

## Research Article

# ***Neochrysocharis okazakii* (Hymenoptera: Eulophidae) as a Major Parasitoid Wasp of Stone Leek Leaf Miner *Liriomyza chinensis* (Diptera: Agromyzidae) in Central Vietnam**

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*Liriomyza chinensis* is a major pest of Welsh onion *Allium fistulosum* in Asia but little is known about the abundance of its natural enemies. A field survey was made to explore the major parasitoids of *L. chinensis* leaf miner in central Vietnam. An eulophid parasitoid, *Neochrysocharis okazakii*, comprised more than 95% of parasitoids reared from leaf miner larvae collected in the onion field and 98.3% of leaf miner parasitoids found during searches of onion plants. The mean number of female *N. okazakii* on plants was greater in onion fields with a higher density of *L. chinensis*, and, during searches, a greater proportion of *N. okazakii* was found on onion with more *L. chinensis* mines, suggesting density-dependent parasitoid aggregation. Melanized dead larvae of *L. chinensis* were more frequently found in onion fields with more parasitoids, demonstrating that melanized leaf miners are a good indicator of parasitoid activity. Mean instant rate of host encounter by *N. okazakii* in the field was estimated at 0.077, and the likelihood of a parasitoid finding a host increased with host density. Taken together, these results show that *N. okazakii* is the major parasitoid of *L. chinensis*. Usefulness of this parasitoid in stone leek leaf miner management is discussed.

## 1. Introduction

Welsh onion *Allium fistulosum* (Liliaceae or Alliaceae) (= green bunching onion or Japanese bunching onion) is a vegetable crop widely cultivated in the world [1, 2]. It is an important part of the fresh market in Asia, particularly in Vietnam, China, and Japan [3, 4]. Welsh onion is grown throughout the majority of region in those countries. Welsh onion is typically grown as an annual though it is a perennial crop.

Pest management is crucial to stable production of marketable Welsh onions. Chemical control, that is, the use of synthetic pesticides, is currently the sole measure (except “hand-picking”) for combating pests of Welsh onion in Vietnam [5]. The situation is similar in other Asian countries including Japan. However, relying too much on chemicals often causes the development of pesticide-resistance and the

resurgence of pest populations [6–8]. This holds true for Welsh onion production in Vietnam [5].

Stone leek leaf miner *Liriomyza chinensis* (Diptera: Agromyzidae) is a common and serious pest of Welsh onion in East and Southeast Asia including Vietnam [9–12]. It often causes unacceptable damage to the marketable parts of green onion. Previous surveys in central and southern Vietnam have shown that almost 100 percent of all leaf miners attacking Welsh onion are *L. chinensis* and that heavy infestation by *L. chinensis* is detected in many Welsh onion fields where insecticides have frequently been applied [6]. Thus, insecticide-based control does not satisfactorily work.

In general, leaf mining insects host many species of parasitoid wasps, and parasitoid wasps can effectively reduce pest leaf miner populations if chemicals that are harmful to them are not used [13–17]. Biological control by preventative or curative releases of commercialized parasitoids may be ideal

in *L. chinensis* management. This approach, however, is not acceptable for Vietnamese farmers because such parasitoids are too expensive for them. Instead, conservation biological control is known as a useful, low-cost tactic [8, 18, 19]. One of the mainstays of IPM of stone leek leaf miner in Vietnam could be conservation biological control.

Very little is known about the impact of parasitoids on *L. chinensis* populations. To date, only a few studies have investigated the parasitoid species complex of *L. chinensis* [11, 20, 21]. The present study was undertaken to determine the major parasitoid species of onion leaf miner in central Vietnam. Field studies were conducted to examine (1) the parasitoid species complex and (2) the relationship between pest infestation levels and parasitoid abundance and activity in the field. The importance of parasitoids and conservation biological control in stone leek leaf miner management in Vietnam is discussed.

## 2. Materials and Methods

Field studies were conducted from February to March 2005. Study sites are located at Hue and Huong An, lowland areas in central Vietnam. In these sites, Welsh onion is a year-round vegetable, and different growing stages were concurrently present. Welsh onion fields selected for study were conventional farmer's fields with onion plants of vegetative and scape elongation stages; farmers exclusively used Cartap as the chemical control tool to manage insect pests, and no herbicides were applied. A variety of vegetable and ornamental crops such as corn, lettuce, green mustard, soybean, snap bean, sweet potato, and chrysanthemum were grown around study fields.

Leaf miner infestation and parasitoid activity levels were examined in 15 farmer's fields. Inspection of the entire plant was made to search leaf mines. Each field was sampled at four sites. Up to a total of 50 plants per field with 10–15 plants per site were randomly sampled. The numbers of leaf mines and melanized dead leaf miner larvae were counted for each plant. The mean numbers of leaf mines and melanized larvae per plant were used as indices of pest and parasitoid activity at each field.

Also, adult parasitoids on Welsh onion leaves were searched. Preliminary observations had shown that fewer adult parasitoids of leaf miners were observed in the afternoon, possibly because unstable weather conditions were commonly encountered at that time. Because parasitoid activity might be influenced by weather condition and time, field observations were made between 9:00 and 11:00 o'clock on fine days. The number of parasitoids was counted by inspecting the entire onion plant. Female parasitoids were collected for identification. Each field was examined on at least three times (= days). In each field, four sites were chosen, and up to a total of 50 plants per field with 10–15 plants per site were randomly examined.

In addition, direct observations of parasitoid behavior were made to determine if leaf miner parasitoids observed in study fields were associated with *L. chinensis*. Thirteen fields were used in this observation study. When a female parasitoid was found, its behavior was observed and classified as

attacking, searching (antennal drumming), resting, walking, and hopping.

In order to examine parasitoid species that emerged from host larvae, the hosts were collected from Welsh onion fields and reared at the laboratory of Hue University. Collections were made at Hue from March to July 2005. Incubators were not available so humidity and room temperature could not be controlled. Consequently, the quality of onion leaves was not optimal for leaf miner development. Also, stone leek leaf miner is very different from other *Liriomyza* species in its feeding habit. Mines of the former are rather straight, and the visible parts of mines are short and interrupted because larvae alternately mine the outer and inner surface of onion leaves. Mature larvae of *L. chinensis* are often found within onion leaves. For these reasons, we were unable to determine the exact percentage of parasitism. Instead, the number of parasitoid wasps that emerged and the species composition were determined to identify the main parasitoid(s) of *L. chinensis*.

Data were analyzed with the aid of JMP version 9.0 [22]. Regression analysis was done to examine relationships between the two variables of interest.

## 3. Results

Infestation by *Liriomyza chinensis* was observed in all study fields. Infestation level (i.e., mean number of leaf mines per plant) was variable among fields, ranging from 0.5 to 38.7. The wide range of infestation levels allowed analysis of the relationship between infestation levels and parasitoid presence. In all, 132 adult hymenopteran parasitoids were detected in the field. Among them, 2 species of Ichneumonidae, three species of Braconidae, and one species of Chrysididae were not associated with dipteran leaf miners. Based on the morphology, the remaining 121 adult parasitoids were associated with leaf miners. Among the leaf miner parasitoids, 119 individuals were *Neochrysocharis okazakii* Kamijo (Hymenoptera: Eulophidae), which was found mostly frequently on onion leaves. This parasitoid was detected in 11 out of 15 onion fields.

The mean number of *N. okazakii* was significantly higher in Welsh onion fields with higher leaf mine densities per plant ( $N = 15$ ,  $r^2 = 0.52$ ,  $F = 13.84$ ,  $df = 1$ , and  $P = 0.0026$ ) (Figure 1). Next, we examined the relationship between the mean number of melanized dead leaf miners per plant and that of *N. okazakii* in fields. A significantly positive relationship was detected between the two ( $N = 15$ ,  $r^2 = 0.45$ ,  $F = 10.47$ ,  $df = 1$ , and  $P = 0.0065$ ) (Figure 2(a)). Also, more melanized leaf miners occurred in green onion fields with higher numbers of leaf mines per plant ( $N = 15$ ,  $r^2 = 0.57$ ,  $F = 17.54$ ,  $df = 1$ , and  $P = 0.0011$ ) (Figure 2(b)). The proportion of melanized leaf miners/leaf mines/plant tended to increase with increasing mean number of *N. okazakii* observed ( $N = 15$ ,  $r^2 = 0.23$ ,  $F = 3.79$ ,  $df = 1$ , and  $P = 0.074$ ) (Figure 3).

In all, the behavior of 91 female *N. okazakii* was observed in the fields. Female *N. okazakii* were often found to hop from leaf to leaf (16.4%) or rest on the leaf (49.5%). They

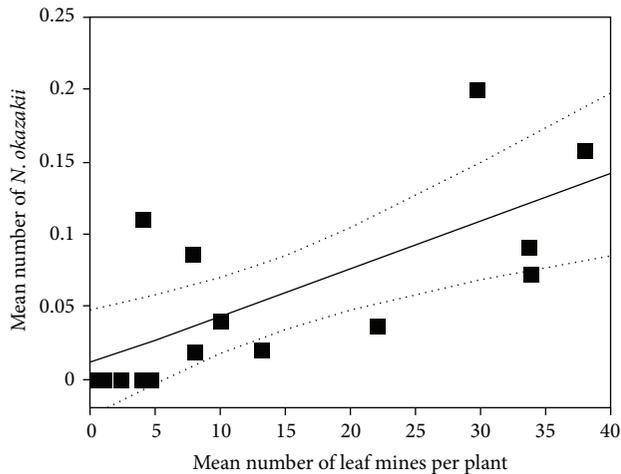


FIGURE 1: Relationship between levels of leaf miner infestation of green onion fields and mean numbers of *Neochrysocharis okazakii* found per plant. Dot lines indicate 95% confidence intervals.

frequently drummed the leaf with their antennae, a behavior associated with searching a host (26.3%). In several cases, it was observed that female parasitoids attacked larvae of *L. chinensis* (7.7%). This value (0.077) can be regarded as an instant rate of host encounter in the field. Because leaf miner species other than *L. chinensis* were not found in study fields, it is likely that *N. okazakii* was associated exclusively with *L. chinensis* in Welsh onion fields.

The proportion of female parasitoids searching for hosts in a given field increased with infestation level in that field ( $N = 13$ ,  $r^2 = 0.42$ ,  $F = 6.38$ ,  $df = 1$ , and  $P = 0.031$ ) (Figure 4). Logistic regression analysis was done to examine the relationship between host finding success of *N. okazakii* and leaf miner density. In this analysis, the relationship between parasitoid behavior (attacking a host or not) on an onion plant and the number of leaf mines on that onion plant was examined. The analysis showed that successful host finding, that is, the likelihood of females attacking a host, tended to increase with leaf miner density per plant ( $N = 91$ ,  $r^2 = 0.104$ ,  $\chi^2 = 5.12$ , and  $P = 0.024$ ) (Figure 5).

In all, 216 parasitoid individuals emerged in the laboratory from onion leaves containing *L. chinensis*. Only three species of hymenopteran parasitoids were obtained, and the majority of them (96.8%) were *N. okazakii*. The other two species were *N. formosa* (Westwood) and *Diglyphus isaea* (Walker). Thus, *N. okazakii* was the major parasitoid of stone leek leaf miner in study areas.

#### 4. Discussion

In the present study, direct field observations were first made for the presence of leaf miner parasitoids searching for hosts in Welsh onion fields. Additional surveys were done to examine the parasitoid complex of stone leek leaf miner by collecting onion leaves containing the leaf miner larvae. Both field and laboratory surveys together demonstrated that

more than 95% of parasitoids detected were *Neochrysocharis okazakii*. Our results show that *N. okazakii* is the most important parasitoid species attacking *Liriomyza chinensis* in lowland areas of central Vietnam. *Neochrysocharis okazakii* is a solitary endoparasitoid (Ueno, personal observations). Although information on the life history of *N. okazakii* is limited, available literature suggests that *N. okazakii* is polyphagous like other eulophid parasitoids attacking *Liriomyza* spp. [23, 24]. *Neochrysocharis okazakii* has been found emerging from other leaf miner species, such as *Liriomyza trifolii* and *L. sativae* in Japan, although *N. okazakii* is not considered a major parasitoid of such leaf miner species [12, 17, 23–26].

In the present field observations, parasitoids found attacking stone leek leaf miner were exclusively *N. okazakii*. During the same period as our study, parasitoid abundance was also examined in vegetable fields (other than Welsh onion) where insecticides, mostly Cartap, were applied, and at least four parasitoid species including polyphagous eulophids were searching for leaf mines caused by *L. sativae* on tomato, yard-long bean, and pumpkin (Ueno and Tran, personal observations). Hence, *N. okazakii* is not the only leaf miner parasitoid that occurs in lowland areas of central Vietnam during spring months. Nevertheless, in our study, *Liriomyza chinensis* did not host parasitoids other than *N. okazakii*. Similarly, Tran et al. [21] found that *N. okazakii* occupies more than 90% of parasitoids emerging from onion leaves infested by *L. chinensis*. However, on other sprayed vegetable fields such as tomato and bean fields, the relative abundance of *N. okazakii* among parasitoid species complexes is less than 10% [21]. Species diversity of parasitoids may be very poor for stone leek leaf miner in Vietnam. Curiously, species diversity of parasitoids was also poorer on *L. chinensis* than for *L. sativae* and *Chromatomyia horticola* (Goureau) in China [20].

The low diversity of parasitoids in our study may reflect the fact that *L. chinensis*, which is a specialist of *Allium* spp., attracts a few parasitoid species. Alternatively, the characteristic odor of green onion may have a repellent effect on other parasitoids, or the plant may be a poor environment because of its architecture or lack of refuge from direct sunlight. More likely, it was simply due to the fact that insecticides had been applied [27]. In our study, we could not sample unsprayed fields because all fields had been sprayed with Cartap and could not assess the diversity of parasitoids in unsprayed onion fields. Tokumaru [11] recently reported that 6 species of parasitoid wasps are associated with onion leaf miner in unsprayed fields in Japan. This fact may imply that *N. okazakii* population in our study field showed a level of resistance to Cartap whereas the other parasitoids did not. In our fields, other pests like *Spodoptera exigua* (Hübner) and *S. litura* F. were also abundant. This is curious because Cartap is an insecticide against which resistance is slow to develop [28]. It appears that, for unknown reasons, the applied pesticide did not work well in our study area. Although the effect of Cartap cannot completely be excluded, we believe it did not strongly affect the main result presented here. Factors affecting the makeup of parasitoid communities across different crops remain to be determined in future research.

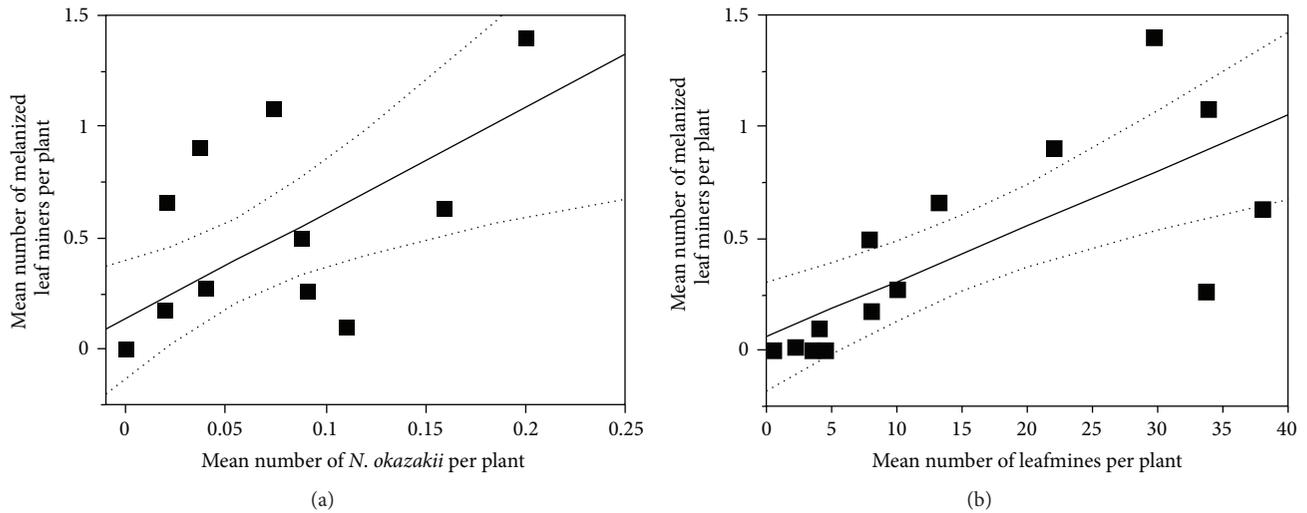


FIGURE 2: Relationships between mean numbers of melanized leaf miner larvae per plant and levels of leaf miner infestation of green onion fields (a) or mean numbers of *Neochrysocharis okazakii* found per plant (b). Dot lines indicate 95% confidence intervals.

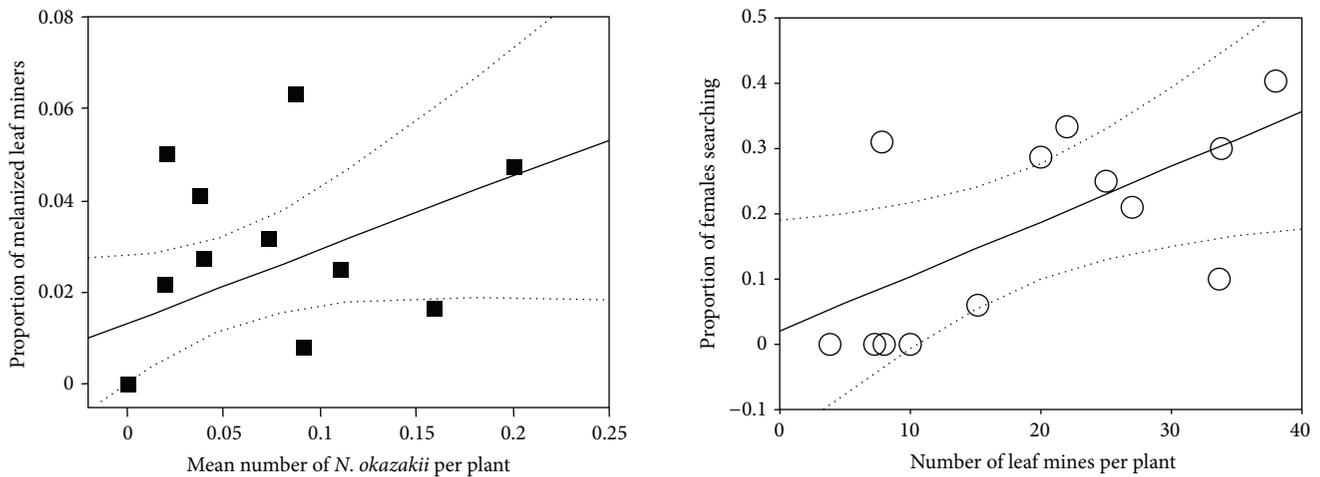


FIGURE 3: Relationship between mean numbers of melanized leaf miner larvae per plant and mean numbers of *Neochrysocharis okazakii* per plant. Dot lines indicate 95% confidence intervals.

FIGURE 4: Relationship between levels of leaf miner infestation and proportions of female *Neochrysocharis okazakii* searching for hosts. Dot lines indicate 95% confidence intervals.

The present study has shown that melanized dead leaf miner larvae can be a good indicator for the presence and abundance of parasitoids in Welsh onion fields. Melanized leaf miners are visibly prominent, and the presence for dead leaf miners can readily be recognized. Destructive host feeding by female parasitoids probably causes quick mortality of leaf miners, making their body color black due to melanization; *L. chinensis* larvae attacked and oviposited by *N. okazakii* do not normally turn to black after parasitism. The presence of many melanized leaf miners is a clear sign that *N. okazakii* is abundant and can be used when monitoring parasitoid activity in the fields.

Very few studies have evaluated the activity of female parasitoids of leaf miners in the field. The majority of studies assessing parasitoid performance so far have been

based on parasitism rates in the field. In the present study, direct observation was made in the field to examine the relationship between densities of host leaf miner and adult parasitoid. The results showed that more adult females of *N. okazakii* are observed and found searching in onion fields with higher *L. chinensis* densities, suggesting that female *N. okazakii* are attracted to Welsh onion fields with high host densities. A density-dependent response to host of parasitoid may support a successful biological control program [29]. *Neochrysocharis okazakii* may prove to be a good biocontrol agent of the leaf miner in onion fields of Vietnam.

The instant rate of host encounter was estimated at 0.077 under field conditions. Because very few studies have estimated successful host encounter rate by leaf miner parasitoids in the field, it is difficult to judge whether the value is relatively high or low compared with other leaf miner

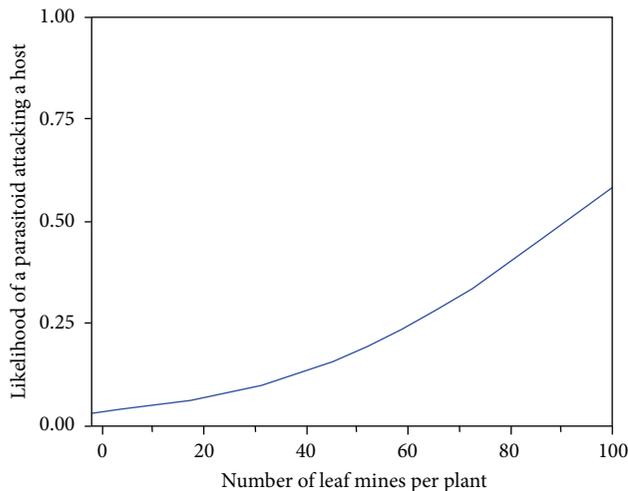


FIGURE 5: Relationship between leaf mine density on an onion plant and likelihood of a female *Neochrysocharis okazakii* successfully finding a host on that plant. A logistic regression curve is illustrated. Data plots are not shown for simplicity.

parasitoids including commercialized species such as *Diglyphus isaea* (Walker), *Neochrysocharis formosa* (Westwood), and *Dacnusa sibirica* Telenga. Nonetheless, our approach in the present study may be useful in assessing the foraging effectiveness of other leaf miner parasitoids.

Pest management strategies should not rely on a single control practice. IPM practices that integrate a variety of tactics should be used [6–8, 30]. The inclusion of different management tactics is required in Welsh onion production in Vietnam. For economic reasons, Vietnamese farmers cannot support high cost pest management practices, and the adoption of close attention should be given to producing the highest possible yield at the lowest cost. In particular, the adoption of low-cost practices that avoid the build-up of insect pests should be emphasized in the country. The conservation of natural enemies is generally simple and cost-effective and may be an important and readily available practice [18, 19]. For successful conservation biological control, farmers' education is necessary together with scientific studies for evaluating the impact of parasitoids on *L. chinensis*.

To date, the importance of natural enemies has not been studied in Welsh onion fields in Vietnam. It is not known whether parasitoids can keep onion leaf miner density below damaging levels if disruptive insecticides are not used. The importance and impact of parasitoids, including *N. okazakii*, should be examined in unsprayed fields. No tests have been conducted to determine which pesticides can be used safely without disrupting *N. okazakii* populations. Also, the possibility that *N. okazakii* demonstrates a level of insecticide resistance is worthy of testing. These tests are essential for the practice of conservation biological control because the use of selective insecticides should not negatively impact the activity of the parasitoid. The usefulness of conservation biological control is worth accessing for Welsh onion production in Vietnam.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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