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Influence of food resource on the development of *Liriomyza trifolii* Burgess 1880 (Diptera-Agromyzidae)

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In order to limit the devastation caused in the celery (*Apium graveolens* L., 1753) plots by *Liriomyza trifolii* Burgess in Nkolondom, (Southern Cameroon), farmers proceed to the anarchic use of synthetic insecticides in addition to the pruning of the infested leaves as sanitary harvests. These infested leaves resulting from sanitary harvests are immediately abandoned in the furrows. The main objective of the present study was to assess the efficiency of pruning as a control method versus *L. trifolii*. The durations of the development of the pre-imaginal stages, the fitness and the sex-ratio of *L. trifolii* we are were evaluated using both whole plants and pruned parasitized leaves, respectively in the laboratory and the celery plot during the warm-dry and cold-humid seasons. The results obtained showed that the average duration of the pre-imaginal development cycle of *L.trifolii* varied between 16.53 ± 0.26 days in the laboratory with cut leaves during warm-dry period to 21.98 ± 0.3 days with entire plants in the garden during cold-humid period. It was also shown that from infested cut leaves emerged leafminers were able to cause serious damages to healthy celery plants in the plot. No significant difference was observed between the cut leaves sex ratio (1/0.96) and that of the whole plants (1/0.91); (χ^2 = 2.38; df = 1; P=0.12). The *L. trifolii* sex ratio which is slightly biased toward females was not affected by the food resource.

Key words: *Apium graveolens*, *Liriomyza trifolii*, leafminer, pruning, sex ratio, fitness.

INTRODUCTION

The Celery, *Apium graveolens* L., 1753 (Apiaceae) is one of the main market crops produced in urban and suburb areas in Southern Cameroon (Damesse, 2003; Mvogo, 2005). At Nkolondom, in the northern outskirt of Yaoundé, farmer estimated today 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/m^2 (Simon et al., 2010). This vegetable is mostly cultivated in Nkolondom, involving about 60% of farmers (Damesse, 2003; Mvogo, 2005).

Despite the fact that celery is always known at Nkolondom as the most gainful vegetable crop, its

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production undergoes some constraints and difficulties due to the bad cropping management leading to an impoverishment of the soil and the intensive and permanent character of this agricultural type leads to outbreak of insect pests. Among the insect pests, *Liriomyza trifolii* Burgess 1880 (Diptera: Agromyzidae) is the major threat (Nguimdo, 2007) and considered as economically important (Parrella, 1987; Kang et al., 2009). Larvae of these flies feed on the parenchyma of celery leaves, making it dry out and then unsuitable for the consumption. *L. trifolii* is very prolific and adults can easily spread gradually on the whole garden when host plant is present. In many farms, frequent outbreaks of *L. trifolii* caused severe damages which sometimes constrain the farmers to leave their activities. This case was observed at Mvog-Dzigui, a nearby village of Yaoundé, where producers were discouraged and obliged to abandon their celery's plots (Mvogo, 2005). At Nkolondom, in order to control *L. trifolii* population on celery plots, farmers use several techniques including chemical treatments and sanitary harvests. The main problem in this area are related to the anarchic use of chemical products that may induce leafminers resistance to pesticides and a sanitary harvest that's the impact on the biological cycle of the pest remains unknown.

The present study is a contribution to the mastery of *L. trifolii* ecology in the celery's plots in Yaounde neighbourhoods. More specifically, we seek to assess: (1) the duration of the first three development phases of *L. trifolii*; (2) the sex-ratio of *L. trifolii* and (3) the influence of the food resource on the fitness of *L. trifolii*.

MATERIALS AND METHODS

Description of the site

The study was conducted at Nkolondom (03°57'07"N; 11°29'27"E) and at the University of Yaoundé1 (03°51'34"N; 11°33'00"E), particularly in the laboratory of Zoology and on experimental celery plot established near the laboratory building. Both sites are located on the Southern Plateau, with a bimodal humid tropical rainfall regime (Suchel, 1988). However, in their agronomic environments, Nkolondom is surrounded by various market crop plots along a small stream and located in an agronomic landscape. Contrarily to Nkolondom, the University is located in an urban landscape and surrounded by buildings and grasslands. The soils of Yaoundé and its neighbourhoods are lateritic on interfluve (Vicat and Bilong, 1998). On a phytogeographic aspect, Nkolondom is in forest-savannah transition zone (Letouzey, 1968). This region has suffered from heavy anthropogenic disturbances and the remaining natural vegetation is preserved on hill slopes.

METHODOLOGY

Three experimentations were carried out during the present study from August to September 2008 (cold-humid period), from December 2008 to January 2009 (warm-dry period-1) and from February 2009 to March 2009 (warm-dry period-2). The first and the second experimentations were carried out in the laboratory and the experimental plot respectively during the cold-humid and the first warm-dry-1 periods. The third experimentation was carried out in the plot during the warm-dry period-2. During each experimentation, the values of the temperature and the hygrometry of periods of the study were measured using a Wireless Weather Station; Model: H10515/DCF; Version10/2007. These parameters were recorded every day at two hourly intervals between 6 a.m and 6 p.m during the entire study periods. The average daily temperature and average daily hygrometry were then calculated from the seven measurements. This helped to know if the experimental environments differ or not from one period to another.

Duration of development phases of *L. trifolii*

During the first experimentation, five hundred celery seedlings and infested leaves were respectively collected in a nursery and celery farmers' plots at Nkolondom and carried to the University. At the laboratory, the seedlings were transplanted in 120 ml plastic pots filled with manure. Leafminers larvae present in infested leaves were well-attended in the laboratory successively inside transparent rearing boxes (7× 10 × 17cm) and Petri dishes (4 and 6 cm of diameter) until leafminers' imaginal molt. The flies were then counted according to their sex (segregated on the base of the structure of the two last tergites setulae of female that are conspicuously dense and elongated compared to males (Ortiz, 2009)) and used to infest celery plants. Three males, three females and five plants were introduced in each appropriate transparent bucket for 12 h per day in the laboratory. A hundred of these infested plants were kept in the laboratory and the rest was transplanted on celery experimental plot of four ridges. We used only middle plot's ridges plants to avoid border effect characterize by abundance of parasite on the plot's border. In the laboratory or in the plot, the leaf which has the most impacts of egg-laying or of feeding was cut with a pruning shears from 50 plants randomly selected. These leaves are collected and put in five rearing boxes (ten leaves per box) covered with a mosquito net. The boxes with damage leaves were placed in the furrow located between ridges of each plot. On the remainder plants of the laboratory and 50 others randomly selected in the plot, the leaf which has the most impact of egg-laying or of feeding was put in a muff sewn with a special mosquito net of 0.5 mm mesh size. After that, the development of *L. trifolii* larvae was monitored on both cut leaves and whole plants. Once pupae were obtained, they were placed individually in labeled rearing Petri dish at the ambient temperature which mean was around 25°C until imago's emergence. The label indicated the food resource and the milieu of experimentation. A yearly roman calendar was used in order to estimate the duration of each development phase in each milieu are as follow:

(i) The embryonic phase (from laying to hatching marked by the appearance of the galleries);
(ii) The larval phase (from the appearance of the gallery to the exit of the larvae (pre-pupae) from the gallery);
(iii) The pupal phase (from pre-pupae to emergence of imagos).
(iv) The pre-imaginal development duration

The second experimentation was carried out similarly to the first one but in the plot during the warm-dry period -1. Independently to the assessment of the durations of development phases, the pupae obtained from the whole plants and the cut leaves during this experimentation were counted and measured separately using a stereomicroscope provided with a micrometer. The variations of pupae's morphometric characteristics were: diameter (0.44 to 0.92 mm); length (1 to 1.98 mm). The basis of classification depended on the fact that during some preliminaries experiments, we realized that from some pupae did not emerged flies. We measure the length and the diameter of those pupae and from that determined

the morphometric characteristics of the two batches. These pupae were classified as follow.

(i) Batch 1; diameter > 0.73 mm, length > 1.74 mm;
(ii) Batch 2; diameter ≤0.73 mm, length ≤1.74 mm.

Then, many comparisons shall be more focused on cut leaves' bathes because it is the hypothetical food resource. A comparison was done to know exactly whether the diameter and length of pupae belonging respectively to cut leaves' batch 1 and batch 2 are different. If it was the case, from the laboratory and the plot, four batches were finally obtained based on the measurements and the foods resources of the pupae. The pupae of each batch was labeled and placed individually in rearing Petri dish at the ambient temperature until imagos' emergence. The label indicated the food resource quality and the batch.

A comparison was done made to know exactly whether the cold-humid period is different to the warm-dry period according to the values of their temperature and their hygrometry. If it was the case, we shall after the first and second experimentations compare development phase's durations and the pre-imaginal development durations according to the experimental environments, the food resources and the seasonal periods of the study.

Once these pre-imaginal development durations obtained, we evaluate the pre-imaginal mean duration of *L. trifolii* obtained respectively in the plot with the cut leaves and entire plant according to the entire year ((pre-imaginal development duration of the warm-dry period + pre-imaginal development duration of the cold-humid period)/2). With this previous result, we calculate the number of generations of *L. trifolii* during the year (365 days/pre-imaginal mean duration).

The fitness of *L. trifolii*

The third experimentation is conducted only in the plot during the warm-dry period -2. After the pupal phase of the second experimentation three lots of 100 plants are respectively infested by the leafminers emerged in the plot from the batch 1 (346 pupae) and batch 2 (58 pupae) of the cut leaves and the batch 1 (196 pupae) of the whole plants. Twenty four leafminers (twelve males and twelve females) from each batch were used for infestation. Those plants are labeled according to the batch of the infesting leafminers. The development of *L. trifolii* is then monitored on the whole plants only as on the first experimentations. Larvae observable through their galleries, pupae and imagos are progressively counted according to each label. The fitness of *L. trifolii* was determined through the larval survival rate ((number of pupae / number of larvae) x 100) and the imago emergence rate ((number of imagos / number pupae) x 100). Two comparisons of larval survival rates and imago emergence rates were done in the aim to know whether the fitness of flies obtained (1) from the cut leaves batches or (2) from the batches -1 of cut leaves and whole plants were respectively different.

Sex-ratio of *L. trifolii*

At the end of the third experimentation, adult flies were counted according to their sex. The numbers obtained allowed us to determine two sex-ratios (number of females /number of males). The whole plants' sex-ratio (obtained with flies emerged from whole plants) which was relative to the natural infestation was then compared to the cut leaves' one (obtained with flies emerged from cut leaves). In fact, the percentage of cut leaves' males and females were compared to the whole plants' males and females to know whether the sex-ratio of *L. trifolii* population was affected by the foods resources.

Data analysis

The development durations of each phase of *L. trifolii* were expressed as mean ± standard deviations and compared using the Wald-χ^2 (General Linear Model procedure) according to the experimental environments, the food resources and the periods of study. We first test the difference in temperature and hygrometry distributions to know whether drastic change existed between the cold-humid and warm-dry periods according to experimental environments using Kolmogorov-Smirnov test (K). Meanwhile, the Pearson correlation test (χ^2) was used to find linear relationship between temperature, humidity and the development stage's entire development durations of *L. trifolii*. The comparisons between the pre-imaginal development durations of *L. trifolii* according to the experimental environments, the food resources and the seasonal periods of the study were then tested using the Mann-Whitney test (U). The lengths or diameters of pupae from batches 1 and 2 were compared using a Turkey-test (T). The fitness of the flies from batches 1 and 2 were also compared using the Chi-square test (χ^2). The Chi-square test (χ^2) was once more used to compare the natural and the experimental sex-ratios. Analyses were done using SPSS (20.0) and the results were appreciated at 5% interval confidence.

RESULTS

L. trifolii development durations

Distribution of the temperature and the hygrometry through the periods of study between the laboratory and the plot

Significant variations of temperature (K = 1; P <0.0001) and hygrometry (K = 1; P <0.0001) existed between the laboratory and the plot during the warm-dry period-1. Similar differences between both sites (temperature: K = 0.931; P <0.0001) (hygrometry: K = 1; P <0.0001) were also observed during the cold-humid period (Table 1). Those results prove that temperature and hygrometry were radically different between the laboratory and the plot during the cold-humid period or the warm dry period.

Distribution of the temperature and the hygrometry between the warm-dry-1 and warm-dry-2 periods in the plot

No significant differences of temperature (K = 1.40; P = 0.65) and hygrometry (K = 1.52; P = 0.41) existed in the plot between the warm-dry-1 and the warm-dry-2 periods (Table 2).

Influence of the experimental environments on L. trifolii development phases durations independently to the food resource and the seasonal period

Three comparisons were made between *L. trifolii* developments durations observed in the different experimental environments during our study (Table 3).

Table 1. Comparison of temperature and hygrometry through the periods of study between the laboratory and the plot.

Different samplings	Frequency	Minimum	Maximum	Mean±ESD	K	P
T°LW	30	27.69	31.4	29.109±0.82[a]	1.000	< 0.0001***
T°PW	30	22.87	24.81	24.114±0.45[b]		
H°LW	30	54.12	61.2	56.113±1.55[a]	1.000	< 0.0001***
H°PW	30	71.63	76.47	73.187±1.28[b]		
H°LC	30	73.12	78.63	76.459±1.47[a]	1.000	< 0.0001***
H°PC	30	81.16	86.74	84.787±1.60[b]		
T°LC	30	22.12	24.16	23.231±0.57[a]	0.733	< 0.0001***
T°PC	30	19.8	23.47	21.329±1.08[b]		

*Highly significant, Std= standard deviation.Means followed with the same small letters marked at upper corner of standard deviation are not statistically different. Legend: T=temperature; H=hygrometry; L=laboratory; P=plot; W=warm-dry period; C=cold-humid period.

Table 2. Comparison of temperature and hygrometry through the warm-dry-1 and warm-dry-2 periods in the plot.

Different samplings	Frequency	Min	Max	Mean±ESD	K	P
T°PW-2	28	23.6	24.9	24.11±0.44[a]	1.4	= 0.65*
T°PW-1	28	22.87	24,81	24.18±0.45[b]		
H°PW-2	28	71.3	76.81	73.31±1.30[a]	1.52	= 0.41*
H°PW-1	28	71.63	76,47	73.18±1.28[b]		

*Not significant, Std= standard deviation. Means followed with the same small letters marked at upper corner of standard deviation are not statistically different. Legend: T=temperature; H=hygrometry; P=plot; W-1=warm-dry period-1 W-2=warm-dry period-2.

Table 3. Comparison of the development stage durations of *L. trifolii* according to the experimental environments independently to the food resource and the seasonal period.

Development stage	Sites	Min	Max	Mean	Wald- χ^2
Embryonic	Plot	4.11	4.24	4.17±0.033[a]	Wald- χ^2 = 255.14; df = 1; P<0.0001***
stage	Laboratory	3.38	3.5	3.44±0.032[b]	
Larval	Plot	4.48	4.75	4.62±0.068[a]	Wald- χ^2 = 5.04; df = 1; P = 0.02**
stage	Laboratory	4.28	4.53	4.40±0.066[b]	
Pupal	Plot	9.23	9.93	9.58±0.17[a]	Wald- χ^2 = 0.02; df = 1; P=0.87*
stage	Laboratory	9.21	9.87	9.54±0.17[a]	

*** Highly significant; ** significant; * non significant, Std= standard deviation. Means followed with the same small letters marked at upper corner of standard deviation are not statistically different

Significant differences were assessed between: (i) the development durations of embryonic phase obtained in the experimental plot, Mean = 4.17±0.33 days) and in the laboratory (Mean = 3.44±0.032 days); (Wald- χ^2 =255.14; df = 1; P<0.0001); (ii) the development durations of larval phase obtained in the experimental plot Mean = 4.62±0.68 days) and in the laboratory (Mean = 4.40±0.066 days); (Wald- χ^2 = 5.04; df = 1; P = 0.025). Meanwhile, no significant differences were observed between the development durations of pupal phase obtained in the experimental plot Mean = 9.58±0.177days) and in the laboratory Mean = 9.54±0.17 days) (Wald- χ^2 = 0.02; df = 1; P = 0.87).

Influence of food resources on L. trifolii development phases durations independently to the experimental environment and the seasonal period

Another series of comparisons were made between *L. trifolii* development phases' durations according to the food resource during our study (Table 4). Significant differences were observed between:

(i) The development durations of embryonic stage of leafminers obtained from cut leaves (Mean = 3.21±0.35 days) and whole plants (Mean = 4.4±0.029 days); (Wald- χ^2 = 683.02; df = 1; P<0.0001);

Table 4. Comparison of the development phases durations of *L. trifolii* according to the foods resources and independently to the experimental environment and the seasonal period.

Development stage	Food resource	Min	Max	Mean	Wald- χ^2
Embryonic stage	Whole plants	4.35	4.40	4.17 ± 0.03^a	Wald- χ^2 = 683.02; df = 1; P<0.0001***
	Cut leaves	3.14	3.28	3.21 ± 0.03^b	
Larval stage	Cut leaves	3.96	4.25	4.10 ± 0.06^a	Wald- χ^2 = 73.13; df = 1;
	Whole plants	4.80	5.03	4.92 ± 0.06^b	P = 0.0001***
Pupal stage	Cut leaves	8.03	8.77	8.40 ± 0.18^a	Wald- χ^2 = 88.37; df = 1; P<0.0001***
	Whole plants	10.41	11.03	10.72 ± 15^b	

*** Highly significant, Std= standard deviation, Means followed with the same small letters marked at upper corner of standard deviation are not statistically different.

Table 5. Comparison of the development phases' durations of *L. trifolii* according to the periods of study and independently to the environment conditions and the food resource.

Development stages	Study period	Development duration			Statistical comparison based on Wald- χ^2 test
		Min Min	Max	Mean ± Std	
Embryonic stage	Warm-dry	3.21	3.34	3.27 ± 0.03^a	Wald- χ^2 = 543.71; df = 1; P<0.0001***
	Cold-humid	4.28	4.4	4.34 ± 0.03^b	
Larval stage	Warm-dry	3.90	4.15	4.02 ± 0.06^a	Wald- χ^2 = 105.04; df = 1; P<0.0001***
	Cold-humid	4.86	5.13	5.00 ± 0.06^b	
Pupalstage	Warm-dry	7.93	8.59	8.26 ± 0.19^a	Wald- χ^2 = 112.05; df = 1; P<0.0001***
	Cold-humid	10.51	11.21	10.86 ± 0.17^b	

*** Highly significant, Std= standard deviation, Means followed with the same small letters marked at upper corner of standard deviation are not statistically different.

(ii) The development durations of larval phase of leafminers obtained from cut leaves (Mean = 4.10±0.73 days) and whole plants (Mean = 4.92±0.061 days); (Wald- χ^2 = 73.13; df = 1; P = 0.0001);
(iii) The development durations of pupal phase of leafminers obtained from cut leaves (Mean = 8.40±0.189 days and whole plants Mean = 10.72±0.157days); (W=88.37; df = 1; P<0.0001).

Influence of seasons on the duration development stages *L. trifolii*

The series of comparisons were made between *L. trifolii* development phase durations according to the seasonal periods of the study, without consideration of neither environment, nor food resource (Table 5). Significant differences were observed between:

(i) The development durations of embryonic phase of leafminers during the warm-dry period (Mean = 3.27±0.32 days) and the cold-humid period (Mean = 4.34±0.033 days); (Wald - χ^2 = 543.71; df = 1; P<0.0001);
(ii) The development durations of larval phase of leafminers during the warm-dry period (Mean = 4.02±0.66 days) and the cold-humid period (Mean = 5.00±0.068 days) (Wald- χ^2 = 105.04; df = 1; P<0.0001);
(iii) The development durations of pupal phase of leafminers during the warm-dry period (Mean = 8.26±0.170 days) and the cold-humid period (Mean = 10.86±0.178 days); (Wald- χ^2 = 112.05; df = 1; P<0.0001).

Comparison of the pre-imaginal durations of L. trifolii according to the seasonal periods of the study

Correlation between the daily means temperature or hygrometry and the pre-imaginal development duration of *L. trifolii* in the laboratory and the plot: The Pearson correlation coefficient showed that the display of previous weather conditions during our study had variable incidences over the *L. trifolii* development cycle duration (Table 6). In the laboratory during the warm-dry periods, the temperature was positively and significantly (r = 0.01; P = 0.008) correlated with the *L. trifolii* pre-imaginal development durations. Under the same conditions, the hygrometry was negatively but not significantly (r = -0.04, P = 0.22) correlated with this duration. Significant and negative correlations (r = -0.17; P <0.001) were highlighted between temperature and development duration in the laboratory in cold-humid period. In the

Table 6. Correlation between the daily means temperature or hygrometry and the pre-imaginal development duration of *L. trifolii* in the laboratory and the plot.

Experimental environments		Study period	Pearson correlation	
Temperature	Laboratory	Warm-dry	r = 0.01 ;	P = 0.008**
		Cold-humid	r = -0.175 ;	P < 0.0001***
	Plot	Warm-dry	r = 0.085 ;	P = 0.019**
		Cold-humid	r = 0.44 ;	P < 0.0001***
Hygrometry	Laboratory	Warm-dry	r = -0.047 ;	P = 0.223*
		Cold-humid	r = -0.58 ;	P = 0.105*
	Plot	Warm-dry	r = -0.292 ;	P < 0.0001***
		Cold-humid	r = 0.412 ;	P < 0.0001***

*** Highly significant; ** significant; * non significant.

Table 7. Comparison of the whole durations of the three development phases of *L. trifolii.*

Experimental environments	Study period	Foods resources	Development durations			U-test
			Min	Max	Mean	
Laboratory	Warm-hot	Cut leaves	13	22	16.53±0.26^{a}	U = 3843; df = 1; P<0.0001***
		Whole plants	14	25	17.97±0.03^{b}	
	Cold-humid	Cut leaves	15	26	19.98±0.27^{a}	U = 5768,5; df = 1; P<0.0001***
		Whole plants	15	26	20.8±0.21^{b}	
Plot	Warm-hot	Cut leaves	14	22	17.51±0.03^{a}	U = 4802; df = 1; P<0.0001***
		Whole plants	15	25	18.58±0.19^{b}	
	Cold-humid	Cut leaves	15	29	20.39±0.4^{a}	U = 3726; df = 1; P<0.0001***
		Whole plants	15	29	21.98±0.3^{b}	

*** Highly significant, Std= standard deviation. Means followed with the same small letters marked at upper corner of standard deviation are not statistically different.

same site and at the same periods, the humidity was negatively and not significantly correlated (r = -0.58; P = 0,105) to the *L. trifolii* pre-imaginal development durations.

In the plot during the cold-humid period temperature (r = 0.44; P <0.0001) and hygrometry (r = 0.41, P <0.0001) were positively and significantly correlated with *L. trifolii* pre-imaginal development durations.

In the same site during the warm-dry periods, the temperature was positively and significantly correlated (r = 0.08; P = 0.01) with *L. trifolii* development phases durations. The hygrometry was negatively and significantly correlated with *L. trifolii* pre-imaginal development durations (r = - 0.29; P < 0.0001).

Comparison of the pre-imaginal development durations of L. trifolii

The series of comparisons are made between the whole duration of the three development phases of *L. trifolii* observed during the seasonal periods of the study (Table 7). During the warm-dry period, the pre-imaginal development duration of *L. trifolii* varied significantly (U = 3843; P<0.0001) between the cut leaves (Mean = 16.53 ± 0.26 days) and whole plants in the laboratory (Mean = 17.97 ± 0.03 days). In the same site during the cold-humid period, this parameter also varied significantly as previously (5768.5; P<0.0001) between cut leaves (Mean = 19.98 ± 0.26 days) and whole plants (Mean = 20.80 ± 0.21 days).

During the warm-dry period, the pre-imaginal development duration varied significantly (U = 4802; P<0.0001) between the cut leaves (Mean = 17, 51 ± 0.03 days) and the whole plants (Mean = 18.58 ± 0.19 days) in the plot. In the same site during the cold-humid period, that duration also varied significantly (U = 3726; P < 0.001) between the cut leaves (Mean = 20.39 ± 0.4 days) and the whole plants (Mean = 21.98 ± 0.3 days). According to the previous results, the pre-imaginal mean duration of *L. trifolii* obtained in the plot during the entire year were respectively 18.95 ± 0.35 days and 20.28 ± 0.11 days with the cut leaves and entire plants. With these pre-imaginal mean durations we are expected to

Table 8. Number of pupae from different batches obtained at the end of the second experimentation.

Batches from experimentation 2 (Warm-dry-1period)		
Batch 1: whole plant	Batch 1: cut leaves	Batch 2: cut leaves
346	196	58

Legend: P= Plants; E= entire; L= leaves; C= cut.

Table 9. Comparison of the fitness of flies obtained from the batch 1 and the batch 2 of the cut leaves.

Variables	Batch 1	Batch 2	χ^2-test
Larval survival rate (%)	80.7	19.3	$\chi^{2\,=}$ 10.85; df = 1; P<0.0001***
Adult emergence rate (%)	83.9	16.1	χ^2=10.16; df = 1 ; P<0.0001***

*** Highly significant.

have more less 19 and 20 generations of *L. trifolii* respectively with the cut leaves and entire plants during the year.

Fitness of L. trifolii

The results obtained at the end of the second experimentation showed that many pupae were obtained with entire plant than cut leaves during the warm-dry period (Table 8).

Comparison between the lengths and the diameters of pupae of the two batches

The lengths and the diameters of pupae of the two batches were compared using the Turkey test. That comparison showed significant differences between:

(i) The lengths of pupae obtained from batch 1 and batch 2 of cut leaves in the plot during the warm-dry period 1 (t = 0.084; P<0.0001).
(ii) The diameters of pupae obtained from batch 1 and batch 2 of cut leaves in the plot during the warm-dry period 1 (t = 0.045; P<0.0001).

Fitness of the flies obtained from the batch 1 and the batch 2 of the cut leaves

The fitness of the flies obtained from batch 1 and batch 2 of the cut leaves are compared using Chi-square test (χ^2) (Table 9). This comparison showed significant differences between:

(i) Larval survival rate (χ^2 = 10.85; df = 1; P = 0.001);
(ii) Imago emergence rate (χ^2 = 10.16; df = 1; P = 0.001).

Fitness of the flies obtained from batch 1 of the cut leaves and batch 1 of the whole plants

The comparison of the fitness of the flies obtained from batch 1 of the cut leaves and batch 1 of the whole plants showed no differences (Table 10) between:

(i) Larval survival rate (χ^2 = 6.25; df = 1; P = 0.012);
(ii) Imago emergence rate (χ^2 = 0.23; df = 1; P = 0.62).

Variation of sex-ratio of L. trifolii

We have considered that the numbers or percentages of leafminers obtained on cut leaves (males: 117 or 49%; females: 121 or 51%) were theoretical when those obtained on whole plants (males: 320 or 48%; females: 348 or 52%) were observed. Then, the comparison done between the cut leaves sex ratio (1/0.96) and that of the whole plants (1/0.91) showed that no significant difference (Table 11) was observed between the cut leaves and the whole plants sex ratios (χ^2 = 2.38; df = 1; P=0.12). The *L. trifolii* sex ratio which is slightly biased toward females was not affected by the food resource.

DISCUSSION

The duration different development stages of *L. trifolii* varied respectively between indoor environment (laboratory) and outdoor (the plot), the warm-dry period and the cold-humid period, the cut leaves and the whole plants. The consequence of the previous results was the variation of the pre-imaginal development durations. The duration of embryonic and larval stages were significantly shorter indoor than outdoor. On the contrary to outdoor, indoor environment was closed and maintained high and more constant temperature, condition that seems more

Table 10. Comparison between the fitness of the flies obtained from the batch 1 of the cut leaves and the batch 1 of the whole plants.

Variables	Batch 1 (leaves)	Batch 1 (plants)	χ^2-test
Larval survival rate (%)	34.8	65.2	$\chi^2 = 6.25$; df = 1; P = 0.12*
Imago emergence rate (%)	42.5	54.8	$\chi^2 = 0.23$; df = 1; P = 0.62*

*Not significant.

Table 11. Comparison between the natural and the experimental sex-ratio.

Variables	Male	Female	Total	Sex-ratio	χ^2-test
Cut leaves (Experimental)	117 (49%)	121 (51%)	238	1/0.96	$\chi^2 = 2.38$; df = 1; P=0.12
Whole plants (Natural)	320 (48%)	348 (52%)	668	1/0.91	

*Not significant.

suitable for the development of *L. trifolii*. Our observations are thus similar to those of Poe (1981) who showed that at constant temperature of 30°C, the *L. trifolii* larvae achieve their development in 4 days while at 20°C it takes 7 days. On the other hand, various authors including Spencer (1973), Nedstam, (1985), Minkenberg and Lenteren (1986) and Lee et al. (1990a) showed that embryonic development of *Liriomyza bryoniae* (Kaltenbach) requires 4-8 days at mean temperature of 20.6°C. Our results showed that the duration of larval stage varied from 4 to 7 days, and were similar to those of Harris and Tate (1933) who obtained larval stage duration between 4-7 days at mean temperature above 24°C). The *L. trifolii* pupal stage durations did not varied in the laboratory compare to the plot. That result may be due to the fact that pupae were less susceptible to the slight variation of temperature observed during the study than eggs and larvae. During similar studies, the *Liriomyza sativae* Blanchard pupal stages took 7-14 days at temperature comprised between 20 and 30°C (Leibee, 1982).

The *L. trifolii* embryonic, larval and pupal phases durations are significantly shorter with the cut leaves than the whole plants. This result may be due to the fact that the cut leaves are poor in food resource availability than whole plants. Therefore, the limited amount of nutrients in cut leaves due to the non-renewal of the sap may induce the acceleration of the embryonic phase before the depletion of the limited amount of nutrients in the cut leaves. Bruno (1986) considered that temperature and food resource availability are the main factors that affect different stages duration of insect development. Poe (1981) showed that on chrysanthemum, the cycle of *L. trifolii* is achieved in 24 days at 20°C *L. trifolii* is very prolific during the warm-dry period than the cold-humid period according to the significant shorter of its embryonic, larval and pupal phases durations. That result may be due to the action of temperature and hygrometry. During the warm-dry period, the actions of temperature and hygrometry seem to be antagonist even if there is a domination of the temperature. Perhaps the temperature contributed to activate the enzymatic reactions of embryos, larvae and pupae which results in short development durations. Contrarily, hygrometry assured to the leaves a permanent hydration through the phenomenon of revival, a phenomenon limiting the water stress whose consequence is the deceleration of embryonic, larval and pupal growth. During the cold-humid period, the antagonism persisted; but the temperature is not sufficient to dominate as previously the effect of hygrometry. The consequence is the deceleration of embryonic, larval and pupal growth which resulted in long *L. trifolii* development stages durations. Our results concerning the temperature confirmed the ones of Minkenberg (1988) who showed that at 25°C with *L. trifolii* on celery, the embryonic stage requires 2.5 days, 4.6 days for larval stage and 9.3 days for pupal stage. According to Leibee (1982), emergence of adults of all species of the genus *Liriomyza* occurs 7-14 days after pupation, at temperatures between 20 and 30°C.

In this study, the means durations of embryonic, larval stage and pupal stage varied respectively from 3.14 to 4.40 days, 3.90 to 5.13 days and 7.90 to 11.21 days. Maybe, when the egg of *L. trifolii* was particularly influenced by some parameters as experimental environments, foods resources and periods of study, it programs the duration of each development phase. The consequence of that programmation is the significant variation of the whole duration of the pre-imaginal development phases of *L. trifolii* according to the experimental environments, the foods resources and the periods of study. These results are in accordance with those of Mauchamp (1988) who observed that different variations in the *L. trifolii* growth cycle are explained by the fact that insects are cold-blooded animals and their body temperature closely follows room temperature. When the temperature decreases, the activity of the cells decreases. Leibee (1984) showed that on celery whole

plants, *L. trifolii* closes its cycle (from the laying to adult emergence) in 12 days at 35°C, 19 days in 25°C, 26 days at 20°C, 54 days at 15°C. Capinera (2006) showed that *L. sativae* development cycle lasts 25 days at 15°C and 15 days at 30°C. Significant differences are obtained between measurements of pupae of batch 1 and batch 2.

Morphometric characters of pupae were: diameter (0.44 to 0.92 mm); length (1 to 1.98 mm). Similar results (1.3-2.3 × 0.5-0.75 mm) were obtained on *L. sativae* (CABI et l'OEPP, 1990) According to their measurements; pupae obtained from batch 1 were different than those of batch 2. In the same way, significant differences are obtained between the fitness of flies obtained from batch 1 and batch 2 of the cut leaves. These differences are showing that the performances of flies obtained from batch 1 are less compare to those of the batch 2. Indeed, contrarily to the whole plants, the larval which fed in the marginal areas leaflets of cut leaves died or were poorly developed as a result of drying or withering of this food parts. This has resulted in a decrease in the number of larvae which performed pupation in cut leaves. Furthermore, from some larvae (diameter > 0.73 mm, length > 1.74 mm) poorly developed which nevertheless performed small sizes pupae, imago did not emerge because of their desiccation. It thus appears from these results that high temperatures of the warm-dry period desiccate the pupae of small sizes. Our results corroborate those of some authors who showed that extreme temperatures were harmful to the overall *L. trifolii* development. Capinera (2005) has estimated that in *L. trifolii*, the development stops at temperatures below 7.5°C or above 12.9°C, depending on the development stage and the host plant; the optimum temperature is around 25°C; above 30°C larval mortality increases. None difference is obtained between the fitness of flies obtained from batch 1 of the cut leaves and batch 1 of the whole plants. These differences are showing that the performances of flies obtained from cut leaves' batch 1 are the same than to those of the entire plants' batch 1. It is worthwhile to note that despite the limited amount of nutrients and the water stress in the cut leaves, many *L. trifolii* have completed their embryonic, larval and pupal stages with success as those obtained with whole plants. These results also showed that the cut leave, synonym of pruning has three drawbacks:

(i) It increase the number of generations of the pest (from 18 with the entire plants to 19 with the cut leaves) in gardens during the year especially during the hot period;
(ii) It does not inhibit the fitness of all adults such as we have great fitness of flies obtained from 50 cut leaves' pupae;
(iii) It reduces the accessibility of some parasitoid larvae to leafminers covered with other cut leaves abandoned in the furrow.

The pruning in the garden contributes to reduce the number of the offsprings and did not affect the fitness of all the leafminers. Great sizes adults resulting from any food resource showed that under natural conditions they are able to provide an offspring that can cause severe devastations in celery plots. Our results on the measurements or the sizes of individuals confirmed those obtained from studies on parasitoids by Van den Assem et al. (1989) and Godfray (1994) which showed that size is a parameter determining the fitness of a parasitoid. In Aphidiidae, it was demonstrated that fertility is proportional to the size of the individual (Elliot et al., 1994). Two reasons showed that a bigger female parasitoid is better: it lives longer and it is more fertile, so that allows it to produce more offsprings and thus increase its fitness (van den Assem et al., 1989).

The increase of the pre-imaginal mortality is so important on cut leaves such as they look like another plant than whole plants. However, the data showed that the pre-imaginal mortality affected similarly males and females. Despite that mortality, no significant difference was observed between the cut leaves sex ratio (1/0.96) and that of the whole plants (1/0.91). Furthermore, the *L. trifolii* sex ratio which is slightly biased toward females.
Our results are collateral to those of some authors who found that the *L. trifolii* sex ratio was also slightly biased toward female with respectively 1:0.75 for castor; 1: 0.8 for cotton; 1:0.6 for cowpea and 1:0.7 for tomato (Sushila Nadagouda et al., 1997).

Conclusion

The present work has shown the capacity of pruning leaves to ensure the entire development of *L. trifolii* from early stages to adult with good fitness. The pruning followed by the abandonment of the infested leaves in the furrow certainly eliminates the pest for a few moments, but cannot be considered as a promotable control method for the protection of celery. It induces the reduction of the development cycle duration and then increase the number of pest generations during the year. Moreover, several individuals obtained throughout that practice have retained an excellent fitness and later infested healthy plants. More efforts will be required in view of establishing an Integrated Pest Management (IPM) which will take into account the preservation of the environment and health of consumers.

Conflict of Interest

The authors have not declared any conflict of interest.

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