An assessment of population fluctuations of citrus pest woolly whitefly
*Aleurothrixus floccosus* (Maskell, 1896) (Homoptera, Aleyrodidae) and its
parasitoid *Cales noacki* Howard, 1907 (Hymenoptera, Aphelinidae): A case
study from Northwestern Algeria

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Received March 19, 2018; accepted June 19, 2018.


**ABSTRACT**

The aim of this study was to assess the effectiveness of the parasitoid *Cales noacki* Howard, 1907 (Hymenoptera, Aphelinidae) in the biological control of the citrus pest woolly whitefly, *Aleurothrixus floccosus* (Maskell,1896) in Northwestern Algeria. In particular the pest and parasitoid population fluctuations under different environmental conditions were investigated. The study was conducted by examination and sampling of whitefly adult populations on young shoots and catches as well as its parasitoid during a 12-month period by counting the parasite and infested-live larvae. Results showed remarkable variations in abundance indices and infestation rates of larvae and adults that depended on the growth flush in foliage and meteorological conditions of the region. The woolly whitefly manifested three flight periods coinciding with three growth flushes of orange sap; in autumn towards the end of September followed by mid-November with the highest average abundance of 10 individuals per square cm; in summer and the third flight period in mid-May. Phases of growth flush seemed to have an effect on the temporal distribution of adults over the sampling period from July to June. Pest emergence appeared favorable at 12-20 °C as a minimum interval temperature whereas the maximum varied between 25-33 °C with humidity levels of 50 % and 75 %. As for the incidence of natural enemies on whiteflies, despite their abundance, their impact was not optimum due to the large fluctuations in number of whiteflies. Although the pest-parasitoid complex appears to be important in the orchard, the biological regulation exerted by *C. noacki* while effective is insufficient for the complete neutralization of citrus woolly whitefly *A. floccosus*. An integrated approach is needed using additional natural enemies associated with the woolly whitefly.

**Key words:** citrus; biological control; population dynamics; *Aleurothrixus floccosus*; *Cales noacki*; phenology; Chief Northwestern Algeria

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**IZVLEČEK**

OVREDNOTENJE NIHANJA POPULACIJ ŠKODLJIVCA CITRUSOV, ŠČITKARJA *Aleurothrixus floccosus* (Maskell, 1896), IN NJEGOVEGA PARAZITOIDA *Cales noacki* Howard, 1907: VZORČNA ŠTUDIJA IZ SEVEROZAHODNE ALŽIRIJE

Namen raziskave je bil oceniti učinkovitost parazitoida *Cales noacki* Howard, 1907 (Hymenoptera, Aphelinidae) pri biotičnem zatiranju škodljivca citrusov, ščitkarja *Aleurothrixus floccosus* (Maskell, 1896), v severozahodni Alžiriji. Čeprav je prihajalo slišno, da je nastalo znanje populacij škodljivca in parazitoida v odvisnosti od različnih okoljskih razmer. V raziskavi so bile pregledane in vzorčene populace odraslih in mladih poganjkih ščitkarja na mladih poganjkih in v pasteh ter njihovi parazitoidi v obdobju 12-mesecev s štijem parazitoida in napadenih, z živih ščitkarjev. Rezultati so pokazali, da je prihajalo značilno z neznanimi poklici in napadenosti ščitkarjev, kar je bilo obdobja od dinamiče rasti listov in meteoroloških razmer območja. Preučevanje ščitkarjev je imelo tri rodove, ki so opisali s tremi viški rasti in prehodov sopkov pomarančevca in sicer konec septembra, v sredini novembra z največjim številom 10 osebkov na vejico, in tretji, v sredini maja. Obdobja rasti pomarančevca so vplivali na časovno pojavljanje odraslih osebkov v obdobju od julija do junija. Najnižji ustrezen temperaturni interval za pojav ščitkovega je bil med 12 in 20 °C, najvišji pa med 25 in 33 °C pri relativni vlago 50 % in 75 %. Pojav parazitoidov ščitkarjev kljub njihovi številnosti ni bil optimalan zaradi velikega nihanja populacije ščitkovega. Čeprav je kompleks škodljivca in parazitoida pomembno v sadovnjaku, je biotično uravnavanje z parazitoidom *C. noacki* nezadostno za celovito nevtralizacijo ščitkarja na citrusih. Zato je potreben integriran pristop z uporabo drugih parazitoidov ščitkarja *Aleurothrixus floccosus*.

**Ključne besede:** citrus; biotično varstvo; populacijska dinamika; *Aleurothrixus floccosus*; *Cales noacki*; fenologija; Chief; severozahodna Alžirija

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INTRODUCTION

Citrus (Citrus spp.) constitutes the major group of fruits including oranges, grapefruits, trifoliate orange, mandarins, pummelo, citranges and lemon (Gebreslasie & Meresa, 2018). It serves as the main source of vitamins, mineral elements and sugar; hence, it controls the building process of the human body (Oviasogie et al., 2015). In Algeria, Chlef is the second most important citrus growing region with more than 5000 ha of which more than 90 % old plantations (Bellabas, 2011).

Gebreslasie and Meresa, (2018) reported that a decline in productivity of citrus can be attributed to several factors including fungal, bacterial, viral, nematode, and insect pests.

Citrus trees are attacked by several insect pests including the woolly whitefly, Aleurothrixus floccosus (Maskell, 1896) and the citrus whitefly, Dialeurodes citri (Ashmead, 1885) causing considerable economic damage (Yigit & Canhilal, 2005; Uygun & Satar, 2008; Giliomee & Millar, 2009; Tello Mercado et al., 2014; Abrol, 2015). The woolly whitefly was introduced accidentally in 1981 in western Algeria from Spain or Morocco (Doumandji & Doumandji-Mitché, 1986). Citrus woolly whitefly's populations are pervasive and cause infestations with serious damages to citrus sector (Biche, 2012; Mahmoudi et al., 2017).

A. floccosus Maskell is characterized with wings covered by white waxy substance. The adult male is slightly smaller than female attaining about 1.5 mm (Tello Mercado et al., 2014). The woolly whitefly produces copious amounts of sticky honeydew from the lower surface of leaves of lemon and other Citrus species (Grout et al., 2012).

Chemical control of crop pests is known for the negative effects on human health and environment as well as having low efficacy against A. floccosus (Katsoyannos et al., 1997; Nega, 2011). This resistance against insecticide is due to the wool-like wax filaments which cover 3rd and 4th instar nymphs and pupae of A. floccosus impeding penetration of insecticides (Katsoyannos et al., 1997). Integrated management of fruit pests including biological control is crucial to the economic success of many regions in the world (Kissayi et al., 2017).

The parasitoid Cales noacki Howard, 1907, (Hymenoptera, Aphelinidae) is an effective natural enemy and biological control agent of this pest (; Grout et al., 2012; Telli & Yiğit, 2012; Abrol, 2015).

There are few extensive reports which address the biological interaction of the woolly whitefly and its parasitoid C. noacki in the world (Ulusoğlu et al., 2003; ; Telli & Yiğit, 2012; Tello Mercado et al., 2014; Kissayi et al., 2017; Gebreslasie and Meresa, 2018).

The objective of this work was to assess the effectiveness of the parasitoid biological control agent Cales noacki Howard (Hymenoptera, Aphelinidae) against the citrus pest woolly whitefly, Aleurothrixus floccosus Maskell in Northwestern Algeria. The infestation rates of orange trees by A. floccosus and the parasitism rates by C. noacki were investigated in order to determine the favorable conditions for population growth and reproduction of A. floccosus.

The relation between adults’ emergence and the appearance of the growth flush (i.e. young shoots) was followed to explain the close relationship between the number of generations per year and the growth flush. In addition, the parasitism activity by C. noacki was evaluated.

2 MATERIALS AND METHODS

2.1 Description of the case study area

This study was conducted in an orchard, occupying 4 ha, belonging to the citrus growing region of Chlef (36°05'53.3"N 1°13'02.4"E) situated in the northwest of Algeria (Figure 1). This area has a semi-arid bioclimatic stage with mild winters and dry periods extending from mid-April to October’s end. The 12-year-old orchard was planted with orange trees Citrus sinensis L. of the Thomson Navel variety. Orchard maintenance (pruning, manual weeding and fertilisation) were done at the right time without phytosanitary treatments.
2.2 Population sampling of *A. floccosus*

The case study orchard was visited periodically each fortnight from July 2012 to June 2013. Ten whitefly-infested trees were randomly sampled at each fortnight. Four branches on different orientations carrying young shoots and elderly leaves per tree were observed for the sampling of adults and live larvae. In a related study Telli & Yiğit, (2012) considered the distribution of woolly whitefly on the four directions of citrus trees in Turkey.

Adult whiteflies as well as other circulating insects (i.e. entomofauna) within the orchard particularly the natural enemies of woolly whitefly, were caught through sticky traps. Three rectangular yellow plates Horiver-type (25 x 10 cm) were deposit within the canopy at a height of 1.5 m at a rate of one trap per tree at a distance of 50 m (Ekbom & Rumei, 1990). These traps were used to count the number of generations of several pests (Toorani & Abbasipour, 2017). In addition, a total of 50 leaves were randomly collected in the plot at each survey to investigate larvae, pupae and parasitized larvae per 1 cm².

2.3 Infestation rates by *A. floccosus*

The infestation rates of the global populations (T) were calculated according to the Townsend-Heuberger formula (Townsend & Heuberger, 1943) as follow:

\[
T (%) = \left[\frac{(\text{number of branches with presence of whiteflies} \times \text{corresponding presence index})}{\text{sum of branches with presence x 4}}\right] \times 100
\]

The presence index was deduced from a scale of 0 to 4 and corresponds to intervals of abundance expressed as a percentage.

For the case of whiteflies:

- (Index 1) < 25 % of presence
- (Index 2) 25-50 % of presence
- (Index 3) 50-75 % of presence
- (Index 4) > 75 % of presence

2.4 Parasitism Rates by *C. noacki*

The parasitism rates were evaluated at each survey throughout the sampling period. Parasitized larvae were counted on a leaf area of 1 cm² under binocular magnifying glass at × 40 and the results were expressed in percent relatively to the total larvae on the observed surface.

The confirmation of the species of parasitoid emerged (*C. noacki*) was made from leaves carrying parasitized larvae collected and placed in rectangular boxes of dimensions (15 cm × 8 cm × 5 cm) containing cotton impregnated for emergence. Identification of the parasitoid was carried out using several identification keys (Doumandji & Doumandji-Mitchie, 1986; Goulet & Huber, 1993).

2.5 Phenology of orange tree in relation to climatic data

The periods of orange tree’s growth flush during the study period were determined on the basis of the phenological growth stages described by Agusti et al. (1995). Meteorological data including rainfall amounts, average temperature values and relative humidity were obtained from the Boukadir meteorological station (5 km west of the orchard) belonging to the National Meteorological Office in Chlef.

2.6 Statistical analysis

The data were subjected to analysis of variance (ANOVA) for variable with normal distribution whereas the variables with abnormal distribution were subjected to the general linear model (G.L.M.). Differences at P < 0.05 were considered to be significant. The statistical analysis was accomplished using the computer software SPSS 16.0 for Windows (Chicago, IL, USA).
3 RESULTS

3.1 Fluctuations in abundance of A. floccosus

3.1.1 Temporal variations in abundance indices of larval & adult populations of woolly whitefly

The results of temporal variations in abundance indices of larval and adult populations of A. floccosus on the branches of C. sinensis of the studied orchard are given in the Figure 2.

The evolution of adult abundance indices of A. floccosus according to the three growth flush (1\textsuperscript{st} gf, 2\textsuperscript{nd} gf, 3\textsuperscript{rd} gf), showed that the appearance of adults abundance peaks corresponding to the emergence of adults from pupae coincide with the appearance of young leaves of growth flush.

According to our survey, larvae index presents three significant abundance’s peaks during the period of mid-November 2013, late February, and mid-June 2014 (Figure 2). In general, the analysis of fluctuations in adult abundance indices showed the existence of three peaks the most important among them was recorded in November 2013.

The evolution of adult A. floccosus caught through yellow traps revealed a stable distribution from July to late November, with a total ranging from 100 to 230 individuals per trap per month, except for mid-November attaining more than 275 individuals / trap / month.

In the last two months of the survey, a continuous evolution was observed in the adults recording about 600 adults / trap / month. The lowest enumeration of adults caught was recorded at end of March not exceeding 25 adults / traps / month (Figure 3). The first adults emerging from the pupae were noted in mid-June, end-October 2013 and early April 2014.

The analysis of the relationship between adult catches of woolly whitefly and orange's growth flush indicated that summer growth flush (1\textsuperscript{st} gf) and autumn growth flush (3\textsuperscript{rd} gf) coincided with the period of adult large trapping.
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The evolution of A. floccosus biological stage was determined by counting the number of live larvae, pupariums on 1 cm² in 50 leaves. The results are shown in Figure 3. As can be seen from this figure, the live larvae are present throughout the year at all stages of foliage. In contrast, the pupae were almost absent during the period ranging from November 2013 to April 2014.

Furthermore, in 2013, from July to October, the density of the pupae of woolly whitefly was higher than those of live larvae. From November (maximum density) to the end of April, only live larvae were present. The density of larvae exhibited its second peak at the end of May followed by a significant change in the number of nymphs (Figure 3).

3.1.2 Variation in infestation rates of woolly whitefly

The larvae of the whitefly infested the leaves throughout the sampling period. A first phase of infestation by A. floccosus larvae was characterized by rate infestation ranging between 19 % to 39 % from early July 2013 to Mid-January 2014.

A second phase with more abundant infestation was observed in mid-February with an infestation rate of more than 80 %, then it reduced gradually to remain at a rate of 20 % during the month of April, a third stage of infestation was noted at the end of the sampling with a peak of more than 60 % (Figure 4).
Adults infesting the canopy, has shown three phases of infestation, the first phase displayed the highest infestation rate, exceeding 65% in early September, coinciding with the 3rd growth flush (3rd gf) of 2013; the second period recorded low infestation rate from November to December and then decrease progressively to its lowest level from February to March; at the end of the sampling period, a rise in the infestation rate to a maximum of 40% was observed (Figure 4).

3.1.3 Effect of exposure on spatiotemporal distribution of populations of *A. floccosus*

The evolution of abundance fluctuations of adult populations on branches with young leaves showed a preference for exposure to layed eggs on the underside of young leaves.

Indeed, adults emerging during the three flight periods recorded through the sampling period exhibited a remarkable preference for the Northern exposure for setting in the deposition of eggs, the southern exposure was sought by adults during the 2nd and 3rd peaks as well as the 1st peak with less importance (Figure 5).

On the other hand, it was noted that the larval distribution was not affected by either stage of leaves (old or young) not by their exposure during the sampling year (Figure 6).

The impact of different exposures/seasons on larval and adult distribution was analysed using ANOVA. The northern exposure of the canopy exhibited a significant effect on the distribution of adults on foliage comparatively to other exposures (p < 0.05). Similarly, the distribution of *A. floccosus* adults appeared to be influenced by the follow-up period (p < 0.05). However, the distributions of the larval populations seemed to be similar (p > 0.05) on different exposures.

![Figure 5: Effect of exposure on the distribution of adult populations (N: North, W: west, E: east, S: south)](image)

![Figure 6: Effect of exposure on distribution of larval populations (N: North, W: west, E: east, S: south)](image)

3.1.4 Evolution of parasitism by *C. noacki*

The temporal abundance and evolution of parasitism by *C. noacki* was followed during the sampling period. The results are presented in Figures 7.

As can be seen the parasitoid specific to *A. floccosus*, *Cales noacki*, showed three peaks of abundance, the most important was recorded at the end of sampling period during June 2014 with 16 individuals / trap / month (Figure 7). Nevertheless, for the rest of sampling period, few parasitoid adults were caught, not exceeding two individuals / trap / month.
Figure 7 shows *C. noacki*’s parasitism activity recorded throughout the sampling period, the highest activity was registered in July 2013 with more than 75% of parasitism. This rate decreased gradually to a stable interval between 5% and 30% during the other months of the year. This rate of parasitism is calculated according to the number of parasitized larvae in relation to the total number of larvae per cm².

**Figure 7**: Evolution of parasitism rate by *C. noacki* emerged from *A. floccosus* & adult catches in traps on orange trees

### 4 DISCUSSION

Ulusoy et al., (2003) reported that the woolly whitefly does not have a preference for specific citrus varieties. However, it has been observed mainly on orange trees in the Mediterranean region (Barbagallo et al., 1986) where considerable damage for citriculture has been reported especially in Italy (Del-Bene & Gargani, 1991).

Furthermore, there are numerous studies on the population dynamics of *Aleurothrixus floccosus* (Onillon, 1973; Gebreslasie & Meresa, 2018).

In Algeria, few studies on this species have been carried out (Doumandji-Mitchie & Doumandji, 1988; Berkani, 1989). The current study has shown that there are three generations during a one year period. In contrast, Biche (2012) estimated that the woolly whitefly evolves in four to five generations per year, almost uninterrupted throughout the year with a simple slowdown noted during the colder months. The slowdown in infestation rates of larvae and adults of *A. floccosus* during the colder Algerian months of January to April was also noted in the present investigation (Figure 4).

Depending on the phonological characteristics of the *Citrus* (i.e. persistence of foliage, rhythmicity of appearance of growth flush), the dynamics of the pest populations are influenced by the host plant because of the qualitative and quantitative evolution of the resources offered (Bran & Onillon, 1978; Kissayi et al., 2017). This species spends the winter at the third or fourth instar larvae and sometimes at the egg stage, laid in the autumn by the last generation of adults.

Additionally, under the specific climatic conditions of the citrus region of Chlef, it has been noted that the first spring generation appears with the young shoots of the first growth flush of orange trees as a result of the first laying of the year. Ripa et al., (1999) and Giliomene & Millar, (2009) estimated that the underside of young leaves is preferred by the whitefly to feed and oviposit.

In another study, Onillon (1973) reported, that in early spring, the adults of the woolly whitefly prefer the young bitter-orange tree shoots resulting from a total transfer of the imaginal population on leaves of the first growth flush. These results are consistent with those cited by Abbassi (1980).

A second summer generation was recorded in the months of August coinciding with the 2nd summer growth flush. The latest generation and the largest in adults’ number started with the new leaf outputs of the latest growth flush. However, Katsoyannos et al., (1997) have counted six overlapping generations in Athens. Grout et al., (2012), estimate that new infestations of *A. floccosus* are, habitually, associated with growth flushes.
The period from January to March corresponds to the hibernation period of the woolly whitefly in the current case study region where there was sometimes a total absence of adults; the lower faces of leaves were observed to be encrusted by larvae and exuviae, covered with sooty mould.

These findings were in accordance with those mentioned by Onillon (1973) and supported by the reports of Gebreslasie and Meresa, (2018).

The difference in the number of generations of *A. floccosus* between the region of Mitidja where it evolves in four to five generations (Doumandji-Mitiche & Doumandji, (1988); Biche, 2012) and the region of Chlef (three generations) can be explained by the specific climatic conditions of the Chlef province.

Indeed, the mean of minimum temperatures of the case study Chlef region varied between 12.20 °C and the maximum oscillating between 25-33 °C which was shown as favorable for the emergence of adults to trigger egg laying on the lower side of young leaves.

In support of this, France et al., (2011) estimated that the favorable average temperature for the development and reproduction of *A. floccosus* varies between 10.8 °C and 28.5 °C in northern Chile which is in the same range as observed in the current study.

The present results suggest that the rainfall pattern does not affect the whitefly population dynamics. The ideal moisture levels coinciding with the flight periods of adults varied between 50 % and 75 %.

Furthermore the yellow sticky traps can be considered as an effective tool for monitoring the population dynamics of crop pests, especially whiteflies (Prema et al., 2018). Adults caught by yellow traps accurately reflects the flight periods of woolly whitefly. The flight peaks effectively correspond to the generations of *A. floccosus*. In a related study, Prema et al., (2018) used different colored sticky traps including blue, white, yellow, red and green to monitor the movement of thrips (i.e. sucking pests similar to whiteflies) and the number of thrips catches at different growth stages of cotton growth. Results of their field trials confirmed that yellow sticky traps attracted more thrips compared to other colors.

In the existing study, in summer the number of adults caught reached more than 600 individuals per trap/month, in contrast to what was observed *in situ* where the autumn period was characterized by a large number of emerged adults.

This result could be due to the fact that the yellow sticky traps enable the capture of adults natives in the case study orchard as well as of the entomofauna circulating at the level of the orchards close to the study station.

The live larvae enumerated on 1 cm² of foliage showed that the latter occurred on the lower side of the leaves throughout the year, which explains the large number of adults caught by the traps or counted in the foliage.

The rate of parasitism by *C. noacki* was very high at 78 % during the month of July. In contrast this rate did not exceed 30 % during the other sampling periods. Katsyannos et al., (1998), consider that in Greece, *C. noacki* is an effective natural enemy against woolly whitefly with parasitism rates exceeding 82 %.

In another report, Telli & Yiğit, (2012) estimated that rates of parasitism by *C. noacki* in Turkey ranged from 70 % to 88 %. Similarly, rates of parasitism reaching 90 % on clementine trees and 75 % on orange trees were reported by Doumandji and Doumandji-Mitiche (1986) for the Mitidja region in Algeria.

In a related study, a survey of *Aphelinidae* (Hymenoptera: Chalcidoidea) species associated with crop and forest pests in Morocco was conducted by Kissayi et al., (2017). The authors concluded that the knowledge of *Aphelinidae* especially in North Africa countries is still incomplete. They also argued that further study is required to implementing biological control of crop pests and taxonomic studies of parasitic wasps for sustainable agriculture in countries of the North Africa region.

The spatiotemporal distribution of the adults depends of climatic conditions, the phenology of the orange tree (growth flush) and exposure or orientation of the leaf. It has been demonstrated that the north and south exposure are the most populated by the adults of the woolly whitefly; this can be explained by the presence of a large number of young leaves favoring the laying and the installation of *A. floccosus* (Onillon, 1973; Meagher, 2008).

Additionally, the evolution of the curve of the larvae parasitized by *C. noacki* showed that the parasitism activity is very intense during the summer and spring period and is remarkably low during the winter period, which may be due to unfavorable climatic conditions. The decline in the rates of parasitism in the present work observed between November and the beginning of May can be explained by the influence of the wind shear at 3.9 - 4.4 m s⁻¹ which was able to involuntary unhooking off the endoparasitic larvae, as they are fixed less firmly on the plant than those that are healthy.
Whereas, Ulusoy et al. (2003) found that the decline in parasitism may be due to the effect of frost, which destroyed parasitized larvae that lack the cottony mass that protects live healthy larvae.

In Algeria, several releases of the parasitoid C. noacki were carried out in 1985 and 1986, by Doumandji and Doumandji-Mitiche (1986). The parasitoid was able to settle in Mitidja and was able to limit the outbreak of A. floccosus. The presence of this parasitoid in the Chlef region can be explained by the adaptability of C. noacki which can makes it an effective tool for biological regulation against the woolly whitefly.

A similar case study of the insect pest complex of citrus, their natural enemies and their management at Keren in Eritrea has been reported by Husain et al., (2017). Furthermore, Gebreslasie and Meresa, (2018) noted that many pests can attack citrus. This suggests that an integrated approach may be needed in the protection of citrus orchards in Algeria. While Husain et al., (2017) focused primarily on chemical control; this may not be the best way forward due to environmental concerns. Integrated biological control of citrus pests using various parasitoids is recommended.

5 CONCLUSIONS

The present study was the first detailed characterization of the relationship between woolly whitefly A. floccosus, the parasitoid C. noacki and the appearance of new citrus shoots in orange trees (‘Thomson Navel’) in the Chlef Northwestern Algerian region. The woolly whitefly pest prefers young shoots to settle and lay eggs.

Results showed remarkable variations in abundance indices and infestation rates of larvae and adults that depended on the growth flush in foliage and meteorological conditions of the region. The woolly whitefly manifested three flight periods coinciding with three growth flushes of orange sap; in autumn towards the end of September followed by mid-November with the highest average abundance of 10 individuals per branch and a third flight period in mid-May.

Phases of growth flush seemed to have an effect on the temporal distribution of adults over the sampling period from July to June. Pest emergence appeared favorable at 12-20 °C as the minimum interval temperature whereas the maximum varied between 25-33 °C with humidity levels of 50 % and 75 %. In the current case study region, as for the incidence of C. noacki on the woolly whitefly, their impact was not optimum due to the large fluctuations in number of whiteflies. Although the pest-parasitoid complex appears to be important in the orchard, the biological regulation exerted by C. noacki is insufficient for the complete neutralization of A. floccosus. An integrated approach is needed using additional natural enemies associated with the woolly whitefly.

6 REFERENCES


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