CHAPTER 8

Biological Control of Invasive Citrus Leafminer, *Phyllocnistis citrella*, in Florida and Implications for Citrus Pest Management

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NON-TECHNICAL SUMMARY

The citrus leaf miner (CLM), *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), is a significant threat to citrus crops in Florida. Its larvae damage leaves, shoots, and sometimes fruit by making serpentine mines in the cuticle, which can also affect the appearance and health of the trees. It also exacerbates the spread of the devastating citrus canker disease by providing opportunities for the pathogen to infect surfaces damaged by the larvae. Classical biological control to reconstruct the natural enemy complexes of CLM was initiated soon after it invaded Florida in 1993. Among the three exotic species of parasitoids introduced from Asia, successful establishment and impact are documented for one species, *Ageniaspis citricola* (Hymenoptera: Encyrtidae), with parasitism rates of 70–100% being reported at different times and locations. Native parasitoids and predators also contribute to CLM suppression. However, the efficacy of these beneficial organisms has been significantly reduced over the last 17 years by the increased use of insecticides needed to suppress Asian citrus psyllid (*Diaphorina citri*), the vector of the devastating disease known as huanglongbing or citrus greening disease, in commercial citrus. Effective natural enemies of CLM cause mortality in both commercial orchards and urban citrus planting, reducing the need for chemical control. Assessments of the impacts of the introduced parasitoids on CLM and their interactions with other natural enemies, the environment, and chemical control, can help in making integrated pest management decisions in Florida citrus.

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

The citrus leaf miner (CLM; **Fig. 1**), *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), is a serious pest of citrus and other plants in the Rutaceae. It is a major pest of citrus in Eastern Hemisphere countries, where it is native to southern Asia. The CLM has spread to all citrus-growing regions of the world (Meyrick, 1909; Janse,

Qureshi, J. A. 2022. Biological control of invasive citrus leafminer, *Phyllocnistis citrella*, in Florida and implications for citrus pest management, pp. 81–91. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

1917; Clausen, 1931, 1933; CIE, 1970, 1986; Vari and Kroon, 1986; Beattie, 1989; Hoy and Nguyen, 1997) and has been recorded in several countries from Asia, Africa, and the Pacific region (Heppner, 1993). In the United States, CLM was intercepted in 1914 on citrus and other horticulture imports of the Atalantia company from the Philippines (Sasscer, 1915). However, the first record of CLM establishment in the continental United States, or elsewhere in the New World, was in southern Florida in May 1993 (Heppner, 1993). An infestation level of 90% mining of leaves was reported in Homestead, Florida on about 200 acres of Persian limes, and in a single growing season, CLM had spread throughout peninsular Florida, infesting nurseries, groves, and dooryard citrus. The CLM is now well established in Florida and has spread to the other Gulf Coast states. Considered an important pest in citrus nurseries and on either young or top-grafted trees, CLM causes feeding damage in both young and mature trees, which promotes the spread of citrus canker disease. By 1994, CLM was present in Alabama, Louisiana, and Texas (Nagamine and Heu, 2003), and by 1995, it was detected in Central America, western Mexico, and several Caribbean islands (Jones, 2001). By 2000, CLM had reached southern California, likely from Mexico (Grafton-Cardwell, 2009). By 2002, CLM had been detected in the five main Hawaiian Islands (Nagamine and Heu, 2003). In the Mediterranean basin, CLM was found in 1994 and has since spread rapidly. It is also spreading in Central and South America and has been reported from southern Africa and West Africa (CABI, 2021).

Figure 1. Adult of the citrus leafminer, *Phyllocnistis citrella.* (Lyle J. Buss, University of Florida)

Nature of the Problem

Citrus leafminer adults are very small moths (**Fig. 1**) (2 mm long, with 4 mm wingspans) that are active during the early morning and early evening hours. Females lay individual eggs, usually next to the midvein on underside of young leaves; eggs hatch in 2–10 days. Females may lay more than one egg per leaf when flush growth is scarce or when leafminer numbers are high. Larvae (**Fig. 2a**) make serpentine mines under the leaf cuticle, and there can be one to several larvae and mines per leaf, depending upon the pest level (**Fig. 2b**). Gottwald et al. (2002) reported that a single larva can consume 1–7 cm^2 (0.2–1 in^2) leaf area, and 2–3 larvae per leaf can consume half the leaf area. Four or more mines per leaf causes leaf distortion and drop (Peña and Duncan, 1994). Damaged leaves lose water, followed by curling, necrosis, chlorosis, leaf deformation, and reduction of photosynthetic activity (Peña et al., 2000). Pupation generally takes place inside the mine in a pupal chamber at the leaf margin (**Fig. 2c**). Succulent leaves with thin cuticles are most favorable for larval mining (Latif and Yunus, 1951), which is why infestations on young plants are more intense than those on mature trees (**Fig. 3a,b**) (Uygun et al., 2000; Garcia-Marí et al., 2002). Heppner (1995) also reported heavy infestations and severe damage to the growth of young plants and possible damage to the fruit rind, particularly for grapefruit. The extent of damage in terms of length of the mines varies greatly among different host plants, with the longest mines on elephant lemon (*Citrus medica*) and the shortest mines on lime (*Citrus aurantifolia*) (Pandey and Pandey, 1964). The life cycle of CLM varies with different citrus species and temperatures (Shevale and Pokharkar, 1992; Patel and Patel, 2001). In laboratory and field tests in Egypt, young flush leaves (1–5 days old) of several varieties of grapefruit (*Citrus paradisi*), mandarin (*Citrus reticulata*), sweet orange (*Citrus sinensis*), Baladi orange, navel orange and acid lime (*Citrus medica* var. *limonum*) were susceptible to CLM infestation (Mogahed, 1999). *Citrus jambhiri*, *Citrus karna*, and *Citrus limonia* were also susceptible to CLM damage, while the trifoliate orange, *Poncirus trifoliata,* and its

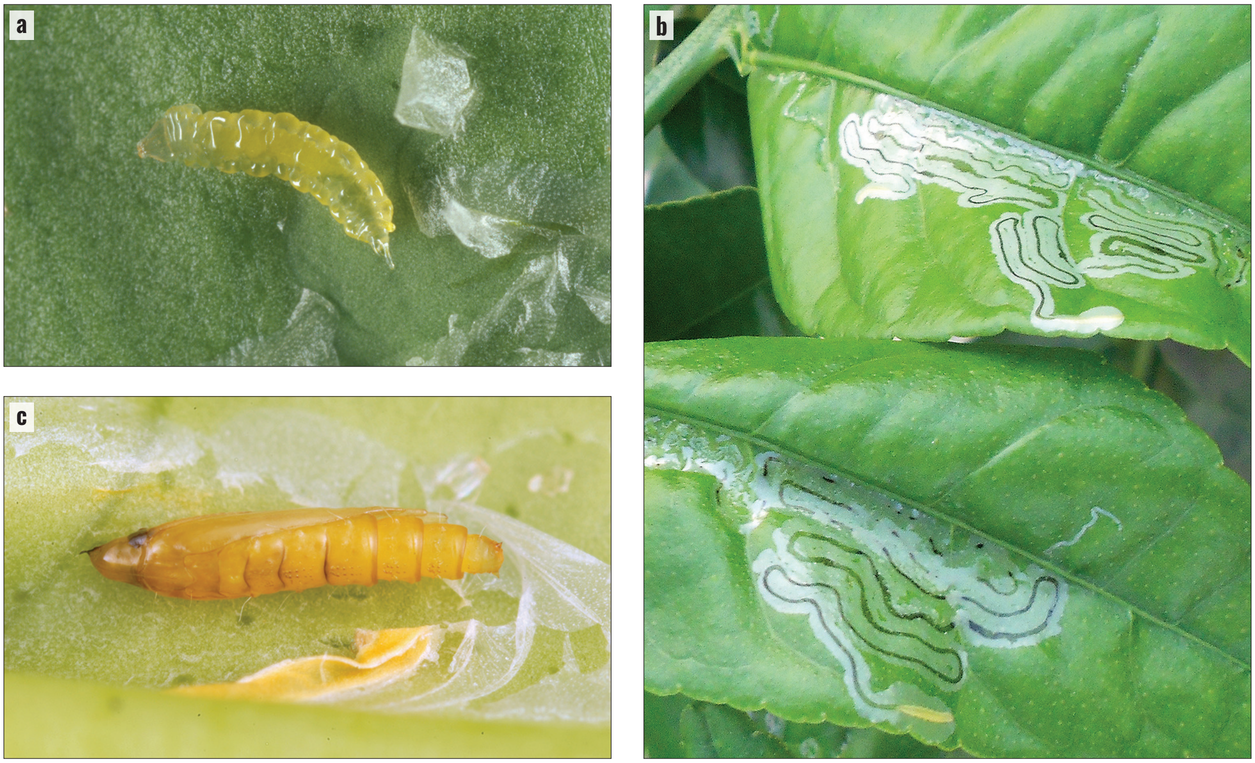

Figure 2. Citrus leafminer, *Phyllocnistis citrella* (a) larva; (b) larvae and serpentine mines in citrus leaves; (c) pupa. (a,c: Lyle J. Buss, University of Florida; b: Mongi Zekri, University of Florida)

hybrids were least susceptible. Grapefruit, lime, and oranges were also reported susceptible in Argentina (Goane et al., 2008). Negative effects on yield from CLM infestations are uncommon in mature trees, but reductions have been reported for heavily infested limes. The most serious effect of CLM infestation is indirect, with CLM making plants more susceptible to plant pathogens, such as *Xanthomonas axonopodis* pv *citri*, the bacterium responsible for Asiatic citrus canker (Sohi and Sandhu, 1968; Cook, 1988; Gottwald et al., 2002; Junior et al., 2006; Canteros et al., 2017). Leaf tissues damaged by larval feeding become more susceptible to *Xac*. Citrus canker was first found in Florida around 1912, and despite multiple eradication attempts it remains established in the state, causing significant losses in citrus crops.

Figure 3. Citrus leafminer, *Phyllocnistis citrella*, infestation on young plant growth. (a,b: Mongi Zekri, University of Florida)

WHY CONTROL THIS INVASIVE SPECIES?

Citrus leafminer larvae cause feeding damage to almost all types of citrus, including oranges, grapefruit, mandarins, lemons, limes, and their close relatives such as kumquat and calamondin. Mature trees generally tolerate CLM feeding damage; however, young plants in nurseries and orchards are more seriously affected. The larval feeding tunnels, called mines, are generally in the lower surface of the leaves but may include the upper surface in heavy infestations. The damaged leaves are curled and distorted, which slows growth of young trees. In heavily infested young trees, new shoots are visibly distorted, and damage reduces photosynthesis. Citrus leafminer damage to fruits may make them less marketable (Heppner and Fasulo, 2010). Controlling CLM is also critical to prevent the pest from increasing the rates of citrus canker disease. The larvae, through their feeding and movement, can move the bacterium of citrus canker throughout the mine and intensify infestation (Gottwald et al., 2002; Belasque et al., 2009). Citrus canker initially is visible as lesions on leaves, stems, and fruits, followed by defoliation, blemished fruits, premature fruit drop, twig dieback, and tree decline (Diez et al., 2006), which affects both the quality and quantity of fruit (**Fig. 4**). Following the 1912 detection of citrus canker in Florida, it was declared eradicated, including in adjacent states, by 1933 (Loucks, 1934; Dopson, 1964). In 1986, citrus canker was discovered a second time in Florida, and despite multiple eradication attempts it is still present in the state (Schoulties et al., 1987; Stall and Civerolo 1991; Schubert et al., 1996; Gottwald et al., 1997). Costs of those eradication efforts included the removal or cutting back of over 1.56 million commercial citrus trees and nearly 600,000 infected and exposed dooryard trees (Gottwald et al., 2002). These regulatory actions received considerable press attention and faced legal challenges, which had a far-reaching political and socioeconomic impact in Florida, with implications for national and international trade (CABI and EPPO, 1997; APHIS USDA, 1999). Most citrus varieties grown in Florida are moderate to highly susceptible to citrus canker disease. Several factors such as wind, rain, actions of people, and feeding by CLM contribute to the spread of the disease. The spring and summer rains, when combined with wind speeds of more than 8 m/s (18 mph), can greatly increase damage from the disease (Serizawa and Inoue, 1974). After the invasion of CLM in Florida in 1993, the incidence of citrus canker increased due to feeding damage by CLM larvae (Gottwald et al., 1997). Feeding by CLM larvae on the epidermal cell layer forms galleries beneath the foliar cuticle, and any splits in the cuticle can lead to the formation of large lesions due to the direct exposure of mesophyll tissues to the pathogen of citrus canker. The combination of canker pathogens and the CLM can lead to significant field infection and spread of the disease, even on highly resistant cultivars and species related to citrus such as calamondin and kumquat (Gottwald et al., 2002).

Figure 4. Citrus canker symptoms on leaves and fruit. (Mongi Zekri, University of Florida)

THE ECOLOGY OF THE PROBLEM

The degree and severity of CLM damage vary with the availability of new shoots on citrus trees. In Florida, most mature trees do not produce new growth during the winter months from November to February, and CLM populations are low to negligible even on young citrus during this period. Because of the strong impact of biological control by the predators and parasitoids on CLM, and the use of insecticides in the conventional citrus during the year, particularly for suppression of the Asian citrus psyllid (the vector of the huanglongbing [HLB] or citrus greening disease), populations of CLM decline in density toward the end of the year. The scarcity of new growth and the lower temperatures in winter impede reproduction and population increase by CLM. Citrus trees have their major flush of new growth each year in spring, followed by smaller, less predictable flushes of growth in summer and early fall. However, CLM infestation is low to negligible in the spring flush, and levels do not start to increase until April or May, with peak populations in summer and early fall. Similar patterns of lower damage levels during winter have been reported in Argentina (Diez et al., 2006) and during spring in China and Australia (Binglin and Mingdu, 1996). One generation is produced about every three weeks in summer and fall in Florida. Citrus leafminer attacks all types of citrus and its close relatives (Rutaceae), whose flushing patterns vary with citrus varieties and tree age (Knapp et al., 1993). Other factors may also influence the timing and magnitude of flush production. Climatic changes may result in warm weather or rain events in winter that promote the production of new growth, stimulating increases in winter CLM populations. Positive effects of temperature, humidity, and rainfall on CLM density have been reported by Patel and Patel (2001). Also, trees that are infected with the causal pathogen of HLB can experience changes in citrus plant physiology that include irregular production of flushes, which may also affect levels of CLM infestations. Practices intended to reduce the unwanted flushes of new growth, such as managing irrigation and fertilization, may also be useful to reduce the CLM feeding and spread of citrus canker.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Following the discovery of CLM in Florida in 1993, its population expanded and high density populations developed in most of the citrus-producing areas of the state within six months (Knapp et al., 1993). Initial efforts were based on repeated applications of various insecticides and had little effect, and concern developed that CLM would greatly harm citrus groves and nurseries. A classical biological control program against CLM, intended to establish natural enemies from its native region or other regions where effective parasitoids of CLM were known, was initiated soon after CLM's invasion. Successful management of CLM through use of native or introduced parasitoids has been reported from Australia, India, Japan, and Israel (Ishii, 1953; Batra and Sandhu, 1981; Argov and Rossler, 1996; Peña et al., 1996).

In 1994, the parasitic wasp *Ageniaspis citricola* (Hymenoptera: Encyrtidae; **Fig. 5**), originally from Thailand but obtained from Australia where it had been released previously, was released at 52 sites in southwest Florida as part of a statewide program of biological control against CLM (Hoy and Nguyen, 1997; Pomerinke and Stansly, 1998; Hoy and Nguyen, 2003). Before its introduction to Florida, *A. citricola* was imported from Thailand into Australia, where it underwent risk assessment (Neale et al., 1995). Based on those host range data and an assessment of the climatic similarity between Queensland (where the parasitoid had established successfully) and Florida, permission was obtained to import *A. citricola* into quarantine in Florida. Marjorie Hoy hand-carried large numbers of *A. citricola* adults and pupae from Australia into Florida on April 25, 1994. In view of the lack of space and hosts in the quarantine facilities in

Figure 5. Adult of the parasitoid *Ageniaspis citricola.* (Salman Alshami, University of Florida)

Florida, and the risk analysis information provided by Australian scientists, a request for direct release of *A. citricola* in Florida was submitted to the Division of Plant Industry and approved, which was followed by immediate submission to the USDA-APHIS. John LaSalle at the British National Museum confirmed the identity of the parasitoids from Australia, which facilitated the permission to release *A. citricola* from that source into Florida. First releases of adults *A. citricola* against CLM were made in April 1994 (Hoy and Nguyen, 1997). Females of *A. citricola* lay their eggs into either the eggs or first-instar larvae of CLM, and pupae are produced within the pupal chamber made by the CLM host (**Fig. 6**) (Edwards and Hoy, 1998). *Ageniaspis citricola* is polyembryonic, and females typically deposit two eggs per oviposition event, one of which develops into a male. The second egg splits, producing two daughters (Zappalà and Hoy, 2004); this reproductive strategy may contribute to the success of this parasitoid when host populations are low as each brood can mate with its own siblings. *Ageniaspis citricola* was able to overwinter in Florida despite frost events, and its recovery in spring of 1995 and again in 1996 confirmed its establishment. Parasitism levels by *A. citricola* at monitored groves increased from 2% in May 1994 to 86% in October 1995, apparently unhindered by competition from native parasitoids. In contrast, apparent parasitism of CLM from local parasitoids fell from 30% to 2% during the same period (Pomerinke and Stansly, 1998). Dispersal of *A. citricola* from release sites was reported to occur as far as 48 km (30 mi) from the nearest release point, most likely aided by wind (Pomerinke and Stansly, 1998). Climatic conditions in Florida proved suitable for *A. citricola*. This parasitoid soon became a permanent component of the biological control of CLM in all major citrus-producing areas of the state. Survivorship of *A. citricola* was greatest at 80–95% RH in studies in southwest Florida in 1995 and 1996 (Edwards and Hoy, 1998). Parasitism of CLM by *A. citricola* steadily increased at most sites following the initial release, whereas the proportion of host pupal chambers with local parasitoids declined. A second population of what was thought to be *A. citricola* was imported from Taiwan and released in Florida. This was later determined to be a distinct but cryptic species. However, no evidence of its establishment was found (Hoy et al., 2000; Alvarez and Hoy, 2002).

Figure 6. Pupae of the parasitoid *Ageniaspis citricola.* (Salman Alshami, University of Florida)

Cirrospilus (=*quadristriatus*) *ingenuus* (Hymenoptera: Eulophidae), another Asian parasitoid of CLM, was introduced from Australia and released in Florida in 1994 (Hoy and Nguyen 1994, 1997; Smith and Hoy, 1995). The natural range of *C. ingenuus* includes Australia, China, India, Indonesia, Japan, Malaysia, Oman, Taiwan, and Thailand (Schauff et al., 1998; Zhu et al., 2002). This species was known previously as *Scotolinx quadristriatus* (Waterhouse, 1998) and *Cirrospilus quadristriatus* (Subba Rao and Ramamani, 1966). The introductions of this species into Australia, Cyprus, Israel, Morocco, Oman, Syria, Tunisia, and Turkey were under the name of *C. quadristriatus* (Schauff et al., 1998). Evans (1999) synonymized *C. quadristriatus* with *Cirrospilus ingenuus*, and the latter was the name used in Schauff et al. (1998) and LaSalle et al. (1999). *Cirrospilus ingenuus* is a solitary ectoparasitoid of late-instar larvae or prepupae of the CLM, and it generally is restricted to this host. Females may deposit more than one egg in a mine, and its larvae feed on the larvae or prepupae of CLM, but only one adult parasitoid emerges per mine. Ujiye and Adachi (1995) reported a female-biased sex ratio, with approximately 60% female progeny. Adult longevity is about two weeks, and a generation requires two to three weeks, depending upon temperature (Smith and Hoy, 1995).

Initial monitoring in 1994–1996 of CLM populations at locations across Florida failed to detect *C. ingenuus* (Hoy and Nguyen, unpub. data), although it was reported established later in November 1997 and January 1998 around Homestead, Florida (LaSalle et al., 1999). *Cirrospilus* sp. (unidentified specimens) was detected at low levels (<5% parasitism) during the fall of 2017 in Fort Pierce, Florida (Khalid and Qureshi, unpub. data). This parasitoid also caused mortality through probing of the host with the ovipositor (without oviposition) and then feeding on host hemolymph (termed "host feeding") (Neale et al., 1995).

Cirrospilus is a large genus, with approximately 130 species of parasitoids of various lepidopteran or dipteran leafminers (Zhu et al., 2002). Besides *C. ingenuus*, *Cirrospilus* species that have been reported attacking CLM include *C. cinctiventris, C. diallus, C. jiangxiensis, C. longifasciatus, C. lyncus, C. nigriscutellaris, C. nigrivariegatus, C. phyllocnistis, C. pictus, C. variegatus, C. vittatus* sp. nr. *lyncus*, as well as other undescribed species from Honduras, Argentina, Japan, and Colombia (Schauff et al., 1998).

Semielacher petiolatus (Hymenoptera: Eulophidae) is another larval parasitoid of CLM, and it also is capable of host feeding (Argov and Rossler, 1998; Mineo and Mineo, 1999a,b). It has been found attacking the CLM in Australia (Boucek, 1988; Smith et al., 1997) and in the Solomon Islands (Schauff et al., 1998), and it is considered endemic in those regions. It has also been introduced into other areas where CLM invaded, including Cyprus, Israel, Morocco, Oman, Syria, Tunisia, Turkey, Egypt, Greece, and Spain (Schauff et al., 1998). In 1998, *S. petiolatus* was found in Italy for the first time, but the path of its introduction there is unknown (Mineo et al., 1998). By 2001, it appeared to be the most efficient parasitoid of CLM in Italy, with parasitism rates up to 80%. In 2002, *S. petiolatus* was recovered in all the citrus-growing areas of Sicily, with its greatest abundance on CLM in early summer (June–August). In contrast, parasitism rates by an introduced eulophid, *Citrostichus phyllocnistoides*, were higher in the latter part of the growing season (September–October) (Siscaro et al., 2002). *Semielacher petiolatus* was imported and evaluated in quarantine in Florida, but not released because the potential risk of disrupting biological control by *A. citricola* was considered higher than the potential benefit of establishing *S. petiolatus* in Florida (Lim and Hoy, 2005; Lim et al., 2006). *Semielacher petiolatus* appears to be able to attack leafminer species other than the CLM although at low rates.

HOW WELL DID IT WORK?

Ageniaspis citricola has many of the attributes of an effective natural enemy (Rosen and Huffaker, 1983). These include relatively high host specificity (Neale et al., 1995), the ability to locate low-density host populations, and to discriminate between previously healthy and parasitized hosts (Edwards and Hoy, 1998; Zappalà and Hoy, 2004. However, it is not effective in regions with low relative humidity (Yoder and Hoy, 1998), and its population growth lags behind that of CLM populations in the spring in Florida (Villanueva-Jimenez et al., 2000). *Ageniaspis citricola* populations can increase from very low densities to detectable levels by the second flush cycle in Florida and, if not disrupted by drought or pesticide applications, can cause up to 100% parasitism of CLM by the fall, which significantly reduces the overwintering population of CLM (Villanueva-Jimenez et al., 2000; Zappalà et al., unpub. data). However, the biological control of CLM is significantly reduced by the frequent the use of foliar sprays, as occurred in Florida in an effort to suppress Asian citrus psyllid (the vector of HLB disease) after HLB was detected in Florida in 2005.

Although *C. ingenuus* was not detected in initial surveys conducted after its release, it did establish and was recovered from the CLM in November 1997 and January 1998 near Homestead, Florida (Knapp et al., 1999). *Cirrospilus* sp. parasitoids were also detected at low levels (<5% parasitism) in the fall of 2017 in Fort Pierce, Florida. However, establishment has not been confirmed for *C. phyllocnistoides*, another parasitoid that was imported and released against CLM in Florida (J. Qureshi, unpub. data). Both *C. ingenuus* and *C. phyllocnistoides* are also known to probe the host with the ovipositor and engage in host feeding, an additional source of mortality besides parasitism. Additional work is therefore needed in both commercial orchards and urban areas to measure the impacts of the parasitoids introduced for control of CLM populations in Florida.

BENEFITS OF BIOLOGICAL CONTROL OF CITRUS LEAFMINER

The biological control program against CLM provided significant benefits to the Florida citrus industry by establishing a sustainable, inexpensive form of management for CLM, a pest that not only caused direct feeding damage to citrus but also increased the spread of citrus canker. The natural enemies of CLM introduced by this program also help reduce the spread of both the pest and pathogen between commercial and urban areas. Suppression of CLM also reduces citrus production costs by reducing the need for pesticide applications, thus also supporting pesticide-resistance management in citrus. Resistance management is becoming increasingly important for citrus production in Florida due to the widespread occurrence of Asian citrus psyllid and HLB disease. The increased use of insecticidal sprays against Asian citrus psyllid also affects other pests, increasing the risk of pest resistance in many pests found in citrus. In areas without canker disease, natural enemies can probably provide sufficient control of CLM in both tree nurseries and mature groves. However, in situations where canker or the vector-HLB complex exists, extensive use of insecticides is needed. The release of host specific-natural enemies from climatically similar regions is a successful pest control tactic as seen with the introduction of *A. citricola* into Florida. Despite the increased use of insecticides in commercial production systems during the past two decades to suppress the vector of HLB, biological control still provides effective control of CLM in commercial citrus orchards and urban environments.

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