increased abundance of secondary pests such as armored scales and katydids because of impacts on natural enemy communities. They identified a need to exploit seasonal population dynamics to improve the level of the pest management. Therefore, the first objective of this study was to improve our understanding of seasonal pest dynamics and to identify potential control points in different production zones through monthly sampling over the course of a year.

MATERIALS AND METHODS

The study was conducted in Costa Rica, in two of the main production zones for *D. marginata* bound for the export market to the United States: San Carlos, in the Northern portion of the country and Guápiles-Guácimo, in the Atlantic zone, both zones with differences in climatic, landscape and crop management characteristics (Figure 2.1). The first zone has a mean altitude of 170 m, mean temperature, and precipitation of 25ºC and 3500mm respectively. The Atlantic sites have a mean altitude of 262 m, mean annual temperature of 25ºC and precipitation of 4500 mm. In both zones, rainfall is most abundant in two periods during the year: May through July and November through January (Figure 2.2).

Figure 2.1. Location of study zones in Costa Rica.
Source: http://maps.lohallas.com

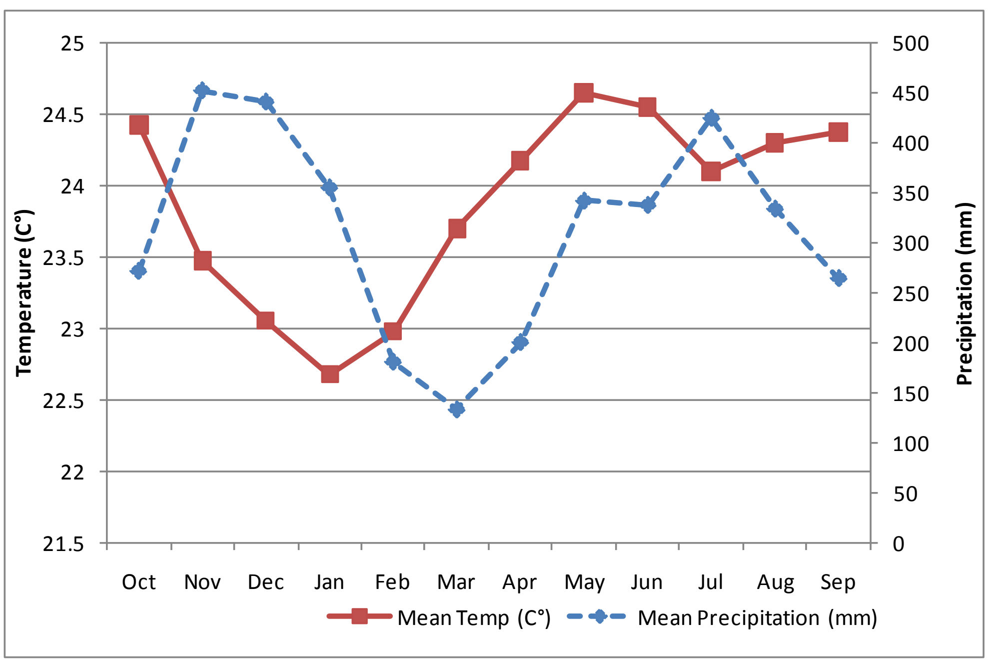

Figure 2.2. Monthly precipitation and temperature (means from eight years, 2000-2008) for the Northern zone (Ciudad Quesada, San Carlos) and Atlantic zone of Costa Rica (Limón) (IMN 2008),

The plantations in the Atlantic zone are located mostly in flat lands drained by ditches previously used by banana plantations. Many of the farms in this area apply herbicides and insecticides frequently to manage pests. The Northern area has a more irregular topography and the *D. marginata* is grown mixed with patches of forest and other ornamental crops, such as Croton, *Schefflera*, and other species of Dracaena. Relative to the Atlantic zone, herbicide, insecticide and fertilizer use is less frequent (Prado *et al.* 2008).

In order to monitor pest abundance throughout the year, we selected three commercial plots of approximately 2100 m^2 for each of the three most commonly grown varieties of *D. marginata* (green, bicolor and magenta), nine plots in each zone for a total of 18 plots. Plot sizes fitted within the size range of 800 to 10000 m^2 used by Prado *et al.* (2008). Plots were distributed over a total of seven farms: two in the Atlantic zone and five in the Northern. The mean number of pests per plot and the mean number of commercial tips per plot infested with quarantine insect pests (leafhopper eggs, leafhopper nymphs, katydid eggs, snails and armored scales) were estimated for each sampling date. The abundance of armored scales was only reported as number of infested tips due to the difficulty to accurately count individuals in the field.

Sampling method

Sampling points within the plots were identified using a modification of the systematic sampling method described by Prado *et al.* (2008). We used a 10 m x 10 m grid sampling at each point of intersection for a total of 32 sampling points per plot. Each sample consisted of nine commercially marketable tips of *D. marginata* randomly taken from the plants found within a one meter radius from each intersection within the grid. Three tips were sampled at three heights (strata) within the plants (>50 cm, 50 to 100cm and <100cm), to achieve a total of nine tips per sampling point. The abundance of leafhopper eggs and nymphs, katydid eggs, presence of armored scales and snails, on marketable tips was assessed during each sampling period. The leaves found with pests were removed from the sampled tips to avoid recounting during successive evaluations. The removal of infested leaves was considered to have no significant effect on the overall pest population due to the foliage density on the sampling plots. The plots were sampled monthly over a period of one year, completing a total of 12 sampling dates. The sampling process ran from October 2006 to September 2007.

Statistical design and analysis of field data

To investigate factors contributing to populations of pests observed in the field, the study was organized in a factorial design in which the sources of variation were the Production Zone (with two levels: Atlantic and Northern areas), Variety (green, magenta and bicolor) and Sampling Date (twelve monthly samplings). We performed an analysis of variance (ANOVA) using linear mixed model theory to take into account the repeated measures in time with the statistical program Infostat (Di Rienzo et al. 2010). Means were compared using Fisher's LSD with a significance of P=0.05. We used general correlation functions to fit the correlations over time and likelihood ratio test (LRT) and Akaike (AIC) and Bayesian (BIC) Information Criteria to evaluate the best correlation model (Di Rienzo et al. 2010; Balzarini et al. 2004).

Estimating Rates of Interception at US Ports

To determine if our estimates for monthly pest abundance on marketable tips in the field was related to the monthly interception rates at the port of Miami, we used the information extracted from the USDA APHIS PIN database provided to the author by representatives of the Department of Plant Health from the Ministry of Agriculture of Costa Rica (SFE-MAG). The database provides detailed information on dates, exporter identification, number of plant tips per intercepted shipment, plant genus or species and quarantine pest problems found. Shipment statistics for *D. marginata* were clustered with other foliage ornamentals in the export data base from Costa Rica making it impossible to determine the real number of shipments sent to the United States during the period of this study. In order to establish the proportional relationship of interception rates to export volume we used the mean number of plant tips contained in each of the intercepted shipments as an index of the monthly volume of export.

To determine the relationship between interceptions of quarantine pests at the Port of Miami and populations in the field we conducted two different analyses. We first determined if there was a relationship between the average monthly density of tips infested with at least one pest in the production field and the number of interceptions in Miami. This analysis does not account for how changes in the volume of tips shipped could influence the number of detections at the port. For this reason we conducted a second analysis using the ratio of number of shipments intercepted each month to adjust our estimate of average number of plants contained in each shipment. Linear regression analysis was performed to determine the presence of a significant relationship with the statistical package InfoStat. We assume that populations in the sampled fields were representative of other fields in the country. We tested this assumption by examining how patterns of pest abundance, in relation to season and variety, corresponded to those found in the study carried out by Prado et al. (2008), where a much larger number of different sized plots were sampled in two different moments: dry season (January-March) and rainy season (late April through August).

RESULTS

We observed a wide variation, for the combined results of both zones, in the relative abundance of leafhopper eggs, leafhopper nymphs, katydid eggs, snails and the proportion of *D. marginata* tips positive with the presence of these pests and armored scales throughout the year. The number of katydid eggs ($F = 2.74$; $df = 11, 132$; $p < 0.0032$) and the proportion of plant tips with this pest ($F = 2.32$; $df = 11,132$; $p < 0.0121$) were very low but were influenced significantly by the sampling date with highest abundance in November in both cases (Tables 2.1 and 2.2).

Leafhopper eggs from the species *Oncometopia clarior* and *Caldwelliola reservata* were predominantly found in all sampled sites, whereas species such as *Diestostema* sp. and *Gypona* sp. were only occasionally found. The small size and cryptic oviposition pattern of *Empoasca* sp. made it impossible to quantify their eggs during field evaluations; however, the presence of *Empoasca* nymphs on *D. marginata* plants in all sampling sites indicates that this is one of the more abundant species.

Table 2.1. Mean number[1] of pest per tip and month in 18 commercial plots of *Dracaena marginata* in Costa Rica from October 2006 to September 2007.

	Pest mean[1] ± SE							
Date	Leafhopper eggs		Nymphs		Katydid eggs[2]		Snails	
Jan	0.058a	±0.059	0.062 a	±0.058	0.00074b	±0.0024	0.0005.6a	±0.0024
Feb	0.027 a	±0.024	0.027 a	±0.024	0.0030ab	±0.009.6	0.0004.1a	±0.012
Mar	0.023 a	±0.024	0.023 a	±0.024	0.0028ab	±0.007.5	0.0004.1a	±0.016
Apr	0.025 a	±0.023	0.026 a	±0.026	0.0019ab	±0.007.1	0.0001.9a	±0.00082
May	0.026 a	±0.026	0.026 a	±0.026	0.00040ab	±0.001.2	0a	±0
Jun	0.051 a	±0.051	0.051 a	±0.052	0.0033ab	±0.01.2	0.0005.6a	±0.0024
Jul	0.037 a	±0.035	0.037 a	±0.035	0b	±0	0.0002.5a	±0.0073
Aug	0.029 a	±0.035	0.028 a	±0.035	0.00056b	±0.002.4	0.00019a	±0.00080
Sep	0.027 a	±0.042	0.025 a	±0.040	0.00042b	±0.001.2	0.00019a	±0.00082
Oct	0.021 a	±0.025	0.021 a	±0.026	0.00094ab	±0.002.5	0.00041a	±0.010
Nov	0.008 a	±0.012	0.029 a	±0.081	0.0027a	±0.003.7	0.00096a	±0.0025
Dec	0.019 a	±0.045	0.019 a	±0.045	0.00042b	±0.001.2	0.00011a	±0.0032

[1] Each plot has an average of 30 sampling points. A sample is composed by nine marketable plant tips taken from each sampling point on a ten by ten meter grid.

[2]Means within the same column followed by the same letter are not significantly different (P<0.05; LSD).

Table 2.2. Proportion of tips per plot infested with leafhopper eggs and nymphs, armored scales, snails and katydid eggs, found during monthly sampling of 18 commercial plots of *Dracaena marginata* in Costa Rica October 2006 to September 2007.

	Pest mean[1] ± SE											
Date	Leafhopper eggs		Nymphs		Armored Scales		Katydid eggs[2]		Snails		Proportion of parasitized eggs[3]	
Jan	0.026	±0.023a	0.071	±0.065	0.004	±0.008a	0.001	±0.002 b	0.001	±0.002a	0.16	±0.27c
Feb	0.027	±0.024a	0.065	±0.037	0.004	±0.010a	0.003	±0.010 ab	0.004	±0.012a	0.25	±0.31 c
Mar	0.023	±0.023a	0.073	±0.076	0.004	±0.011a	0.003	±0.008 ab	0.004	±0.016a	0.25	±0.38 c
Apr	0.025	±0.023a	0.072	±0.033	0.006	±0.014a	0.002	±0.007 ab	0.000	±0.001a	0.42	±0.50 c
May	0.026	±0.025a	0.067	±0.080	0.005	±0.011a	0.000	±0.001 b	0.000	±0a	0.60	±0.33 abc
Jun	0.051	±0.051a	0.031	±0.029	0.013	±0.032a	0.003	±0.012 ab	0.001	±0.002a	0.72	±0.38 abc
Jul	0.037	±0.035a	0.066	±0.080	0.006	±0.011a	0.000	±0 b	0.002	±0.007a	0.60	±0.34 bc
Aug	0.029	±0.035a	0.094	±0.055	0.005	±0.009a	0.001	±0.002 b	0.000	±0.001a	0.72	±0.16 abc
Sep	0.027	±0.041a	0.140	±0.090	0.004	±0.011a	0.000	±0.001 b	0.000	±0.001a	0.70	±0.21 abc
Oct	0.021	±0.025a	0.093	±0.087	0.014	±0.023a	0.001	±0.002 ab	0.004	±0.010a	0.73	±0.38 abc
Nov	0.008	±0.012a	0.089	±0.096	0.021	±0.019a	0.003	±0.003 a	0.001	±0.002a	0.95	±0.08 a
Dec	0.013	±0.021a	0.042	±0.039	0.013	±0.023a	0.000	±0.001 b	0.001	±0.003a	0.87	±0.22 ab

[1] Each plot has an average of 30 sampling points. A sample is composed by nine marketable plant tips taken from each sampling point on a ten by ten meter grid.
[2]Means within the same column followed by the same letter are not significantly different (P<0.05; LSD).
[3]Calculated as the total number of eggs per month divided by the total parasitized eggs.

Leafhopper egg masses were more abundant in January followed by June with 0.058 and 0.051 egg masses per plant tip respectively. There was an interaction between crop variety and sampling date ($F = 3.38$; $df = 22.132$; $p < 0.0001$), the higher population found in January came from *D. marginata* var. magenta whereas the second peak in June was found on bicolor (Figure 2.3). There was an interaction between zone, sampling date and variety that significantly affected the number of leafhopper nymphs per tip ($F = 2.32$; df = 22,132; $P < 0.0018$). The highest amount of nymphs (0.64 nymphs per tip) was observed in December, on the Green variety, in the Northern zone followed by Magenta (0.63 nymphs per tip) in the same zone and date. The variety Bicolor, in the Atlantic zone, showed peaks of population in November, February, April, June and August that were above the rest of the varieties (Figure 2.4).

The population of leafhopper nymphs was higher in January and June with 0.062 and 0.051 nymphs per tip respectively (Table 2.1), with significant differences between varieties per sampling date (F = 3.39; df = 2.132; p < 0.0001). The highest population was observed on magenta in January followed by bicolor in June (Figure 2.4). Snail population was significantly affected by the combination of sampling date, plant variety and production zone finding higher numbers on bicolor in February and March (F = 1.67; df = 2,131; p < 0.0404) and two smaller peaks in bicolor-October and bicolor-July in the northern zone (Figure 2.5)

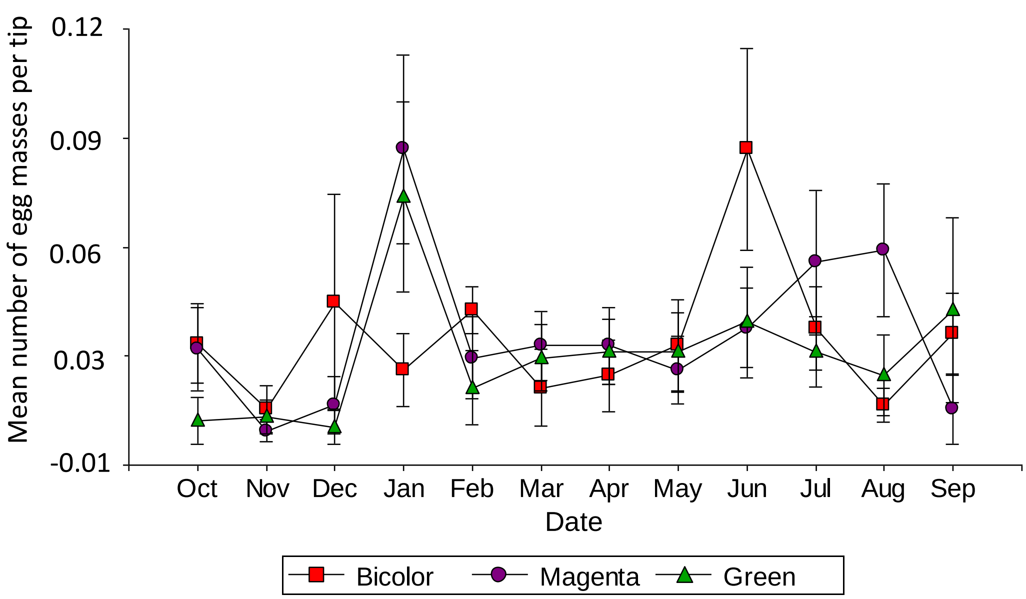

Figure 2.3. Average number of leafhopper eggs masses (*Oncometopia clarior* and *Caldwelliola reservata*) per tip of three varieties of *Dracaena marginata* (bicolor, magenta and green) monthly collected from 18 commercial plots in Costa Rica, from October 2006 through September 2007.

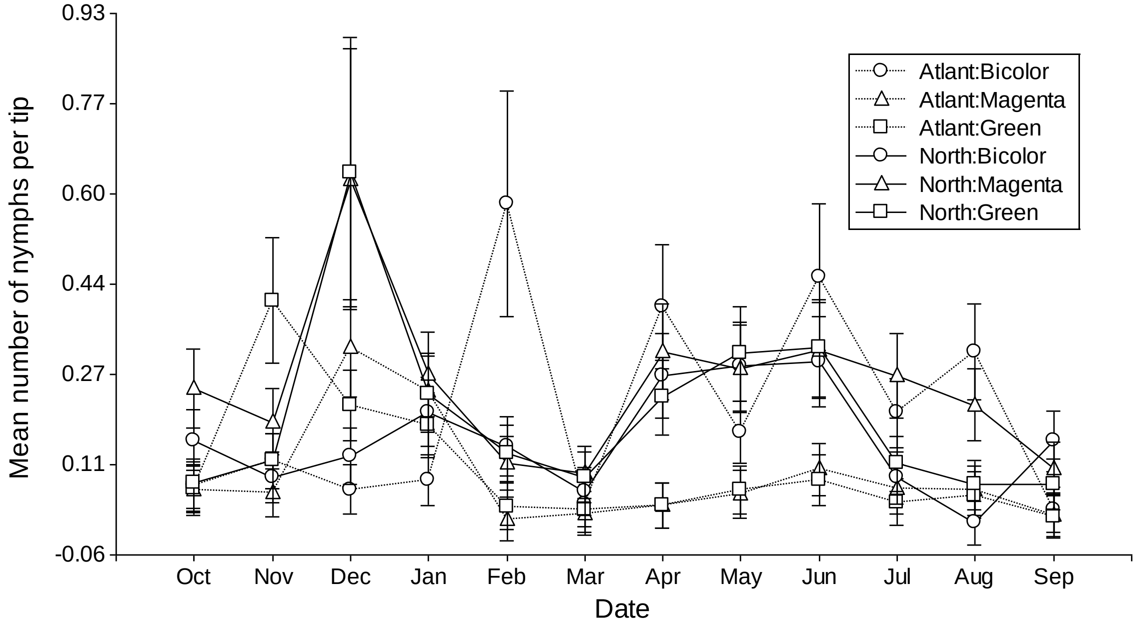

Figure 2.4. Average number of leafhopper nymphs per tip of three varieties of *Dracaena marginata* (Bicolor, Magenta and Green) collected monthly from 18 commercial plots in Costa Rica, from October 2006 through September 2007.

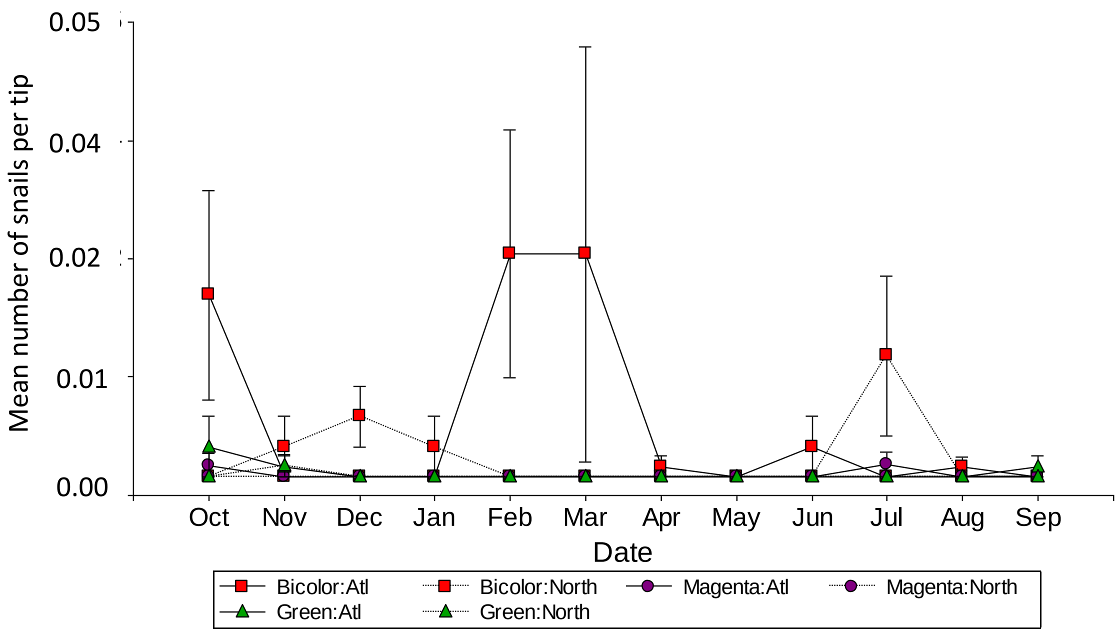

Figure 2.5. Average number of snails, *Succienea costarricana* (Gastropoda, Stylommatophora, Succineidae), per sampled tip, found on three varieties of *Dracaena marginata*, in two production regions in Costa Rica from October 2006 through September 2007.

The proportion of pest infested tips followed a similar pattern to that observed for the mean number of pest per tip. The peaks of infested tips were found for bicolor with leafhopper eggs in June ($F = 2.81$; df = 22,132; $p < 0.0001$, Figure 2.6), leafhopper nymphs on bicolor in June ($F = 3.02$; df = 22,132; $p < 0.0001$, Figure 2.7), snails on bicolor in February for the Atlantic zone ($F = 1.68$; df = 22,132; $p < 0.0386$, Figure 2.5) and armored scales on bicolor in June ($F = 4.38$; df = 22,132; $p < 0.0001$, Figure 2.8 and Table 2.2).

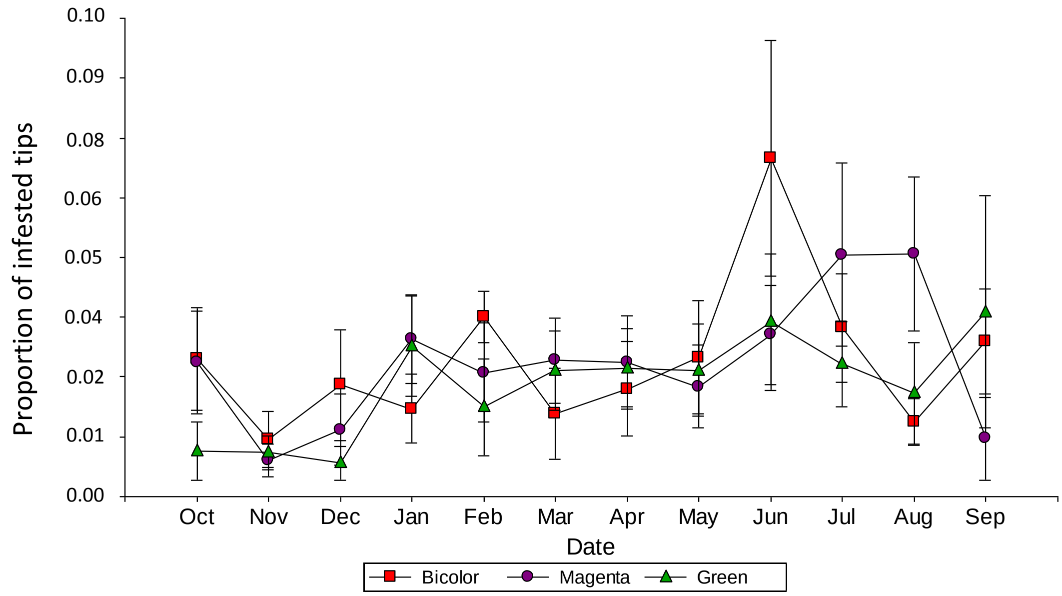

Figure 2.6. Average proportion of shoot tips infested with leafhopper eggs (*Oncometopia clarior* and *Caldwelliola reservata*) on three varieties of *Dracaena marginata* (bicolor, magenta and green) monthly collected from 18 commercial plots in Costa Rica, from October 2006 through September 2007.

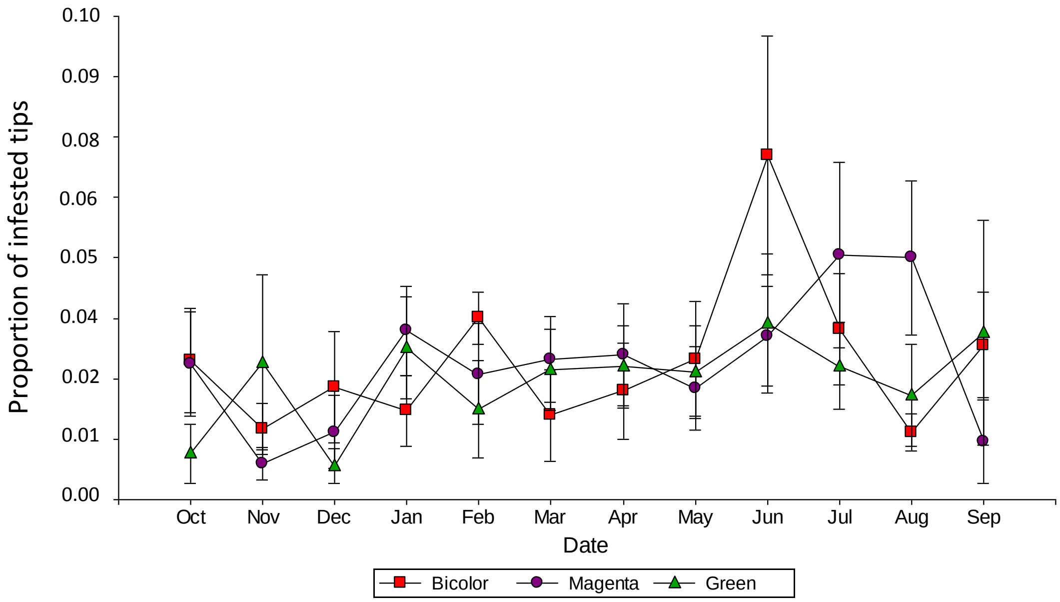

Figure 2.7. Average proportion of shoot tips infested with leafhopper nymphs (*Empoasca* sp., *Oncometopia clarior* and *Caldwelliola reservata*) found in three commonly grown varieties of *Dracaena marginata* per month collected from 18 commercial plots in Costa Rica from November 2006 to October 2007.

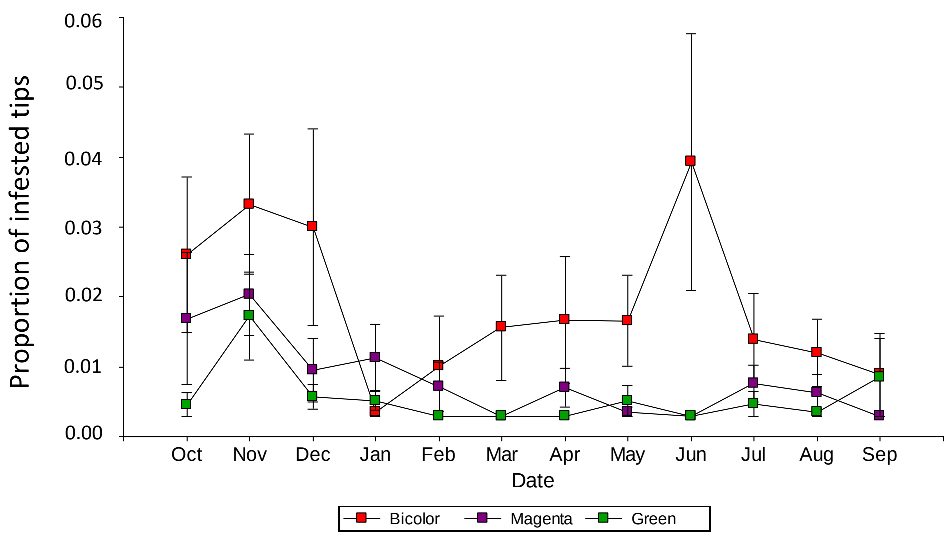

Figure 2.8. Average proportion of shoot tips infested with armored scales on three varieties of *Dracaena marginata* (bicolor, magenta and green) monthly collected from 18 commercial plots in Costa Rica, from October 2006 through September 2007.

Leafhopper eggs were parasitized by *Gonatocerus* spp. (Hymenoptera:Mymaridae) in all the productions zones and crop varieties studied. The percentage of parasitism was higher during the last three months of 2006 but dropped drastically reaching the lowest point in January 2007, when the leafhopper egg population was at its peak. However, there was no correlation between the number of eggs and the percentage of parasitism ($r_s(22)=0.48$, $p=0.1100$) After leafhopper egg population dropped in February, the curve of parasitism gradually increased throughout February-March and closely followed the trend of the relative abundance curve of leafhopper eggs from May to September (Figure 2.9). The differences in parasitism were significant between months ($F = 6.9$; df =2.25; $p = 0.0041$) (Table 2.2) and followed a tendency similar to the precipitation curve (Figure 2.2) but without correlation between the variables ($r_s(22) = 0.52$, $p = 0.0800$) and no apparent relation with the changes in mean temperature. Although the trend of the parasitism curve was similar for the three varieties of the crop, there was a significant difference on the level of parasitism ($F = 9.27$, df = 11,25 $p = 0.0001$) showing higher percentages on bicolor (Figure 2.10).

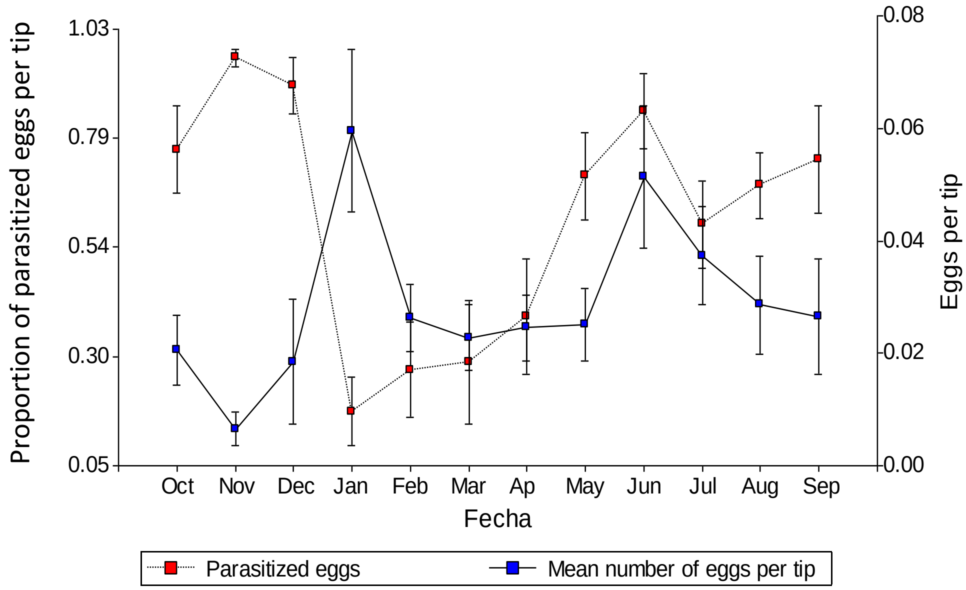

Figure 2.9. Average number of leafhopper eggs (*Oncometopia clarior* and *Caldwelliola reservata*) per shoot tip of sampled *D. marginata* plants and average proportion of parasitized eggs sampling date, from 18 commercial production plots in Costa Rica, monthly sampled from October 2006 through September 2007.

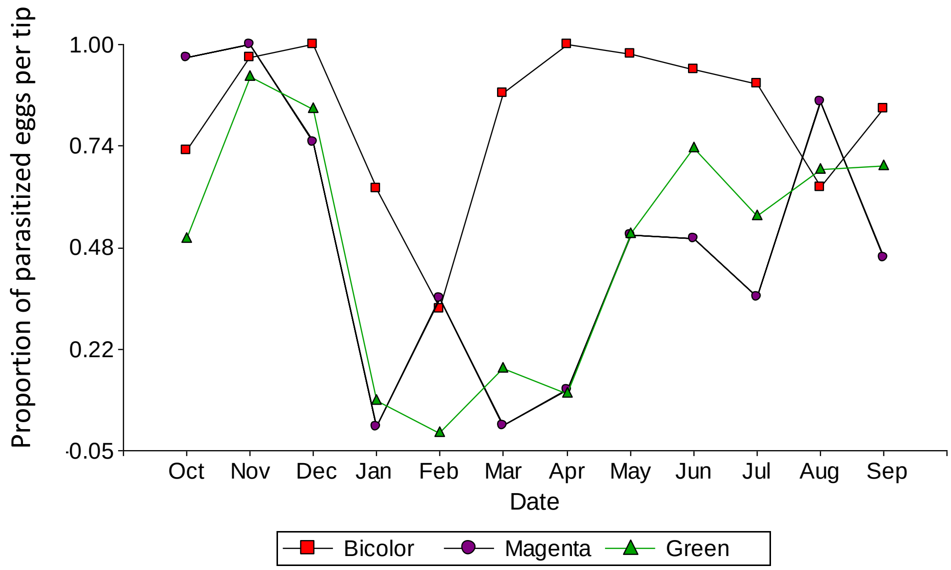

Figure 2.10. Average proportion of leafhopper eggs parasitized per sampling date on three varieties of *Dracaena marginata* (bicolor, magenta and green) monthly collected from 18 commercial plots in Costa Rica, from October 2006 through September 2007 in two production zones in Costa.

The proportion of tips infested with any of the studied pests showed a significant interaction between sampling date and variety (F = 2.58; df = 22,132; p = 0.0005). The highest variation was observed for the variety bicolor which showed a significant increase in the number of infested tips found in June, where 19% of the evaluated tips were positive for at least one on the pests in the study (Figure 2.11). The lowest proportion of infested tips for the three varieties was: January for bicolor with 3%, September for magenta with 2% and December for green with infestation in only 1% of the sampled tips.

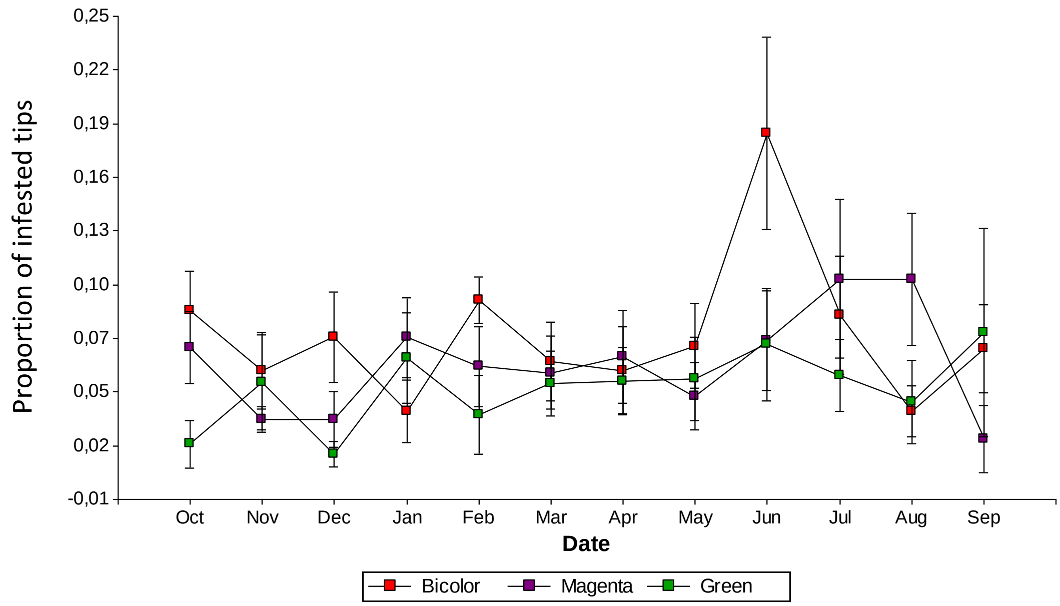

Figure 2.11. Average proportion of shoot tips infested with leafhoppers, katydids, armored scales and snails found in three commonly grown varieties of *Dracaena marginata* per month collected from 18 commercial production plots in Costa Rica from November 2006 to October 2007.

The number of shipments intercepted for any of the regulated pests between December 2006 and September 2007 ranged between 4 and 55 per month. The curve of intercepted shipments increased gradually through the first five months of 2007 reaching 24 in May. Numbers of interceptions decreased in June and July but then increased again in the following months to reach the highest point in September (Figure 2.12). The proportion of plant tips infested in the field coincided with the number of interceptions at the ports only in December when both values were at their lowest point. The highest proportion of infested tips in the field was observed in June and July a time when the interceptions were declining to its lowest point as well as the shipment size (Figure 2.12). Spearman's Rank correlation analysis showed no relation between the proportion of infested tips in the field and the number of interceptions ($r_s = 0.09$; df = 22; p = 0.76) but a significant correlation between shipment size index and interceptions ($r_s = 0.69$; df = 22; p = 0.02).

The linear regression analysis of the proportion of tips with pests in the field *vs* the ratio of number of interceptions divided by the mean size of the monthly shipments showed a significant interaction between the variable ($R^2 = 0.35$, F = 5.34; df = 1,10; p = 0.0435). The figure, however, shows how most of the observations that contribute to this relationship have levels of infested tips in the field clustered around 6% (Figure 2.13).

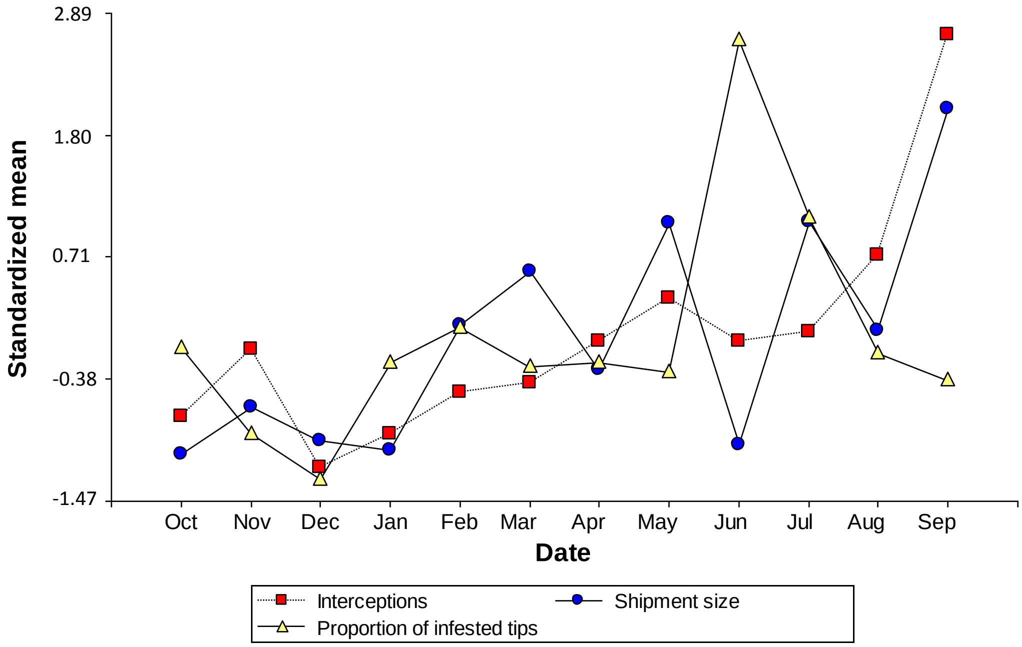

Figure 2.12. Curves of standardized number of interceptions of *D. marginata* shipments, average number of tips per intercepted shipment (Shipment size) and pest abundance in the field represented as the proportion of plant tips infested with the studied pest, from October 2006 to September 2007.

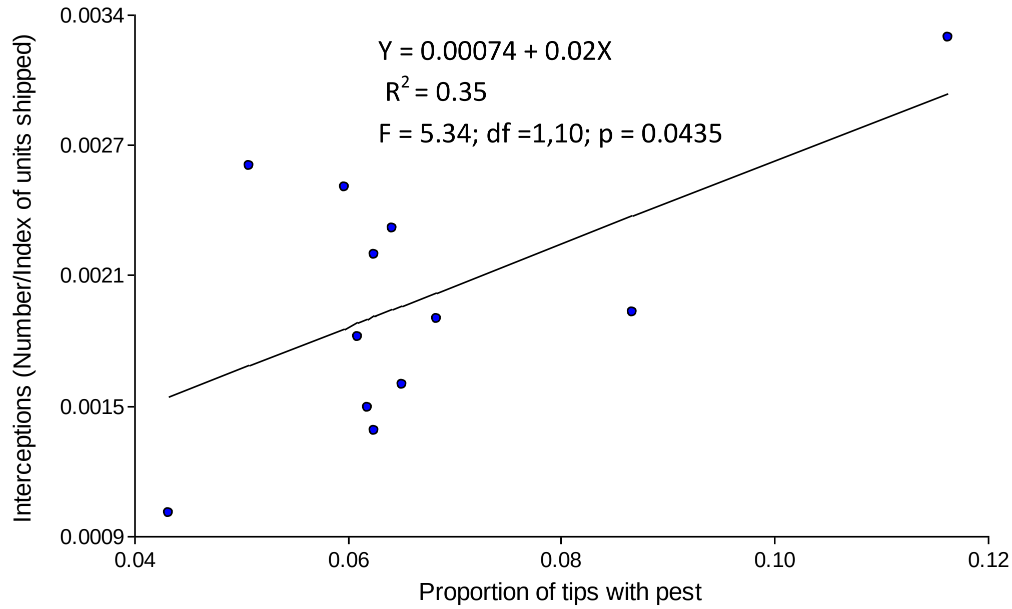

Figure 2.13. Regression curve for the proportion of *D. marginata* tips infested with pests in the field and the ratio of the number of interceptions divided by the average size of the monthly shipments (index of units shipped).

DISCUSSION

Each of the four quarantined pests were present on marketable plant tips in *Dracaena* production fields throughout the course of the entire year. For this reason pests of quarantine importance have the potential to enter the export market throughout the year. Therefore, the monthly observations of the current study fills a temporal gap found in a previous study (Prado *et al.* 2008) that identified substantial variation between pest abundance on *Dracaena* during wet and dry seasons in the same growing region in Costa Rica. Distribution of leafhopper egg and nymph abundance fluctuated over time, with peaks observed in January and June for eggs (Figures 2.3) and December for nymphs (Figure 3). This is consistent with Wolda (1978) who suggested that populations of Hemipterans increase during the wet season as a result of an increment on the availability of new leaves where the adults and nymphs can feed. These observations also fit with a behavioral model which suggests that leafhoppers move to actively growing portions of plants that provide the highest quality nutrients for growth and reproduction (Mizell 2008). Although a high percentage of the nymphs accounted for, belonged to the genus *Empoasca,* it was not possible to quantify the population of this genus at the egg level due to their small size (0.5mm) and cryptic position inside *Dracaena* leaves that made them virtually invisible.

The mean parasitism of leafhopper eggs fluctuated from 16 to 95% and its curve closely followed the amount of the precipitation, but with a low coefficient of correlation between the two variables. The two species that primarily composed the egg counts in the field (*Oncometopia clarior* and *Caldwelliola reservata*) shared the same parasitoid: *Gonatocerus* spp. (Hymenoptera:Mymaridae). Although there was no significant correlation between the number of eggs in the field and the percentage of parasitism, the proportion of eggs parasitized in January was lowest when the number of leafhoppers in the field was highest. This condition may respond to the heavy rains that are typical of the months of November, December and January and can wipe out the population of parasitoids. Mizell (2008) described a similar phenomenon for *Homalodisca variegate*, in which this leafhoppers can use the enemy-free period to increase their population.

Seasonal fluctuation of leafhopper egg and nymph abundance differed among the three varieties. The highest populations on Magenta and Green were observed in December for nymphs and January for eggs, when the population on the variety Bicolor was lowest. In contrast, the highest egg population on Bicolor was observed in June when populations in Magenta and Green were lower. Clearly the yellow stripes on the leaves of *D. marginata* var. bicolor might have an influence on insect preference and could explain the June peak. Yellow is one of the colors widely used for insect attraction to sticky traps (Hall and Hunter 2008) and has proven to be a visual stimulant for target selection for several leafhoppers (Patt and Tamou 2007, Todd et al 1990). However, this alone could not explain why leafhoppers on bicolor were lowest in abundance in January. Parasitism may have been a contributing factor during this month when overall rates of parasitism were lower than in January and near zero on the magenta and green varieties. Then attractiveness of the yellow leaf color to parasites and predators could have directed their activity toward these plants and lowered the number of eggs. Although yellow leaf color has been shown to influence plant selection by predatory coccinellids which prefer yellowing stressed maize plants (Lorenzetti *et al.* 1997), this mechanism would require further testing in Dracaena. Similarly, a nutritional explanation would require determining that each variety of *Dracaena* responded differently to monthly weather conditions or changes in management practices

Dracaena tips infested with armored scales were present throughout the year. The variety bicolor maintained the highest incidence of tips infested with armored scales (primarily Florida red scale, *Chrysomphalus aoinidum* (L.)) with a peak in June and November (Figure 2.8). Peak abundance for the other two varieties occurred in November. Other studies of Coccoidea are reported that varieties with leaf yellow variegation tend to have higher densities of armored scales (Sadof and Raupp 1991) and mealybugs (Sadof *et al.* 2003). In these studies they attributed differences on yellow variegated plants to the reduced production of phenolic based defenses resulting from lower rates of photosynthesis.

Both peaks of pest armored scale incidence occurred in months where high precipitation was preceded by long periods of low rainfall. This suggests that during the dry period, armored scales may have a greater rate of survival because fewer neonate scale crawlers were being killed by driving rains prior to settling on the leaf. Due to logistics, it is difficult to find studies implicating rain as a significant population mortality factor (Blank *et al.* 1995). However, there are numerous studies that indicate that factors, which reduce parasitoid longevity and attack rates can increase scale populations (Murdoch *et al.* 2006). Prado *et al.* (2008) found other interesting relationships that can affect armored scale population behavior. They observed a reduction of the armored scales population in *D. marginata* associated with the application of nitrogen rich fertilizers and an increase associated with the use of insecticides. Other authors have also described the resurgence of armored scales triggered by the use of insecticides that eliminate natural enemies of the pest (Rehman *et al.* 2000, Trumper and Holt 1998; McClure 1977).

The presence of snails (*S. costarricana*) was more frequent in the bicolor variety, with two population peaks in February-March and October in the Atlantic zone and another, smaller rise in July for the Northern zone. Both peaks appeared in months with lower precipitation rates (Figure 2.11) suggesting that dry conditions might have had an effect on the snails' behavior. However, this contradicts the expected results according to the nature of snails which generally need highly humid environments and tend to hide on the litter during the hot hours of the day (Monge 1996). Prado *et al.* (2008), found a different distribution pattern in their studies with significantly higher numbers of snails in the Northern zone for the wet season and no differences between varieties. The reduced population observed in general, makes the analysis more sensitive to differences due to abundance of individuals found on the plants. Growing conditions in the Atlantic zone with higher humidity and the frequent use of weed control practices to maintain denuded soils in the plantations, could have increased the population of snails which may tend to migrate from bare soil into the foliage during the dryer months. Clearly, other sampling methods could be explored to overcome the behavioral particularities of the snails, which are more active in the very early hours of the day. Monge (1996) suggests using metaldehyde baits both for monitoring and control of the pest. This may be a complementary practice to the systematic sampling method proposed by Prado *et al.* (2008).

Pest abundance and interception rate

Two sampling protocols are used at ports of entry into the United States for agricultural products in order to prevent quarantine pests from coming into the country. The first protocol, AQIM (Agriculture Quarantine Inspection Monitoring) uses a hypergeomteric approach to adjust the sampling effort to shipment size. This method is designed to detect pest presence with a 95% probability when infestation is 10% or higher. The second protocol is based on a binomial approach and examines approximately only 2% of the shipment. It is more commonly used due to the volume of produce arriving at ports of entry. The hypergeometric sampling protocol is highly effective for detecting quarantine pests at any shipment size whereas the binomial, 2% protocol, increases its efficiency in larger shipments (Venette *et al.* 2002).

To determine the relationship between pest abundance in the field and rates at which shipments exported to the US market are intercepted with pests at the Port of Miami, we examined both the proportion of infested tips and the densities of pests in the field. The number of infested tips is important from the exporter`s perspective, because it directly affects the number of marketable tips. However, in terms of pest management, the actual number of pests present in the field provides a better estimate of the seasonal fluctuation of populations, as well as tools for determining windows of opportunity for strategic application of control measures. The results in Figures 2.3, 2.5, 2.6 and 2.7 show that pest peaks can differ at some point from the proportion of tips where these populations are located. For example, peak densities of leafhopper eggs and nymphs detected in January were not reflected by the proportion of tips infested during the same month. Using the proportion of infested tips in the field we found that the pest threat was present throughout the growing season. Based on pest densities, the risk posed by leafhopper egg and nymph populations were especially high in January and June; armored scales in June; katydid eggs in November; snails in February and March with smaller peaks in October and July.

Despite these observations, pest population dynamics in the field was not related to the interception rate at the Port of Miami, at the levels of pest infestation found on plants in the field. Similarly, no relationship was found between densities of infested tips in the field and interception rates. We believe that this is because inspection methods are capable of detecting very low infestation frequencies. Some of the greatest monthly averages of intercepted shipments were detected when the average level of infestation of plant tips in the field was as low as 6% (Figure 2.12). Although there is a wide range of variability in detection at this density, our findings indicate that the inspection protocols regularly detect infested *Dracaena* tips when < 10% of the crop is infested. This suggests that inspection methods in place at the Port of Miami can exceed the Agriculture Quality Inspection Monitoring (AQIM) standards of being 95% sure of detecting infestations in a shipment that is 10% infested (Venette *et al.* 2002), and, although there was no relation between the proportion of infested tips in the field and the number of interceptions, there was a significant correlation between shipment size index and interceptions ($r_s = 0.69$; df = 22; p = 0.02). This clearly suggests that exporters are incapable of keeping standard quality control measures at higher volumes of exportation.

CONCLUSIONS

We showed that quarantine pests are present in Dracaena production fields throughout the year. Our findings suggest that there is no direct relation between the abundance of pests in the field and the interception rates because growers can selectively reject infested plants before and after the harvest. The most plausible explanation for the observed fluctuations in the interception rate is the volume of plants going through the port of entry. Assuming that the observed average size of intercepted shipments is a reliable indicator of the actual volume of trade, 69% of the interceptions could be explained by the size of the shipment. This relationship suggests that under the observed range of infestation in the field, increasing shipment size results in higher interception rates. This response may be caused by two possible reasons. Growers are either less selective when they have to fill larger shipments or that the volume of plants infested in the packing house exceeds the capacity of the workers to be effective at maintaining quality control. Based on these assumptions, the best recommendation that can be made for reducing the number of interceptions, and the risk of quarantine pest invasions into the importing country, is to adjust the needed staff for sorting, inspecting and cleaning the plant tips, in order to provide the labor required to maintain the same quality throughout time regardless of the shipment volume.

Controlling pest populations in the field will help in the process of reducing interception rates, however, the development of best management practices to reduce interception rates must balance the cost of quality control measures, with the benefit of reducing the number of harvested tips rejected in the packing house during pre-shipment inspections.

The curves of pest populations observed may be particular for the period of evaluation in this study, but show important trends that enable us to better understand the seasonal behavior of the pest complex, and this will help in the process of developing a more structured management strategy. The climatic conditions in the tropics and the heterogeneity of cropping systems and microclimates make it difficult to establish rigid pest forecast systems, but the consecutive monitoring will provide a good prediction tool for specific production zones that will help establishing an efficient pest management strategy.

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CHAPTER 3

Influence of propagated cane size on pest incidence. A case study of *Dracaena marginata* plants managed for the export market in Costa Rica

INTRODUCTION

Dracaena marginata (Fam. Ruscaceae) is an ornamental foliage plant native to Asia and Africa but grown in most tropical regions of the world for export to temperate climates for use as indoor house plants. In Costa Rica different varieties are produced for the export market to the United States. The most commercially viable varieties are bicolor, green and magenta. *D. marginata* is propagated for sale in different forms including bare canes, unrooted tips, branches and rooted plants (Acuña *et al.* 1991) and is grown at altitudes from a few meters above sea level up to 1200 m. Plants are generally grown under full sun exposure (Acuña *et al.* 1991). Predominantly small to medium size stake holders, ranging from one to five hectares, manage the production as a family business. Dracaenas are part of the ornamental products which represent 4% of the Gross Domestic Product in Costa Rica.

Although the ornamental export market has grown from $70 million in 2005 to $85 million in 2008 (Arce *et al.* 2009) the future of *D. marginata* has been jeopardized by the repeated interceptions of quarantine pests found in the shipped plants. Propagative *Dracaena* cuttings bound for the US market cannot be shipped if the distance from the base of the cane to the growing tip is longer than eighteen inches (46 cm). This regulation is based on the assumption that taller plants are more prone to pest incidence due to their longer exposure to pest infestations under field conditions. According to a USDA-APHIS pest risk assessment, over 70% of the interceptions on *D. marginata* shipments arriving to the US from Costa Rica are due to the presence of katydids (Orthoptera: Tettigoniidae), armored scales (Hemiptera: Diaspididae), leafhoppers (Hemiptera: Cicadellidae) and the snail *Succinea costaricana* von Martens (Gastropoda, Stylommatophora, Succineidae). Among these pests, leafhoppers are responsible for approximately 41% of the total interceptions (Colpetzer 2005).

Although most of these invertebrate pests are commonly found in *D. marginata* plantations throughout the different growing areas in Costa Rica, very little is known about their population dynamics and the actual relationship between plant size and pest incidence. This study aims to examine pest abundance on commercial *D. marginata* plants of different sizes under field conditions, to determine whether pest abundance is influenced by plant size.

MATERIALS AND METHODS

An observational trial was carried out on commercial plantations of *D. marginata* in order to determine if the size of harvested propagated plant material has an effect on pest distribution and abundance in *Dracaena marginata* plants under field conditions. Commercial plantations were identified in each of the two production zones of Costa Rica, the Northern area near La Tigra in San Carlos and the Atlantic Area near Guápiles and Siquirres. Fifty two plots within fourteen farms were selected randomly among those which were in the process of preparing shipments of *D. marginata* with different cane sizes, and where the crop met the appropriate age criteria for pest sampling (shoot tips of 14 to 16 weeks old). Each selected farm had at least one plot of plants with propagated stem canes of 18 inches (46 cm) or smaller and a plot of plants with canes larger than 18 inches (46 cm). All propagated canes used in this study fell within three size categories: 6 to18 inches (15 to 46 cm), 18 to 32 inches (46 to 81cm), and taller than 32 inches (81cm).

The study had a completely randomized design in which the treatments were represented by the combination of Production Zone (with two levels: Atlantic and Northern zone), Season (with two levels: dry and rainy) and Plant Size (with three ranges: from 6 to 18 inches, between 18 and 32 inches and taller than 18 inches). The mean number of pests per plot and the mean number of commercial tips per plot with pests on each category (leafhopper egg masses, leafhopper nymphs, katydid eggs, snails and armored scales) were estimated for each plant size category. The abundance of armored scales was reported only as the number of infested tips due to the difficulty of performing accurate counts of individuals in the field. The eggs of katydids and leafhoppers were categorized as viable, parasitized, parasitized-hatched and viable-hatched.

Analysis of variance (ANOVA) was performed on the data using mixed model theory to determine if cane size, growing zone and, or season affected pest abundance. The analysis was performed with the statistical program InfoStat (Di Rienzo *et al.* 2011), using Fisher's

LSD procedure with a significance of 0.05 to compare the means and Akaike (AIC) and Bayesian (BIC) Information Criteria to select the best model (Balzarini *et al.* 2004).

Sampling method

The sampling points within the plot were defined using a 10 by 10 meter grid as reported by Prado *et al.* (2008). At each sampling point the tips of nine propagated cane tips were selected at random from plants within a radius of 1.5 meters from the center of the sampling point. Each experimental plot measured approximately 2100 m^2 with 32 sampling points. The evaluations were carried out when the cane tips were between the 14^{th} and 16^{th} week of growth of the commercial 16 week propagation cycle in order to represent the total exposure to pests during production. There were two evaluations during the year in each production zone, one during the dry season (November to April) and the second during the rainy season (May to October).

RESULTS

The main leafhopper species found laying their eggs in *D. marginata* were *Oncometopia clarior*, *Caldwelliola reservata* and *Empoasca* sp. The three species were commonly found in both production zones sampled during the study, and are reported as total leafhoppers throughout this paper making no distinction at the species level. Micro-hymenopteran wasps from the genus *Gonatocerus* (Hymenoptera:Mymaridae) were found parasitizing *Oncometopia clarior* and *Cladwelliola reservata* eggs in all of the sampled plots. Overall, cane size alone (F = 0.64; df = 2, 42; p = 0.5321) and zone (F = 0.08; df =1, 42; p = 0.7770) did not have significant effects on the number of leafhopper egg masses found on shoot tips. In contrast, there were significantly more egg masses found in the dry season than in the wet season (F = 8.76; df = 1, 42; p = 0.0050).

There was an interaction between sampling season and plant size (F = 3.28; df = 2,42; p = 0.0476) showing significantly higher incidence of eggs in the plants categorized in the small size range (6 to 18 inches) during the dry season (Figure 3.1) with an average of 0.1 egg masses per plant tip. There were also fewer egg masses (0.05 egg masses per plant tip) on the plants in the large category (36 inches and larger), closely followed by plants in the medium size range with 0.06 egg masses per plant tip. However for the rainy season, the incidence was similar for all three plant sizes (Table 3.1).

Table 3.1. Average number of leafhopper egg masses and proportion of parasitized eggs per plant tip found on *D. marginata* tips of three sizes (small, medium and large) during the dry and rainy season of 2007 in Costa Rica.

		Number of egg masses				Proportion of egg masses parasitized			
Season	Cane Size[1]	N	Mean	SE		n	Mean	SE	
Dry	Small	13	0.10	0.01	a	13	0.64	0.33	a
Dry	Medium	5	0.06	0.02	ab	5	0.64	0.37	a
Dry	Large	9	0.05	0.01	bc	7	0.74	0.40	a
Rainy	Small	14	0.02	0.01	c	10	0.58	0.32	a
Rainy	Medium	8	0.02	0.01	c	7	0.5	0.44	a
Rainy	Large	3	0.02	0.02	bc	3	0.53	0.17	a

Different letters indicate significant differences (Fisher LSD, p<0.05)

[1]Small = 6- 18 in (15-46 cm) ; Medium = 18-32 in (46-81 cm); Large> 32 to 60 in (81 to 152 cm)

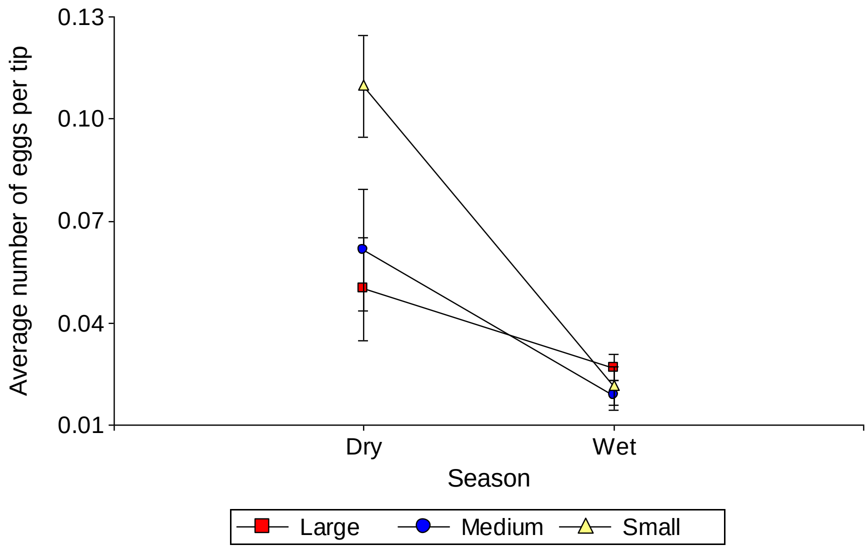

Figure 3.1. Average number of leafhopper eggs per plant tip on shoots of different size found during the rainy and dry season, in 52 plots within 14 commercial *Dracaena marginata* plantations in Costa Rica, 2007.

The proportion of parasitized eggs ranged between 0.5 and 0.74 but there were no statistical differences associated with propagated cane size (F = 0.40; df = 2,35; p = 0.6763), production zone (F = 0.14; df = 1,35; p = 0.7149) nor season (F = 0.14; df =1,35; p = 0.7145) (Table 3.2). The abundance of leafhopper nymphs was not influenced by the size of the plant tip (F = 1.28; df = 2,42; p = 0.2895) nor the growing zone (F = 3.59; df = 1,42; p = 0.0651) but showed a significant difference (F = 5.18; df = 1,42; p = 0.0280) produced by the interaction between growing zone and season showing its highest population in the Northern zone during the dry season (Figure 3.2). Although the difference in abundance of nymphs per cane size was not statistically different, the highest populations were found on plant tips with cane sizes in the small category whereas the population in the large plant tips were equal or smaller than the population in medium size plant tips (Table 3.3).

Table 3.2. Average number of pest per tip (± standard deviation), in three size categories of commercial *D. marginata* plants, from two production zones in Costa Rica. The incidence of armored scales was recorded as number of infested plant tips per sampling point due to the difficulty of carrying out accurate individual insect counts in the field. Costa Rica, 2007.

zone	Cane Size[1]	n	Leafhopper (Lh) egg masses	proportion of Lh eggs parasitized	Armored scales	Katydid eggs	Snails
Atlantic	Small	11	0.04 ±0.05	0.57 ±0.45	0.02 ±0.02	0.0003 ±0.001	0.003 ±0.01
Atlantic	Medium	6	0.03 ±0.03	0.44 ±0.45	0.03 ±0.05	0.0029 ±0.010	NF
Atlantic	Large	4	0.04 ±0.03	0.73 ±0.49	0.07 ±0.08	0.0009 ±0.002	NF
North	Small	16	0.08 ±0.06	0.64 ±0.22	0.03 ±0.03	0.0019 ±0.003	NF
North	Medium	7	0.04 ±0.04	0.67 ±0.35	0.04 ±0.05	0.0020 ±0.003	NF
North	Large	8	0.04 ±0.05	0.64 ±0.29	0.05 ±0.06	0.0004 ±0.001	NF
P- for effects of size			0.5321	0.6763	0.5269	0.8688	0.6627
P-for effect of zone			0.7770	0.7149	0.5430	0.2784	0.2758

[1]Small = 6- 18 in (15-46 cm); Medium = 18-32 in (46-81 cm); Large> 32 in (81 cm)
NF: Not found

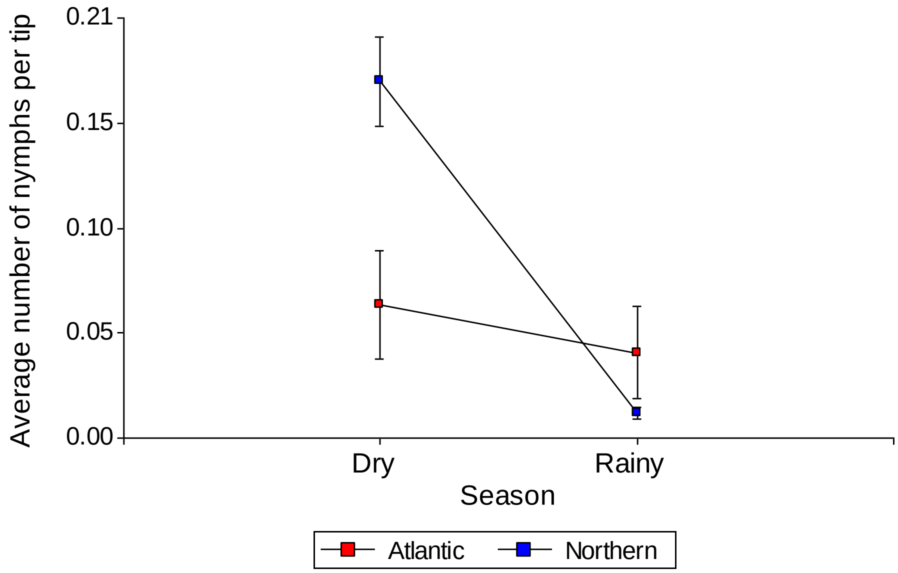

Figure 3.2. Average number of leafhopper nymphs per plant tip found during the rainy and dry season in 52 plots within 14 commercial *D. marginata* plantations in the Atlantic and Northern zone of Costa Rica in 2007.

The population for the other two quarantine pests, katydid eggs and snails, was not significantly different from zero in this study. However, the abundance of their reduced population was not influenced by the size of the propagated plant tip (F = 0.14; df = 2,42; p = 0.8688, F = 0.42; df = 2,42; p = 0.6627 respectively). Logistic regression results showed that the presence of katydid eggs was more likely to occur in the rainy season (χ^2 = 4.92; p = 0.0266). The only time snails (*Succineacostaricana*) were found was in the rainy season.

Table 3.3. Average number of leafhopper nymphs per plant tip found during the rainy and dry season on plants with different cane sizes from commercial *D. marginata* plantations in the Atlantic and Northern zone of Costa Rica, 2007.

Zone	Cane Size[1]	n	Mean	SD
Atlantic	Small	11	0.08 a	0.1
Atlantic	Medium	6	0.03 a	0.05
Atlantic	Large	4	0.03 a	0.05
North	Small	16	0.12 a	0.11
North	Medium	7	0.08 a	0.11
North	Large	8	0.08 a	0.11

[1]Small = 6- 18 in (15-46 cm) ; Medium = 18-32 in (46-81 cm); Large> 32 in (81 cm)

DISCUSSION

Science based regulations for the export market of agricultural products can prevent the introduction of unwanted invasive species to importing countries, by restricting the movement of goods that are likely to contain pests. Regulations must protect the interests of importing countries but also be flexible enough, within acceptable technical parameters, to fit a growing and globalized market. According to Wittenberg and Cock (2001) the effectiveness of management strategies on activities that can lead to the entrance of invasive species, requires concerted bilateral, regional, or global action based on common objectives and jointly agreed international standards. The authors also indicate that "to promote transparency and accountability, legislation should require permit decisions to be made in accordance with scientific evidence". Cases such as the size regulation for *Dracaena* plants from Costa Rica, bound to the US market are among those which need complementary research to provide scientific proof for their support or modification, depending on the evidence given by the results. My examination of different sized propagative material of *D. marginata* grown in Costa Rica indicated that none of the 4 main quarantine pests reported by Colpetzer (2005) were influenced by the size of the shoot tips, thus the current regulation deserves a revision and most probably a modification.

D. marginata pruning practices are designed to induce the continuous production of shoot tips. Plant stems are allowed to grow for variable periods of time, depending on the final length of the cane requested by the market, and then the growing point is cut off to stimulate the production of shoots that will be ready for harvesting in approximately sixteen weeks. At the same time the lower foliage is maintained for photosynthetic purposes (Acuña *et al.* 1991). This management gives origin to plants with different heights but with similar foliage age of the tip. The even distribution of the young leaves in short to tall plants and the known preference of chewing and sucking insects for feeding and laying eggs on young tissue make all plant sizes equally susceptible to pest attack.

The attraction of insects to their hosts is influenced by host age, secondary chemistry (Arimura *et al.* 2005; Munné-Bosch 2007; Muticainem *et al.* 1996) and the developmental stage of the insect (Schoonhoven *et al.* 2005) among other biotic and abiotic factors. *Dracaena marginata* appears to be one of many plant species where the availability and distribution of young leaves determines the abundance and diversity of herbivorous insects (Basset 2001). Structural and physiological similarities of same aged leaves on different sized plants may help to explain the lack of significant differences in number of leafhopper eggs found on shoot tips of different sized plants. According to Schoonhoven *et al.* (2005), as plants age they go through chemical, morphological, and physiological changes that can affect the feeding behavior or oviposition preference of insects. Plant tips that came from small and large plants were phenologically similar because they all sprouted from buds that emerged from the host plant soon after the apical tips were removed. Similarly aged shoot tips should have similar levels of abscisic acid (ABA), a plant hormone that induces stomatal closure thus lowering hydraulic conductivity (Munné-Bosch 2007). ABA concentration has been shown to correlate positively with the production of anti-herbivore defensive compounds that are mediated by production of jasmonic acid (Thaler and Bostock 2004). Thus, current understanding of physiological similarities of phenologically similar shoots may provide one possible explanation for the lack of difference in leafhoppers on larger plants.

Leafhopper populations have been reported to increase during the rainy season in Costa Rica due to the proliferation of new plant tissue used by insects for feeding (Wolda 1978). A preference for young leaves has been reported among all the genera of leafhoppers we have found attack *Dracaena* as well as some of those that are not known to feed on this plant (Hidalgo *et al.* 1999; Pedreira *et al.* 2008; Daane and Williams 2003). Studies of alfalfa demonstrate that oviposition of *Empoasca fabae* is higher on the succulent young stems than less succulent and more lignified tissue (Hoffman and Hogg 1992, Kieckhefer and Medler 1964). Pedreira *et al.* (2008) also report the preference of *Oncometopia facialis* for feeding on young shoots of citrus trees where the concentration of amino acids is higher. The evidence gathered in the course of this study indicates that *Caldwelliola reservata* also prefers young *Dracaena* tissue for feeding. This species laid its eggs on newly opened leaves after the shoots reached the fourth week of growth. Nymphs and adults seek refuge in the crown of the shoot and feed on the sheath of the leaves.

Leafhoppers, on the other hand, may present different nutritional requirements between life stages having to switch host to fulfill their needs. In complement to the optimal oviposition theory (Jaenike 1978), in which leafhoppers should feed and lay their eggs on an optimal host plant, both nymphs and adults discriminate between host quality according to their needs for growth, development, or maintenance. Hence, female leafhoppers will tend to lay their eggs in leaves with high amide concentrations, suitable for adult feeding, but nymphs may tend to move looking for a more amino acid balanced host (Mizell *et al.* 2008).

Prado *et al.* (2008) found significant differences on reduction of leafhopper egg abundance associated with weed control, suggesting that weed cover is a reservoir for leafhoppers that eventually migrate to the lower strata of the crop for feeding and egg laying. However, the application of the weed control approach may produce an opposite response on the egg abundance of leafhoppers with small cryptic eggs such as *Empoasca* sp., as found in a parallel study (Chapter 4). This study shows an increase in the number of eggs found on the crop when removing the weed cover, proving that some species that originally divide their feeding and oviposition among a number of host plants in the plantation, can successfully live on *D. marginata* in absence of a secondary host, with an expectable increment on the number of eggs laid on the crop. However, despite differences in species of leafhoppers, increased size did not increase abundance of leafhopper pests since they tend to follow the same vertical distribution pattern.

The population of armored scales showed no statistical differences between propagated plant sizes, production zones or season. In general the observed population was low ranging between 0.02 to 0.07 infested tips per sampling point. The homogeneity of the foliage age in commercial tips of *D. marginata* decreases the chances of finding marked differences on availability of nutrients such as amino nitrogen known to be important for growth, fecundity and survival of sap sucking insects like armored scales (McNeill and Southwood 1978). Having an even distribution of food resources, the distribution of the pest may be affected by other factors such as presence and distribution of natural enemies and plant-water relations (Hanks and Denno 1993). Because female armored scales are mobile only during the first instar (crawler stage) their dispersal in the field is limited, resulting in patchy distributions of the population in the crop. They spread on infested plant material, through unsettled crawlers within the plant, helped by the wind or other phoretic means such as insects, birds, animals and man (Beardsley and Gonzalez 1975). Prado *et al.* (2008) found an effect of crop management practices over the population of armored scales, as well as for katydids, and leafhoppers. These authors determined that the use of chemical insecticides statistically increased the population of armored scales and katydids, probably due to the elimination of their natural enemies (Trumper and Holt 1998; Waage *et al.* 1985).

Although low in numbers, snails (*S. costaricana*) were found only during the rainy season. Similar behavior has been also reported in previous studies (Prado *et al.* 2008), although other authors concluded that the reproductive peak of the snail *S. costaricana* starts with the beginning of the dry months in Costa Rica (Villalobos and Najera 2004), probably due to a decrease on the rate of mortality due to pathogen infections. However, according to Villalobos *et al.* (1995) *S. costaricana* produces eggs all year round and adults are more active under humid conditions. Population studies indicate that diurnal counts of adult *S. costaricana* on plants underestimate the population by a factor of 17 (Villalobos *et al.* 1995). They also determined that the rainfall pattern did not correlate with the fluctuations in the total number of snails observed. Based on this information it is reasonable to think that the population of snails observed in the current study may have been underestimated. However, the registered data showed no relationship between snail abundance and propagated plant tips. Foliage age and nutritional content of *Dracaena* plants is unlikely to have an effect on snail host preference since they do not feed on fresh plant tissue (Villalobos *et al.* 1995).

The incidence of katydid eggs was very low during the development of this study and showed no relationship with the size of the propagated plant tips or the production zone; however, they were more abundant during the rainy season. Katydids are omnivorous insects that can feed on seeds, flowers, fruits and occasionally act as a biological control agent eating other insects and their eggs, and reducing the seed production on rushes and grasses (Gwynne 2001). Capinera *et al.* (1997) also reports the preference of katydids for grasses on ditch margins where they feed on flowers and disperse seeds from pastures to oviposition sites. In *D. marginata*, katydids were not observed to feed on the plant but used both mature and young leaves to lay their eggs. The increase on the number of katydid eggs observed during the rainy season may then be explained due to the increased grass growth providing more sources of food transformed by the insect in higher reproductive capacity.

CONCLUSION

The abundance of quarantined pests found in fields does not increase with the size of a *Dracaena* cane. Due to physiological differences between young and old plants taller plant tips are, in general, less colonized by the studied pests under the management scheme used by the growers in Costa Rica and under the conditions present during the current study. Thus, the size of the cane from which leaves are growing does not contribute to the likelihood of it containing a quarantined pest. For this reason, phytosanitary regulations, which restrict the movement of *D. marginata* larger than 18 inches are not likely to reduce the rate of quarantine pests from coming into importing countries

It was difficult to draw strong conclusions on the behavior of snail populations (*S. costaricana*) and katydid eggs since their numbers were low during the study. However, an important observation toward the goal of this study was that their distributions showed no relation to size of the plant shoots but tended to be more abundant during the wet season. On the other hand, armored scales were expectedly more abundant during the dry season but their distribution showed no statistical difference between the three plant size ranges studied. The pruning practices maintain homogeneous foliage characteristics, regardless of cane length, which may explain the lack of a relationship between plant size and distribution of the studied pest in Dracaena. Hence, same age foliage located at different plant heights was equally attractive to the pests.

The high percentage of parasitism observed in the field suggests that natural enemies are important for controlling pest populations. More research should be addressed to understand and improve the efficiency of biological control carried out by the micro-hymenopteran *Gnomocerus* spp. since it is controlling a high proportion of the leafhopper population in a natural way.

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CHAPTER 4

Influence of weed community composition on the abundance of *Empoasca* sp. eggs and parasitism level in *Dracaena marginata*.

INTRODUCTION

Dracaena marginata (Fam. Ruscaceae), an ornamental foliage plant native to Asia and Africa, is grown in tropical regions of the world for use as indoor houseplants. Exportation of *Dracaena* plants and other ornamental crops represent 4% of the Gross Domestic Product in Costa Rica and has grown from $70 million in 2005 to $85 million in 2008 (Arce *et al.* 2009). However, this activity is jeopardized by the number of interceptions due to quarantine pests detected in shipped plants at the ports of entry to the US.

According to Coltpetzer (2005), 41% of the interceptions of *D. marginata* at the ports of entry to the US from 1984 to 2004 were due to presence of leafhopper eggs or nymphs, making this insect group a highly relevant quarantine pest for this crop. *Oncometopia clarior* and *Caldwelliola reservata* are known to lay their eggs underneath the waxy cuticle of the *Dracaena* plants but are still visible during inspection. Smaller leafhopper species such as *Empoasca* sp., lay their eggs inside the leaf tissue and due to their small size (approximately 0.5 mm) and cryptic location within the leaf blade make them difficult to detect by growers during inspection and sorting at the packinghouses. Nymphs hatching out of these eggs during transportation of *D. marginata* shipments may be one of the main sources of leafhoppers detected by US authorities on *D. marginata* shipments from Costa Rica. Studies carried out for the establishment of a Clean Stock Program for *D. marginata* in Costa Rica identified *Empoasca* sp. as the group of leafhoppers responsible for laying cryptic eggs in the main exported varieties (green, bicolor and magenta) (CSP 2005). It was demonstrated that eggs laid in a single plant can be numerous, with up to fifty nymphs hatching out of a caged asymptomatic plant. This situation and the broad host range of *Empoasca* (Lamp et al, 1997; King & Saunders, 1984), make it necessary to develop tools for detection and monitoring of populations and managing the abundance of these pests.

Weed communities can have a dramatic influence on the abundance of highly vagile polyphagous herbivores in the family cicadellidae. On a previous study, Villalobos (2007) studied weed community composition on *Dracaena* agroecosystems and arranged the species within five tentative functional groups based on their succulence, leaf shape, pubescence, presence of aromatic compounds, nitrogen fixation and presence of extra floral and floral nectaries (Table 4). This classification was used as a reference for subsequent studies aimed at determining the influence of weed community composition and the oviposition behavior of a cryptic leafhopper.

Table 4.1. Summary of the functional traits and functional groups (FG) of weeds identified in *Dracaena* agroecosystems in Costa Rica. Modified from Villabolos (2006).

Functional trait	FG1	FG2	FG3	FG4	FG5
Phenology	Perennial and annual	Perennial	Mainly annual plants (68%)	Mostly perennial (78%)	Mostly annuals (75%)
Stem	Erect	Erect	Mostly sub-erect (42%) and erect (37%)	Mostly erect (83%)	Erect
Succulence*	74%	81%	79%	76%	83%
Leaf shape	Elliptic-ovate-obovate	Peltate-orbicular (50%), deltoid (25%), elliptic-ovate (25%)	Variable leaf shape, mainly lanceolate-oblanceolate (42%)	Mostly acicular (72%)	Lanceolate-oblanceolate
Pubescence	Present in most of the sp. (93%)	Only present in some sp. (25%)	Absent (52%); Present (47%)	Present in most of the sp. (78%)	Present in most of the sp. (75%)
Aromatic compouds	No	Most of the sp. (75%)	Present in 42% of the sp.	No	Present in few sp. (17%)
Nitrogen fixation	No	No	Only few sp. (10%)	No	No
Extra-floral nectaries	Only some sp. (30%)	No	Only some sp. (26%)	No	Only few sp. (8%)
Floral nectaries	Yes (100%)	Yes (100%)	Most of the sp. (95%)	No	Yes
Entomophilous pollination	Most of the sp. (75%)	Most of the sp. (75%)	Yes (100%)	Only few sp. (5%)	Most of the sp. (83%)

*Succulence is shown as the average percentage of water content in the tissue.
** Percentage for other functional traits (%) represents the proportion of species (sp.) within the functional group that show the trait.

This study had two main objectives. First, it sought to improve our capacity to detect *Empoasca* eggs in *D. marginata* leaves by evaluating different staining procedures. Second, using the procedure that allowed us to clear the leaf tissue and stain visible the cryptic eggs, we aimed to evaluate the influence of weed community composition on the abundance of *Empoasca* sp. eggs and parasitism levels in a *D. marginata* field.

MATERIALS AND METHODS

A series of staining methods reported for detecting *Agromyzid* and *Empoasca* eggs (Moorthy *et al.* 1988; Nuessly *et al.* 1995; Lu 1987), *Homoptera* eggs (Khan 1986), parasitization (Consoli *et al.* 2000) and leafhoppers salivary sheaths (Backus *et al.* 1988), were tested on *D. marginata* leaves to determine their suitability for detecting *Empoasca* eggs in this plant species. In the first stage we evaluated the capability of the methods for clearing the leaf tissues and contrasting structures, then incorporated modifications to the methods in order to improve their performance.

Adult *Empoasca* sp. were captured in two *D. marginata* fields, in one commercial farm, and caged with a potted *D. marginata* plant. Groups of six insects were selected from the more active specimens in the cage and confined in a fine mesh bag made with polyester fiber (Tergal ®) manufactured by Fabrica Maria S.A, Caleras, Mexico. A single *Dracaena* leaf was introduced in the bag and sealed with a paper clip without detaching the leaf from the plant (Figure 4.1). Insects were kept in confinement for a seven day period to assure eggs would be present in the leaf tissue. These egg-infested leaves were then used for testing the efficacy of the different staining procedures listed in Table 4.2. Effectiveness of each staining measure was evaluated visually by comparing their capacity to clear plant tissue and to reveal a brightly stained egg.

Figure 4.1. Confinement of *Empoasca* sp. for oviposition, using fine mesh, polyester cloth.

Table 4.2. Staining methods tested for detection of *Empoasca* sp. eggs in *D. marginata* leaves.

Method	Reference	Clearing agent	Staining agent	Counter Staining
1	Moorthy *et al.* (1988)	Carnoy (6:4:3 ethyl alcohol, chloroform).+ 20 g phenol and 5 g chloral hydrate in 100 ml lactic acid	1% aqueous acid fuchsin	Malachita green
2	Khan (1986)	Boiling water, then 95% ethyl alcohol	1% aqueous acid fuchsin	Tub water
3	Nuessly (1995) Lu(1987)	Phenol	Lactophenol-acid fuchsin	Lactophenol-cotton blue
4	Neergaard (1997)	Chloroform, Methyl alcohol, Lactic acid (1:1:1)	Clearing solution + 0.5% acid fuchsin	70% ethyl alcohol

Effect of weed cover community and *Empoasca* egg abundance in *D. marginata*

The effect of weed cover on *Empoasca* egg laying was studied in one experimental field of *D. marginata* var. green, which had been managed in order to establish seven treatments with specific weed cover communities (Table 4.3). These treatments ranged from weed free to complete cover with weeds endemic to the research farm, and were designed to compare pest abundance on Dracaena in the presence of weed groups that were previously studied by Villalobos (2007) and Perez (2007). Other treatments included common weeds, proportionally higher in density and with potential use by the pest for feeding and resting such as *Spermacoce latifolia* (Perez 2007). Additional treatments included weeds with contrasting functional traits such as nectaries and pollen production that could attract natural enemies, presence of erect stems that add to habitat complexity and provide refuge for predators (Finke and Denno 2002), or weeds that might be attractive to leafhoppers due to their succulence, capacity for nitrogen fixation, acicular (needle-like) leaves and absence of trichomes (Villalobos 2007; Linkimer 2011).

The weed cover experiment was arranged in a randomized complete block design with ten replicates, seven treatments and experimental units of 10x10 m. The position of the treatments in the field was defined after a baseline survey using a 5x5m area to determine abundance of the different weed species of interest. The sampling was performed across the entire production area using a $50cm^2$ frame. Once the position of the experimental units was defined, a combination of manual and chemical controls were performed periodically for a period of 7 months in order to establish the desired weed cover composition for each treatment. Weed cover composition was defined as established when at least 70% of the plant species present belonged to the desired families. Weed cover was estimated by taking random samples from each experimental unit using the $50cm^2$ frame and manually counting the plant species in the sampling area. The treatments were set as described in Table 4.3.

In order to determine the effect of the weed cover treatment on the oviposition behavior of *Empoasca* sp., three plants were randomly selected from the central area of each plot and were pruned to induce the growth of new same age shoots tips. Shoot tips were allowed to grow for six weeks and then cut for evaluation in the laboratory. Tips were evaluated at this age because they were mostly of juvenile foliage that was more susceptible to attack by leafhoppers, and could be processed more easily due to the smaller leaf size. Collected tips (three per plot) were labeled and individually processed using the staining methodology selected in the previous phase. Ten leaves of representative sizes from each stained tip were examined under a light microscope to determine the number of *Empoasca* eggs that were unhatched, had emerged normally, contained a parasitoid, or had contained a parasitoid that had emerged. Poisson regression with orthogonal contrasts was performed on the average number of eggs per tip and average number of parasitized eggs per tip, within the different categories, using generalized linear models with the statistical program InfoStat (Di Rienzo *et al.* 2011).

Table 4.3. Treatments for selective weed community composition in *D. marginata* experimental plots.

Treatment	Rational for treatment selection	Management strategy	Final cover composition and functional groups* (FG)
Weedy	Unmanaged populations of endemic weeds to allow a natural assortment of species	Free growth of weeds allowed	*Spermacoce latifolia*(55%) (FG1); *Lindernia diffusa*(12%) (FG3); *Youngia japónica*(5%)(FG5); *Phyllanthus urinaria* (4%)(FG3); *Peperomia pellucida*(4%)(FG3); **Other species (20%)
Weed free	Absence of weed cover to eliminate any possible influence on pest behavior.	Weeds eliminated using herbicides: Glyphosate (Ranger 24 SL) and Difenil-eter-oxifluorfen (Goal 24 EC), additionally using manual control when necessary.	
Drymaria cordata cover	*Drymaria cordata* was found to repel leafhoppers (Perez 2007) and growers have used it as cover crop. Lacks vertical structure to provide refuge for leafhopper predators (Denno and Finke 2002)	Other weeds were controlled using Glyphosate (Ranger 24 SL) and Fluazifop-p-butil (Fusilade 12.5 EC) with additional manual control when necessary.	*Drymaria cordata*(51%)(FG3); *Cardamine bonariensis*(14%)(FG3); *Peperomia pellucida* (8%)(FG3) Other species (27%)
Poaceae Cover	Reported as Leafhopper host (Maes and Godoy 1993).	Broad leaved weeds were eliminated with herbicides: Picloram-Fluroxypyr (Plenum 16 EW), Metsufluron methyl (Met-Weed 60 WG.), and manual control when necessary.	*Eleusine indica*(72%)(FG4); *Digitaria* (15%)(FG4) Other species (13%)
Cyperaceae Cover	Classified by Villalobos (2007) in the same functional group with T4 but described as repellent to leafhoppers by Perez (2007)	Other weeds were controlled with herbicides: Terbutrina (Igram 50 SC), Picloram-Fluroxypyr (Plenum 16 EW), Fluazifop-p-butil (Fusilade 12.5 EC), and manual control when necessary.	*Cyperus* sp. (40%)(FG4); *Cyperus tenuis*(37%)(FG4); *Kyllinga brevifolia* (21%)(FG4) Other species (2%)
Rubiaceae Cover	Described by Perez (2007) as attractive to leafhoppers only for resting and occasional feeding.	Predominantly weeds from the Rubiaceae family (*Spermacoce latifolia*). Other groups were controlled with Fluazifop-p-butil (Fusilade 12.5 EC) and manual control when necessary.	*Spermacoce latifolia* (88%)(FG1); *Phyllanthus urinaria*(10%)(FG3) Other species (2%)
Flowering Broad leaf	Mixture of endemic broad leaf weeds with flowers and nectaries that can attract natural enemies of the pests.	Herbicide Fluazifop-p-butil (Fusilade 12.5 EC) and manual control were used to maintain the weed selection.	*Lindernia diffusa*(52%)(FG3); *Youngia japónica*(24%)(FG5); *Lindernia crustaceae*(11%)(FG3) Other species (13%)

*Plants characterized and classified by Villalobos (2007) in five functional groups based on succulence, leaf shape, pubescence, volatile production, nitrogen fixation, extra floral and floral nectaries presence (see text for detail composition of the functional groups). Percentages refer the relative abundance of the individual species respect to the other weed species on the treatment.

**Other species with population below 5% were grouped in this category.

RESULTS

Three of the four staining methods (Methods 1, 3 and 4 in Table 4.2) were capable of clearing and staining the *Dracaena* leaf tissue after 72 hours of immersion. Boiling water (Method 2) was unsuccessful as it made the leaves more opaque and dark making it more difficult to detect the presence of eggs (Figure 4.2).

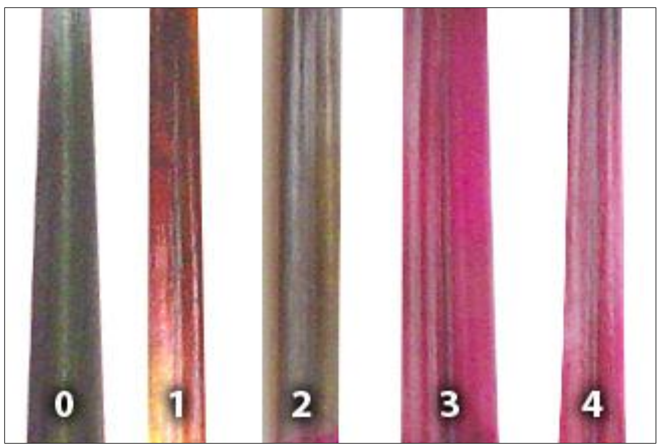

Figure 4.2. Visual scale of leaf tissue clearing and staining with staining methods tested in this study: 0-Untreated leaf, 1-Method 1(Carnoy and 1% aqueous acid fuchsin), 2- Method 2 (Boiling water,, ethyl alcohol and 1% aqueous acid fuchsin), 3- Method 3 (Phenol and Lactophenol-acid fuchsin) and 4- Method 4 (chloroform, methyl alcohol, lactophenol, and 0.5% acid fuchsin).

Eggs and their surface structures were easily visible using staining Method 4 in which the leaves were submerged in a solution of chloroform, methyl alcohol and lactic acid (1:1:1) plus 0.5% acid fuchsin for 72 hours, then allowed to clear for 24 hours in 70% ethyl alcohol. This procedure colored the *Empoasca* eggs bright pink and gave good contrast for easy detection in the stained leaf (Figure 4.3a). This stain also made it possible to ascertain whether or not the egg had hatched (Figure 4.3c), was hatching (Figure 4.3b) or if parasitic wasps had emerged leaving a round exit hole on the leaf surface (Figure 4.3d).

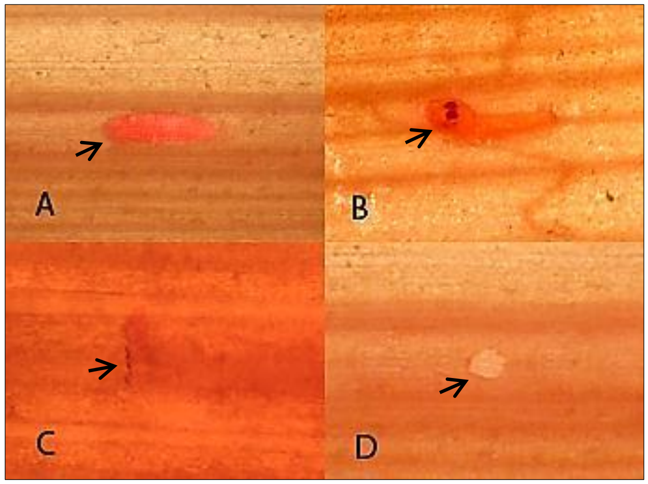

Figure 4.3. *D. marginata* leaf with stained unhatched egg (A), *Empoasca* hatching from egg (B), Visible fissure resulting from *Empoasca* emerging from the leaf tissue (C), parasitoid exit hole coming out of an *Empoasca* egg (D).

A Poisson regression analysis of the data indicated that weed cover composition significantly affected the incidence of *Empoasca* eggs on *Dracaena* leaves (X^2 = 23.33; df = 6,63; p = 0.0007).

Orthogonal contrasts of the treatments (Table 4.4) showed that the number of eggs per tip is significantly higher on the plots without weeds in comparison to the mean number observed on the rest of plots with different weed cover combinations (X^2 = 22.57; df = 1,63; p <0.0001). Plant tips from the weed free treatment had on average 4.07 eggs, which was the highest infestation observed, against 2.24 eggs on average for the other treatments together. The second highest incidence observed was for the *Drymaria cordata* cover treatment with 3.4 eggs per plant tip compared to the average of 1.63 on the other treatments excluding the weed free (X^2 = 5.68; df = 1,63; p = 0.0171). The comparison of the number of eggs on the natural weed cover treatment (Weedy) and the mean number of eggs on the other treatments, excluding Weed Free, also gave significant differences (X^2 = 8.78; df = 1.63 ; p=0.0030) with an average of 2.83 and 2.12 eggs per tip respectively. The combined number of eggs on the treatments with *Drymaria* and Flowering Broad Leaf cover was significantly higher than the number observed on the treatment Rubiaceae ($X^2 = 16.82$; df = 1.63 ; p <0.0001) with 2.53 and 1.33 eggs per tip respectively. The combination of broad leaf treatments (*Drymaria*, Rubiaceae and Flowering Broad Leaf) showed statistical differences on the number of eggs compared to the combination of narrow leaf treatments (Poaceae and Cyperaceae) ($X^2 = 6.12$; df = 1.63; p = 0.0133). More eggs were found on the broad leaf group (2.13 eggs per tip) than in the narrow leaf treatments (2.10 eggs per tip). There was no statistical difference for the number of eggs between the Cyperaceae and Poaceae cover treatments ($X^2 = 0.67$; df = 1.63; p = 0.4118).

There was no significant difference on the number of parasitized eggs found across treatments ($X^2 = 4.34$; df = 6,63; p = 0.6308). The highest number of eggs per plant tip was observed on the weed free treatment followed by *Drymaria cordata* and natural weed cover (weedy) with 4.07, 3.40 and 2.83 eggs per tip, respectively (Figure 4.4.).

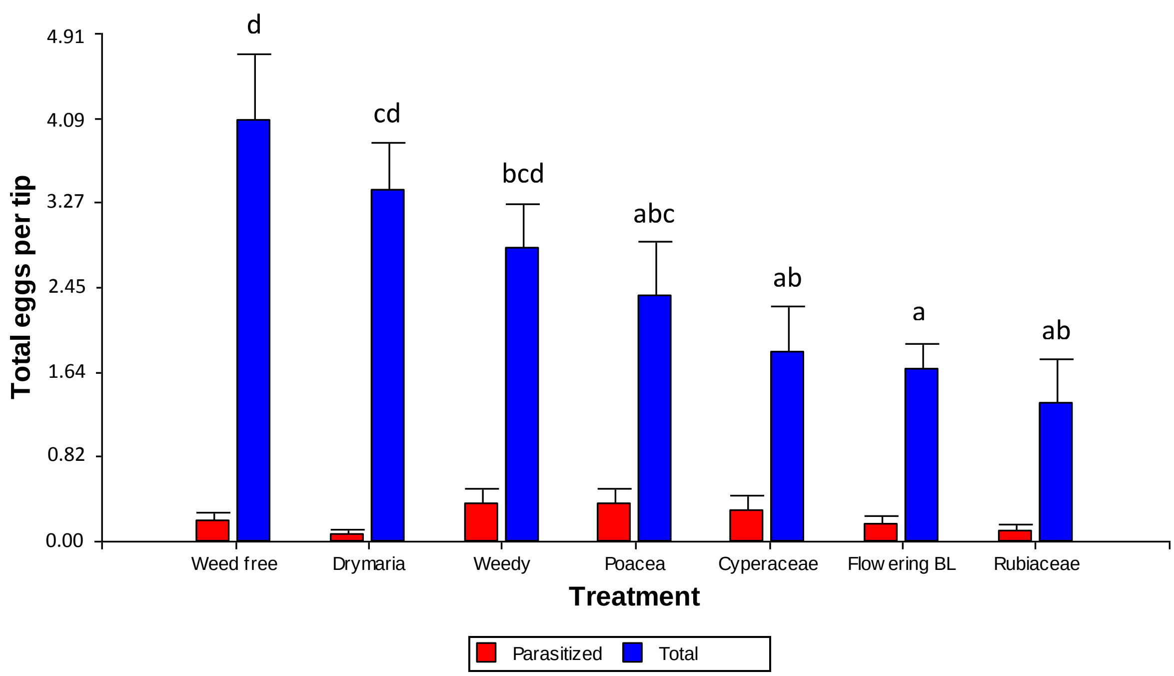

Figure 4.4. Average total number of eggs of *Empoasca* sp. and average number of parasitized eggs per tip, in six weeks old tips growing in *D. marginata* plots with seven different weed cover composition: Natural weed cover composition (Weedy), without weeds (Weed free), Plants from the family Caryophyllaceae (*Drymaria cordata*), Poaceae, Cyperaceae, Rubiaceae and flowering broad leaf weeds (Flowering BL), in Guapiles, Costa Rica, 2007. (See Table 4.3 for more explanation).

Table 4.4. Orthogonal contrasts for the mean total number of *Empoasca* eggs per plant tip in six weeks old tips growing in *D. marginata* plots with seven different weed cover composition: Natural weed cover composition (Weedy), without weeds (Weed free), Plants from the family Caryophyllaceae (*Drymaria cordata*), Poaceae, Cyperaceae, Rubiaceae and flowering broad leaf weeds (Flowering BL), in Guapiles, Costa Rica, 2007.

Contrast	df (Num)	df (Denom)	[i]Mean1	*Mean2	X^2	p-value
Weed Free ***vs*** All other treatments	1	63	4.07	2.24	22.57	<0.0001
Weedy ***vs*** Other treatments except Weed Free	1	63	2.83	2.12	8.78	0.0030
[ii]Broad leaf ***vs*** [iii]Grasses	1	63	2.13	2.10	6.12	0.0133
Drymaria ***vs*** Flowering BL	1	63	3.40	1.67	5.68	0.0171

[i]Means 1 and 2 correspond to the means of the first and second parameter compared on the contrast.
[ii]Broad Leaf = *Drymaria*+Rubiaceae+Flowering Bl
[iii]Grasses = Poaceae+Cyperaceae

DISCUSSION

Studying the behavior of small insects with cryptic oviposition habits is not possible without the appropriate methods and tools. Detecting *Empoasca* sp. eggs in *D. marginata* requires an effective staining procedure that allows for the quantification of oviposition rates. Detecting the presence of eggs inside *Dracaena* leaves will make it possible to determine the distribution patterns of the population in the field facilitate the selection of management strategies, and allow an improveed evaluation of the performance of the applied control measures.

In this study, I found that the hardness of *D. marginata* leaves and its waxy cuticle made it difficult to clear the tissue; However, three of the staining procedures tested were shown to be effective allowing the inspection of the inner tissue under the light microscope. Method 4 (Table 4.1), using 0.5% acid fuchsin with a mixture of 1:1:1 chloroform, methyl alcohol and lactic acid, was preferred over Method 1 (Moorthy et al. 1988) and Method 3 (Lactophenol-acid fuchsin used by Nuessly *et al*.1995) for clearing the leaf tissue because it did not expose the user to phenol. Eggs were stained bright pink making them easily observed under the light microscope.

Using this staining method we were able to detect that changes in weed community composition significantly affected the number of *Empoasca* eggs laid on *D. marginata* leaves, based on direct counts. The highest incidence of eggs was observed on the leaves of *Dracaena* maintained without a weed cover (weed free treatment). This may be the result of the reduction of alternate hosts for egg laying, leaving only *Dracaena* as a possible host. *Empoasca* is a polyphagous insect that will feed and reproduce on a wide number of suitable hosts (Poos and Wheeler 1943). *D. marginata* has been identified as one of the four most frequent genera associated with leafhopper feeding and egg laying on its leaves (CSP 2008). The reduction of plant diversity may force the insects to use the crop as its main host, thus increasing the rate of oviposition. Perez (2007) observed that *Oncometopia clarior*, another polyphagous species of leafhopper associated *with D. marginata*, also preferred this species for feeding, resting and as an oviposition site. The orthogonal contrast analysis confirms that the number of eggs laid by *Empoasca* on the crop in absence of a weed cover, is significantly higher than the mean number of eggs in the other treatments where a variety of weed species combinations were available for oviposition. This suggests that a *Dracaena* plantation that includes alternate weed hosts presents a more diffuse resource for *Empoasca* females in search of oviposition sites. As such, egg densities per *Dracaena* tip are lower because eggs are distributed over the tips of *Dracaena* and other plant hosts. Dilution of egg abundance per colonized unit has been observed for other highly mobile pests (Yamamura 1999 and references therein).

The treatment with *Drymaria cordata* cover had the second highest incidence of *Empoasca* eggs laid on the *Dracaena* leaves. During a host preference trial, Perez (2007) determined that *D. cordata* was not used for feeding or resting by *Oncometopia clarior*, one of the leafhopper species associated with *D. marginata* plantations in Costa Rica. The same weed was used by Hilje and Stanley (2007) as a covercrop in tomato resulting in a reduction of the number of incoming white flies into the crop. Lamp *et al.* (1994) conducted extensive work to define the pattern among the host plants of *Empoasca fabae*, but did not find any host belonging to the same family of *D. cordata* (Caryophyllaceae) among 220 species reported. Because of the low preference of leafhoppers for this weed cover we expected their presence to deter their populations and decrease the incidence In contrast it showed to bear significantly higher number of eggs than the flowering broadleaf cover. A feasible explanation for this behavior might be given by the low height of *Drymaria* and the low habitat complexity it provides as a cover plant. Finke and Denno (2002) found high Intra-guild predation rate of mirid bugs by wolf spiders, both planthopper predators, in structurally simple habitats but, this antagonism decreased in complexly structured vegetation by providing mirids with refuge from spider, incrementing the collective effect on against planthopper populations. This suggests that intra-guild predation induced by the lack of refuge for predators in plots with *Drymaria* cover, might have reduced the efficacy of natural enemies for reducing the *Empoasca* population resulting in an increment of the eggs laid in the crop.

Dracaena tips from the weedy treatment were the next highest in egg abundance. One of the major species on this treatment was *Spermacoce latisfacia* (Rubiaceae), which was also the main component of the Rubiaceae cover treatment. Perez (2007) determined that this weed was visited and occasionally used for feeding by the leafhopper *O. clarior*, suggesting that it is attractive to this group of insects, although not as an oviposition site. Based on this observation, the observed increase in the number of eggs laid on the crop in this treatment may be caused by an increased population of *Empoasca* attracted by the weed cover but then laying their eggs on the crop.

The family Poaceae has been pointed out as a possible source of infestation of *Empoasca* sp. to crops since adult *Empoasca* are commonly found feeding on a variety of grasses. However, Lamp *et al.* (1994) in their list of hosts for *Empoasca fabae* classified all of the species in the class Liliopsida, including grasses (Poaceae) and sedges (Cyperaceae) as non-hosts due to the inability of this insect to produce offspring on any of these plants, probably due to the inaccessibility of nutrients for the nymphal stage. The authors also indicated that in no choice tests, adults of *Empoasca* will survive in various grasses but will not lay eggs. In this study, plots covered predominantly with plants from the Poaceae family showed medium levels of oviposition, on the *Dracaena* plants, compared to other treatments. The treatments with Rubiaceae, Cyperaceae, both classified by Lamp *et al.* (1994) as non-hosts, and flowering broad leaf cover, containing mainly plants on the Scrophulariaceae family (*Lindernia diffusa*), showed the lowest oviposition incidence. This plant family was also described by Lamp *et al.* (1994) as non-hosts for *Empoasca fabae* due to their production of prominent chemical defenses. The comparison of the mean population of eggs on broad leaf weeds and narrow leaf weeds, including Poaceceae and Cyperaceae families, showed statistical differences with higher numbers in broad leaf weeds

It was expected that flowering broad leaf weeds would have a positive effect on the abundance of parasitic wasps but the level of parasitism was similar even in the weed free treatment. The existence of extrafloral nectaries in *D. marginata* (Keeler 1985; Bentely 1977) may explain why there was no difference on the distribution of the parasitoids in the experimental field.

CONCLUSION

Although the leaf staining procedures and microscopic examination of the samples was a laborious task, it was found to be feasible and an efficient method for quantifying oviposition of the small and cryptic eggs of *Empoasca* sp. in *Dracaena marginata*. The selected technique allowed for the identification of behavioral patterns that may be considered while developing management strategies for this specific pest.

There was a clear effect of the presence of weed cover and its composition on the incidence of *Empoasca* eggs on *D. marginata* leaves. The results suggest that selective management of cover crops may contribute to the reduction of the pest population.

The weed free plots had the highest average number of eggs per tip followed by the treatment with *Drymaria cordata* cover. Considering that *D. marginata* has shown to be a suitable host to *Empoasca* sp. for feeding and oviposition, the absence of weed cover might have had an adverse effect on the crop, limiting the host availability for the pest therefore increasing oviposition. In plots with a diverse weed cover, the charge of eggs was possibly laid in more than one host decreasing the incidence in *D. marginata*.

Drymaria cordata, sometimes used as cover crop in *D. marginata* plantations, showed an increase in the number of *Empoasca* eggs laid on the crop. This behavior may be induced by the short stature of the weed, which might have reduced optional hosts through competition of other cover crops.

Despite the lack of influence of the weed cover on the occurrence of biological control by parasitic wasps, there was a significant effect on the reduction of oviposition linked to the weed cover type. Flowering broad leaves and Rubiaceae cover help to decrease the number of *Empoasca* eggs laid on *D. marginata*. This can be an effect a more diverse source of hosts for oviposition and feeding that is translated in a more balanced system. It also may indicate that some of the weed covers offer higher habitat complexity decreasing intra-guild predation and allowing a better pest control by the natural enemies.

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CHAPTER 5

Evaluation of chemical insecticides for reducing the risk of *Empoasca* sp. infestation on commercial tips of *Dracaena marginata*.

INTRODUCTION

The highly polyphagous leafhopper *Empoasca* sp. has been found to use *Dracaena marginata* as a host (CSP 2008); feeding and laying eggs inside its leaf tissue. Whereas in other crops such as potato, grapevines and soybean, *Empoasca* sp. is important due to potential yield reductions or disease spreading (McPherson et al. 2008; Kaplan et al. 2008; Galeto et al. 2011; Lamp et al. 2011).The damage in *D. marginata* is mainly cosmetic and visible only when the crop is under a high infestation. *D. marginata* is a foliage ornamental plant grown predominantly by small producers in Costa Rica bound for the export market to the United States. Due to the quarantine importance of leafhoppers, the presence of any stage of its life cycle on shipments sent to the US market are a cause for interception and application of sanitation actions by the plant health authorities of this country.

Different from the eggs of other leafhopper species such as *Oncometopia clarior* and *Caldwelliola reservata* (Chapters 2 and 3) which are commonly found on *D. marginata*, the eggs of *Empoasca* are impossible to detect without appropriate staining procedures and the use of light microscope. These eggs are very small (about 1mm long) and are laid inside the leaf tissue (Chapter 4). The difficulty for detecting *Empoasca* eggs during inspection at the packing house, poses a high risk of introducing this species into importing countries or cause the interception of the shipments when nymphs hatch during the transportation process and are detected at the ports of entry. An option to get over this problem is to find effective treatments capable of killing the eggs or the first instar nymphs during emergence. The objective of this study was to test the efficacy of a group of insecticides for killing the emerging nymphs from infested tips of *D. marginata* and to determine their residual efficacy and persistence to prevent oviposition in the field.

MATERIALS AND METHODS

In order to measure the effectiveness of five chemical insecticides to control nymphs of *Empoasca* sp. emerging from infested *D. marginata* leaves, tips were collected from field grown plants, six weeks into their propagation cycle, then planted into 250ml pots and kept under greenhouse conditions. Acetate sheets were bent and capped with a plastic lid to form a cylindrical cage around each potted tip (Figure 5.1a). Groups of ten unsexed adult *Empoasca* leafhoppers were collected from a *Dracaena* field and transferred into the acetate cages for a period of seven days to assure oviposition. After this time the insects and cages were removed to facilitate the application of the treatments on each plant.

Five commercial insecticides were selected to include characteristics such as systemic translocation capacity or contact action and persistence (Table 5.1). The insecticides were applied to the plants using a hand sprayer until run-off and allowed to dry for one hour. Each plant was then placed on individual trays and randomized on the tables keeping them at least 1 meter apart. The surface of the trays was impregnated with vegetable oil to capture nymphs in case they jumped away from the plant (Figure 5.1b)

The experiment was arranged in a completely randomized design with five replicates and the experimental unit was a single *D. marginata* potted tip.

Table 5.1. List of insecticides and doses of AI used to determine their capacity to kill emerging nymphs on leaves of six week old *D. marginata* tips, exposed to *Empoasca* sp. for 7 days for oviposition.

Product	AI	Concentration (gm AI/liter)
Sevin®XLR 48 SC	Carbaryl	7.92
Vertimec ® 0.18EC	Abamectin	0.0135
Confidor ® 70 WG	Imidacloprid	0.21
Muralla® 10 EC	Cyfluthrin + Imidacloprid	0.25
Decis® 5 SC	Deltamethrin	0.025
Applaud® 25 WP	Buprofezin	0.125

Evaluated variables

The number of emerging nymphs was counted every other day for a period of 14 days. They were categorized as either live or dead during hatching. After 14 days, the tips were collected, labeled and stained, using chloroform, methyl alcohol, lactophenol (1:1:1), and 0.5% acid fuchsin for 72 hours, then cleared in ethyl alcohol for 24 hours (Neergaard 1997) (Chapter 3). After staining, the leaves were detached and counted and were examined under the microscope to detect unhatched eggs. An analysis of variance was performed on the average number of unhatched eggs per tip and the mean cumulative number of live and dead nymphs using generalized linear models with the statistical program Infostat (Di Rienso *et al.* 2008).

Figure 5.1. Acetate cage used to confine *D. marginata* tips inoculated with adult *Empoasca* leafhoppers in order to force oviposition on their leaves (A) and, arrangement of the trial using *D. marginata* tips infested with *Empoasca* eggs and sprayed with the chemical insecticide treatments (B).

To study the persistence of insecticides applied to *D. marginata* against the adult stage of *Empoasca* sp., the insect growth regulator Applaud® 25 WP (Buprofezin) at 0.5ml/l and the other products and concentrations found in Table 5.1, were applied to six weeks old Dracaena tips. Groups of adult *Empoasca* were exposed to the treated plants at four different times with two weeks difference between exposures. The exposure times were: 0 weeks (insects exposed to the plants the same day of the application, approximately 2 hours after spraying), 2 weeks, 4 weeks, and 6 weeks after application. Mortality was recorded after 48 hours of exposure and analysis of variance was performed using generalized linear models with the statistical program Infostat (Di Rienzo *et al.* 2011). The experimental design was completely randomized with three replicates of one potted plant and five adult *Empoasca* per replicate.

RESULTS

There was a significant difference between treatments for the number of dead nymphs observed (F=19.50; df=5,23; p<0.0001), as well as the number of nymphs surviving the treatments (F=27.50; df=5,23; p<0.0001). However, there was no difference in the number of unhatched eggs remaining on the leaves after 14 days of incubation (F=0.58; df=5,23; p=0.7124) or the total *Empoasca*, which was represented by the number of nymphs and eggs per tip (F=2.18; df=5,24; p=0.0898). The insecticides Confidor ® 70 WG and Muralla® 10 EC produced 100% mortality at the end of the evaluation period, followed by Sevin®XLR 48 SC and Decis® 5 SC with 99 and 93% mortality respectively, whereas Vertimec ® 0.18EC showed no difference from the unsprayed control with 38 and 35% respectively (Table 5.2). The number of dead nymphs varied from 0.6 per tip, in the unsprayed control, to 12.8 in the tips applied with Sevin®XLR 48 SC. The number of dead nymphs was not significantly different among the treatments Confidor ® 70 WG, Decis® 5 SC, Muralla® 10 EC and Sevin®XLR 48 SC although they ranged from 5.6 to 12.8 dead nymphs per tip (Figure 5.2 and Table 5.2). The highest survival of nymphs occurred on the unsprayed Control with 14.4 nymphs per tip but was not significantly different from the survival on plants sprayed with Vertimec ® 0.18EC on which an average of 11.2 nymphs per tip survived (Table 5.2)

There was a significant interaction between the insecticide applied and the weeks after exposure when adult *Empoasca* were introduced to the plants (F=17.0; df=18,56; p<0.0001). This indicates significant differences in the persistence of the products and their capacity for killing nymphs during emergence. All the treatments, except the unsprayed control, produced 100% mortality to the insects exposed the same week when the plants were initially sprayed (week 0). The treatment with Applaud (Buprofezin) showed the lowest persistence causing only 26.7% mortality in average two weeks after the initial application (week 2) and 6.7% on weeks 4 and 6 (Table 5.3). The level of mortality on the treatment Vertimec ® 0.18EC decreased to 73.3% on week 2 and continued decreasing on weeks 4 and 6 with 53.3 and 33.3 respectively. The treatments Confidor ® 70 WG, Decis® 5 SC, Muralla® 10 EC and Sevin®XLR 48 SC kept levels of mortality of 100% or near 100% throughout weeks 0, 2 and 4 but started to show strong variations on the level of mortality on week 6 (Table 5.3). Data on Table 5.3 and the curves on Figure 5.3 show a sudden drop on the mortality produced by treatment Confidor ® 70 WG falling from 100% on week 4 to 6.7% on week 6 and Decis® 5 SC which produced only 46.7% mortality, whereas Sevin®XLR 48 SC and Muralla® 10 EC produced both 93.3% mortality at this time.

Table 5.2. Mean number of unhatched eggs, dead and live nymphs and proportion of mortality observed after 14 days of incubation of *D. marginata* tips previously exposed to *Empoasca* sp. adults for oviposition then sprayed with chemical insecticides.

Treatment	n	Unhatched eggs		Dead nymphs		Live nymphs		Total *Empoasca*[1]		Unhatched proportion[2]	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Imidacloprid	5	2.80 a	2.39	6.80 c	4.97	0.00 ab	0.00	9.60 a	5.81	0.26 a	0.23
Deltamethrin	5	3.60 a	1.95	5.60 c	1.67	0.80 b	0.84	10.00 a	2.92	0.35 a	0.18
Cyfluthrin + Imidacloprid	5	2.20 a	2.28	5.60 c	3.21	0.00 ab	0.00	7.80 a	4.38	0.28 a	0.21
Carbaryl	5	5.80 a	7.01	12.80 c	6.98	0.20 a	0.45	18.80 a	13.72	0.24 a	0.17
Abamectin	5	5.60 a	6.73	2.00 b	1.73	11.20 c	7.79	18.80 a	15.30	0.23 a	0.19
Untreated Ctrl	5	6.20 a	2.77	0.60 a	1.34	14.40 c	7.30	21.20 a	7.89	0.32 a	0.17

[1] Total *Empoasca* was estimated as the sum of unhatched eggs + dead nymphs + live nymphs, for each treatment.

[2] Unhatched proportion was estimated dividing the sum of unhatched eggs by total *Empoasca*.

Means within the same column followed by the same letter have no significant difference between them (P<0.05; LSD).

Table 5.3 Mortality of adult *Empoasca* sp. exposed to *D. marginata* tips after zero, two, four and six weeks of application of six insecticides to the plant foliage.

		Week 0		Week 2		Week 4		Week 6	
Treatment	n	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Buprofezin	3	100 f	0	26.7 c	11.5	6.7 ab	11.5	6.7 ab	11.5
Imidacloprid	3	100 f	0	100 f	0	100 f	0	6.7 ab	11.5
Deltamethrin	3	100 f	0	93.3 f	11.5	100 f	0	46.7 d	11.5
Cyfluthrin + Imidacloprid	3	100 f	0	100 f	0	100 f	0	93.3 f	11.5
Carbaryl	3	100 f	0	100 f	0	100 f	0	93.3 f	11.5
Abamectin	3	100 f	0	73.3 e	11.5	53.3 c	11.5	33.3 d	11.5
Untrated ctrl	3	0 a	0	0 a	0	6.7 ab	11.5	13.3 ab	11.5

Means followed by the same letter have no significant difference between them (P<0.05; LSD).

DISCUSSION

Our findings demonstrate that it is possible to reduce the risk of shipping viable *Empoasca* on living *Dracaeana* through the judicious use of pesticides that reduce rates of oviposition and kill nymphs that emerge from eggs that are laid. Four of the insecticides used in this trial were capable of killing 100% or nearly 100% of the nymphs emerging from the infested tips. Although Confidor and Muralla have systemic translocation, the lack of statistical difference on the number of unhatched eggs between insecticidal treatments and the unsprayed control indicates that there was no ovicidal effect caused by the systemic products. Mortality was observed due to persistence of the insecticides on the *Dracaena* leaves. In the preparation of this trial, adult, male and female *Empoasca* leafhoppers were caged for 7 days with single *Dracaena* tips to ensure the presence of eggs on the plant leaves before applying the treatments. This means that the eggs at the end of the 14 days evaluation period were between 15 and 21 days old. Since the time needed for *Empoasca* eggs to hatch under tropical conditions in between 12 and 15 days (King and Saunders 1984), we can assume that the unhatched eggs observed in this trial were not viable. Nevertheless, they were used for estimating the total mortality since they were part of the total potential offspring. The number of unhatched eggs was similar among all the treatments, being the untreated control among the treatments with the higher number observed. This result may suggest that *Empoasca* produces a high proportion of unfertile eggs, possibly due to sub-ideal nutritional conditions, as suggested by Mitzel *et al*. (2005).

The evaluation of the effect of insecticides on *Empoasca* leafhoppers emerging from the eggs shows that nymphs hatching from plant tips treated with Vertimec had almost the same survival rate as the untreated control. Although this abamectin insecticide is considered to be slightly persistent with residual activity from 5 to 15 days (Nornberg et al. 2011), it was ineffective against *Empoasca* in this trial. The capacity of Sevin®XLR 48 SC (Carbaryl) and Decis® 5 SC (Deltamethrin) to produce 99 and 93% mortality of leafhopper nymphs demonstrate that they have good potential as components of an *Empoasca* management strategy on *Dracaena* for the export market. Sevin® has already been used to control *Empoasca* populations in other crops. Root and Gowan (1978), on their study about the effect of insecticides on the structure of the fauna associated with potatoes, reported a sharp reduction of the population of *Empoasca fabae* after biweekly application of Carbaryl (Sevin®). Confidor ® 70 WG (Imidacloprid) and Muralla® 10 EC (Cyflutrin + Imidacloprid) gave 100% control of the emerging nymphs, appearing as the best treatments. These two products share the same active ingredient (Imidacloprid) that has previously shown to be effective on controlling *Empoasca*. Kapadia and Butani (2009) reported persistence of the effectiveness of Imidacloprid 70 WS when used in fenugreek as a seed coating against *Empoasca spinosa* (Dworowaska and Sohi) and continued to control the insect after 90 days of seed germination. Sarkar *et al*. (1999) studied the persistence of Imidacloprid in water, finding that the concentration of Confidor 200 SL drops 17 to 35% in 15 days and the dissipation increases to 45 to 62% in 30 days in pH values ranging from 4 to 9. Whereas Timmeren et al. (2011) compared neonicotinoid insecticides against *Emposca fabae* in wine grapes finding differences between products and delivery route (foliar of soil application). Despite an initial difference where foliar application showed earlier control, both delivery routes gave long-lasting leafhopper control, with minimum nymph survival after 27 days of the application.

The trial testing the persistence of the insecticides on the *Dracaena* leaves are consistent with the results obtained in the emergence control trial. After 4 weeks of applied, the products Confidor ® 70 WG, Muralla® 10 EC, Sevin®XLR 48 SC and Decis® 5 SC continued to cause 100% mortality to *Empoasca* adults when exposed to the treated leaves.

Vertimec showed significant loss of effect after 2 weeks of application and continued to decrease mortality in the following weeks. The efficacy of Confidor and Decis fell abruptly on the 6th week whereas Muralla and Sevin continued to maintain mortality levels of about 100%. The insect growth regulator included in this trial (Applaud® 25 WP (Buprofezin)) was the treatment with the lowest efficacy losing nearly 75% of its effect on the first 2 weeks. This product had shown good results in other studies controlling *Empoasca*. Moreno and Nakano (2002) observed a high insecticidal effect of Buprofezin on first instar nymphs of *Empoasca kraemeri*. They determined a CL_{50} of 0.112mg/l exposing the nymphs to leaves of *Phaseolus vulgaris* 2 days after the application. Buprofezin interferes with the synthesis of chitin (Uchida et al., 1985) and can affect oviposition and nymph emergence (Shibuya, 1984). Moreno and Nakano (2002) also observed a reduction on the oviposition rate from 120 eggs per female on untreated leaves to 0.9 and 0.1 eggs per female on leaves treated with 10 and 50 mg/l Buprofezin respectively. The background information on this insecticide suggests a good potential that was not observed in the present study.

CONCLUSIONS

Four of the insecticides tested in this study (Confidor ® 70 WG, Muralla® 10 EC, Sevin®XLR 48 SC and Decis® 5 SC were capable of killing 100% of the emerging nymphs of *Empoasca* from infested *D. marginata* tips. The effect of these four products persisted for a period of four weeks; double the time needed for the *Empoasca* eggs to hatch. Two of the products (Muralla® 10 EC and Sevin®XLR 48 SC) showed the longest persistence causing mortality of 93% six weeks after the application. These findings are important because suggest that applications of these products prior to harvest, will significantly reduce the risk of exporting live *Empoasca* nymphs on living shoot tips.

According to the results, the best recommendation, in order to eliminate the risk of surviving *Empoasca* eggs on *Dracaena* tips for the export market, is the application of Muralla® or Sevin® four weeks before harvesting. This will prevent new eggs to be laid and will kill all the nymphs emerging from previously laid eggs.

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CHAPTER 6

Concluding Chapter

INTRODUCTION

Generating knowledge about the pest complex found in *Dracaena marginata* has been an important tool for developing better crop production strategies as well as helping formulate management plans for reducing the risk of quarantine species. Filling knowledge gaps also contributes greatly to the process of decision making when it comes to plant health regulations.

This study started off with the main objective of generating information on the dynamics of four quarantine pests in this crop (leafhoppers, armored scales, katydids and snails) in order to understand the role of key factors such as seasonal abundance and the effects of weed cover composition and plant size on pest incidence.

The leading questions of the research were focused on relevant issues, important for the future of the *D. marginata* as export crop. The first question was: Are there windows of opportunity during the year where the application of management practices can make more efficient the reduction of quarantine pest populations? With this approach we basically were aiming to detect fluctuations on pest abundance attempting to define the causes of this behavior and the possibility of manipulating it to cause further reductions on the population. The second question was: Is there a relation between the size of the commercial plant tip and the incidence of pests? This question responded to the need for generating robust scientific information on pest abundance and plant size in order to determine if there is a valid justification for the size restriction existing in the United States market for *D. marginata*. The information here provided is meant to be an input for the decision maker when it comes to the revision of this regulation.

The predominance of leafhoppers on the interception reports of *D. marginata* (Colpetzer 2005) and the fact that *Empoasca* sp is one of the main species found laying their eggs on the crop (CSP 2008), was the motivation for the third question: Is there and effect of the weed cover composition on the incidence of *Empoasca* egg laid in the *Dracaena* leaf tissue? The polyphagia of this leafhopper well known (King and Saunders 1984) but there is also information on weeds, within the *D. marginata* production areas, that have proven some level of attraction or repellence on leafhoppers (Perez 2007) suggesting an opportunity for managing the weed cover composition as another method for reducing *Empoasca* population. This is especially important since the small size and cryptic location of the eggs make them impossible to detect during the sorting and packing process. However, the nymphs emerging from these eggs during shipping are detectable and possible one of the common causes of interception.

The selection of staining procedures for detecting eggs inside the leaves and the screening of different chemical insecticides, were also included as part of a control strategy to further decrease the risk of survival of nymphs emerging from viable eggs during the shipping. The success of such procedures may eventually be considered as and standard mitigation procedure in the *D. marginata* export process.

PRINCIPAL FINDINGS AND REMARKS

The main four groups of pests found on this study matched the reported by Colpetzer (2005) (katydids, leafhoppers, armored scales ad snails). Leafhoppers showed to be the more abundant group with higher number of species. These pests are present throughout the year posing a constant threat of interception for the exported shipments of *D. marginata. Oncometopia clarior*, *Caldwelliola reservata* and *Empoasca* sp were the leafhoppers most commonly found. The eggs of the first two species were count for pest abundance, but *Empoasca* eggs were impossible to detect en the field due to their small size and cryptic location. This indicates that the real infestation of eggs in the field is potentially higher than reported in this study.

June was a month of coincidence for peaks of population for leafhopper eggs, nymphs and scale insects. Other peaks were observed showing different population dynamics among the four pests (Snails: February, March and October; leafhopper eggs and nymphs: another peak in January; armored scales: October-December; Katydids: November). The variation found on population dynamics and previous findings on contrasting response of the four pests to management practices (Prado *et al.* 2008), suggest the need for identifying individual management strategies that could be incorporated into an IPM program to increase efficacy on their control. Further monitoring of pest population dynamics will provide complementary data needed for confirming the seasonality of population peaks. This information will allow the producer to schedule control measures for those periods in the year when pest population outbreaks are expected to appear. This timely application of pest control will reduce management costs and will prevent populations to reach economically damaging levels.

There was no correlation between the number of interceptions and the abundance of pest in the field during the period of evaluation (October 2006 through September 2007), possibly due to the capacity of growers for selecting and cleaning the plants before shipping. On the other hand, the size of the shipment was the best explaining factor affecting the rate of interceptions. 69% of the interceptions were explained by the shipment size, suggesting that the capacity of exporters for performing an effective quality control of their plants decreases with increment of the volume of the shipments. In order to reduce this problem, it is necessary to adjust the availability of hand labor to perform appropriate inspection and cleaning of the plants according to the volume of plants in process.

There were no statistical differences on the abundance of leafhopper eggs, leafhopper nymphs, katydid eggs, armored scales or snails, associated to the size of the *Dracaena* plant tip. However, there were trends showing higher incidence of leafhopper eggs on smaller tips (18 inches and smaller) than in taller plants. This may have been an effect of the proximity of the smaller tips to the weedy ground cover where the population of leafhoppers is higher. Weeds play an important role on insect population dynamics, within and outside the crop area. Some insects feed exclusively from weeds but others would move into the crop in absence of their preferred weed species (Capinera 2005).

The weed species composition on the ground cover affected the abundance of *Empoasca* eggs laid on *D. marginata* leaves. This evaluation was made possible by using an effective method for clearing the leave tissue and staining the egg with a mixture of chloroform, lactic acid, methyl alcohol (1:1:1) and 0.5% acid fuchsin. The staining process took 78 hours due to the hardness of the leaf, but it gave a good contrast under the light microscope. The egg count showed the highest incidence of oviposition observed where the weed cover had been removed and the ground was bared. This is an important observation with practical implications since *Empoasca* may be using some of the weed species for feeding and also for laying eggs. The complete elimination of the divers weed cover, forces *Empoasca* to move and lay its eggs into the crop. Instead of denuding the ground, selective management of the weed cover may result in a cover that helps to reduce the leafhopper population. This effect was observed with the Rubeaceae cover, which showed the lowest number of *Empasca* eggs per *Dracaena* tip. This cover was selected because it had been described by Perez (2007) as attractive for leafhoppers for feeding and resting. In order to establish this weed cover, other weed species were controlled other species applying the herbicide Fluazifop-p-butil (Fusilade 12.5 EC) and carrying out manual control when necessary.

D. marginata plants growing together with *Drymaria* cover showed the second highest incidence of eggs after the weed free treatment. This weed was characterized by Perez (2008) as no attractive for leafhoppers, suggesting that Drymaria is not a suitable host for Empoasca, resulting on the preference of *D. marginata* for feeding and laying eggs.

These observations demonstrated that weed cover composition and hence weed cover management are feasible strategies to follow within IPM strategies for reducing the incidence of Empoasca eggs on Dracaena leaves. Complementarily, the use of chemical insecticides offered another field of action for reducing the threat of viable *Empoasca* eggs laid on the commercial *Dracaena* tips for the export market. A series of commercial insecticides (Confidor ® 70 WG, Muralla® 10 EC, Sevin®XLR 48 SC and Decis® 5 SC) were evaluated on their capacity to kill emerging *Empoasca* nymphs from harvested plant tips, and their protective effect to prevent *Dracaena* leaves from being used by *Empoasca* for laying their eggs, after being sprayed with the products. Four of the insecticides (Muralla® 10 EC and Sevin®XLR 48 SC) killed 100% of the emerging nymphs during a period of four weeks, and two products showed a high persistence killing up to 93% of the *Empoasca* adults attempting to feed on the leaves six weeks after application. According to these results, the combination of selective weed management and the application of appropriate insecticides will greatly reduce the risk of shipping *D. marginata* plants infested with the cryptic leafhopper *Empoasca* sp. The response of other leafhopper species associated with *D. marginata* will need to be assessed in future studies. However, the information generated in this study can be used as tools for the elaboration of strategies for reducing pest abundance in the field or as remediation alternatives to prevent the dissemination of quarantine pests into countries importing *D. marginata*.

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