

ARGYRESTHIA ATLANTICELLA REBEL (INSECTA: LEPIDOPTERA) AN EXCLUDED AGENT FOR MYRICA FAYA AITON (MYRICACEAE) BIOCONTROL

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ARQUIPÉLAGO



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Myrica faya (Myricaceae), is a small tree, considered as an Ibero-macaronesian endemic, and classified as an aggressive weed in Hawaii, after its introduction by Portuguese immigrants. *Argyresthia atlanticella* (Lepidoptera, Yponomeutidae), was found associated with *Myrica faya* in the 1980's, in the Azores Islands, and was considered a potential biological control agent for that plant species. Larvae develop on *M. faya* male flowers and green fruits from April until August, and have the potential to decrease seed set. In the 1990's, field studies showed that *A. atlanticella* was also associated with *Erica scoparia* ssp. *azorica* and *Vaccinium cylindraceum* (Ericaceae). Adults and larvae were found associated with *E. scoparia* ssp. *azorica* throughout the year. In the laboratory, *A. atlanticella* oviposited on *E. scoparia* ssp. *azorica* shoots, and developed to fertile adults. In view of these results, this species was excluded as a candidate for the biological control of *M. faya* in Hawaii.

SILVA, LUÍS, GEORGE MARKIN & JOÃO TAVARES 1995. *Argyresthia atlanticella* Rebel (Insecta: Lepidoptera) um inimigo natural excluído para o controlo biológico de *Myrica faya* Aiton (Myricaceae). *Arquipélago*. Ciências Biológicas e Marinhas 13A: 105-113. Angra do Heroísmo. ISSN 0870-6581.

Myrica faya (Myricaceae), um arbusto ou pequena árvore, considerada como um endemismo Ibero-macaronésico, foi classificada como infestante no Hawaii, após a sua introdução por imigrantes portugueses. *Argyresthia atlanticella* (Lepidoptera, Yponomeutidae), foi encontrada associada a *Myrica faya* nas ilhas dos Açores na década de 1980, tendo sido considerada como um potencial agente de luta biológica contra aquela planta. Os estados larvares desenvolvem-se sobre flores masculinas e frutos de *M. faya*, de Abril até Agosto, tendo sido considerados como um meio de diminuir o potencial reprodutor do hospedeiro. No início da década de 1990, estudos de campo revelaram que *A. atlanticella* também se encontrava associada a *Erica scoparia* ssp. *azorica* e *Vaccinium cylindraceum* (Ericaceae). Adultos e larvas foram encontrados sobre *E. scoparia* ssp. *azorica* ao longo de todo o ano. Em testes de oviposição, *A. atlanticella* preferia os rebentos de *E. scoparia* ssp. *azorica* aos de *M. faya*. O insecto completa o seu ciclo de vida sobre *E. scoparia* ssp. *azorica*. Face a estes resultados, excluiu-se *A. atlanticella* como espécie a utilizar no controlo biológico de *M. faya* no Hawaii.

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INTRODUCTION

Myrica faya Aiton (Myricaceae), a shrub or small tree that is considered as an ibero-macaronesian endemic (QUEIRÓS 1987), was introduced to Hawaii in the 1800's by Portuguese immigrants (KIM 1969). By the 1950's *M. faya* was considered a noxious weed, invading rangelands, pasturelands and the natural forests of Hawaii. In the 1950's (YAMAYOSHI 1954; KRAUSS 1964) and later, in the 1980's a biological control program was initiated to search for natural enemies (HODGES & GARDNER 1985; GARDNER et al. 1988; GARDNER & HODGES 1990; MARKIN 1990; SILVA 1992). In the Azores Islands, *M. faya* natural enemies were surveyed and evaluated from October 1991 to October 1993.

Argyresthia atlanticella Rebel (Lepidoptera, Yponomeutidae), was found feeding on *Myrica faya* in the Azores in the 1980's and at that time was considered as a potential biological control agent. GARDNER et al. (1988) noted a staminate flower feeder and referred to it as "red caterpillar" or "pollen feeding lep". Adults reared from *M. faya* in the Azores were sent for identification to the International Institute of Entomology (CAB International) and were identified as *Argyresthia atlanticella* ? Rebel. The identification was later confirmed by Passos de Carvalho (Estação Agronómica Nacional, Oeiras, Portugal, pers. commn) and by the authors following the original description given by REBEL (1940). This species is considered as endemic to the Azores Islands. Other references to this species are: São Miguel, Pico, Faial, Flores (REBEL 1940); Terceira (CARVALHO 1982); Graciosa, São Jorge (CARVALHO 1992); Santa Maria (VIEIRA & PINTUREAU 1993); and Corvo (VIEIRA 1994). In this paper, we describe the biology and host range of *Argyresthia atlanticella*, and evaluate its potential as a candidate for the biological control of *Myrica faya* in Hawaii.

METHODS

Field sampling

Study sites

Sampling was performed on a weekly basis at two sites in São Miguel island: Pico das

Camarinhas (150 m); and Lombadas (550 m). Additional studies were made at other places in São Miguel, São Jorge, Faial, Pico and Terceira islands.

At Lombadas *M. faya* is associated with *Pittosporum undulatum* Ventenat, *Erica scoparia* ssp. *azorica* Huchstetter, *Hedychium gardnerianum* Sheppard and *Pteridium aquilinum* (L.) Kuhn, these two species covering free space among shrubs and trees. The following plants are also abundant: *Blechnum spicant* (L.) Roth, *Polygonum capitatum* D. Don, *Potentilla erecta* (L.) Räuschel, *Erigeron karvinskianus* DC. and *Lysimachia nemorum* L.. A layer of humus mainly composed of *M. faya* leaves covers a compact clay soil horizon.

At Pico das Camarinhas, near the coast, the following plants are abundant: *M. faya*, *P. undulatum*, *E. scoparia* ssp. *azorica*, *Festuca petraea* Guthn. ex Seub., *Rubus inermis* Pourr., *Ligustrum henryi* Hemsley, *Phytolacca americana* L.. A layer of humus mainly composed of *M. faya* and *P. undulatum* leaves covers an organic enriched soil horizon, followed by volcanic rock, exposed in many places.

M. faya density is the same for the two places (4000 plants/ha) but *P. undulatum* is more abundant at Pico das Camarinhas, reaching a higher density than *M. faya*. *Erica scoparia* ssp. *azorica* is the third most abundant species. Mean basal diameter of *M. faya* at Lombadas and Pico das Camarinhas was 7.2 cm and 6.6 cm, respectively. Male and female flowers are found from April to August, and fruits from May to November. A massive emergence of seedlings is observed at Pico das Camarinhas in Spring. At Lombadas some seedlings were found in open areas.

Annual mean temperature at both places is 14.9°C. At Lombadas fog is frequent, and vegetation is covered with condensation droplets, while at Pico das Camarinhas strong salty winds are frequent.

Sampling

An adaptation of the beating/tray method (VAN EMDEN 1972) was used, by shaking 100 branches of *M. faya*, to collect adults and larvae with an

entomological net and a suction apparatus. When fruits or flowers were present, 30 terminal shoots were collected and searched for larvae and pupae.

Sex-ratios were calculated. Females were dissected to determine the number of spermatophores and the development of the ovary. They were divided into three groups, using an adaptation of the categorizations given by DAUMAL (1987): 1) immature ovaries, transparent ovarioles with or without undifferentiated eggs without sculptures; 2) functional ovaries, eggs well developed with sculptures, or eggs less developed without sculptures; and 3) degenerated ovaries, empty or nearly empty, only with some sculptured eggs.

Life-cycle

Development of the insect was followed under controlled conditions from egg to adult (20°C, photophase 16 hours) using *M. faya* or *E. scoparia* ssp. *azorica* flowers. Duration of larval and nymphal stages and longevity of the adult were determined. Eggs, larvae and pupae were measured, including the head capsule width of the larvae, using Quantimet 500 (Leica) coupled to a stereomicroscope.

Three different sized cages and varied numbers of adults were used to determine the best conditions for mating and oviposition, namely: 1) a wood cage (60x50x40 cm) with a glass top and nylon mesh on the back ("bouquets" of *M. faya* and *E. scoparia* ssp. *azorica*, shoots, leaves or plyed paper, and 50 adults per cage were added); 2) plastic cages (18x24x10 cm), with similar "bouquets" and 10 adults per cage; 3) and plastic cages (5 cm high, 8 cm diameter) with a fine copper mesh in the lid, small shoots of *M. faya* and *E. scoparia* ssp. *azorica*, and 10 adults per cage. Plant material was changed every other day.

Feeding tests

Test A. Five last instar larvae were placed in Petri dishes with the following food sources: flowers and leaves of *E. scoparia* ssp. *azorica*,

M. faya male flowers or green fruits, *Potentilla erecta* flowers and *Polygonum capitatum* flowers. Five replicates were used per treatment.

Test B. Five first instar larvae were placed in a Petri dish with flowers of the following plants as food: *E. scoparia* ssp. *azorica*, *M. faya*, *Potentilla erecta* and *Polygonum capitatum*. Five replicates were used per treatment. The proportion of larvae that survived to the adult stage was calculated and differences among treatments were evaluated with a χ^2 test (SCHERRER 1984).

In both tests plants were presented separately. Tested plants were chosen among those with flowers in the natural habitat of the insect, since no records existed of attacks to agricultural plants and no other species of Myricaceae were available. Larval feeding behaviour was observed using a VCR camera coupled to a stereomicroscope.

Oviposition tests

Test C. In March 1994, terminal shoots of *E. scoparia* ssp. *azorica* (with flowers) and *Myrica faya* (without flowers), and 10 unsexed adults were placed in cylindrical plastic cages (5 cm high, 8 cm diameter) with a cotton plug containing a 10% sugar solution. Twelve replicates were prepared.

Test D and E. In April 1994, test C was repeated using *Myrica* with male flowers (D) or green fruits (E) both with flowering shoots of *Erica*. Ten replicates were used.

The numbers of live adults and eggs laid were recorded every other day. The number of eggs per female was calculated. Differences between the number of eggs laid on each plant were evaluated using the Wilcoxon-Mann-Whitney test (SCHERRER 1984).

Test F. In July 1994, a multiple choice oviposition test was performed. The following plants were used: *Myrica faya*, *E. s. azorica*, *Calluna vulgaris* (L.) Hull, *Vaccinium cylindraceum*, *Myrsine africana* L., *Polygonum capitatum*, and *Juniperus brevifolia* (Seubert) Antoine. Tested plants were chosen among those

found to be natural hosts, and related plants found in the natural habitat of the insect, since no records existed of attacks to agricultural plants and no other species of Myricaceae were available. A 10 cm shoot of each test-plant was placed in a transparent plastic 2 l box, with a fine copper mesh at the lid. The cut end of each shoot was inserted into a portion of water soaked sponge. Three cotton plugs soaked in 10% sugar solution were also added to each box. Forty adults of *A. atlanticella* were placed in each box. Eight replicates were prepared.

Adults used in the tests were collected at Lombadas, in the morning of the test, by beating the foliage and collecting the falling insects with an entomological net and suction apparatus. Tests were performed at 20°C with a photophase of 16 hours. After one week, eggs were counted on each plant and differences between plants were evaluated using the Kruskal-Wallis non-parametric test followed by a multicomparison test (SCHERRER 1984), since variances differed significantly and the statistical distribution of the eggs in the shoots was unknown.

RESULTS

Field sampling

Our observations include the following:

São Miguel: Lombadas and Pico das Camarinhas (larvae and adults on *Erica* and *Myrica* all around the year 1992 & 1993), Salto do Cavalo (adults, July 1992), Lagoa do Fogo (adults, July 1992), Furnas (adults and larvae on *Erica*, February 1993), Lagoa do Fogo (adults and larvae on *Erica*, larvae on flowers, leaves and shoots of *Vaccinium cylindraceum*, June 1994).

São Jorge: Pico do Areeiro (adults, June 1992).

Faial: Varadouro (adults and larvae on *Erica*, June 1993), Caldeira (adults and larvae on *Erica*, larvae on flowers of *Vaccinium cylindraceum*, adults and larvae on *Juniperus brevifolia*, June 1993).

Terceira: Macela (adults and larvae over *Erica*, June 1994), Pico Alto (adults and larvae on *Erica*, June 1994), Caldeira de Guilherme Moniz

(adults and larvae on *Erica*, June 1994), Caldeira de Santa Bárbara (larvae on leaves and shoots of *Vaccinium cylindraceum*, June 1994), Lagoa do Negro (larvae on *Juniperus brevifolia*, June 1994).

Adults were observed flying in July/August, and females with functional ovaries were predominantly found in summer (Fig. 1) so mating may occur predominantly in that season. Mean number of spermatophores per female was 2.1 (range: 0-8).

Larval infestation levels over *M. faya* exhibit two peaks (Fig. 2) one associated with male flowers and the other with green fruits of which no more than 10% are damaged (Fig. 3).

Adults and larvae of *A. atlanticella* are found on *Erica scoparia* ssp. *azorica* and *Myrica faya* throughout the year. In February, larvae mine *E. scoparia* ssp. *azorica* flower buds and in March they begin to attack *M. faya* flowers. In April and May larvae are found abundantly on *M. faya* male flowers and less frequently on female flowers. By that time, *Erica* flowers are drying but larvae are still found associated with young shoots. Association with *M. faya* male flowers end with pollen release and senescence. Later, in June and July, larvae are found on the green fruits as well as *Erica scoparia* ssp. *azorica* shoots, *Vaccinium cylindraceum* flowers and shoots, and *Juniperus brevifolia* immature galbuli.

Life-cycle

Egg

The egg is cylindrical (0.212 ± 0.006 mm diameter, 0.329 ± 0.015 mm long). It is milky-white; the colour does not change during embryogenesis (except for the appearance of the larval head capsule). The larva emerges after about 6 days at 20°C.

Larvae

The first and second larval stages are milky-white, except for the head capsule and the cephalic and anal plates which are dark-brown.

The third and fourth larval stages have a red pattern at the dorsum.

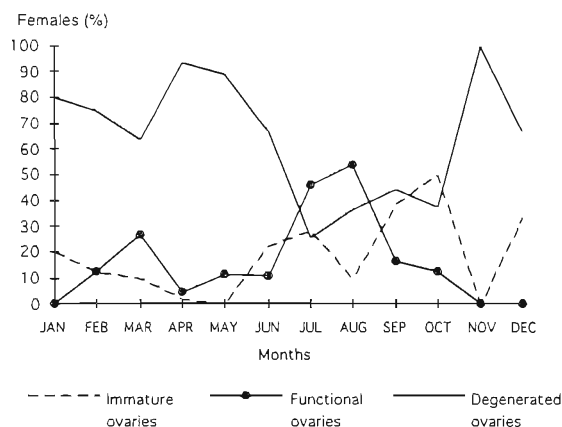


Fig. 1. Ovary development of *Argyresthia atlanticella* females captured on São Miguel island, in 1993.

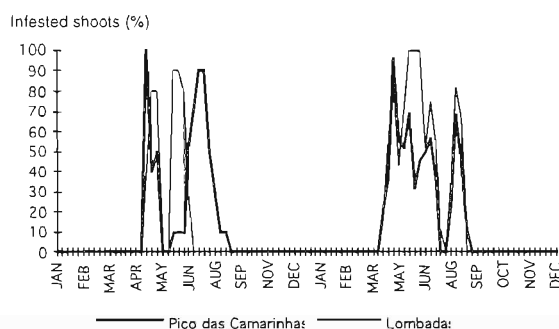


Fig. 2. Proportion of *Myrica faya* shoots infested by *Argyresthia atlanticella* larvae, in 1992 and 1993, at Lombadas and Pico das Camarinhas (São Miguel).

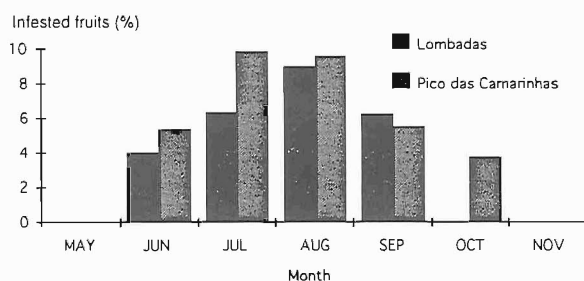


Fig. 3. Proportion of *Myrica faya* fruits infested with *Argyresthia atlanticella* larvae, in 1992, at Lombadas and Pico das Camarinhas (São Miguel).

The larvae measure from 0.5 to 5.5 mm in length. The widths of the cephalic capsules of the four larval stages are: 0.124 ± 0.002 ; 0.185 ± 0.003 ; 0.264 ± 0.003 ; and 0.388 ± 0.006 mm, respectively. Durations of each larval stage at 20°C are: 5.1; 4.8; 4.2; and 4.9 days, respectively.

First instar larvae search actively for a flower or a leaf into which they can mine. They penetrate the leaf or flower using their mandibles, eliminating fecal pellets and detritus through a hole through the cuticle. The accumulation of frass extruding from the hole is an excellent marker for locating larvae.

During the second instar the insect may remain inside the flower, changing to a fresh one when necessary. Alternatively, they may remain in the exterior forming a shelter by connecting different flowers or leaves with silk treads, or by building an incomplete cocoon. Third and fourth instars remain on the surface of the plant, and develop an incomplete cocoon where they hide when threatened.

All larval stages are very active and may suspend from the plant by silk treads. First instar larvae die rapidly from desiccation if a suitable host is not found.

Cocoon

The cocoon is compact and covered by an external and looser sheet. It measures 2.82 ± 0.16 mm long and 1.15 ± 0.06 mm in diameter. The pupal stage lasts 13.4 days at 20°C.

Imago

Virgin adults have a life span of 32.4 (range: 8-60) days. They were originally described by REBEL (1940). The typical morph has a white head and two longitudinal white marks along the front wings (Fig. 4). Other morphs with brown head and the white marks of the wings more or less reduced, or even completely lacking are found.

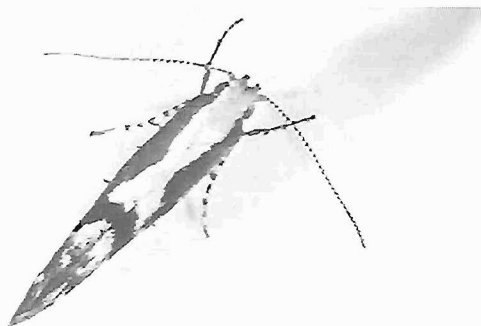


Fig. 4. The typical morph of *Argyresthia atlanticella*, 4 mm.

Sexes differ externally at the tip of the abdomen. In lateral view, male valvae form a round tip, while in females the tip is truncated, and may exhibit the terminal portion of the ovipositor. Fecundity in the laboratory was low, about 12 eggs per female, and dissected females showed about 20 well developed eggs. Oviposition occurs mainly during the first two weeks (Fig. 5). Mated adults have a shorter life span (Fig. 6).

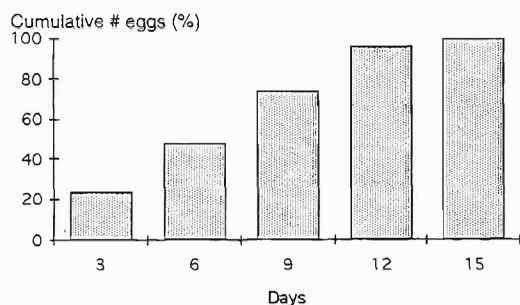


Fig. 5. Oviposition of *Argyresthia atlanticella* after mating (cumulative number of eggs).

Mating and oviposition were observed when 10 adults were added to a plastic box with 5 cm high by 8 cm of diameter with a copper mesh in the lid and using shoots of *Erica* and *Myrica* as oviposition substratum. Eggs were never observed on the walls of the box.

Adults rested on *Erica* and *Myrica* and were observed feeding on a cotton plug soaked with 10% sugar solution. Mating was observed during

both day and night and may take longer than four hours.

Besides one of the dissected females that was found to be parasitized by a nematode (Mermithidae), no other natural enemies were found.

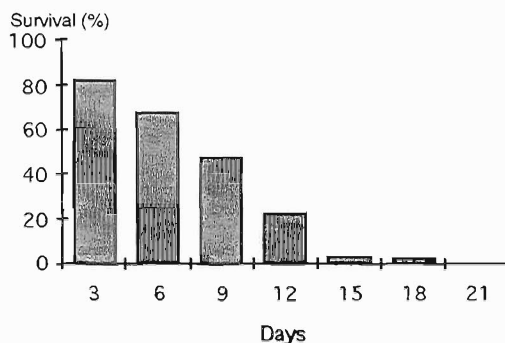


Fig. 6. Survival of *Argyresthia atlanticella* after mating.

Feeding tests

Tests A and B. Last instar larvae developed to the adult stage feeding on the following diets: *Erica scoparia* ssp. *azorica* flowers and leaves, *M. faya* flowers and green fruits, and *Potentilla erecta* and *Polygonum capitatum* flowers.

First instar larvae developed to adult stage feeding on *E. scoparia* ssp. *azorica*, *M. faya* and *Polygonum capitatum* flowers (Table 1).

Oviposition tests

Tests C, D and E

Eggs were generally found in the axils of the leaves or in crevices on the shoot, and more rarely on flowers. Significant differences (Wilcoxon-Mann-Whitney test, $p < 0.05$) were found between the number of eggs laid on each plant, with higher numbers for *Erica* (Table 2). An higher porportion of eggs was found in *M. faya* flowering shoots when compared with shoots without flowers or with fruits.

Table 1

Proportion of *Argyresthia atlanticella* larvae that reach the adult stage when submitted from the first instar to different types of food (flowers of four species of plants).

Test plant	% of survival to the adult
<i>Myrica faya</i>	28 *
<i>Erica scoparia</i> ssp. <i>azorica</i>	36 *
<i>Polygonum capitatum</i>	16 *
<i>Potentilla erecta</i>	0

* No significant differences ($p > 0.05$) between plants (χ^2 test).

Table 2

Number of *Argyresthia atlanticella* eggs laid in shoots of *Myrica faya* (faya) and *Erica scoparia* ssp. *azorica* (heather), when in presence of both plants. Heather with flowers; faya without flowers and fruits (Test C), with flowers (Test D) or with fruits (Test E).

	Test C		Test D		Test E	
	faya	heather	faya	heather	faya	heather
Total	119	785	112	268	8	231
Mean	9.9	65.4	11.2	26.8	0.8	23.1
Maximum	27	163	32	65	2	81
Minimum	0	17	0	2	0	4
Replicates	12	12	10	10	10	10

Significant differences ($p < 0.05$) were found between faya and heather for the three experiments (Willcoxon-Mann-Whitney test).

DISCUSSION

First instar larvae of *Argyresthia atlanticella* were found associated with *Erica scoparia* ssp. *azorica* in various islands of the Azores, even when no *M. faya* plants were present. Larvae were also collected on *Vaccinium cylindraceum*, and developed to the adult stage feeding on leaves and flowers (they feed initially on anthers and later on the ovary). Larvae were also captured in *Juniperus brevifolia* galbulae.

Furthermore, during winter adults and last instar larvae of *Argyresthia atlanticella* are regularly found on *Erica scoparia* ssp. *azorica*. In fact, adults seem to use the plant as a shelter to avoid rain and wind, since *Erica* with its nanophilous leaves acts as a protection against the wind and intersects the atmospheric humidity.

Test F

In multiple choice oviposition tests the insect prefers *E. scoparia* ssp. *azorica* as a substratum for oviposition, followed by a group of three plants, *M. faya*, *C. vulgaris* and *V. cylindraceum*, chosen in lower degree (Table 3). The remaining plants are seldom chosen as substratum for oviposition.

Table 3

Multiple choice oviposition test. *Argyresthia atlanticella* eggs laid (average, standard error and total) on each plant, when in the presence of seven different plants.

Test plant	Eggs laid		
	Average	s. e.	Total
<i>Myrica faya</i> (a)	18.75	7.57	150
<i>Erica scoparia</i> ssp. <i>azorica</i> (b)	40.88	10.9	327
<i>Vaccinium cylindraceum</i> (a)	6.12	1.48	49
<i>Calluna vulgaris</i> (a)	30.75	11.3	246
<i>Polygonum capitatum</i> (c)	1.00	0.63	8
<i>Myrsine africana</i> (c)	2.00	1.07	16
<i>Juniperus brevifolia</i> (c)	0.50	0.27	4

a, b, c: significant differences ($p < 0.05$) between different letters (Kruskal-Wallis test, followed by a multiple comparison test). s.e. standard error.

In this way this plant species would be used not only as food source and an oviposition substratum but also as a shelter, for adults during the cold and rainy seasons.

Feeding tests show that this insect can develop on plants of several different families. Larval feeding behaviour, mining *Erica scoparia* ssp. *azorica* flowers and leaves, suggests a strong association with that plant that was preferred in oviposition tests.

Argyresthia atlanticella feeds on *M. faya* male flowers and green fruits but the insect also completes its life cycle feeding and ovipositing on plants of other families.

BALACHOWSKY (1966) suggested the completion of one generation each year for the species of *Argyresthia* from Europe (univoltine species). On the contrary, in the Azores, A.

atlanticella shows several superimposed generations (multivoltine species), since first instar larvae are found during *Erica* flowering (beginning in February), *Myrica* flowering (beginning in March), and later, in *Myrica* green fruits and *Vaccinium cylindraceum* flowers (June-July). In July and August, adults are seen flying during the day. In winter, adults remain at rest over the vegetation. In that season, some last instar larvae are also collected.

Several species of the same genus are considered as pests (BALACHOWSKY 1966). BIGOT et al. (1988) referred *Argyresthia reticulata* Staudinger on *Juniperus thurifera* Linnaeus in Morocco. *Argyresthia chrysidella* Peyerimhoff was found in France on *Juniperus oxycedrus*. SHARMA et al. (1988) and MIRZOYAN & GRIGORYAN (1987) describe *Argyresthia conjugella* Zeller as a pest of apple-trees, *Prunus padus* and *Sorbus aucuparia*; and *Argyresthia ephippella* Fabricius of *Prunus padus*, *Sorbus aucuparia*, *Corylus avellana* and *Lonicera xylosteum*. BAILLY et al. (1990) noted *Argyresthia pruniella* Clerck as a pest in cherry flowers, new leaves and immature fruits, and *Argyresthia trifasciata* Staudinger that causes dessication of the shoot tip of several Cupressaceae.

According to BALACHOWSKY (1966), about 50% of the European species of *Argyresthia* are associated with Coniferae, and many species associated with Cupressus spp. and *Juniperus* spp. are also found in North America and Japan.

In conclusion, *Argyresthia atlanticella* is a polyphagous species so that the insect should not be used as a biological control agent in Hawaii. On the other hand, this is an endemic and highly polymorphic, phytophagous insect associated with endemic plants. In this regard the insect interactions with its hosts and its genetic variability would deserve further studies.

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