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Pre-release evaluation of *Semielacher petiolatus* (Hymenoptera: Eulophidae) in quarantine for the control of citrus leafminer: Host discrimination, relative humidity tolerance, and alternative hosts

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Abstract

To evaluate the potential benefits and risks associated with releasing *Semielacher petiolatus* Girault (Hymenoptera: Eulophidae) in a classical biological control project directed against the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in Florida, we evaluated the ability of *S. petiolatus* females to discriminate between hosts previously parasitized by *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) and unparasitized hosts. In laboratory trials, *S. petiolatus* females did not discriminate between hosts previously parasitized and oviposited and fed on each host category equally. Hatch rate of *S. petiolatus* eggs on hosts previously parasitized by *A. citricola* was normal and development time was not different. However, mortality of immatures was significantly higher on previously parasitized hosts when compared to hosts that were not parasitized by *A. citricola*, and size of adult females reared on hosts previously parasitized was reduced. The relative survivorship of *S. petiolatus* adults compared with adults of *P. citrella* and another parasitoid, *A. citricola*, under three relative humidities (RHs) (55, 76, and 95% at 28 °C) indicated that *S. petiolatus* survives longer than *A. citricola* at all RHs tested, but did not survive as long as the citrus leafminer. Finally, the leafminer *Liriomyza trifolii* Burgess (Diptera: Agromyzidae) was evaluated as a possible host for *S. petiolatus* but no progeny were produced in choice and no-choice tests. The lack of discrimination raises the concern that *S. petiolatus* could disrupt the efficacy of *A. citricola*, which is already established in Florida, without providing substantial reduction of citrus leafminer populations during early spring.

Keywords: *Phyllocnistis citrella*; *Semielacher petiolatus*; *Ageniaspis citricola*; *Liriomyza trifolii*; Host discrimination; Relative humidity; Alternative host; Classical biological control; Risk assessment

1. Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is an important citrus pest that is native to Southeast Asia. The citrus leafminer was first discovered in Florida in 1993 and was able to spread throughout the approximately 344,000 hectares of citrus within a few months after its arrival (Heppner, 1993). Adult females of citrus leafminer deposit eggs singly upon tender young citrus foliage (flush), and larvae immediately enter the leaf and start feeding on epidermal cells, producing broad serpentine mines (Hoy and Nguyen, 1997). Mining of immature foliage reduces growth rates and yields, and the mines serve as foci for the establishment of the bacterium causing citrus canker

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(Gottwald et al., 2001; Graham et al., 1996; Liu et al., 1999; Ujiye, 2000).

A classical biological control project was initiated in February 1994 in Florida and two parasitoids, *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) and *Cirrospilus ingenuus* (=*quadristriatus*) Gahan (Hymenoptera: Eulophidae), were imported from Queensland, Australia and released (Hoy and Nguyen, 1997; Hoy et al., 1995; Smith and Hoy, 1995). Monitoring of citrus leafminer populations throughout the state failed to detect *C. ingenuus* after the release (Hoy and Nguyen, unpublished), although it apparently did establish near Homestead, Florida (LaSalle et al., 1999). Subsequent to its release, Nguyen and Hoy (unpublished) discovered *C. ingenuus* did not discriminate between hosts parasitized and unparasitized by *A. citricola* and devoured pupae of *A. citricola* in the pupal chamber of the citrus leafminer. *A. citricola* established, multiplied, dispersed, and overwintered in the majority of the Florida release sites. In some sites, parasitism of citrus leafminer pupae was as high as 99% only 15 months after initial releases, and parasitism levels of 60–80% were common (Browning et al., 2002; Hoy et al., 1995; Hoy and Nguyen, 1997; Pomerinke and Stansly, 1998). *A. citricola*, in combination with native natural enemies (Browning and Peña, 1995), significantly reduced citrus leafminer populations (Hoy et al., 1995; Peña et al., 1996; Pomerinke and Stansly, 1998). *A. citricola* has many attributes of an effective and low-risk natural enemy; it apparently is host specific and able to discriminate between citrus leafminer hosts previously parasitized by *A. citricola* and is also able to locate low density populations in the field (Hoy and Nguyen, 1997; Zappalà and Hoy, 2004). It is relatively well adapted to the Florida climate, although adults do not survive well under low relative humidity (RH) conditions (Yoder and Hoy, 1998) and during several recent drought years *A. citricola* populations lagged behind those of their host in Florida, particularly during the spring (Zappalà et al., unpublished). This raised the question as to whether another parasitoid should be considered for release to augment the control exerted by *A. citricola* and the native natural enemies in Florida.

One candidate that appeared to be worthy of evaluation is *Semielacher petiolatus* Girault (Hymenoptera: Eulophidae). Because this parasitoid had established in Mediterranean climates, it was thought to be more tolerant than *A. citricola* of dry conditions but this needed confirmation and no information was available regarding its potential to disrupt the effectiveness of *A. citricola*. *S. petiolatus* was originally described from Australia (Girault, 1915) and introduced into several Mediterranean countries as a natural enemy of the citrus leafminer (Massa et al., 2001; Schauff et al., 1998). It initially became an abundant parasitoid of citrus leafminer in Sicily (Massa et al., 2001; Mineo, 1999; Mineo and Mineo, 1999), although it failed to establish in Spain (Garcia-Marí et al., 2004). Massa et al. (2001) reported that *S. petiolatus* parasitized three other Lepidoptera (*Cosmopterix pulchrimella* Chambers, *Stigmella aurella* Fabr., and *Dialectica scalariella* Zeller) and two dipterans (*Liriomyza* sp. and *Chromatomyia horticola* Goureau) on diverse host plants in Italy and Jordan. A broad host range raises concerns about non-target effects in classical biological control programs, although the use of alternative hosts could be a positive factor if it allows the parasitoid to maintain higher populations during intervals when the target pest population is low.

Semielacher petiolatus is a solitary ectoparasitoid that deposits eggs on the second-, third-, and fourth-instar larvae of citrus leafminer (Argov and Rössler, 1998; Ateyyat, 2002; Lim and Hoy, 2005; Mineo and Mineo, 1999). Adults of *S. petiolatus* were imported from Italy in 2003 into the high security quarantine at the Division of Plant Industry, Department of Agriculture and Consumer Services in Gainesville and subsequently transferred to the quarantine facility at University of Florida, Gainesville. To evaluate the potential effectiveness of *S. petiolatus* as a natural enemy of citrus leafminer in Florida, the development time of both sexes, adult longevity, total fecundity, host mortality caused by host feeding and/or parasitism, and host-size choice of *S. petiolatus* were evaluated under quarantine conditions (Lim and Hoy, 2005). Under laboratory conditions, adults of *S. petiolatus* are relatively long lived (25–26 days at 25 °C), develop from egg to adult in 8.4–9.5 days, and kill hosts both by host feeding and oviposition (Lim and Hoy, 2005). However, under these conditions, *S. petiolatus* females self-superparasitized at a relatively high rate (22%), which raised the issue of whether it could discriminate between hosts that were unparasitized or parasitized by *A. citricola*. If *S. petiolatus* does not discriminate between hosts previously parasitized by *A. citricola* (which attacks eggs and first-instar hosts), then *S. petiolatus* could kill multiple individuals of the polyembryonic *A. citricola* and potentially reduce the effectiveness of *A. citricola* in Florida.

To aid in our decision whether to release *S. petiolatus*, we evaluated the ability of *S. petiolatus* to discriminate between unparasitized citrus leafminers and leafminers parasitized by the endoparasitoid *A. citricola*. In addition, we compared the ability of adults of citrus leafminer, *A. citricola* and *S. petiolatus* to survive at different RHs as an indicator of the likelihood that the ectoparasitoid *S. petiolatus* could be effective during dry springs when *A. citricola* is less effective than normal (Hoy et al., 2003; Villanueva-Jimènez et al., 2000). We propose that *S. petiolatus* should not be released unless females can discriminate between unparasitized hosts and hosts previously parasitized by *A. citricola*, and that *S. petiolatus* should survive better at low RH conditions than *A. citricola*. Finally, we determine whether an abundant dip-