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Impact of chemical pest control on *Liriomyza trifolii* (Burgess, 1880) and its parasitoids populations in celery’s plots at Nkolondom (Yaounde - Cameroon)

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Celery (*Apium graveolens*) Linné, 1753 is one of the main market-gardening crops in the urban and periurban areas of Cameroon. Its production is threatened by the leafminer *Liriomyza trifolii* Burgess, 1880 (Diptera: Agromyzidae) whose larvae bore into the leaves' parenchyma, making them unsuitable for consumption and therefore for sale. These damages would have been more injurious if there was no insecticidal treatment and any natural enemies of this pest. The present study aimed at assessing the influence of insecticidal treatments on populations of *L. trifolii* and its parasitoids *Neochrysocharis* sp. and *Opius* sp. in four experimental celery plots (three treated and one control). The harvest and incubation of leaves bearing *L. trifolii* larvae present in celery leaves showed that according to the variations in the leaves' infestation rate and the average number of leafminers' larvae per leaf, CYPERCAL and KART were the most efficient insecticides in *L. trifolii* control. Then, the absolute abundance of *Opius* sp. unlikely to that of *Neochrysocharis* sp. was null for the treated celery plots, great for the control plot (297 individuals) during the dry season.

**Key words:** *Apium graveolens*, *Liriomyza trifolii*, parasitoids, *Opius* sp., *Neochrysocharis* sp. insecticides

**INTRODUCTION**

In Cameroon, *Apium graveolens* Linné, also called celery, is one of the major market gardening crops, cultivated by about 60% of farmers in urban and peri-urban areas (Mvogo, 2005). It is known to be the most gainful vegetable crop. At Nkolondom, in the northern outskirt of Yaoundé, farmers estimated that plants as celery, nightshade and leek provide respectively incomes of about 1800 CFA/m², 1400 CFA/m² and 1000 CFA/m² per crop cycle (Prolinnova Cameroun, 2011). The celery’s yield in individual leafs cropping was 14.3 kg/m² (Simon et al., 2010). However, the intensification and permanent character of its cultivation has led to the emergence and outbreaks of various pathogens and insect pests in the gardens. Among insect pests, the leafminer *Liriomyza trifolii* Burgess, 1880 (Diptera: Agromyzidae) is the most harmful. Indeed, the punctures of the female flies when laying eggs and the tunnels made on the foliar parenchyma by larvae may lead to the desiccation of leaves and are potential gateways for opportunist pathogenic microorganisms (Spencer, 1973; Minkemerg and van Lenteren, 1986; Parrella and Jones, 1987; Maier, 2001). Consequently, frequent outbreaks of *L. trifolii* on celery have led to the abandon of its production in various
localities in the Center Region of Cameroon, confirming the statement that the introduction of *L. trifolii* in Africa, Europe and North-Western America has resulted in serious economic losses on various gardening and ornamental plants (Spencer, 1973, 1982; Parrella, 1987; Kang et al., 2009).

Currently the chemical control remains the main pest control strategy in agriculture worldwide (Cooper and Dobson, 2007), and their use has increased of about 50% over the last 30 years, leading to about 25 million tons applied per year (Anonymous, 2006). At Nkolondom, producers had significantly reduced the negative impact of *L. trifolii* in celery gardening by using large spectrum chemical pesticides. But the practice is characterized by a misuse of synthetic insecticides, the amounts of active molecule being often insufficient or excessive and the last-time between two treatments or between treatments and harvest not respected (Djiéto-Lordon and Aléné, 2006).

Despite this predominance of the chemical control in agriculture, Edwards-Jones (1988) pointed out the harmfulness of these products for the environment and the non-target organisms. Furthermore, this strategy maintains farmers financially dependent of the price fluctuations, while misuse of pesticides may lead to development of resistant forms in pest populations (Saha and Mukhopadhyay, 2013), making them inefficient. It also increases the risk for the residues accumulation in crops product and in the environment (Anonymous, 2006). Furthermore, the lack of selectivity in target-organisms by the use of pesticides usually leads to the emergence of other pests which may cause greater damages than the target ones (Anonymous, 2006). In certain cases, it has been observed development of chemical resistance in leafminers as they passed from the secondary pest statute to the main one in gardens (Saha and Mukhopadhyay, 2013). Specific examples are those of *Liriomyza sativae* (Hills and Taylor, 1951), *L. trifolii* (Reitz et al., 1999), *Phyllonorycter* spp. on fruit trees (Maier, 2001) and several other tomato miners (Gelenter and Trumble, 1999). Also, pesticides’ misuse may lead to the accumulation of chemical residues in soil, water, or agricultural product and then may alter the environment or the health of consumers (Bertheloot et al., 2008).

Such drawbacks led researchers to develop alternative pest management strategies that seem to be the most sustainable way to achieve plant protection (Leibee, 1981; Trumble, 1985; Keil and Parrella, 1987). Among different control methods involved in integrated Pest Management (IPM), the biological control use natural enemies of pests, including pathogens, predators or parasitoids. In several countries, agronomists and researchers use biological agents in pest control, in order to limit expenditure due to the purchase of chemicals and the speedy development of resistance in some growing areas (Keil and Parrella, 1987), the emphasis given to IPM programs using biological control agents has increased (Trumble, 1985). This has been the case for *L. trifolii* control (Albert et al., 2000). Parasitoids of leafminers are predominantly polyphagous and may include at any moment a new species of leaf miners in their host range (Salvo and Valladares, 2007).

The aim of this study is to contribute to the development of an IPM programs using parasitoids against the major pest of celery in Nkolondom. This contribution is based on the assessment of the impact of insecticides on the *L. trifolii*'s populations and parasitoids.

MATERIALS AND METHODS

Study period and sites

The study was conducted from November 2009 to March 2010 in two sites in and around Yaoundé (1) the Campus of the University of Yaoundé I (03° 51' 34"N, 11° 33' 00"E) (hereafter termed Campus); (2) Nkolondom (03° 57' 07"N, 11° 29' 27"E), in the northern outskirts of Yaoundé. Both sites are located on the Southern Plateau, with a bimodal humid tropical rainfall regime (Suchel, 1988). However, they differ in their agronomic environments. The plot of Nkolondom (surrounded by various market crop parcels along a small stream) is located in an agronomic landscape contrarily to the Campus site (surrounded by buildings and grasslands) that is located in an urban landscape. On a phytogeography aspect, Nkolondom is located in the forest-savannah transition zone (Letouzey, 1968). Currently, this region has suffered heavy anthropogenic disturbances and few remanent of the natural vegetation are preserved on hill side.

Insecticides involved in the study

Three insecticides commonly found in local markets and used by gardeners in pest control were involved in the present study. According to their packings we obtained some informations about their actions against pests. These insecticides were: KARATE MAX 2.5 WG (hereafter called KARATE; Syngenta Agro S.A.S. France, formulation: liquid; active ingredient: Lambda-cyhalothrine, contact insecticide); CYPERCAL 720 EC (hereafter called CYPERCAL; Syngenta Agro S.A.S. France; formulation: liquid) active ingredients: Cypermethrine 600 g /l, contact insecticide + Dimethoate 120 g / l, systemic insecticide); KART 500 (hereafter called KART; Villa Crop Protection (Pty) Ltd. Reg Co. N° 1992/002474/07 MPY. Reg. Nr. CP/Posbus 10413, Manor Aston, Netherlands; formulation: powder) active ingredient: Chlorhydralate 500 g/kg.
systemic insecticide). CYPERCAL and KART are insecticides homologated in Cameroon for the perennial crops while Karate is indicated for the market-gardening. All these insecticides are widely used by farmers of Nkolondom. According to them, CYPERCAL and KARATE were introduced in early 1990’s; but Cypercal was initially less used than karate because it hight cost. KART was recently introduced in Nkolondom, in early 2000s.

**Experimental design**

At Nkolondom, four experimental plots were set up. Each plot was made of six plates of 4 m x 1 m, separated by furrows of about 0.5 m wide. Treatments on plots were organized as follow: Plot 1 treated with CYPERCAL; Plot 2 treated with KARATE; Plot 3 treated with KART and Plot 4 used as control, received water instead of treatment. Insecticide treatments began about six weeks after sowing. Plots’ management and insecticidal treatments followed farmer’s procedures.

**Sampling method**

Sampling began a week after sowing, and was conducted once per week in each plot and ended after the last commercial harvest. For each sampling, thirty randomly selected equidistant plants were examined per plot. On each plant, all leaves were carefully examined and the total number of leaves as well as the number of infested leaves noted. One infested leaf was collected per plant and brought to the Laboratory of Zoology (University of Yaoundé I) for further observations and manipulations. Treatments began six weeks after sowing and ended two weeks before the last sampling. Commercial harvest, pruning or insecticidal treatment, were programmed when necessary after a sampling, according to the farmers’ procedure.

In the laboratory, infested leaves were examined in order to count larvae (mummified and alive) they carried. Mummified larvae were kept in Petri dishes while living ones were kept in 7 cm X 10 cm X 17 cm plastic boxes covered with mosquitos’ nets and followed up till to the pupation. As mummified larvae, pupae were individually incubated in Petri dishes up to the emergence of imagos. After identification, *L. trifolii*, parasitoids *Neochrysocharis* sp. and *Opius* sp. were regularly counted per species, per plot and per sampling date. Pupae from which adults did not emerge were dissected to identify potential mummies of parasitoids.

**Data analysis**

The foliar mean number of *L. trifolii* larvae per leaf and the different ratio were expressed as means ± standard deviations and comparisons performed using the Wald-χ², procedure general Linear Model (GLM) between different plots. Analyses were conducted using SPSS (20.0) software and the results were appreciated at 5% confidence interval. Comparisons among celery’s plots were done through the weekly cumulated variation of *L. trifolii*’s foliar attack rate, *L. trifolii*’s number of larvae/leaf, *Opius*’s infestation rate, *Neochrysocharis*’s infestation rate, *Opius*’s mortality rate and *Neochrysocharis*’s mortality rate.

**RESULTS**

Table 1 showed different values obtained during the experimentations. These values were then used to calculate some important rates and mean relative to the present study.

**A. graveolens**’s leaves’ infestation rate

During the first six weeks after sowing, the weekly rate of *A. graveolens*’s leaves infested by *L. trifolii* increased up to about 47% in all the plots. Then, with the beginning of control essays, these rates increased up to

<table>
<thead>
<tr>
<th>Total number of leaves</th>
<th>Control</th>
<th>KARATE</th>
<th>CYPERCAL</th>
<th>KART</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1635</td>
<td>1998</td>
<td>2133</td>
<td>2128</td>
</tr>
<tr>
<td>Total number of infested leaves</td>
<td>1564</td>
<td>1491</td>
<td>537</td>
<td>585</td>
</tr>
<tr>
<td>Total number of <em>L. trifolii</em> larvae</td>
<td>5910</td>
<td>7651</td>
<td>1666</td>
<td>1616</td>
</tr>
<tr>
<td>Total number of infested larvae by <em>Opius</em> sp.</td>
<td>2111</td>
<td>901</td>
<td>111</td>
<td>119</td>
</tr>
<tr>
<td>Total number of dead larvae infested by <em>Opius</em> sp.</td>
<td>23</td>
<td>39</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total number of infested larvae by <em>Neochrysocharis</em> sp.</td>
<td>3408</td>
<td>5676</td>
<td>1170</td>
<td>177</td>
</tr>
<tr>
<td>Total number of dead larvae infested by <em>Neochrysocharis</em> sp.</td>
<td>2305</td>
<td>3561</td>
<td>485</td>
<td>563</td>
</tr>
</tbody>
</table>
95% in the control plot and 74% in the KARATE plot at the end of the sampling period. Furthermore, these rates decreased moderately, reaching 25% on the CYPERCAL plot and 27% on KART plots at the end of the sampling period. Pair-wise comparisons showed significant effect of insecticides effects on the mean rates of leaves infested by L. trifolii (Table 2).

**Table 2.** Comparison of the weekly rates of A. graveolens's leaves infested by L. trifolii on experimental plots.

<table>
<thead>
<tr>
<th>Different plots</th>
<th>N</th>
<th>Means rates of infested leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22</td>
<td>57.55 ± 4.1 *</td>
</tr>
<tr>
<td>KARATE</td>
<td></td>
<td>47.09 ± 4.1 a</td>
</tr>
<tr>
<td>CYPERCAL</td>
<td></td>
<td>26.92 ± 4.1 b</td>
</tr>
<tr>
<td>KART</td>
<td></td>
<td>27.83 ± 4.1 c</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different (Wald-χ² = 40.26; df = 3; P = 10⁻⁹); N = Number of samplings.

**Table 3.** Comparison of the mean number of L. trifolii larvae per leaf on experimental plots.

<table>
<thead>
<tr>
<th>Different plots</th>
<th>N</th>
<th>Means number of L. trifolii larvae per leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>1.41 ± 0.13 a</td>
</tr>
<tr>
<td>KARATE</td>
<td></td>
<td>1.81 ± 0.14 a</td>
</tr>
<tr>
<td>CYPERCAL</td>
<td></td>
<td>0.08 ± 0.13 b</td>
</tr>
<tr>
<td>KART</td>
<td></td>
<td>0.08 ± 0.13 b</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different (Wald-χ² = 124.17; df = 3; P = 10⁻⁹); N = Number of samplings.

**Table 4.** Comparison of the weekly infestation rates of L. trifolii larvae by Opius sp. on experimental plots.

<table>
<thead>
<tr>
<th>Different plots</th>
<th>N</th>
<th>Means infestation rates of L. trifolii larvae by Opius sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22</td>
<td>25.03 ± 1.4 a</td>
</tr>
<tr>
<td>KARATE</td>
<td></td>
<td>15.28 ± 1.4 abc</td>
</tr>
<tr>
<td>CYPERCAL</td>
<td></td>
<td>10.26 ± 1.4 abc</td>
</tr>
<tr>
<td>KART</td>
<td></td>
<td>10.58 ± 1.4 abc</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different (Wald-χ² = 64.01; df = 3; P = 10⁻⁹); N = Number of samplings.

**Table 5.** Comparison of the mean mortality rates of Opius sp. on experimental plots.

<table>
<thead>
<tr>
<th>Different plots</th>
<th>N</th>
<th>Means mortality rates of Opius sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>1.58 ± 0.39 a</td>
</tr>
<tr>
<td>KARATE</td>
<td></td>
<td>3.69 ± 0.39 abc</td>
</tr>
<tr>
<td>CYPERCAL</td>
<td></td>
<td>4.32 ± 0.39 abc</td>
</tr>
<tr>
<td>KART</td>
<td></td>
<td>4.12 ± 0.39 abc</td>
</tr>
</tbody>
</table>

Means following of the same letters are not significantly different (Wald-χ² = 30.86; df = 3; P = 10⁻⁹); N = Number of samplings.

**Infestation rates of L. trifolii larvae by Opius sp.**

The weekly infestation rates of Opius sp. on L. trifolii's larvae increased progressively and similarly on the four plots during the first six weeks after sowing reaching almost 20%. Then, three patterns appeared: (i) a continuous increase of the infestation rates on the control plot where it reached 36% at the end of the sampling period; (ii) a slight and progressive decrease on the KARATE plot where it reached 12% at the end of the sampling period and (iii) an obvious decrease on both CYPERCAL and KART plots where it reached respectively 7% and 6% at the end of the sampling period. Pair-wise comparisons showed significant differences between the insecticides effects on the mean infestation rates of L. trifolii's larvae by Opius sp., suggesting that chemical treatments was harmful to the Opius sp. populations (Table 4).

**Variation of the mean mortality rates of Opius sp.**

The weekly mortality rate of Opius sp. increased up to 4% in every plot during the first six weeks after sowing. Then, these weekly mortality rates increased continuously and reached maximal values of 6.12% on the CYPERCAL plot, 5.66% on the KARATE plot, and 5.71% on the KART plot. Later, these rates decreased continuously on the whole parcel reaching 1.08% on the Control plot, 4.32% on the KARATE plot, 5.40% in CYPERCAL plot and 5.04% on KART plot, at the end of the sampling period. The pair-wise comparisons showed significant difference between the insecticides effects on the mean mortality rates of Opius sp. (Table 5).

**Variation of the weekly infestation rates of L. trifolii's larvae by Neochrysocharis sp.**

The weekly infestation rates of L. trifolii's larvae by Neochrysocharis sp. increased up to 50% on all the plots during the first six weeks after sowing. Then, it
increased very slightly and reached 57.66% in the Control plot, 70.22% on CYPERCAL plot, 72.83% on KART plot and 74.22% on the KARATE plot at the end of the sampling period. Pair-wise comparisons showed no significant difference between the insecticides effects on the infestation rates of *L. trifolii*’s larvae by *Neochrysocharis* sp. (Table 6).

**Table 6.** Comparison of weekly mortality of *L. trifolii*’s pupae due to *Neochrysocharis* sp. on experimental plots

<table>
<thead>
<tr>
<th>Different plots</th>
<th>N</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>49.45±3.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>KARATE</td>
<td>22</td>
<td>56.76±3.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYPERCAL</td>
<td></td>
<td>54.34±3.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>KART</td>
<td></td>
<td>55.63±3.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by the same letters are not significantly different (Wald-<sup>χ</sup><sup>2</sup> = 2.18, df =3; P =0.53); N = Number of samplings.

**Variation of the mortality rates of *Neochrysocharis* sp.**

The variation of the weekly mortality rates of *Neochrysocharis* sp. increased up to 22% in every plot during the first six weeks after sowing. Then, the rate increased continuously and reached 67.63% in the Control plot, 62.70% on CYPERCAL plot, 41.45% on KART plot and 47.83% on the KARATE plot at the end of the sampling period. Pair-wise comparisons showed no significant difference between the insecticides effects on mortality rates of *Neochrysocharis* sp. (Table 7).

**Table 7.** Comparison of the weekly mortality rates of *Neochrysocharis* sp. on experimental plots.

<table>
<thead>
<tr>
<th>Different plots</th>
<th>N</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>39.54±3.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>KARATE</td>
<td>22</td>
<td>37.22±3.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYPERCAL</td>
<td></td>
<td>28.19±3.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>KART</td>
<td></td>
<td>30.24±3.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means following the same letters are not significantly different (Wald-<sup>χ</sup><sup>2</sup> = 6.57; df =3; P =0.08); N = Number of samplings.

**DISCUSSION**

The variation of the rates leaves affected by *L. trifolii* and that of the mean number of *L. trifolii* larvae per leaf showed significant treatment effect, with respectively Karate and Control plot more affected than the two others. Damages on Karate plot were more important than on the control one, indicating that Karate was no more efficient in the management of *L. trifolii* at Nkolondom. Considering that pruning and commercial harvest processes were similar for all the plots, the variations observed may be exclusively related to the efficiency of different treatment essays. According to their packing, the systemic mode of action of Cypercal and Kart makes them more efficient since it leads to killing of *L. trifolii*’s larvae which are contaminated by ingesting the chemicals when feeding on *A. graveolens*’s leaves. Cypercal seemed more efficient than Kart probably because of its double mode of action (systemic and by contact). However, the contact action seems less efficient than the systemic; as observed in the case of the Karate plot.

The infestation rate of *L. trifolii*’s larvae by *Opinus* sp. showed a highly treatment effect, with reduced very low populations size on the Cypercal and Kart plots than on the Control. The decrease of *Opinus* sp. population on the threatened plots, compared to that of the Control plot, may be explained either by its contamination by residual pesticide accumulated in the host tissues, especially in the case of the systemic pesticides (Cypercal or Kart) or by the contamination of adults by contact pesticides on the host plant’s leaves such as in the contact pesticides like Karate.

Systemic insecticides can potentially contaminate floral and extrafloral nectar when systemically distributed throughout (Lord et al., 1968) and cause high mortality to nectar feeding parasitoid (Cate et al., 1972). Several contact insecticides (organochlorines such as cypermethrine or pyrethroids such as Lambdacyhalotrine) used against leafminers or other pests are highly toxic to their parasitoids (Reitz et al., 2013).

According to Hernández et al. (2010), the effects of lambdacyhalotrine (a contact insecticide) on adult parasitoids could differ from a species to another: it was toxic to *Geogaripus nigrimanus* Simon (a larvo-pupal parasitoid of *Liriomyza* spp.) but have no effect on *Neochrysocharis formosa* Westwood.

The insecticides’ influence on the mortality rate of *Opinus* sp. was highlighted by its low mortality rate on the Control plot. This result confirms the non-selectiveness of insecticides both on pests and on non-target organisms which are sometimes natural enemies of some pests (Bertheloot et al., 2008). Several studies, based on different crops systems show that non-treated crops or those submitted to low dosages of insecticides have an important percentage of parasitoids (Chen et al., 2003a). In addition, beyond the mortality effect, sub-lethal doses can severely reduce performance (flying food research, fertility and longevity) of bio-control agents (Roger et al., 1995). Furthermore, bio-control programs based on *Trichogramma* spp. and other hymenopteran parasitoids are weakly used in North America or in intensive agriculture in Europe because of the general environmental pollution by insecticides (Li, 1994).

There was no significant treatment effect in the
infestation rate of *L. trifolii* larvae by *Neochrysocharis* sp. Indeed, *Neochrysocharis* sp. was always present in all the plots, even if, it could be noted that at the end of the experimentation, *Neochrysocharis* sp. infestation rates were slightly higher on the treated plots comparatively to the Control plot. This permanent presence of *Neochrysocharis* sp. in the treated plots as well as in the Control could be explained by some hypotheses: - *Neochrysocharis* sp. may mummify the *L. trifolii* larvae previously parasitized by *Opius* sp.; - larvae of *Neochrysocharis* sp. would have fed on less polluted host organs or would have developed genes of resistance ensuring degradation and elimination through its feces a great quantity of toxic substances from pesticides. Such observations are reported by several authors on the survival of parasitoids to some insecticides including permethrin (Poe et al., 1978), methomyl (Trumble, 1985), and several other compounds (Lange et al., 1980).

According to Rathman et al. (1990), *Diglyphus begini* Ashmead (Hymenoptera: Eulophidae) showed a tolerance to oxamyl, methomyl, permethrin and fenvalerate. Thus, our data on *Neochrysocharis* sp. corroborate those of Rogers and Dewdney (2012) who stated that more than 500 insects and mites have currently developed such a resistance.

However, there was no significant treatment effect in the mortality rate of *Neochrysocharis* sp. on *L. trifolii* larvae. It may be due to the fact that pests usually detoxify the active matter and convert it into more or less toxic metabolite which can affect the internal parasitoid (Liu, 2012). In such cases during our experimentation, neither host nor parasitoid survives. Meanwhile, Stapel (1999) suggested that in order to develop an integrated pest management, the determination of compatibility among the types of insecticides, their usage and the sensibility by different development stages of biological control agents is imperative. Unfortunately, this type of test is usually performed only on one developmental stage of the beneficial insect (often adults) and yet, other development stages are concerned (for example endoparasitoid larvae may be contaminated according to our study). This constitutes the main shortcoming (Jones et al., 1998). Thereby, when a parasitoid is resistant to a given insecticide, this insecticide is appropriate in the Integrated Pest Management (IPM) programs (Reitz et al., 2013).

Despite the impact of both parasitoids and/or insecticides, *L. trifolii* succeed to maintain noticeable population level on each plot. This situation may depend on the fact that the pruning and the commercial harvest were evenly done on all plots, so the variation of *L. trifolii* pressure (leaves infestation rates and mean number of larvae per leaf) between treated and non-threatened plots was exclusively due to the efficiency of the insecticides; then, the Karate seemed to be the less efficient. The efficiency of this insecticide, used in Nkolondon since 1990, decreased progressively, probably because of the development of resistant forms among the pests. Similar evolution has been observed elsewhere on *L. trifolii* undergoing different pesticides (Saito et al., 1992), such as in Texas (USA), where a long-time protection of pepper, based on lambda-cyhalotrine (Karate), result in a resurgence of leafminers in fall 2007 and in no reduction in spring 2008 (Hernández et al., 2010). Martin et al. (2000) showed that a massive use of pyrethroids to fight against caterpillars of *Heliothis - Helicoverpa* complex causes the first cases of resistance in Australia (1983), then in Turkey and Thailand (1984-1985) where the cultivation of cotton has fallen sharply. Various pests have shown a formidable ability to develop resistance with respect to conventional insecticides (Saha and Mukhopadhyay, 2013). Moreover, Reitz et al. (2013) suggests a list of ineffective materials including almost all the insecticides classes developed up to now. Others works show that the resistance is developed because a small number of individuals survive after each treatment, multiplying and transmitting their resistance gene to the next generation. Once the resistant forms are dominant in a population, the pesticide becomes ineffective (Kershon, 2000). Now, Cypercal 720 EC seems to offer better results, but it is more probably that *L. trifolii* develop further a resistance in relation to this insecticide because of its use without interruption at Nkolondon.

Despite the presence of the two parasitoids (*Opius* sp. and *Neochrysocharis* sp.) in the control plot, almost all the leaves were attacked by *L. trifolii*. This may be due to an interspecific competition between the two parasitoids, which negatively affect their individual effectiveness (Turnbull, 1967; Bader et al., 2006).

As Cobblah et al. (2012) suggest the use of *Diglyphus isaea* Walker, *Dacnusa sibrica* Telenga in the control of *L. trifolii* in market crops gardens, parasitoids assessed in the present study may be used, despite the negative effect of insecticides on *Opius* sp., as bio-control agents in an integrated pest management program against *L. trifolii*.

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