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Abundance of *Epiphyas postvittana* (Walker, 1863) in forestry nurseries of São Miguel Island (Azores, Portugal) (Lepidoptera: Tortricidae)

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Abstract

Epiphyas postvittana (Walker, 1863) is an invasive polyphagous pest for the Azores and its bioecology and the potential natural enemies were unknown. We evaluated the temporal profile of this species larval abundance and the number of males captured in sex pheromone traps, hypothesizing that both profiles were similar. The study was carried out on seven endemic host plants and one native species grown in two forest nurseries (Furnas and Nordeste) in São Miguel Island over two years from 2018 to 2019. A total of 827 plants attacked by *E. postvittana* were observed in Furnas nursery (2018: 503, 2019: 324) and 1227 in the Nordeste (2018: 649, 2019: 578), including the presence of 525 larvae distributed by the Furnas (2018: 178, 2019: 79) and from the Nordeste (2018: 131, 2019: 137). In 2019, the average weekly number of males captured in the sex pheromone traps (total 31 weeks) were higher in Furnas (mean ± SE: 9.68 ± 1.982) than in Nordeste (3.33 ± 0.651). In synthesis, (i) the population density varied throughout the year and as a function of the host plant species in production; (ii) the abundance profile of larval and adults suggests has at least three to four generations per year and that adults are active year-round, experiencing some delayed development during the winter; (iii) a low larval density does not represent very serious damage to Azorean endemic plants, but is reflected in the population density of its natural enemies; (iv) some biological control agents are present in the field, parasitizing the larvae (i.e., Braconidae species of *Meteorus ictericus* (Nees, 1811) and *Microgaster opheltes* Nixon, 1968); (v) finally, the knowledge of the population dynamics and its natural enemies needs further and long-term study.
KEYWORDS: Lepidoptera, Tortricidae, *Epiphyas postvittana*, endemic woody plants, forestry nurseries, Laurel Forest, Azores Islands, Portugal

Abundancia de *Epiphyas postvittana* (Walker, 1863) en viveros forestales de la isla de São Miguel (Azores, Portugal) (Lepidoptera: Tortricidae)

Resumen

Epiphyas postvittana (Walker, 1863) es una plaga invasora polífaga en las Azores, pero su bioecología y enemigos naturales eran desconocidos. Evaluamos el perfil temporal de la abundancia de larvas de esta especie y el número de machos capturados en trampas de feromonas sexuales, con la hipótesis de que ambos perfiles eran similares. El estudio se llevó a cabo en siete plantas nutricias y una nativa cultivadas en dos viveros forestales (Furnas y Nordeste) en la isla de São Miguel durante dos años consecutivos de 2018 a 2019. Se observaron un total de 827 plantas atacadas por *E. postvittana* en el vivero de Furnas (2018: 503, 2019: 324) y 1.227 en el Nordeste (2018: 649, 2019: 578), incluyendo la presencia de 525 larvas distribuidas por el Furnas (2018: 178, 2019: 79) y del Nordeste (2018: 131, 2019: 137). En 2019, la media semanal de machos capturados en las trampas de feromonas sexuales (total 31 semanas) fue mayor en Furnas (media ± SE: 9,68 ± 1,982) que en Nordeste (3,33 ± 0,651). En síntesis, (i) la

densidad poblacional varió a lo largo del año y en función de la especie de planta nutricia en producción; (ii) el perfil de abundancia de larvas y adultos sugiere que tiene al menos de tres a cuatro generaciones por año y que los adultos están activos todo el año, experimentando cierto retraso en su desarrollo durante el invierno; (iii) una baja densidad larvaria no representa un daño muy grave para las plantas endémicas de las Azores, sino que se refleja en la densidad de población de sus enemigos naturales; (iv) algunos agentes de control biológico están presentes en el campo, parasitando las larvas (por ejemplo, especies de Braconidae de *Meteorus ictericus* (Nees, 1811) y *Microgaster opheltes* Nixon, 1968); (v) finalmente, el conocimiento de la dinámica de la población y de sus enemigos naturales necesitan más estudios a largo plazo.
PALABRAS CLAVE: Lepidoptera, Tortricidae, *Epiphyas postvittana*, plantas leñosas endémicas, viveros forestales, bosque de laurel, islas Azores, Portugal.

Introduction

In the Azores archipelago the Laurel forests already dominated the landscape and could have occupied more than 2/3 of the territory. However, the Azorean islands were extremely impacted by human activities, mainly associated with a dramatic land-use changes (only about 5% of the original forests still remain; TRIANTIS *et al*., 2010), habitat degradation and the introduction of exotic and invasive species (CARDOSO *et al*., 2010; TRIANTIS *et al*., 2010; BORGES *et al*., 2013; TERZOPOULOU *et al*., 2015).

Currently, the Official Forestry Services rear Azorean endemic plant species, which seeks the restoration of the Laurel Forest and areas with high erosion risk or sensitive from the hydrological point of view, awareness-raising activities, and support forestation by private landowners (ROSAGRO *et al*., 2019). In São Miguel Island, most of these plants are reared in two forestry nurseries located in Povoação (Furnas) and Nordeste counties. Since 2008, nursery production of endemic woody plants has increased significantly, reaching in Furnas and the Nordeste, respectively, 55,000 and 80,000 plants annually (ROSAGRO *et al*., 2019). Among about 80 endemic plants that inhabit the Azorean archipelago (VIEIRA *et al*., 2020), eight species integrating the IUCN red list are reared in both forestry nurseries (Table 1).

Table 1.– List of Azorean the endemic plant species reared in Furnas and Nordeste forestry nurseries integrating the IUCN red list.

Common name	Scientific name	Family	IUCN status
Azevinho	*Ilex azorica*	Aquifoliaceae	Least Concern
Cedro-do-mato	*Juniperus brevifolia*	Cupressaceae	Vulnerable
Faia	*Morella faya*	Myricaceae	Least Concern
Folhado	*Viburnum treleasei*	Adoxaceae	Least Concern
Ginja	*Prunus azorica*	Rosaceae	Endangered
Pau-branco	*Picconia azorica*	Oleaceae	Least Concern
Sanguinho	*Frangula azorica*	Rhamnaceae	Least Concern
Urze	*Erica azorica*	Ericaceae	Near Threatened

The use of phytopharmaceutical products is limited to a single fungicide compound, which makes endemic plants vulnerable to attack by insect pests such as aphid and lepidopteran species (ROSAGRO *et al*., 2019). Preliminary studies allowed us to identify a major lepidopteran pest attacking Azorean endemic plants and one native species (Faia) (Table 1): *Epiphyas postvittana* (Walker, 1863) (Tortricidae), an exotic species commonly named the light brown apple moth (LBAM).

Epiphyas postvittana (Fig. 1) has a long list of synonyms (e.g., see VIVES MORENO, 2014; GBIF, 2021). It is native to Southeastern Australia, but has invaded Western Australia, New Zealand, Hawaii, New Caledonia, Australasia and Pacific Islands, Europe, United Kingdom, and California - USA (BROWN *et al*., 2010; SUCKLING & BROCKERHOFF, 2010; CABI, 2021). The species is recorded in numerous areas of biogeographic regions except for Antarctica (BROWN *et al*., 2010). In

the Macaronesia region there are some records of the presence of LBAM, but in the Azores to date it was only cited on São Miguel, Pico, Terceira (VIEIRA & KARSHOLT, 2010; PÉREZ SANTA-RITA *et al*., 2018), and recently recorded to São Jorge and Flores islands (V. Vieira, unpublished). This species is considered introduced to the Macaronesia region, including in the Azores archipelago (VIEIRA & KARSHOLT, 2010).

Fig. 1.– *Epiphyas postvittana*: (A) larva, (B) pupae and (C) adult (Photos: V. Vieira).

In literature, LBAM is multivoltine and an economically important polyphagous pest species. The number of annual generations varies with latitude within its range. There is considerable overlap between generations, with development driven by temperature and larval host plant (BROWN *et al*., 2010; BUERGI, 2012; CABI, 2021). For example, in North America, *E. postvittana* completes 2-4 generations annually. Such in Australia (ZIELONKA *et al*., 2021), populations in California appear to undergo at least four generations and adults are active throughout the year. There is no winter resting stage, although overwintering larvae tend to develop slowly, with a lower threshold of development for all stages of 7.5ºC and an upper threshold of 31ºC (DANTHANARAYANA, 1975); 20ºC is the optimum for development, leading to a life cycle of 25 days (BUERGI, 2012). The variation in its cold response resulted in different forecasts of geographic distribution, which can have important management and regulatory implications (MOREY, 2015). The life cycle of adults lasts 2 to 3 weeks, depending on temperature and hostplant availability (BROWN *et al*., 2010).

Eggs are typically laid in clusters of 3-150 on the upper surface of leaves and take 8 days at 20ºC to hatch (THOMAS, 1975). These give rise to the first generation of larvae. The fully developed larvae are green and have a length from 10 to 20 mm (Fig. 1).

The larvae affect a wide range of horticultural and agricultural plant species in over 100 families (BROCKERHOFF *et al*., 2011; WANG *et al*., 2012). In the southeastern part of Australia and New Zealand, it is a pest on apples, grapes, berries, stone fruits, citrus, vegetable crops and numerous ornamentals (BROWN *et al.*, 2010; WANG *et al.*, 2012). In the Azores it occurs preferably in cultivated and garden areas (PÉREZ SANTA-RITA *et al*., 2018), and attacks the plants reared in forest nurseries. In the first stages, the larva feeds on the undersides of leaves within a silk chamber, later it

continues to feed on leaves, leaf rollers, flowers, or perforate and enter the fruit, causing leaf twisting, browning, and drying out of the needles, inhibition of the stem growth, and leaf damages serve as foci for the establishment of some entomopathogenic microorganisms.

Global knowledge of natural enemies of LBAM is well reported (ADLER, 1991; WEARING *et al*., 1991; HOGG *et al*., 2013) and some spiders, chrysopids and mirids are cited as predators of larvae. Additionally, many hymenopteran parasitoids (braconids, ichneumonids and encyrtids) or tachinid flies attack the larval and egg stages.

The life cycle of LBAM and their natural enemies in the Azores archipelago are unknown. In this study we evaluated the temporal profile in the larval abundance of LBAM in eight endemic host plants grown in two forest nurseries on São Miguel Island. Besides some information on the abundance of *E. postvittana* males captured in sex pheromone traps.

Material and Methods

Study sites

The Azorean archipelago stretches out over 615 km in the North Atlantic Ocean (37-40º N, 25-31º W), 1,584 km west of southern Europe, and 3,900 km east of the North American continent. It comprises nine main islands of recent volcanic origin, distributed in three groups: the western group of Corvo and Flores; the central group of Faial, Pico, Graciosa, São Jorge, and Terceira; and the eastern group of São Miguel and Santa Maria.

The current study was performed in São Miguel Island, the largest island in the archipelago of the Azores, with a surface area of 750 km^2.

To determine the temporal profile of larvae abundance in eight host plants (Table 1), 30 plants of each endemic host species were randomly observed on a weekly basis. One leaf from each plant were collected (with or without larvae if the plant is damage or not) to be analyzed after that in laboratory, in both nurseries at Furnas (37º 46’ 37.790” N -25º 18’ 46.193” W) and Nordeste (37º 49’ 45.128” N -25º 08’ 54.289” W), in São Miguel island. The samplings program occurred during two years between April 2018 and December 2019.

In addition, two populations were monitored weekly from April to December 2019 (total of 31 weeks), using open-sided delta traps baited with a synthetic female sex pheromone lure (containing: E11-14Ac, E9E11-14Ac) to attract males. The numbers of male individuals caught in each trap was recorded weekly. Pheromone lures were replaced once a month in each trap per local between April and December 2019.

Statistical analysis

Before data analysis, given high number of zeros, the number of observed larvae were (x+0.5) transformed to homogenize the variance (ZAR, 2010). None of the data were normally distributed (Shapiro-Wilk test, $p < 0.05$) and did not meet homogeneity of variance assumptions (Levene’s test, $p < 0.05$). Thus, analyses were performed using nonparametric Mann-Whitney U and/or Kruskal-Wallis H tests. In the last case, when significant differences were found, multiple comparisons were performed with unpaired two-samples Mann-Whitney U test applying the Bonferroni correction. All statistical tests were performed using ISPSS® Statistics v. 27, and the significance level was set at = 0.05.

Results and Discussion

The endemic plants reared in the Furnas and Nordeste nurseries, were attacked mainly by two lepidopteran species, being mostly *E. postvittana* (2018: 88.9%, 2019: 99%) and, to a minor

percentage, *Palpita vitrealis* (Walker, 1863) (Lepidoptera: Crambidae) (2018: 12.5%, 2019: 1%). The latter species strongly and exclusively attacked Pau-branco in both nurseries (Furnas: 10.3%, Nordeste: 16.3%), the pattern of which was similar in both years of the survey.

A total of 827 plants attacked by *E. postvittana* were observed in Furnas nursery (2018: 503, 2019: 324) and 1227 in the Nordeste (2018: 649, 2019: 578), including the presence of 525 larvae distributed by the Furnas (2018: 178 (35.4%), 2019: 79 (20.2%)) and from the Nordeste (2018: 131 (24.4%), 2019: 137 (23.70%)) (Table 2, Fig. 2).

Table 2.– Number of Azorean endemic host plants damaged (Total) by *E. postvittana* larvae recorded every week at Furnas and Nordeste nurseries between April 2018 and December 2019. SE= Standard error. L and NL stands for number of plants with or without the presence of larvae, respectively.

Host plants	Furnas 2018			Nordeste 2018			Furnas 2019			Nordeste 2019		
	L	NL	Total	L	NL	Total	L	NL	Total	L	NL	Total
Faia	46	73	119	47	69	116	29	44	73	20	38	58
Urze	3	7	10	2	2	4	0	1	1	0	1	1
Pau-branco	7	55	62	17	330	347	15	123	138	4	189	193
Cedro	1	7	8	3	9	12	3	4	7	1	6	7
Folhado	1	6	7	2	5	7	0	0	0	0	0	0
Sanguinho	24	57	81	26	79	105	30	69	99	38	111	149
Ginga	95	117	212	28	18	46	2	4	6	73	96	169
Azevinho	1	3	4	6	6	12	0	0	0	1	1	2
Total	178	325	503	131	518	649	79	245	324	137	441	578
Mean	22.25	40.63	62.88	16.38	64.75	81.13	9.88	30.63	40.50	17.13	55.25	72.38
SE	11.82	14.78	26.09	5.77	39.38	41.12	4.63	16.01	19.43	9.30	24.76	29.74

Concerning the weekly average abundance of larvae, in 2018, at Furnas nursery it was highest in Ginja (mean ± SE: 2.79 ± 0.577 larvae), Faia (1.35 ± 0.249) and Sanguinho (0.71 ± 0.172), while in the Nordeste it was in Faia (1.38 + 0.280), Sanguinho (0.77 ± 0.174) and Pau-branco (0.5 ± 0.175). In 2019, the highest number of larvae was observed at Furnas in Faia (0.73 + 0.139), Sanguinho (0.75 ± 0.205) followed by Pau-branco (0.38 ± 0.122), while in the Nordeste it was in Ginja (1.81 ± 0.436) followed by Sanguinho (0.94 ± 0.240) and Faia (0.50 ± 0.095). In both the Furnas and Nordeste nurseries, during the two years of observations, Urze, Cedro, Folhado and Azevinho showed a very low average weekly number of larvae (< 0.1 larvae). At the Furnas nursery in 2019, unlike in 2018, the number of larvae found on Ginja was very low due to the small number of plants in production (Fig. 3).

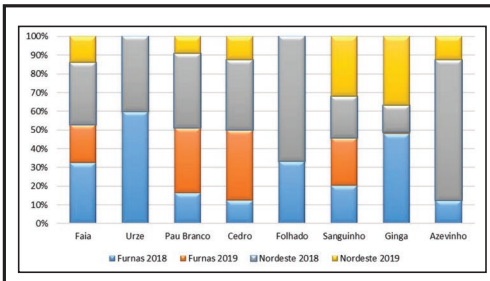

Fig. 2.– Percentage of *E. postvittana* larvae found weekly on the eight host plants at the Furnas and Nordeste nurseries during 2018 and 2019.

The weekly mean abundance of *E. postvittana* larvae only varied significantly for overall host plants during both years of observations on Ginja (Kruskal-Wallis [KW]: x^2 = 35,393; df = 3; p = 0,000) and Azevinho (KW: x^2 = 10,178, df = 3; p = 0,017). Comparing annual larval abundance on Ginja, we found a significant difference only for the Furnas 2018/Furnas 2019 (Mann-Whitney U test: Z = -46.225, p = 0.000), Furnas 2018/Nordeste 2018 (Z = 33.309, p = 0.001) and Furnas 2019/Nordeste 2019 (Z = - 31.963, p = 0.000).

The larval abundance was relatively low at both locations and seems to be dependent on the density of their host plants in production and may show a distinct profile variation from year to year. Although *E. postvittana* fed on all eight nursery-grown plants, it showed a greater preference for young plants of Faia, Sanguinho and Ginja (Fig. 3).

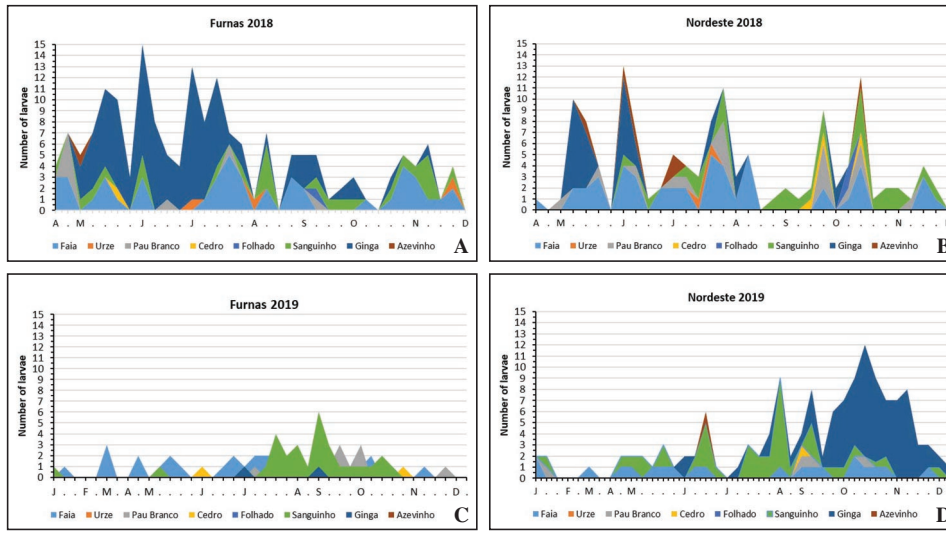

Fig. 3.– Weekly average abundance of *E. postvittana* larvae on eight Azorean endemic host plants in the Furnas and Nordeste nurseries between April 2018 and December 2019. J- January, F- February, M- Mars, A- April, M- May, J- June, J- July, A- August, S- September, O- October, N- November, D- December.

Table 3 shows that the mean weekly abundance of *E. postvittana* males captured in the sex pheromone traps between May and December 2019 (total 31 weeks) was higher at the Furnas nursery (76.14%) than at the Nordeste (23.86%) (Z = -1.960; p = 0.05). However, it was observed that the pattern of adult distribution is relatively similar for both sites (Fig. 4).

The results of the average weekly abundance of *E. postvittana* males seems to indicate overlapping generations depending on the presence/absence of host plants in production (Figs 3 and 4), under relatively stable abiotic conditions, but maintaining the pattern of abundance throughout the year at both larval and adult levels. As observed for continental regions, particularly in Australia (ZIELONKA *et al.*, 2021) and USA - California (BUERGI, 2012), there are at least 3 to 4 annual peaks in the abundance of larvae and adults, suggesting that the populations on São Miguel island appear to undergo at least three to four generations annually; also, adults are active year-round, and there is no winter resting stage.

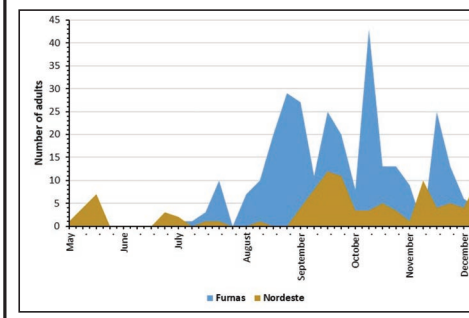

Fig. 4.– Males of *E. postvittana* caught weekly in sex pheromone traps installed in Furnas and Nordeste nurseries between May and December 2019.

Table 3.– Average weekly abundance of *E. postvittana* males collected in the sex pheromone traps, installed in the Furnas and Nordeste nurseries between May and December 2019. N = total number of males.

Local	N	Average	Standard error	Z*	*p*
Furnas	300	9.68	1.982	-1.96	0.05
Nordeste	104	3.36	0.651		

* Mann Whitney U test

Conclusions

Our results provide preliminary information on population dynamics of *E. postvittana* in the Azores, including the temporal profile of larval abundance in seven endemic host plants and one native species grown in two forest nurseries of the São Miguel Island (Furnas and Nordeste) and the abundance of males captured in sex pheromone traps.

The results suggests: (i) the population density varied throughout the year and as a function of the host plant species provided; (ii) the abundance profile in São Miguel Island indicates at the occurrence of at least three to four generations per year and that adults are active year-round, experiencing some delayed development during the winter; (iii) a low larval density does not represent very serious damage to Azorean endemic plants, but is reflected in the population density of its natural enemies; (iv) some biological control agents are present in the field, parasitizing the larvae (i.e. Braconidae species of *Meteorus ictericus* (Nees, 1811) and *Microgaster opheltes* Nixon, 1968); (v) finally, the knowledge of population dynamics and its natural enemies needs further and long-term study.

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