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The Seasonal Population Dynamics of *Corythucha arcuata* (Say, 1832) (Hemiptera: Tingidae) and the Relationship between Meteorological Factors and the Diurnal Flight Intensity of the Adults in Romanian Oak Forests

Flavius Bălăcenoiu [1,2,*], Dieter Carol Simon [1], Constantin Neţoiu [2], Dragoş Toma [1,2] and Ion Cătălin Petriţan [1]

1 Faculty of Silviculture and Forest Engineering, Transilvania University of Braşov, Sirul Beethoven 1, 500123 Braşov, Romania; disim@unitbv.ro (D.C.S.); dragost93@gmail.com (D.T.); petritan@unitbv.ro (I.C.P.)
2 National Institute for Research and Development in Forestry "Marin Dracea", Eroilor 128, 077190 Voluntari, Romania; c_netoiu@yahoo.com
* Correspondence: flavius.balacenoiu@icas.ro

Abstract: To control a forest pest, it is necessary to understand the biotic and/or abiotic factors that can lead to population regulation. Such knowledge is even more critical if the pest is an invasive alien species. This is the case for *Corythucha arcuata* (Say, 1832), commonly known as the oak lace bug (OLB), an alien insect species that has invaded oak forests on a large scale, both in Romania and other European countries. In this study, we set out to examine the relationship between adults of this species and meteorological factors, such as air temperature and humidity. The study lasted for two years (2019–2020) and was performed on three plots in Romania, in Ilfov, Giurgiu, and Călăraşi counties. In the first year, the seasonal dynamics of the OLB populations and the meteorological factors potentially influencing those were studied, whilst in the second year, the dynamics of the diurnal activity of the insect population were examined. We found that *Corythucha arcuata* experienced two population peaks—in July and August—and a diurnal population dynamic that reached a maximum at midday. Data analysis indicated that, for most of the time, the adult activity was influenced by both temperature and humidity. In addition, the population dynamics were not constant over the course of the day, preferring relatively high temperatures and low humidity but not exceeding certain thresholds of thermal discomfort.

Keywords: oak lace bug; ecology; oak forests; pests; invasive alien species

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1. Introduction

Climate change, through temperature increases from one decade to the next, has led to changes in the behaviour of pest insects. These may be manifested, for example, as an expansion in the altitudinal/latitudinal distributions of certain species, by outbreaks of species that previously have not occurred in pest quantities, by reports and the rapid expansion of invasive alien species, by an increase in the frequency and intensity of such outbreaks, by an increase in vitality and implicitly, feeding, by an increase in the number of generations per year, and by disturbances in the food chain [1]. For example, a study on the relationship between future climatic elements in Portugal and the predicted behaviour of *Thaumetopoea pityocampa* (Den. & Schiff.) (Lepidoptera: Notodontidae)—a defoliating insect affecting *Pinus* spp. and *Cedrus* spp.—showed that the insect would become active earlier in the year, in May [2]. Another study, modelling the potential global distribution of one of the most important bark beetles—*Dendroctonus ponderosae* Hopkins. (Coleoptera: Curculionidae)—in North America [3], found that climate change might significantly influence the spread of this species over the next few decades. It has also been suggested that climatic conditions could determine the intensity of outbreaks of *Dendrolimus pini* L.

(Lepidoptera: Lasiocampidae)—a pest affecting *Pinus sylvestris* L. (Pinales, Pinaceae)—and that the pest may become more frequent and widespread the climate warms [4,5].

In recent decades, against this general background, several new invasive alien insect species have been reported in forest ecosystems on the European continent. Kenis and Branco [6] opined that even though European forest ecosystems are less affected by invasive pests than forests on other continents, a greater diversity of invasive forest pests that can cause damage has recently become established on the continent, including species such as *Dryocosmus kuriphilus* Yasumatsu. (Hymenoptera: Cynipidae), *Megaplatypus mutatus* Chapuis. (Coleoptera: Curculionidae), *Anoplophora glabripennis* Motschulsky. and *Anoplophora chinensis* Forster. (both Coleoptera: Cerambycidae).

Among the invasive alien insect species that have begun to affect forest ecosystems in Europe in recent years, worth mentioning is *Corythucha arcuata* (Say, 1832) (Hemiptera: Tingidae). Commonly known as the oak lace bug (OLB); it originated in North American and was accidentally introduced to Europe (Italy) in 2000 [7]. Two years later, in 2002, the species was reported in both Switzerland [8] and Turkey [9]. Recognising its potential for substantial attack and invasion, the OLB was included on the European and Mediterranean Plant Protection Organisation's Alert List in March 2001 [10], remaining there until 2007 [11], when it became clear that administrative efforts could not stop its expansion. In 2012, the species was first reported in Bulgaria [12], and in 2013, it was first observed in three other countries: Hungary [13], Croatia [14], and Serbia [15,16]. To date, the oak lace bug has invaded many European countries, considering that most European and Asian forests have suitable hosts for this species [17]. This insect species feeds on the undersides of the host (usually oak) leaves by breaching the epidermis with piercing and sucking mouthparts, which draw out the cellular sap [18]. In strong infestations, the pest can cause premature defoliation or can increase the host's susceptibility to various diseases or pests [19]. In 2019, in only five European countries (Croatia, Hungary, Romania, the European part of Russia, and Serbia), the OLB infested an estimated total area of over 1.7 million ha of the oak forest [20]. This species has two or even three generations per year, both in its original area [19] and in its invasive range, in Europe [21,22]. It overwinters in the adult stage in bark crevices or under-raised bark. At the beginning of May, the adults start to become active. The adults of the first generation appear in the second part of June, with adults of the second generation appearing in August. Of these, only those that appear by the end of August will develop a third generation, which will retire for overwintering.

In the context of such a biological invasion, understanding the behaviour of the pest can help in determining the conditions favourable for an outbreak so that forest managers can make decisions based on scientific grounds regarding the control of the species. In order to control a pest in an appropriate, scientific way, it is necessary to identify the feedback processes that lead to population regulation [23,24]. In applying integrated pest management, information is needed concerning pest population abundances and the environmental conditions under which they develop, as well as an understanding of the factors that influence the development of such populations [25,26].

From a biological point of view, insects are poikilothermic species, their body temperatures influenced by the ambient temperature. Consequently, meteorological factors are decisive in insect biology, influencing metabolic activity, abundance rate, and dispersion [27,28]. Among the various environmental factors, air temperature is considered one of the most important in affecting insect behaviour [29], having an important role in metabolism, metamorphosis, mobility, and host availability, which, in turn, can cause changes in their numbers and dynamics [30]. Precipitation is also an important factor in the dynamics of pest populations. On the one hand, a dry climate can provide environmental conditions adequate for the development and growth of herbivorous insects, whilst on the other, plants that suffer drought stress may attract other insect species [30]. Additionally, when precipitation is present, and drought occurs out of context, it can affect a pest by physically washing it away during heavy rain, especially those species with small bodies [31,32]; this could be one of the influential factors in OLB behaviour.

Lately, the invasive OLB has been the subject of so much study that, in addition to continual updates on occurrence in different countries, or information on the invasion phenomenon, there is also information on its ecology and its impact on its hosts and possible control methods. For example, for this species, there is information on its feeding preferences [33], its physiological effects on trees [34], and its impact on seed quality [35], as well as methods that can be used for the biological [36–38] and chemical control of this species [39]. Nevertheless, there is still no information available concerning the relationship between this species and the meteorological factors prevailing in the newly invaded territories; consequently, this is the purpose of our study. Considering that meteorological factors are decisive in the metabolic activity of insects, we proposed two hypotheses for the OLB: (1) The dynamics of the population in a growing season may vary depending on meteorological factors, and (2) any meteorological change during the day can lead to changes in their diurnal activities. We expected that both temperature and humidity would decisively influence the OLB population dynamics during a growing season. We also anticipated that any fluctuations in temperature and humidity in a day would influence the diurnal activity of the insects.

2. Materials and Methods

2.1. Study Site and Data Collection

This study was carried out in Romania, which, climatically, falls entirely within the temperate climate region, where two zonal climates—steppe and forest–steppe—and three regional climates—central European (sub-oceanic), eastern European (continental), and southeastern European (with sub-Mediterranean nuances) meet. As the strongest attacks of the OLB invasion of Romania have been recorded in the oak forests of the south-east [40], we established our examination of the relationship between meteorological factors and behaviour of the species in this area. The climate has a local continental influence causing aridity, and a high annual thermal amplitude, low rainfall, and frequent droughts in summer. Consequently, in order to carry out the study in an established area, we installed three monitoring points that were located in different counties—Ilfov, Călărași, and Giurgiu (Figure 1).

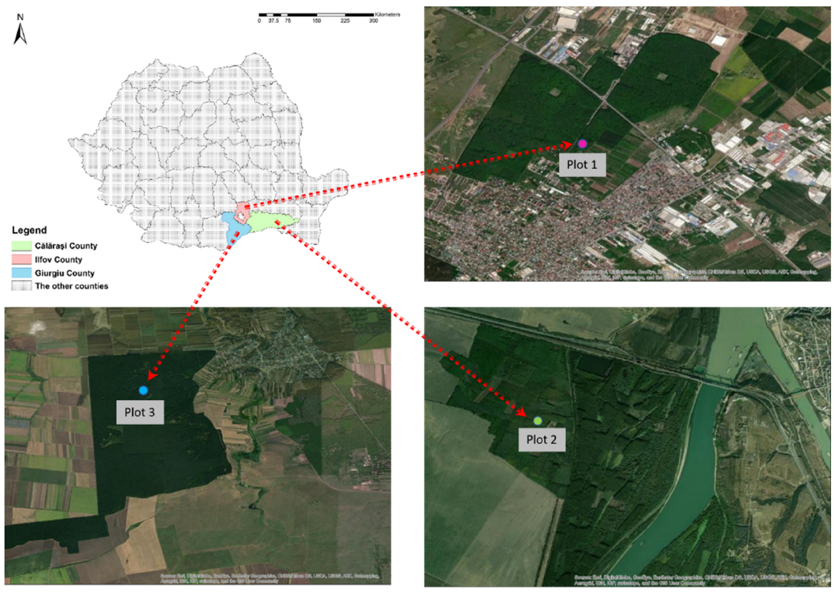


Figure 1. Location of the study area (Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, Aero Grid, IGN, and the GIS User Community).

The plots were located in three oaks stands—Plot 1 in Ștefănești Forest, Plot 2 in Incinta Forest, and Plot 3 in Ceagău Forest. These stands were relatively similar in terms of the composition of the main species, which included common oak (*Quercus robur* L.) (Fagales, Fagaceae) in a mix with turkey oak *Quercus cerris* L.) (Fagales, Fagaceae) and Hungarian oak (*Quercus frainetto* Ten.) (Fagales, Fagaceae). There were some differences between the plots in terms of their environmental conditions, however. Table 1 summarises the characteristics of the three plots, extracted from the management plans for each forest.

Table 1. Description of the three studied plots.

Characteristic	Plot 1	Plot 2	Plot 3
County	Ilfov	Călărași	Giurgiu
Forest district	I.N.C.D.S.	Lehliu	Giurgiu
Forest name	Ștefănești	Incinta	Ceagău
Coordinates	44°30′13′′ N 26°11′02′′ E	44°20′06′′ N 27°59′22′′ E	44°08′11′′ N 26°01′50′′ E
Altitude	80 m	10 m	90 m
First reported OLB	2016	2018	2017
Main species of stand [1]	Q.r.	Q.r. + Q.c.	Q.r. + Q.c. + Q.f.
Second species of stand [2]	T.t. + C.b.	U.m.	C.b.
Regeneration form [3]	N.R.	N.R.	N.R.
Average annual temperature	10.9 °C	13.3 °C	11.3 °C
Average annual rainfall	580 mm	504 mm	553 mm
Stand age (years)	75	30	75
Average stand diameter	28 cm	12 cm	30 cm
Average stand height	23 m	10 m	17 m
Vegetation limiting factor	poor humus content	poor water retention capacity	water deficiency

[1] Q.r. = *Quercus robur*; Q.c. = *Quercus cerris*; Q.f. = *Quercus frainetto*. [2] T.t. = *Tilia tomentosa* Moench. (Malvales, Malvaceae); C.b. = *Carpinus betulus* L. (Fagales, Betulaceae); U.m. = *Ulmus minor* Mill. (Rosales, Ulmaceae). [3] N.R. = natural regeneration from sprout.

The study of the ecological behaviour of the OLB at the three monitoring points was carried out over a period of two years—in 2019 and 2020. Information on the research period and visit frequency of the three points, as well as sampling procedure, are summarised in Table 2. Therefore, in the first year (2019), the seasonal population dynamics were monitored, whilst in the second year (2020), we examined the influence of meteorological factors on their diurnal flight dynamics.

Table 2. Information on sampling procedures and research period from 2019 to 2020.

Description of the Sampling Procedure	2019	2020
Research period	22 May–29 August	18 May–8 September
Frequency of visits	7–10 days	Two visits for each generation
Total number of visits	13 for each plot	6 for each plot
The purpose of each visit	Determining the proportion of in-flight adults	Determining the hourly proportion of in-flight adults (8:00 a.m.–7:00 p.m.)
Sampling methods	Mean number of adults on the leaf	Mean number of adults on the leaf
Number of samples	300 leaves	300 leaves

2.1.1. Seasonal Dynamics of OLB Populations—2019

To study the seasonal population dynamics, during the 2019 growing season (May–August), the three plots were visited frequently; the field season lasted about 13 weeks, and over the season, each site was visited 13 times.

To determine the number of insects in flight, we considered tracking the active flying stage—the adults—of the OLB. In each plot, a monitoring point was established, such that the trees around it represented the understorey of the stand (smaller in size than the average diameter and height), and the canopy, with no (natural) pruning, was easily accessible during the process of monitoring. During each visit, 300 leaves were analysed around the point, and the adults found on each of these were counted. In this way, we could record the total number of adults on 300 leaves and the proportion of in-flight adults ($adults \times leaf^{-1}$). The leaves were chosen randomly, ensuring a uniform distribution of canopy sampling. For this, a 7.5 m high ladder was used, so the canopy was covered up to about 9 m high. As the adults are sensitive, we did not harvest the leaves, in order to avoid the escape of adults. To monitor the meteorological factors at the same time, we recorded the daily average air temperature and daily average air humidity during each visit.

2.1.2. Dynamics of the Diurnal Activity of the OLB Population and Influential Weather Factors—2020

To study the effects of meteorological factors on the diurnal activity of the OLB, the times for field data collection were chosen based on the biological cycle of the species, aiming not to collect data on a day when the population dynamics were influenced by the appearance of adults of a new generation.

In accordance with OLB biology [19,21,22,41], and also our own observations from the previous years (including the results from 2020, presented in Section 3.1), we determined that the most favourable periods for collecting the data would be 18–26 May, 20–30 July, and 31 August–8 September (Table 3). As the periods between which the generations did not overlap were short, there were only 6 days in each period that fieldwork (observations) could be performed. Thus, observations were made on 2 days at each site in each of the three periods (i.e., 2 days/period/site). In this way, it was possible to target the populations of three generations, including those that caused the most significant attack—the active adults from the overwintering population and the first and second generations.

Table 3. Periods during which monitoring was performed in the three studied plots.

Period	Target OLB Generation	Day 1	Day 2	Plot/Forest
18–26 May 2020	Adults from the overwintering population	20 May 18 May 25 May	21 May 19 May 26 May	2–Incinta 3–Ceagău 1–Ștefănești
20–30 July 2020	Adults from the first generation	20 July 22 July 28 July	21 July 23 July 30 July	2–Incinta 3–Ceagău 1–Ștefănești
31 August 31–8 September 2020	Adults from the second generation	31 August 02 September 07 September	01 September 03 September 08 September	2–Incinta 3–Ceagău 1–Ștefănești

The study was carried out at the same three monitoring points in both years, using the same trees, with the same characteristics described in Table 1. On each observation day, the monitoring was performed hourly, for 12 h, between 8:00 a.m. and 7:00 p.m. Insect flight intensity was recorded from hour to hour, at fixed hours (e.g., 8:00, 9:00, etc.), by the same method as in 2019, with the number of adults found on 300 leaves recorded ($adults \times leaf^{-1}$). Given that, as indicated in the introduction, air temperature and air humidity are considered to be the most influential climatic factors on insects, we chose to study the influence of these two factors on the diurnal behaviour of the OLB. For this purpose, the average temperature and the average humidity of the air were recorded every hour, between 8:00 a.m. and 7:00 p.m.

Meteorological data were recorded using Pro v2 Logger sensors (HOBO) and downloaded using HOBOware software (HOBO). In 2019, the sensors were left installed throughout the growing season, set to record the air temperature and humidity every day, hourly. In 2020, the sensors were installed only on the days of data collection and were set to record air temperature and humidity every 10 min.

2.2. Data Analysis

For determining the relationship between the diurnal OLB population dynamics and the influential meteorological factors, a linear mixed-effects model was used to assess the influence of temperature, relative air humidity, site, generation, and hour the sample was taken on the average number of adults on a leaf. The number of adults in flight was selected as the dependent variable, while temperature, relative air humidity, and hour the sample was taken were used as the independent fixed variables, with the site (plot) and generation as random variables.

The model developed is presented as follows:

$$d_{ijk} = a + u_i + v_{ij} + w_k + b \cdot H + c \cdot T + \epsilon_{ijk}$$

where d_{ijk} is the number of adults in flight; a, b, and c are the fixed (population) parameters; H and T are the hour and temperature; v_{ij} is a random parameter specific for jth observation within the ith plot; w_k is a random generation parameter, specific to the observations taken during the kth period; u_i is a vector of random plot parameters specific to the ith plot; ϵ_{ijk} is the residual error.

In order to take the temporal autocorrelation into account, an ARIMA stationary autocorrelation structure was specified in the model. To characterise the relationship between the number of insects in flight and meteorological parameters (temperature and relative air humidity), Spearman's correlation coefficient was computed (the lack of data normality, checked by applying Shapiro's test, did not permit the use of Pearson's correlation coefficient). In order to assess any tendencies of the diurnal dynamics of the OLB population, temperature, and relative humidity, the second-order polynomial curve was used.

The statistical analysis was performed using R v.4.1.2. (R Development Core Team, 2021), whilst the modelling was performed using the 'nlme' package [42].

3. Results

3.1. Seasonal Dynamics of the OLB Populations

The seasonal population dynamics showed that the OLB adults were active throughout the growing season, from the start of our observations (22 May 2019) to the end of August. The proportion of adults in flight followed an increasing pattern during the vegetation season, which corresponded to increasing air temperatures and decreasing air humidity. In all three plots, the number of adults in flight was the lowest in the period of adults from the overwintering population, the highest for the second generation, and the intermediate for the first generation. Given this, the dynamics indicated the following three distinct periods (Figure 2a–f):

- Period 1, starting the second week of May until the second week of June (Figure 2a). It is worth mentioning that, when the observations began (May), the number of adults in flight was already at a maximum level for this period (<1 $adults \times leaf^{-1}$), which decreased through time at Plots 1 and 2, while it remained relatively constant at Plot 3. The data analysis, using standard error values, showed that the variability was small during this period (Figure 2d).
- Period 2, between the last week of June and the second week of July (Figure 2b). During this period, the proportion of insects in flight began with similar values to those in Period 1, with an increase in the population to a peak in the middle of July, and then a decrease to low values. During this period, the standard error values indicated that the variability had begun to increase (Figure 2e).
- Period 3, beginning with the last week in July until the last week of August (Figure 2c). As in Period 2, there was an increase in the number of insects in flight, up to a peak, after which they decreased. The data analysis showed that, during this period, the standard error values were higher than in Periods 1 and 2 (Figure 2f), meaning that the variability in this period was the highest throughout the season.

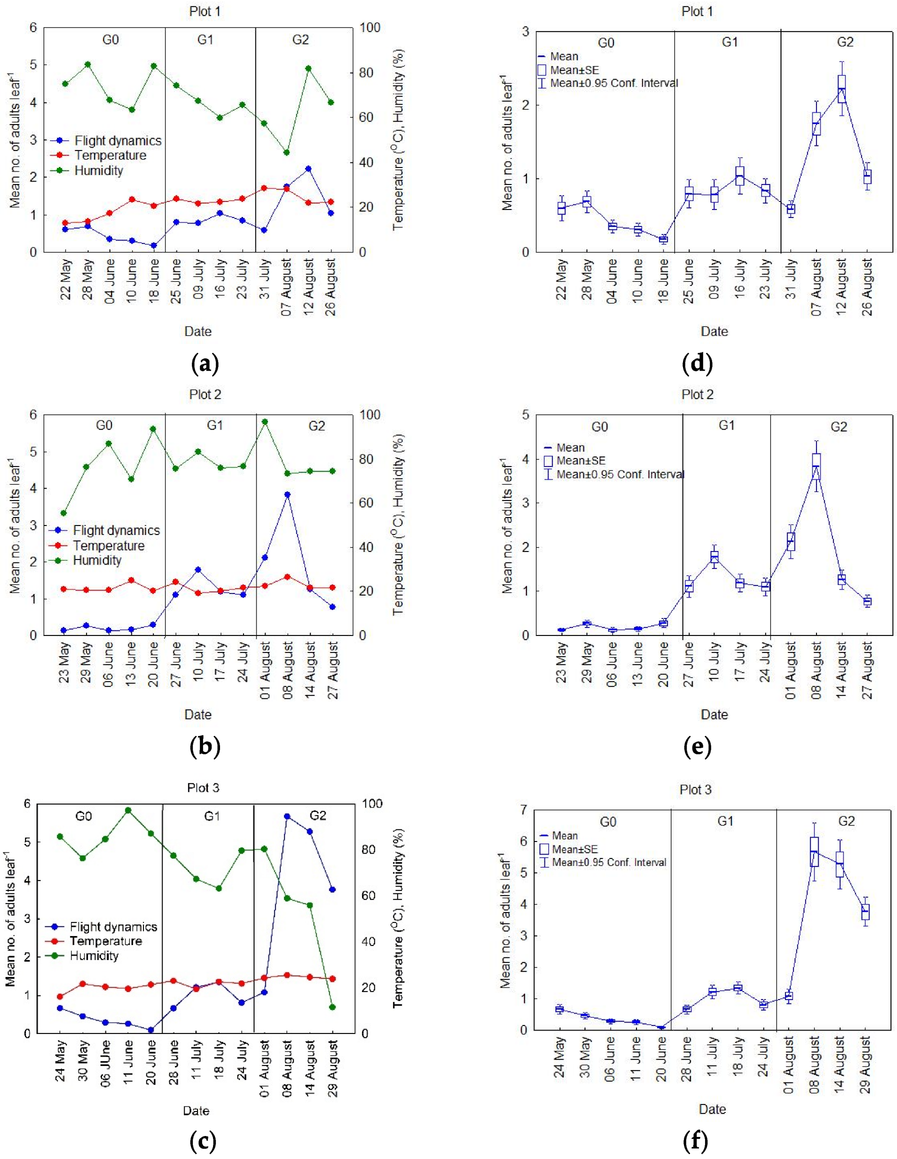


Figure 2. Seasonal dynamics of OLB populations in the studied tree plots: (**a**–**c**) variation in flight dynamics and meteorological factors: (**a**) Plot 1, (**b**) Plot 2, and (**c**) Plot 3. (**d**–**f**) Dynamics of OLB population variability: (**d**) Plot 1, (**e**) Plot 2, and (**f**) Plot 3. G0 = overwintering population; G1 = first generation; G2 = second generation.

3.2. Dynamics of the Diurnal Activity in the OLB Population and Influential Factors

3.2.1. Relationship between the OLB Population Dynamics and the Influential Factors

In the first step, temperature, relative air humidity, and the hour at which the sample was taken were considered to be independent fixed variables, while the intercept was considered to be a random factor that varied within each generation and plot.

The hour at which the sample was taken was introduced in the model as a factor, while the temperature was introduced as a continuous variable. The random effects were found significant at level 0.00001.

The intercept value in this model represented the mean value of the adults in flight recorded at 8 a.m. (considered to be the reference time). Likewise, the estimate values from the other hours were considered to deviate (plus or minus) from the value recorded at 8 a.m. The number of adults in flight was considered to be a dependent variable. However, relative air humidity did not manage to explain the population variability, as the conditional F test showed a nonsignificant value for this variable ($p > 0.05$). Therefore, in the second step, we continued to use temperature and the hour at which the sample was taken, as well as the two levels grouping generation and site. For this model, the Akaike information criterion (AIC) statistics yielded a value of 249. Including the correlation structure (autoregressive moving average—ARMA (1,1)) of the data (from repeated measurements) further improved the model by 65% (AIC = 88; $p < 0.0001$).

The coefficients of the independent variables were significant ($p < 0.001$), emphasising their importance for understanding the diurnal population dynamics of the OLB. The proportion of adults in flight increased with an increase in temperature. The average number of adults/leaves increased by 0.19 for every 1 °C increase (Table 4). Interestingly, the diurnal activity of the population increased until the afternoon (2–3 p.m.), after which it stagnated for 2 h, decreasing after 4 p.m.

Table 4. Results from the application of linear mixed-effect models, including temperature and the hour at which the sample was taken, as fixed variables.

Fixed Effects	Estimate	*p*-Value
Intercept (Time: 8 a.m.)	0.17621	0.0000
Temperature	+0.01936	0.0001
Time: 9 a.m.	Time: 8 a.m. + 0.163521	0.0004
Time: 10 a.m.	Time: 8 a.m. + 0.133903	
Time: 11 a.m.	Time: 8 a.m. + 0.081469	
Time: 12 p.m.	Time: 8 a.m. + 0.108626	
Time: 1 p.m.	Time: 8 a.m. + 0.136774	
Time: 2 p.m.	Time: 8 a.m. + 0.163615	
Time: 3 p.m.	Time: 8 a.m. + 0.1688	
Time: 4 p.m.	Time: 8 a.m. + 0.05681	
Time: 5 p.m.	Time: 8 a.m. − 0.00516	
Time: 6 p.m.	Time: 8 a.m. − 0.02147	
Time: 7 p.m.	Time: 8 a.m. − 0.15106	

3.2.2. Dynamics of the Diurnal Activity in the OLB Population

At the Ștefănești site (Figure 3), on the first day of observing the overwintering adult population, there was a downward trend in the diurnal dynamics, with the highest values in the first part of the day, and no significant relationship between the number of adults in flight and meteorological factors. On the second day, the diurnal dynamics reached a peak at around 2 p.m. and varied significantly with both air temperature (r = 0.68, $p = 0.0034$) and relative air humidity (r = −0.72, $p = 0.0025$). For the first generation, during the first day, there was an inexplicable increasing tendency in the number of adults, which continued in the second half of the day, with no significant relationship identified between the diurnal flight dynamics and the meteorological factors. On the second day, a maximum value was reached around 2 p.m., influenced by air humidity (r = −0.51) and air temperature (r = 0.42), although these correlations were not statistically significant ($p > 0.05$). For the second generation, on both days, there was a maximum of in-flight adults at 12 p.m. (noon), after which there was stagnation and then a decrease with the arrival of evening. On the first day, the number of adults in flight was statistically significant, varying negatively with diurnal air humidity (r = −0.58, $p = 0.0498$), but on the second day, this correlation was no longer significant.

In the Călărași location (Figure 4), for the overwintering adults on both days, there was a maximum of around 12 p.m. (noon). While on the second day, no statistical relationship was found between the proportion of adults in flight and meteorological factors, on the first day, it was observed that the insect population varied negatively with increasing air humidity (r = −0.67, $p = 0.0232$) and positively (but not significantly) with increasing temperature (r = 0.23, $p > 0.05$). For the first generation, on the first day, the population reached a peak at around 4 p.m., with the number of adults in flight being significantly influenced by the weather, varying positively (r = 0.63, $p = 0.0234$) with temperature and negatively (r = −0.87, $p = 0.0201$) with relative humidity. On the second day, the activity was in general agreement with the established pattern, and the population was still influenced (nonsignificantly) by air temperature and relative humidity. For the second generation, it was much more obvious that there was a maximum proportion of adults in flight between 12 p.m. and 2 p.m. On the first day, the proportion of in-flight adults was significantly influenced by the weather, increasing with warming air temperatures (r = 0.81, p = 0.0081) and decreasing with increasing air humidity (r = −0.75, p = 0.0346). The next day, the correlation analysis showed the same trend, but it was not significant, the OLB population varying positively with air temperature (r = 0.44, p > 0.05) and negatively with relative humidity (r = −0.15, p > 0.05).

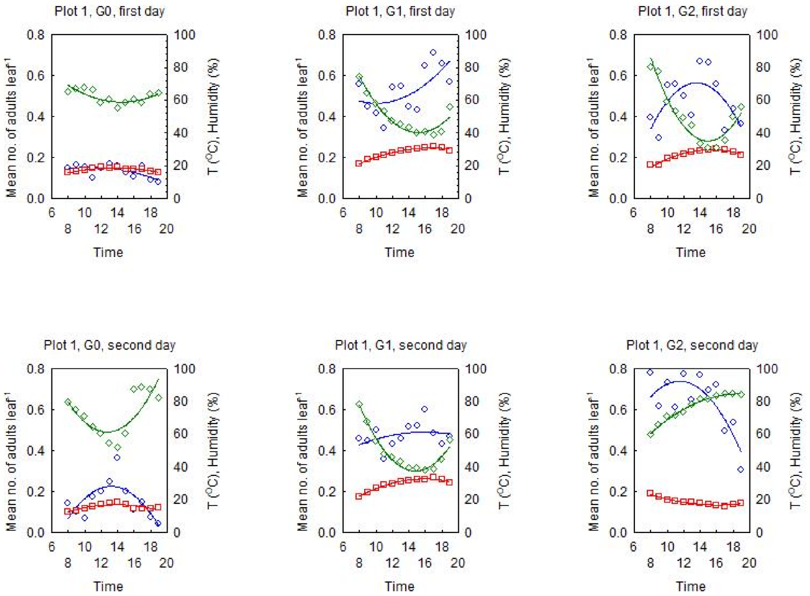


Figure 3. Dynamics of the diurnal activity of the OLB population in Plot 1 (Ștefănești): G0 = overwintering population; G1 = first generation; G2 = second generation; blue = number of adults in flight; green = relative air humidity; red = air temperature.

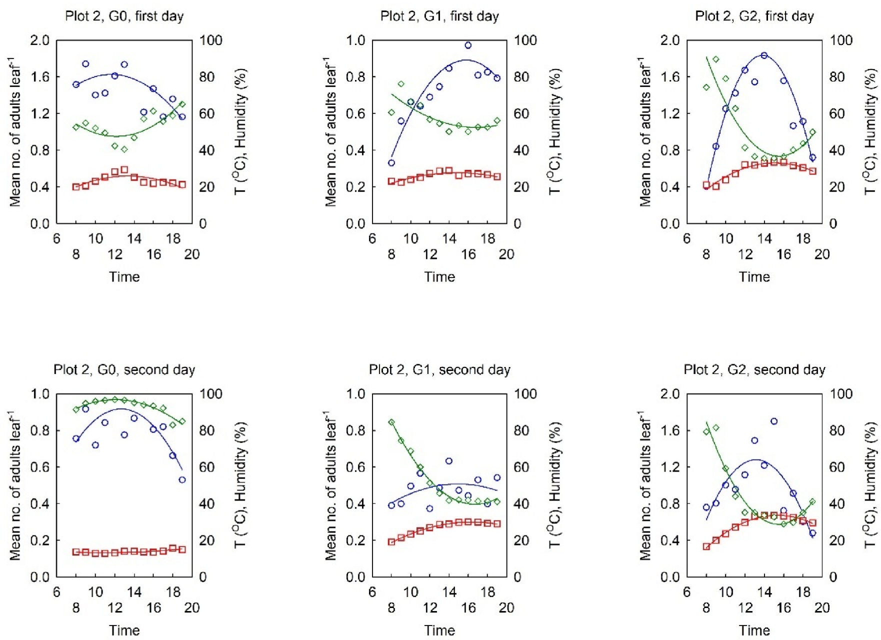


Figure 4. Dynamics of the diurnal activity of the OLB population in Plot 2 (Incinta): G0 = wintered population; G1 = first generation; G2 = second generation; blue = number of adults in flight; green = relative air humidity; red = air temperature.

At the Giurgiu monitoring point (Figure 5), the number of overwintering adults in flight, on both the first and second days, increased until midday (12–2 p.m.), when the peak was reached, after which the population level began to decrease, with no significant meteorological factors being identified. For the first generation, on the first day of observation, the adult diurnal activity followed the same pattern, with a peak reached around 2–4 p.m., but this was significantly influenced by the weather conditions, varying positively with air temperature (r = 0.79, p = 0.0041) and negatively with relative air humidity (r = −0.83, p = 0.0044). On the second day, there was an ascending tendency that was positively influenced by air temperature (r = 0.58, p = 0.0487). The data analysis showed that the number of adults in flight was negatively influenced by relative air humidity (r = −0.51), but that this inverse correlation was not statistically significant (p > 0.05). On both days for the second generation, the OLB population again peaked at midday (12–2 p.m.) but without a significant relationship between the proportion of adults in flight and meteorological factors.

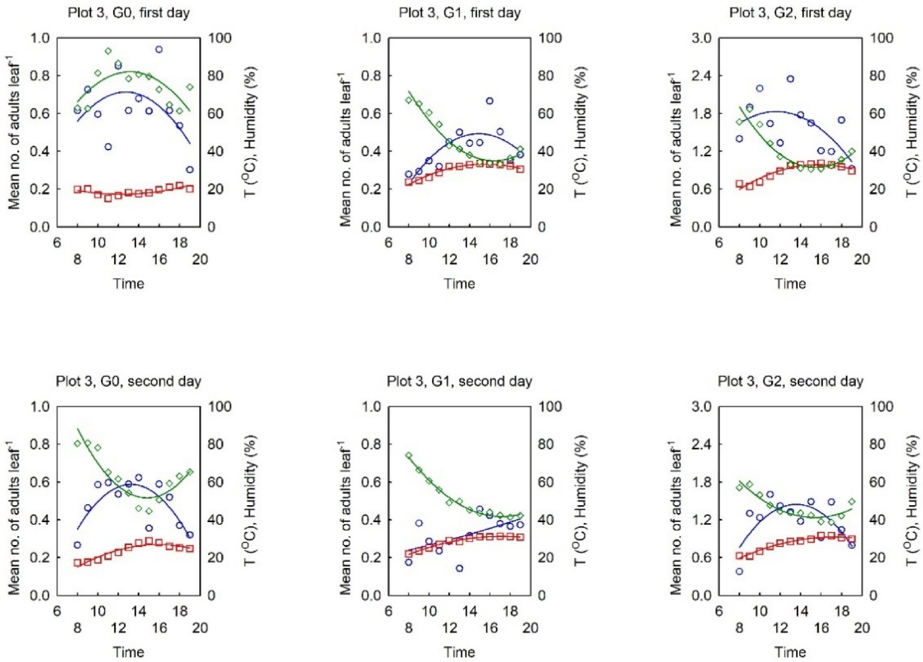
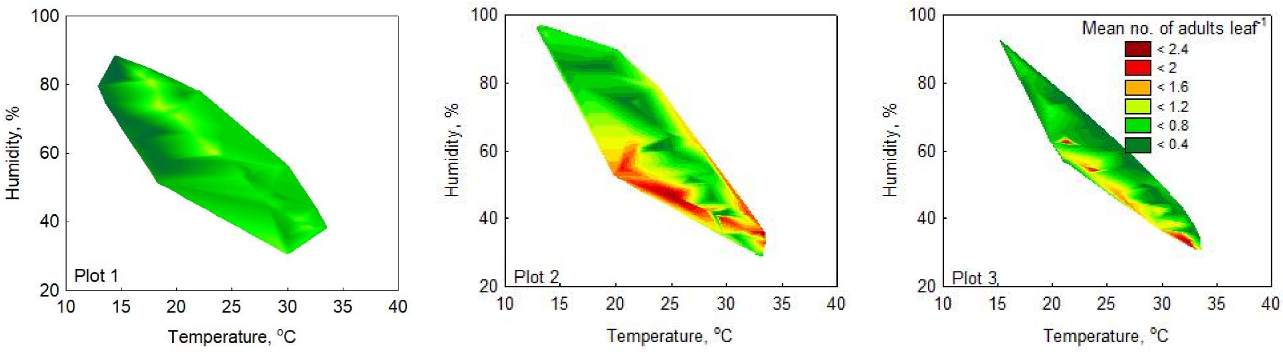


Figure 5. Dynamics of the diurnal activity of the OLB population in Plot 3 (Ceagău): G0 = wintered population; G1 = first generation; G2 = second generation; blue = number of adults in flight; green = relative air humidity; red = air temperature.

3.2.3. Range of Optimal Meteorological Factors for Activity in the OLB Population

The fact that the dynamics of the OLB population were not constant during the day, often following a pattern with a peak around midday, gives us an indication that the OLB had certain preferences in terms of meteorological conditions.

In Plot 1, the Ștefănești monitoring point (Figure 6a), where the population level reached a peak of 0.8 $adults \times leaf^{-1}$, there was no obvious range of meteorological conditions in which the insect showed intense activity. However, it was noted that the number of in-flight OLBs increased with increasing air temperature, from 20 to 30 °C, and decreasing relative air humidity, from 50 to 35%.



(**a**) (**b**) (**c**)

Figure 6. Intensity of OLB activity along a gradient of meteorological conditions for all three studied periods in the three plots: (**a**) Plot 1 (Ștefănești); (**b**) Plot 2 (Incinta); (**c**) Plot 3 (Ceagău).

In Plot 2, at the Incinta monitoring point (Figure 6b), when the air temperature reached a maximum of 18 °C, regardless of the relative air humidity value, the OLB population peaked at 0.8 $adults \times leaf^{-1}$. Once the temperature began to rise, the OLB appeared to show preferred environmental conditions, at air temperatures ranging from 20 to 30 °C and relative air humidity of between 40 and 50%, reaching a level population of up to 2.0 adults on average per leaf. However, even for the same air temperature range, the number of in-flight insects could fall below 0.8 $adults \times leaf^{-1}$ if the air humidity exceeded the preferred threshold (40–50%). This time, with the air temperature passing 30 °C, the population level remained high only when the humidity was maintained at ±40%. While the air humidity decreased, the data analysis showed that the insect population could be reduced to 0.4 $adults \times leaf^{-1}$.

In Plot 3, the Ceagău monitoring point (Figure 6c), the population level was higher than Plots 1 and 2, varying up to a maximum of 2.4 $adults \times leaf^{-1}$. The data analysis suggested that, in order for the OLB to be more active, it was necessary for the temperature to increase at the same time as the relative air humidity decreased. It was also found that, when the air temperature was up to 20 °C, regardless of the relative humidity level, the population was at a maximum of 0.8 $adults \times leaf^{-1}$. With increasing temperatures, from 20 to 30 °C, at high relative humidity values, the population level remained at the same low level; however, with a decrease in humidity to below 50%, the population level reached 1.6 $adults \times leaf^{-1}$ (in isolated cases, even 2 $adults \times leaf^{-1}$). The data interpretation showed that the population level was at its peak (>1.6 to 2.4 $adults \times leaf^{-1}$) when the air temperature was between 30 and 32 °C (maximum 33 °C) and the relative air humidity between 30 and 35%. At the same time, with increasing temperatures, to above 33 °C, the proportion of insects in flight decreased exponentially to values below 0.8 $adults \times leaf^{-1}$, even if the air humidity remained in a favourable range.

4. Discussion

The results of this study show that, in terms of seasonal population dynamics, regardless of the population level in each plot, at each point, there were three distinct periods. If we consider the biology of the OLB in its natural region [19] and in Italy [21], the first period represents the overwintering adults, with the next two periods reflecting the dynamics of the first and second generations, during which time, the foliage of the host trees became discoloured by up to 100% [20]. Accordingly, the constant, low-level population from the first period can be explained in terms of the overwintering adults becoming active, beginning to feed, and laying the eggs for the first generation. The following two increases in the proportion of insects in flight from the next two periods can be explained by the appearance of new adults from the first and second generations, and the overlap of these with adults from the previous generation(s). The population began to decrease when the adults from the previous generation naturally died off, leaving only the adults from the current generation. To the best of our knowledge, we are not aware of studies researching OLB population dynamics in the native range, to be able to compare the results. However, a study in the origin area of *C. arcuata* that followed insect preferences for *Quercus alba* L. [33] showed that the OLB populations have a preference for trees only when their foliage was not exposed to additional water, but they preferred the leaves of trees grown under low or no water deficit. Likewise, in 2020, the dynamics of the OLB population have been studied in three points in Romania (east, west, and south of the country) but by the method of adults captures on yellow sticky traps [43]. Thus, our results are relatively similar to those in the aforementioned study, in which in two out of three points the dynamics of the OLB population had two peaks, one in July and the other in August. It is interesting that the third point in the study in which the population had a single peak, in August, is located in southern Romania, close to our locations. Furthermore, this pattern of OLB seasonal dynamics is consistent with the model of another invasive species that shares similar biology—*Corythucha ciliata* (Say, 1832) (Hemiptera, Tingidae)—that has also been studied in Hungary [44]. It also exhibits two peaks in the two generations in the same periods as *Corythucha arcuata*, with the population being more numerous in the second period than the first. The third generation was not targeted in our study because the adults of this generation do not exhibit intense activity on the leaves; rather, they are looking for a place to overwinter.

Regarding the influence of meteorological parameters on the fluctuating population dynamics in the growing season, there was a close relationship between these and the proportion of insects in flight, but it had no statistical significance. One explanation for the nonsignificant result could be the biology of the insect. Based on our (unpublished) observations, when generations overlap during certain periods, a phenomenon of overpopulation is created in which meteorological factors cannot be differentiated from biological influences. Despite the nonsignificant result, our findings are consistent with those of a previous study [45] that suggested that the development of *Corythucha ciliata*—a similarly invasive species—is positively influenced by the air temperature. That finding was a starting point that guided our study for the year 2020; to exclude the biological factor from the analysis of the relationship between population dynamics and meteorological factors, we avoided collecting data in the periods when the populations overlapped.

For 2020, in which data collection was avoided during intervals of population overlap, an analysis of the mixed-effects model revealed that OLB activity was obviously influenced by meteorological factors—a finding that accords with those of other studies [26,46–48], which have indicated that other species of insects are also influenced by the weather. The analysis also showed that, among meteorological factors, air temperature is crucial for insect activity—a finding that is also consistent with those of previous studies [49–52]. Furthermore, the analysis highlighted another important factor influencing insect activity—the hour of the day when the samples were taken. This finding supports that of a previous study [52] which suggested that the time of day influences the behaviour of insects because of the difference in temperature, as well as the difference in light intensity, both of which can inhibit the insect flight and, implicitly, their level of activity.

The analysis of data from each day, for each generation, for the three individual plots confirmed the hypothesis generated by the mixed-effects model—namely, that the proportion of adults in flight increases until noon and then begins to decrease. With few exceptions, on most days, the pattern of diurnal activity of the OLB resulted in a peak at midday. Clearly, the tendency of the adults was to be active during the warmer parts of the day; however, the air humidity was relatively low. When certain thresholds were exceeded, however, such as when the temperature was too high around noon, thermal discomfort occurred, and the population level was affected. This reasoning is in line with that of a previous study [53], which showed that thermal limits—in this case, higher—could affect the survival, reproduction, dispersion, and colonisation of several species of the Heteroptera.

However, another study [54] showed that, during a single growing season, the survival, fecundity, and longevity of *Corythucha ciliata* adults were not significantly affected by a thermal shock when temperatures rose to up to 41 °C, whilst we found that the diurnal activity of OLB adults is influenced by a certain threshold of thermal discomfort. In this regard, our results may be used to inform future studies concerning other factors that may negatively influence OLB populations in the middle of the day.

According to the data analysis, when the population level was low (e.g., in Plot 1—Ștefănești, where the highest value was 0.8 $adults \times leaf^{-1}$), it was more difficult to identify the range of optimal conditions in which the OLB exhibited the most intense activity. Conversely, in both Plot 2 and 3, more intense activity was obvious when the air temperature exceeded 20 °C. However, when the air temperature reached 30 °C (Plot 3, Giugiu) or 33 °C (Plot 2, Călărași), it was observed that the number of adults in flight was affected by thermal discomfort. The results of this study are consistent with those of Lu et al. [55], who showed that the flight of *Corythucha ciliata* was significantly faster, more durable over time, and extended for longer distances in the same temperature range (20–30 °C). Furthermore, our findings accord with those of a study [45] that showed that the optimal temperature for the *C. ciliata* would be 30 °C, with the longevity of the adults significantly affected by temperatures exceeding a threshold of 30–33 °C. However, Ju et al. [45] investigated the survival of *C. ciliata* at different constant temperatures over time, while our goal was to observe the behaviour of the active adults during the day. In this regard, our findings could be used as a starting point to investigate whether temperatures outside this range (20–30 °C) only affect the diurnal activity of the adults.

An explanation for the fact that OLB prefers certain climatic conditions, with high temperatures and low humidity, but within certain limits, can be explained by a study [33] which showed that, in his native area, adults of *Corythucha arcuata* feds selectively only on leaves that were not supplemented with water and preferred those leaves that were grown under low or no water deficit.

Given that the findings of this study are limited to the environmental conditions of southern Romania, and the weather conditions in the 2019–2020 growing seasons, this study could be used for comparative purposes. It would be worth investigating whether other meteorological factors, such as the speed and direction of the wind or solar radiation, contribute to OLB behaviour. A pan-European project could study the behaviour of the OLB along an invasion gradient. The results of such studies, along with our findings, could help to develop a complex control programme against this pest, especially since Kovač et al. [38] identified the conditions under which this pest could be biologically controlled using an efficient and environmentally friendly method, whilst a pan-European study [56] suggested the circumstances under which the general public, foresters, and other stakeholders would be prepared to support possible OLB control methods. Additionally, our findings concerning the times of day when the population level increases or decreases could be useful for isolated chemical actions aimed at control, especially since the extent to which this pest can be chemically controlled has been demonstrated [39].

5. Conclusions

- *Corythucha arcuata* has a seasonal dynamic that shows two population peaks—one in July and the other in August;
- The diurnal dynamics of OLB activity have a pattern—the number of adults in flight reaches a peak at midday, which varies positively with the diurnal air temperature and negatively with the humidity;
- In many cases, air temperature and humidity significantly influence the dynamics of the OLB population;
- Even if the findings of this study are limited by the environmental conditions in southern Romania, they can be used to inform the direction of future studies on other meteorological or climatic factors that may influence OLB behaviour.

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data curation, F.B. and D.T.; writing—original draft preparation, F.B.; writing—review and editing, F.B., D.C.S., C.N., D.T., and I.C.P.; visualisation, F.B.; supervision, F.B.; project administration, F.B.; funding acquisition, F.B. and I.C.P. All authors have read and agreed to the published version of the manuscript.

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