Abundance and phenology of two pest species, *Cacoecimorpha pronubana* and *Epiphyas postvittana* (Lepidoptera: Tortricidae)

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Abstract

Cacoecimorpha pronubana (Hübner, [1799]) and *Epiphyas postvittana* (Walker, 1863) are economically important polyphagous pest species. The larvae of these moths affect a wide range of horticultural crops. We evaluated the seasonal abundance and population dynamics of these two species, hypothesising that both species of moth exhibit similar patterns in their seasonal abundance and respond to abiotic factors. The study was carried out on a wide range of protected ornamental crops grown on a total area of one hectare at a commercial nursery. The study was completed over 5 consecutive years from 2015 to 2019, where both populations were monitored weekly during the months of moth activity, from April to November, using sex pheromone traps to trap the males of both species. The timing and abundance of catches were analysed in relation to local meteorological data. The mean yearly abundance of adult males was 604 ± 23.89 (mean $\pm$ SE; *C. pronubana*) and 1706 ± 167.18 (*E. postvittana*) also differing significantly between years for both species. There was no influence of any weather measures on the abundance of *E. postvittana*, but sunshine duration (4.84 ± 0.26 h) and temperature ($13.04 \pm 0.57°C$) affected the mean seasonal abundance of *C. pronubana.* There was an overall difference in the seasonal pattern of abundance between the species studied. While we demonstrate a seasonal abundance difference between years for *C. pronubana*, we found no significant seasonal differences for *E. postvittana*. Improved understanding of seasonal abundance, phenological patterns and inter-seasonal variations in population dynamics of these two species may be useful in developing forecasting models that can be used in improving integrated pest management strategies for these two pests.

1 | Introduction

The carnation tortrix moth (CTM), *Cacoecimorpha pronubana* (Lepidoptera: Tortricidae) and the light brown apple moth (LBAM), *Epiphyas postvittana* (Lepidoptera: Tortricidae) are economically important pest species affecting a wide range of horticultural crops (Fisher, 1923; Speyer, 1938; Beitia *et al.,* 2016; New, 2016). Both species of moth are cosmopolitan pests and are naturalised adventive species in the UK (CABI, 2020; Gilligan *et al.*, 2020). Damage to the plant is caused by the larva that feeds on the foliage. It produces silk, which it uses to roll leaves together creating a shelter (Fisher, 1923; Cameron, 1939). CTM can produce at least five generations per year in warmer regions (North Africa), or four generations per year in Spain or Italy. In the UK, the species produces two generations per year overwintering as first or mainly second generation larvae (Fisher, 1923). LBAM produces three to four generations per year in Australia, where the moth is a native species (Danthanarayana, 1983). In the UK, LBAM typically has two generations per year (Suckling & Brockerhoff, 2010). There is, however, no winter resting stage for LBAM, although overwintering larvae tend to develop slowly with a lower threshold of development for all stages of 7.5°C and an upper threshold of 31°C (Danthanarayana, 1975). Both moths can be seen in Britain flying from April to July, and from August to October (Fisher, 1923; Suckling & Brockerhoff, 2010). The population dynamics and the seasonal abundance of these two insect pest species is dependent on numerous biotic and abiotic factors. Eggs and larvae of Tortricidae species are naturally controlled by parasitic wasps and other insects (Price *at al.*, 1980; Hassan, 1993; Thomson & Hoffmann, 2010; Murray, 2014; Rusch *at al.*, 2015; Göttig & Herz, 2016). Eggs and larvae of CTM, for example, can be parasitized by *Trichogramma sp.* and *Apanteles sp.*, respectively (Solomon *et al.,* 2001). Such phenomena, however, are also governed by environmental conditions (e.g. precipitations, sunshine duration, temperature or humidity) upon which performance of both prey and predators depends (Thiéry *et al.,* 2018). For example, at

average temperatures of 15°C and 30°C, the complete life cycle of CTM ranges between 123–147 and 28–44 days, respectively. In addition to slower development, low temperatures and rain may increase larval mortality up to 70–90% (CABI, 2020). Changes in air temperature can also affect the flight duration of the adult moth. It was shown that the longest flight duration for both sexes of LBAM occurs at 20°C and relative humidity of 60%, but an increase of atmospheric humidity can significantly decrease the flight duration of females (Danthanarayana & Gu, 1992). Abiotic factors can also affect the development of natural enemies of Lepidopterans. For example, the pupal survival, parasitism rates, longevity and fecundity of the parasitoid, *Apanteles hemara* (Nixon) (Hymenoptera: Braconidae) significantly decrease with increasing temperature (Agbodzavu *et al.,* 2020).

The choice of control methods of these species of moth depends on their life stage, and whether an integrated pest management (IPM) approach has been adopted. The egg and larval stages of these two pests are controlled mainly by the use of synthetic pesticides (e.g. neonicotinoids and pyrethroids), bio-pesticides (e.g. *Bacillus thuringiensis* Berliner 1915), or natural enemies (Suckling & Brockerhoff, 2010; Wearing *et al.,* 2012; Reddy, 2016). Monitoring is recognised as an essential component of any IPM programme. It helps to evaluate pest population dynamics, their potential outbreaks, improving the timing of the application of control measures and may allow for the selection of the most sustainable control strategy (Wearing *et al.,* 2012). The most efficient monitoring method of both populations of these moths are pheromone traps. Traps are baited with a specific female sex pheromone that attracts male adults of the same species. Traps are used not only for monitoring and data collection purposes (Guda & Capizzi, 1988; Suckling *et al.,* 2015), but also for mass-trapping of males to disrupt mating, thereby reducing offspring production in female adults (Quaglia, 1993; Suckling *et al.,* 1994, 2014).

The objective of this study was to evaluate the seasonal abundance of two species of moth, CTM and LBAM in relation to rainfall, sunshine duration and temperature during a 5-year

study period. We hypothesised that both species would exhibit similar phenological population patterns, in terms of seasonal abundance both within and between seasons, and that the pattern of abundance for both species would be significantly affected by the three abovementioned abiotic factors. Results from this study may be useful in developing forecasting models, which in turn would be useful within integrated pest management programmes for these two herbivorous species in the ornamental crop habitats.

2 | Materials and methods

2.1 | Data collection and pheromone traps

This study was carried out at an ornamental plant nursery in Shropshire, UK (52°37'20'' N, 2°15'20'' W; 119 m a.s.l.) on a wide range of continuously ventilated protected ornamental crops grown in 2–10 L black polythene pots. All sides of polytunnels remained open each season for the entire 5-year study period. Crop plants included species from the families Araliaceae, Buxaceae, Caprifoliaceae, Celastraceae, Escalloniaceae, Garryaceae, Griseliniaceae, Hypericaceae, Lamiaceae, Pittosporaceae, Plantaginaceae, Rhamnaceae, Rosaceae and Rutaceae. The height of the crop plants ranged between 20 cm to 80 cm.

Two populations of moth species – CTM and LBAM were monitored weekly from April to November in each of five years (2015 to 2019). Monitoring was carried out using Agralan® Pheromone Trapping System (Agralan Ltd, Wiltshire, UK), consisting of open-sided delta traps baited with a synthetic female sex pheromone lures. Each trap was baited with one lure containing either CTM (Z11-14Ac, E11-14Ac, Z11-14OH, Z9-14Ac) or LBAM (E11-14Ac, E9E11-14Ac) synthetised female sex pheromone, respectively, to attract males of the same species (Agralan Ltd, Wiltshire, UK).

The numbers of male individuals caught in each trap was recorded weekly. A total of 26 moth traps were deployed, 13 for each species in each year of the study. Traps were placed in polytunnels of a total area of approximately one hectare, within a 16-hectare nursery site. Two traps were used per polytunnel, one trap for each species of moth. Traps were hung on a string just above crop height with a distance of at least 50 m between each trap adhering to spacing instructions from the Agralan ® Pheromone Trapping System supplier (Agralan Ltd, Wiltshire, UK), and similar to other studies (David *et al*., 1982, 1983; Perry & Wall, 1984).

Pheromone lures were replaced four times per year in each trap for each moth species between April and November. Non-target species of moth occasionally caught in traps were also

recorded. Pyrethroid insecticides (deltamethrins) and natural pyrethrins were used throughout each of the growing seasons in order to prevent pest populations from exceeding economic injury levels in each of the crops being monitored (Higley & Wintersteen, 1992; Pedigo & Higley, 1992; Peterson *et al.,* 2018). The economic threshold for both species of moth is a catch of over 30 adult male moths caught per pheromone trap per week (AHDB, 2020). Pest management practices initiated by growers are made regardless of the plant growth stage.

The 'seasonal abundance' term used in this paper indicates the number of adult males caught in traps recorded in each week over the given season. The 'season' term used here indicates the period of 32 weeks that begins from week 14 and ends in week 45 for each calendar year during the study. The 'pattern of the seasonal abundance' phrase used in this paper indicates the seasonal distribution of abundance recorded for adult males caught in traps over the given season, and may be used in order to distinguish certain phenological characteristics in this respect.

2.2 | Meteorological data

Meteorological data for the years 2015–2019 were obtained from the weather station nearest to the study site (~33km), located in RAF Shawbury, UK (52°47'38'' N, 2°39'47'' W; 72 m a.s.l.). Data were supplied by MetOffice, and consisted of daily total rainfall (0900–0900), daily total sunshine (0100–2400), and daily mean temperature (0900–0900) measured in millimetres, hours, and degrees Celsius, respectively. Sunshine data were taken from a Campbell Stokes recorder located at the abovementioned weather station. Data were converted into weekly means for statistical analyses.

2.3 | Statistical analyses

Statistical analysis was performed in order to evaluate the seasonal abundance of each moth species, and to compare their abundance within and between seasons. We also analysed

the correlation between the seasonal abundance of each species and abiotic factors: precipitation, sunshine duration, and temperature. None of the data were normally distributed (Shapiro-Wilk test, all $P < 0.05$) and did not meet homogeneity of variance assumptions (Levene's test, $P < 0.05$). Thus, analyses were performed using non-parametric tests. The unpaired two-samples Wilcoxon rank sum test with continuity correction (Mann-Whitney U-test) was used to compare the overall difference in abundance between the two moth species for the entire study. The Kruskal-Wallis test was used to test intra-seasonal differences in moth abundance between weeks within seasons, as well as inter-seasonal differences in the moth abundance between years. The spearman rank correlation test was used to evaluate the association between the moths and three abiotic factors (estimating a rank-based measure of association with Spearman's rho coefficient). All statistical tests were performed using R version 4.0.2 (R Core Team, 2020), and the significance level was set at α=0.05.

3 | Results

3.1 | Number of male moths recorded

The average yearly abundance of CTM adult males caught over the five-year study period (2015–2019) was 604 ± 23.89 (mean ± SE) which was 2.82 times less than the average yearly abundance of LBAM (1706 ± 167.18). The difference was statistically significant (Wilcoxon test: W = 169, P < 0.0001). The average weekly abundance of adult males per year was (mean ± SE) 45 ± 0.57, 159 ± 0.79, 104 ± 0.81, 183 ± 1.54 and 113 ± 0.72 for CTM, and 69 ± 0.62, 930 ± 4.16, 500 ± 2.78, 102 ± 0.93 and 105 ± 0.57 for LBAM for 2015, 2016, 2017, 2018 and 2019, respectively.

3.2 | Intra-seasonal pattern of abundance

The mean weekly abundance varied significantly overall for both species of moth (Kruskal-Wallis chi-squared [KW]: χ^2 = 20.8, df = 4, P = 0.0003, CTM; KW: χ^2 = 68.8, df = 4, P < 0.0001, LBAM). Comparing the yearly abundance of each moth species we found a significant difference only for the years 2016 and 2017, (Wilcoxon test: W = 95.5, P < 0.0001; and W = 229.0, P = 0.0001) (Figure 1).

3.3 | Inter-seasonal differences in moth abundance (weekly 5-year cumulative)

The average number of male moths caught each week differed significantly for CTM (KW: χ^2 = 71.0, df = 31, P < 0.0001). By contrast, weekly catches throughout the 5-year period did not differ significantly overall for LBAM (KW: χ^2 = 34.5, df = 31, P = 0.304) (Figure 2). A cumulative seasonal abundance for both moths can be observed in Figure 2A and 2B. Two common characteristic patterns of the seasonal abundance between the species are reflected in two generational peaks; observed throughout the seasons, each of the peaks represents the rise in the mean number of males caught. For both moths, there was a gradual rise in trap catches

between weeks 18–22, and then there was a second shorter period where there was a more pronounced increase in trap catches between weeks 29–30 for CTM, and between weeks 31–32 for LBAM.

3.4 | Correlation between cumulative seasonal moth abundance and abiotic factors

While overall abundance for CTM across all years 2015–2019 was not significantly correlated with precipitation (Spearman's rank correlation [SRC]: S = 5568.7, $P = 0.911$, ρ (rho coefficient) = -0.021; Figure 3A), it was significantly correlated with mean sunshine duration (SRC: S = 3046, $P < 0.01$, ρ = 0.44), and with mean temperature (SRC: S = 1882, $P < 0.0001$, ρ = 0.66) (Figure 3B and 3C). Precipitation, mean sunshine, nor temperature were correlated with LBAM abundance in this study (all $P > 0.05$; Figure 3D–3F). With respect to the seasons (means ± SE), sunshine duration (5.46 ± 0.57) affected only CTM in 2018 (SRC: S = 2329, $P = 0.0006$, ρ = 0.57). Temperature was significantly correlated with abundance in both species for some years, CTM in 2015 (12.32 ± 0.49), 2016 (13.01 ± 0.70), 2018 (13.70 ± 0.67) and 2019 (12.82 ± 0.68), (SRC: S = 3512, $P = 0.045$, ρ = 0.36; S = 1681, $P < 0.0001$, ρ = 0.69; S = 2018, $P = 0.0001$, ρ = 0.63; S = 2651, $P = 0.003$, ρ = 0.51) respectively, and LBAM in 2017 (13.34 ± 0.58) (SRC: S = 2641, $P = 0.003$, ρ = 0.52) (Table 1).

3.5 | Correlation between two species of moth in cumulative seasonal abundance

There was a significant correlation between the cumulative seasonal abundance of the two moth species (SRC: S = 2034, $P = 0.0001$, ρ = 0.63), as well as between the overall abundance of both species combined, and individual CTM (SRC: S = 833, $P < 0.0001$, ρ = 0.52), and LBAM (SRC: S = 449, $P < 0.0001$, ρ = 0.92) (Figure 3G–3I).

4 | Discussion

This study analysed the seasonal abundance of two herbivorous pest moth species, CTM and LBAM, in an ornamental crop habitat over a five year study in relation to precipitation, sunshine duration and temperature. Both species exhibited a bimodal patterns of the seasonal abundance, due to their known bivoltine life history. The first generational peak was observed between May and June, week 18–25 (first generation), and the second peak around August, week 30–37 (second generation) for both species. An increase in CTM abundance, however, tended to appear on average 1 or 2 weeks earlier than the increase of LBAM abundance, which is consistent with previous studies (Fisher, 1923; Gratwick, 1992; Fountain & Cross, 2007; Suckling & Brockerhoff, 2010). Fisher (1923) found that the first generation of CTM emerges in spring, after a five to seven months prolonged winter larval stage, and a second generation emerges from eggs laid in the autumn. Our results, however, suggest that the second generation of CTM begins to appear earlier than previous studies have shown, at the end of July, and they remained active through August and September exhibiting a slow and steady decline.

We observed significant differences in population abundance between the species in our study, reflected by significantly higher numbers of LBAM adult males caught, compared with the abundance of CTM adult males. This was consistent for both generational peaks in each season across years, with LBAM consistently exhibiting a larger increase in abundance, than CTM. Significant differences in abundance were also observed intra-seasonally for both species. With respect to a particular year, significant differences were observed between the species only in years 2016 and 2017. It is possible, that the large increase in numbers of LBAM in 2016 and 2017 may reflected changes in abiotic factors, e.g. favourable temperatures during the development of the moth, or milder winter that occurred prior the season in which LBAM performed better than CTM. The same changes in abiotic factors, may not, however, have

benefited CTM, where in reverse, for example, warmer periods during development may increase the sensitivity of a certain life stage that is exposed and lead to negative effects on, for example, reproduction (Wellington, 1960; Zhang *et al*., 2015; Boersma *et al*., 2018).

It is also possible that changes that occurred in the local habitats may have affected the composition of the diversity or abundance of natural enemies of these two pest species, and as a result, indirectly affecting the moth species in our study differently. It was shown, for example, that variable temperatures to which the parasitic wasp, *Trichogramma* (Hymenoptera: Trichogrammatidae) is exposed during its development, significantly reduces fecundity and longevity of the wasp (Firake & Khan, 2014). It is also possible that natural enemies of CTM are better established in the local environment than natural enemies of LBAM (Fisher, 1923; CABI, 2020). The specificity and impact of these natural enemies on each species of moth may also be important (Buxton & Talbot, 2011; Golbaghi *et al*., 2020). While the occurrence of natural predators or diseases could explain the differences we observed between our study species in terms of their seasonal first appearance and abundance, population trends of natural enemies would be required to confirm this.

A large amount of previous research has suggested that LBAM is a relatively abundant, cosmopolitan pest species (Buchanan, 1977; Suckling *et al.,* 1985; Burnip & Suckling, 2001; Fountain & Cross, 2007; Suckling *et al.,* 2014) with significant potential for becoming established in new habitats (He *et al.,* 2012). It is thought that local abundance from season to season is influenced by climate, larval host plants and predation (Danthanarayana; 1983). The abundance of CTM populations, however, remains well established in known habitats (Cavalloro, 1986; Leandro *et al.,* 2002; Ostrauskas *et al.,* 2008; Brown *et al.,* 2010), but the factors that the moth is exposed to in those habitats, such as environmental pollutants (Uhl *et al.,* 2016) weather fluctuations or host plants diversity (Calvo & Molina, 2003) often govern the species own unique dynamics. Nonetheless, due to global temperature rise, CTM also exhibits the potential to migrate into new habitats (LaštůVka, 2009).

We analysed specific differences between years exclusively for each species, and we found that the seasonal abundance for CTM differed significantly between seasons throughout the 5-year study period, while the seasonal abundance for LBAM did not differ significantly between these years. It is possible, that differences between years in seasonal abundance of CTM may be characterised by inter-seasonal variations in phenological patterns reflected by the species susceptibility to certain environmental changes (e.g. weather conditions, host plant habitats). It appears, however, equally possible that LBAM populations within each season are more uniform between years, and perhaps indicates that this species is better adapted to withstand changes, recover quickly, or thrive when conditions become more suitable (Kingsolver, 1989; Kiritani, K., 2013; Khaliq et al., 2014).

Our results also suggest that the abundance of CTM were associated with sunshine duration and temperature, exhibiting moderate positive correlations. Carnation tortrix moths are inactive during the day, but in the early morning sun and towards dusk or summer evenings adults become active, with the male being much more active than females (Fisher, 1923). It is likely, that such activity may be correlated with the release of pheromones by female moths (Witzgall & Frérot, 1989). The phenomenon that CTM exhibited - an increase in number of adult male appearing with an increase in temperature, is well known to occur in insects (Taylor, 1963; Solbreck, 1991; Gilbert & Raworth, 1996). None of the abiotic factors analysed here correlated with the seasonal abundance of LBAM, and seasonal abundance of neither species was correlated with rainfall.

Both species of moth tended to appear in greater numbers when the length of the mean daily sunshine duration ranged approximately between 4–6 hours per day (Figures 2B & 2E). In terms of air temperature, we observed that the adult males from both species were only found within the temperature range of approximately 8°C and 18°C, respectively (Figures 3C & 3F). The lowest temperature threshold we observed for LBAM was similar to other studies (Danthanarayana, 1975; Suckling & Brockerhoff, 2010). The possibility cannot be excluded,

however, that abiotic factors acted on the volatility of the pheromone lures used or the mobility of the male moths, both of which may have led to differences in trap catches (Li *et al*., 2019; Pitcairn *et al*., 1990). Equally, temperature may have been involved in the perception of the sex pheromone through the neural pathways, and not as a result of an increase in motor activity, or in the increased release rate of the pheromones at higher temperatures (Linn *et al*., 1988).

We observed a moderate positive correlations between the cumulative seasonal abundance of the two moth species over the 5-year study period. A similar correlation was observed for CTM when compared to the total combined seasonal abundance of both species, although, the strongest positive correlation was exhibited here by LBAM (Figure 3G–3I).

Results presented here illustrate that the seasonal abundance of these two species are similar. Significant differences, however, were observed in abundance between these two species, both within and between years. Both species of moth qualitatively exhibited two distinct generational peaks for the geographical region studied here.

Our findings from this study on phenology and abundance of these two pest species indicate that patterns measured here have a tendency to occur repeatedly from season to season, but the patterns recorded here differ from previous studies, such as the finding that the start of CTM flight activity is earlier than has previously been reported. These results may be useful in developing local or regional forecasting models, e.g. based on phenology model proposed by Milonas & Savopoulou-Soultani (2006) in improving integrated pest management practices and in developing more efficient crop management strategies for these two species in the ornamental crop habitats.

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Figure legends

Figure 1. Number of male moths recorded for each year between 2015 and 2019, for *Cacoecimorpha pronubana* (CTM) and *Epiphyas postvittana* (LBAM). Statistical analyses were performed using Wilcoxon rank sum test with continuity correction in order to compare the number of moths caught in any given year between the moths; [NS] $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$. Boxes represent the interquartile range (IQR) with a thick horizontal line at the median and whiskers extending to the extrema of the observations falling within 1.5 IQRs of the upper or lower quantiles. Black scattered dots represent data points spread horizontally for each box & whisker in order to show the distribution of data for each moth in a given year.
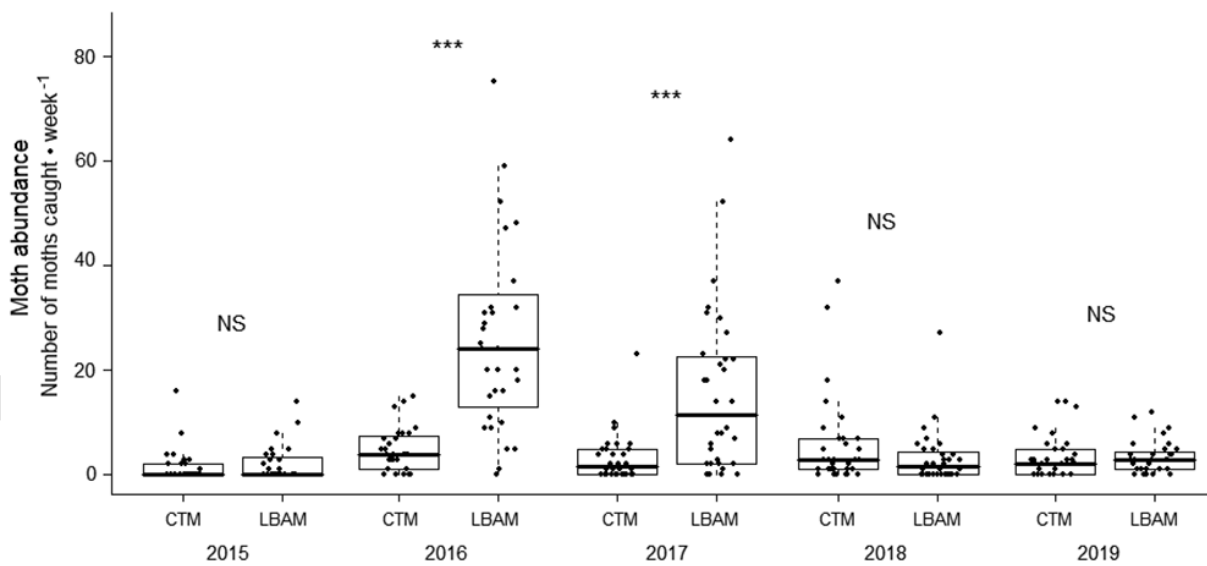

Figure 2. Cumulative seasonal abundance of two tortricid moth species, **A** – *Cacoecimorpha pronubana* (CTM), and **B** – *Epiphyas postvittana* (LBAM). The boxplots show the central tendency of counts per week for all years, represented by the central 50% of the data. The horizontal line in boxes shows the median, the dashed lines show the range, and the dots show the mean. The horizontal line that runs along the x-axis from week 14 to 45 across the box &

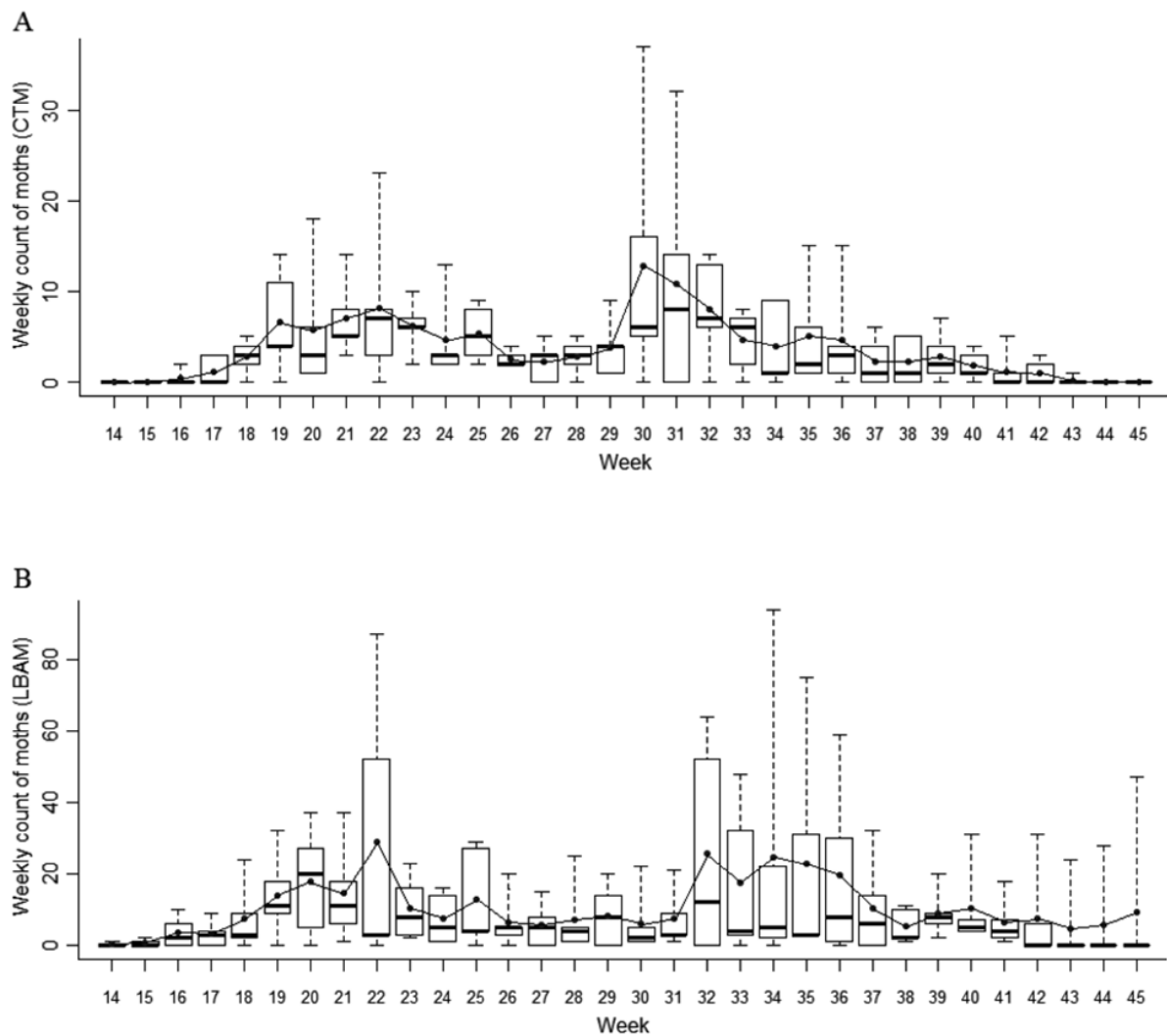whiskers connects the means representing the mean seasonal abundance of the moth for the years 2015–2019.

Figure 3. Scatter plots represent correlation between mean weekly abundance of moths, *Cacoecimorpha pronubana* (CTM) and *Epiphyas postvittana* (LBAM), and three abiotic factors represented by mean weekly precipitation (rainfall in millimetres), sunshine duration (hours) and temperature (degree Celsius) per five-year cumulative period study (2015–2019) (A–F). Figures G, H and I, show correlations of the mean abundance between both moths, and between cumulative data for both moths, and either of the moth.
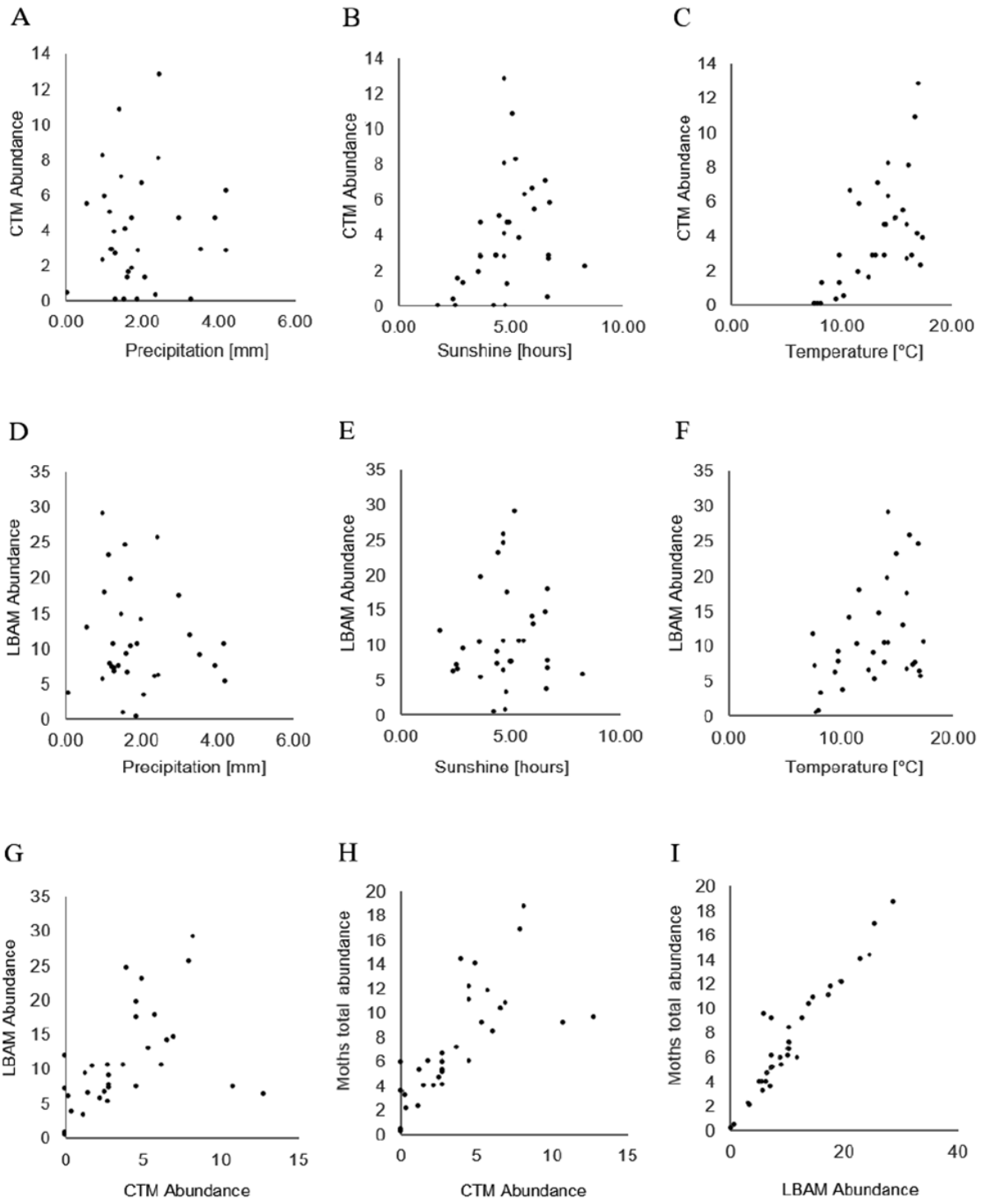
Table 1. Correlation between the mean seasonal abundance of two species of moth, *Cacoecimorpha pronubana* (CTM) and *Epiphyas postvittana* (LBAM) and mean abiotic factors for 5 consecutive years (means ± SE). Spearman rank correlation test was used to evaluate the association between the moths and three types of abiotic factors.

Abiotic factors for given year (means ± SEM)			CTM		LBAM	
			rho	P - value	rho	P - value
Precipitation [mm]	2015	1.68±0.19	0.02	0.909	-0.08	0.665
	2016	1.83±0.31	0.07	0.695	-0.25	0.161
	2017	1.86±0.29	0.09	0.635	-0.03	0.884
	2018	1.57±0.34	-0.12	0.5314	-0.07	0.708
	2019	2.64±0.51	-0.05	0.0778	-0.06	0.744
Sunshine [hours]	2015	5.13±0.41	0.21	0.242	0.11	0.547
	2016	4.88±0.31	0.19	0.302	0.08	0.672
	2017	4.39±0.38	0.21	0.259	0.32	0.071
	2018	5.46±0.57	0.57	0.0006	0.22	0.233
	2019	4.34±0.37	0.33	0.062	0.34	0.055
Temperature [°C]	2015	12.32±0.49	0.36	0.045	0.26	0.148
	2016	13.01±0.70	0.69	<0.0001	0.28	0.118
	2017	13.34±0.58	0.35	0.053	0.52	0.003
	2018	13.70±0.67	0.63	0.0001	0.03	0.866
	2019	12.82±0.68	0.51	0.003	0.34	0.055