Factors affecting oviposition of the box tree moth (*Cydalima perspectalis*) on outdoor box plant trials and in controlled choice chamber experiments

Introduction

Box tree moth (*Cydalima perspectalis*) is an invasive species native to East Asia, which has spread across Northern Europe; it was recorded for the first time in Europe in 2007 in Western Germany and the Netherlands (Leuthardt and Baur 2012). The species was also detected in the UK *C. perspectalis* adults lay egg plaques (Figure 2) on box plants (*Buxus* spp.), first instar caterpillars eat the leaf surface (windowing), whilst older instars eat the whole leaf and can also gradually consume box plant bark, which can result in girdling with the section above the damage drying out and dying back. The egg plaques turn from translucent to orange-brown over time, making older plaque much easier to spot on plants. Adult moths require nectar to prolong their lifespan and will feed off *Nemesia* and *Lavandula* flowers; adults do not cause defoliation damage to box plants.

Currently there are few successful control methods for this species available to the home gardener allowing it to spread rapidly when coupled with international plant trade and the species' dispersal abilities. The species can have up to three generations a year-between May and September- in Europe, causing frequent management of box plants to ensure complete removal of caterpillars (Salisbury *et al*. 2012). The caterpillars go through 6 to 7 instars (larval stages) before pupation (Maruyama and Shinkaji 1991). Pupa are around 20mm in length, and are green with dark dorsal stripes, and darken over time. Adults have multiple morphs, from a normal colouring (Figure 1) with white, iridescent wings with a brown border and two white crescents on each wing; to a melanic morph with almost entirely brown wings with two white crescents again on the wings. The melanic form can be identified in the pupal stage, as the pupa is clearly darker (Göttig 2017).

Figure 1. An adult box tree moth with normal colouring (Plant *et al*. 2019).

Figure 2. Egg plaques on the underside of box leaves (Stephanie Bird).

The aim of the project was to study oviposition in box tree moths at the Field Research Facility, Deer's Farm at RHS Wisley, via two different projects; in order to find ways to minimise the impact this moth has on box plantings. There is pressure to find ways to protect box plants due to its importance in many ornamental gardens, as an understorey plant in some forests in Northern Europe: in boxwood forest in Germany and Switzerland the understorey layer is essential in maintaining a high biodiversity of invertebrates within the forest. One project utilised an outdoor box plant trial of 136 plants managed in different ways, originally set up to explore treatment controls for a box blight fungal pathogen (*Cylindrocladium buxicola*). The other project was recording oviposition preferences on pruned and unpruned box plants in controlled choice chambers.

In other species of Lepidoptera there are common factors that affect oviposition; these factors can be separated by which behavioural stage in oviposition they affect. The behavioural sequence generally includes finding a suitable plant, landing, contact evaluation and finally acceptance or rejection of the plant. Studies in searching for/finding a suitable plant often consider visual cues such as shape, size and colour. Landing and contact evaluation may involve sensory stimuli-physical and chemical properties of the plant surface as well as non-plant stimuli such as other insects, pheromones or eggs (Renwick and Chew 1994). Therefore, shape was assessed as a factor in oviposition, along with various pruning methods, as pruning may release plant volatiles that affect oviposition as well as changing the look of the plant.

Method

Controlled choice chamber experiments

The box tree caterpillars used were raised on *B. sempervirens* box plants inside an outdoor cage at the RHS Field Research Facility covered in fine, insect-proof netting (with mesh size 0.8mm x 0.8mm) from a population established in 2017. The pupae of the second generation were collected in August 2019 and kept separately by sex inside insect rearing chambers. The pupae were sexed using a dissecting microscope, through identification of indentations on their ventral side (UK Lepidoptera Org. 2014). Once the adults emerged, they were mated in small netted chambers then released into tent chambers with proportions 210cm x 140cm (Quechua Arpenaz 4.2 tent), for oviposition on two box plants- one pruned (both shoot tips and leaf tips pruned to resemble a shaped box plant just before being put into tent chambers) and one unpruned. The box plants were in 2L pots and were approximately 60cm in height. The adult moths were fed using *Nemesia* plants whilst being reared, then fed with sugar water in the tents; approximately 50ml of sugar water at 0.02 g ml $^{-1}$. The plants were photographed before use in tent chambers, to enable estimations of plant area.

Originally, the aim was to mate six males and six females in small chambers, then release the females into a tent chamber, providing them with a choice of pruned vs unpruned box plants on which to ovisposit. However, during the first four attempts no egg plaques were laid on box plants during a 48hr period; for the next set of attempts both the male and female moths were released directly into the tent chambers with the box plants, provided with sustenance, and kept for 60hrs. In the third set of experiments 12 male and 12 female moths were released directly into the tent chambers. Plants were examined for egg plaques by the removal and checking of each individual leaf. Where leaves were found with egg plaques, the number of eggs within each plaque were recorded.

Box plant trials

The experimental site was situated at the RHS Field Research Facility), Deer's Farm, near Wisley, Surrey (TQ 064 592), see Figure 3 for an overview of the experimental site. The box plant trial consisted of three separate trial originally set out to test control management strategies against box blight; one based on architecture, one on cultivar susceptibility, one on pruning severity (minimal to heavy pruning and thinning) and one on pruning timing (summer/winter). The architectural trial included both cube and sphere shaping, thinning out of the plants, and differing frequency of pruning out of material if it had been infected with box blight. The cultivar trial included three cultivars: Common box (*B. sempervirens*), *B. suffruticosa*, and *B. microphylla* 'Faulkner', with/without pruning, and fungicide application treatments see Table 1 for a summary of all of the trials and respective treatments.

Table 1. Summary of the Box plant trial designs, including number of plants subject to each combination of treatments

Trial	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Replicas / treatment * level**
Architecture	Shape (2 levels)	Thinning (Y/N)	Trimming frequency (2 levels)	6
Cultivar	Cultivar (3 levels)	Pruning out (Y/N)	Fungicide use (Y/N)	4
Pruning severity	Severity (3 levels)	Fungicide use (Y/N)		6
Pruning subset	Severity (3 levels)	Fungicide use (Y/N)	Pruning timing (Wint./Sum.)	2

Figure 3. Box plant trial experimental site, adjacent to the Field Research Facility, Deer's Farm, Surrey (Stephanie Bird). The architecture trial is in the foreground on the left, the cultivar trial is at the back on the left hand side of the site and the pruning experiments are on the right

The plots were surveyed three times, once in 2019 (May, July and September) to observe three different generations of moths. The surveying noted the extent of any damage to the box plants, and the presence of any caterpillars/adult moths and egg plaques. Each plant section was examined for either 3 (architecture trial) or 4 minutes (all other trials) depending on size of box plants.

Additionally, the plots were surrounded by four pheromone traps (Solabiol BUXatrap Box Tree Moth Trap) at approximately each pole (N, E, S, W) 1m from the edge of the experiment plots, and set out with lures changed according to the manufacturer's instructions. These were checked every weekday for any box tree moths (originally set out 21/05/2019). The pheromone lure used attracts male *C. perspectalis* moths.

Analysis

Statistical analysis was conducted using the core packages within RStudio. For the pruning severity trial, the data for presence of box tree moths was not normally distributed, and could not be normalised by transformation, therefore a non-parametric Kruskal-Wallis test was used. For the pruning timing trial (summer/winter) it was not possible to normalise the data for the presence of box tree moths and so a Mann-Whitney U test was used to compare the medians of the results.

For the number of egg plaques on the cultivar trial and the pruning severity trial, the data could not be normalised, so a Kruskal-Wallis test was used in place of an ANOVA.

Results

Box plant trials

The plants with the highest infestation levels were those of *B. sempervirens*, with 88% of plants being infested with *C. perspectalis* and 2.209 individuals found on each plant on average across all three samples (Table 3). Plants in the architecture plot that had been shaped spherically and been thinned had the lowest levels of infestation, with only 48% of plants being infested, and 0.028 individuals found per plant on average (Table 2).

Table 2. Presence of *C. perspectalis* on architecture box plant trial

Treatment		**Presence of BTM**	**Average number of individuals per plant**	**Proportion of plants with egg plaques**	**Average number of eggs per plaque**
Shape	**Thinning**	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**
Round	**Y**	0.472±0.028	0.889±0.611	0.208±0.125	10.000±0.000
Round	**N**	0.778±0.167	1.556±0.834	0.167±0.083	6.834±0.833
Square	**Y**	0.639±0.028	1.195±0.028	0.167±0.083	10.000±2.000
Square	**N**	0.750±0.139	1.417±1.139	0.000	0.000

Table 3. Presence of *C. perspectalis* on cultivar box plant trial

Cultivar	Presence of BTM Mean±SE	Average number of individuals per plant Mean±SE	Proportion of plants with egg plaques Mean±SE	Average number of eggs per plaque Mean±SE
B. sempervirens	0.875±0.024	2.209±0.273	0.125±0.051	7.500±2.901
B. suffruticosa	0.813±0.040	1.313±0.124	0.063±0.063	2.000±2.000
'Faulkner'	0.625±0.054	0.688±0.354	0.156±0.060	6.875±2.649

Table 4. Presence of *C. perspectalis* on pruning severity box plant trial

Prune severity	Presence of BTM Mean±SE	Average number of individuals per plant Mean±SE	Proportion of plants with egg plaques Mean±SE	Average number of eggs per plaque Mean±SE
Minimum	0.722±0.000	2.056±0.000	0.250±0.000	10.333±0.000
Medium	0.667±0.000	1.056±0.056	0.042±0.042	5.000±5.000
High	0.722±0.000	1.806±0.584	0.083±0.000	10.500±2.500

Table 5. Presence of *C. perspectalis* on pruning timing box plant trial

Pruning season	Presence of BTM Mean±SE	Average number of individuals per plant Mean±SE	Proportion of plants with egg plaques Mean±SE	Average number of eggs per plaque Mean±SE
Summer	0.600±0.131	0.733±0.358	0.000	0.000
Winter	0.667±0.488	1.333±0.513	0.200±0.133	1.700±1.136

There was a significant difference between the percentage of box plants infested with *C. perspectalis* between different cultivars (ANOVA, $F_{2,9}$= 9.99, p=0.005) (Figure 5). A post hoc Tukey test showed there was no significant difference between *B. sempervirens* and *B. suffruticosa* (p=0.553), but there was a significant difference between *B. microphylla* and *B. sempervirens* (p=0.005) and *B. microphylla* 'Faulkner' and *B. suffruticosa* (p=0.026). The *B. microphylla* cultivar had lower infestation levels, with a mean (±SE) percentage infestation of 0.625±0.0538, compared to the *B. sempervirens* or *B. suffruticosa* cultivars, with means of 0.875±0.0242 and 0.8125±0.04, respectively.

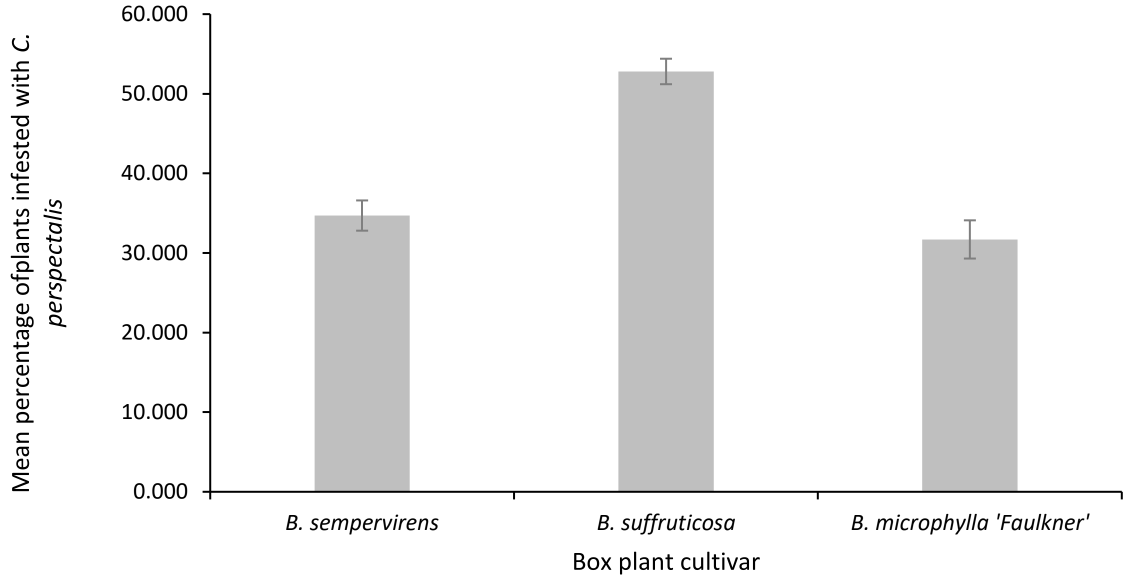

Figure 5. A graph showing the mean percentage of box plants infested by *C. perspectalis* by cultivar, with error bars of standard error of the mean.

There was also a significant difference between the number of individuals found per plant depending on cultivar (ANOVA, $F_{2,9}$=8.13, p=0.01), with the *B. sempervirens* plants having significantly more individuals than *B. microphylla* (p=0.008). *B. suffruticosa* plants had no significant difference in the number of individuals compared to either *B. sempervirens* (p=0.097) or *B. microphylla* (p=0.276). There was no significant difference between the number of egg plaques laid on the plants depending on cultivar (Kruskal-Wallis, H=1.52, p=0.468).

For the architecture plot, neither the shape nor thinning of the plant-or an interaction between them- had a significant effect on the percentage of plants infested with Box Tree caterpillars/moths (ANOVA, Shape $F_{1,4}$=0.40, p=0.561; Thin $F_{1,4}$=3.57, p=0.132; Shape*Thin $F_{1,4}$=0.78, p=0.428). Additionally, the average number of individuals per plant was not significantly affected by shape or trim or an interaction between them as well (ANOVA, Shape $F_{1,4}$=0.01, p=0.919; Thin $F_{1,4}$=0.33, p=0.594; Shape*Thin $F_{1,4}$=0.08, p=0.787). Lastly, the number of egg plaques laid on plants was not affected by the shape, thinning, or an interaction between the two (ANOVA, Shape $F_{1,4}$=1.47, p=0.292; Thin $F_{1,4}$=1.47, p=0.292; Shape*Thin $F_{1,4}$=0.53, p=0.506).

In the pruning severity plot, there was no significant difference between the percentage of plants infested (Kruskal-Wallis, H=4.00, d.f.=2, p=0.135) (Table 4). There was also no significant difference between the number of individuals per plant (ANOVA, $F_{2,2}$=1.27, p=0.440). The number of egg plaques laid on differently pruned plants was also not significant (Kruskal-Wallis, H=3.00, p=0.223).

Considering pruning timing, there was no significant difference in the percentage infestation of the plants comparing those pruned in either summer or winter (Mann-Whitney U, W=225.00, p=0.728), nor in the number of individuals found on each plant on average (Mann-

Whitney U, W=214.50, p=0.411) (Table 5). There were no statistically significant results on the number of egg plaques laid on this plot.

For the pruning timing box plant subset, the data on the number of egg plaques could not be analysed, as no egg plaques were found on the summer pruned plants, so there was nothing to compare the winter plants to.

There was an issue during some of the study where box moths could be seen flying around the plots, and eggs and larvae were found on the plot, but no adults were caught. There was a peak in captures in late August with seven *C. perspectalis* adults being captured in a couple of weeks, which may be linked to the flight of a new generation. It is hard to say why the traps were unsuccessful some of the time, but it could be due to the pheromone lure used losing effectiveness over time, or there being a low proportion of adult males present; the moths seen on the trial could have been mostly females which would not have been attracted.

Controlled choice chamber experiments

The egg plaques of C. perspectalis are very inconspicuous and it was initially thought that there had been no mating success within any of the choice chamber experiments. However, 6 days after the first experiment using 12 male and 12 female moths had been assessed; first instar caterpillars were observed consuming the upper leaf surfaces of both the pruned and unpruned box plants – it should be noted that this renders the data unreliable as initial number of egg plaques / caterpillars cannot be known with certainty. The final pair of chambers using 12 male and 12 female moths was then assessed leaf by leaf under a dissecting microscope, see Table 6 for results. There was not enough data for valid statistical analysis.

Table 6. Oviposition by *C. perspectalis* on pruned and unpruned box plants

			Number of leaves		
	Chamber aspect	**Pruning status**	**Without egg plaques**	**With egg plaques**	**Number of eggs**
Replica 1	S	Pruned	566*	36*	330*
	S	Unpruned	565*	69*	366*
	N	Pruned	All	0	0
	N	Unpruned	All	0	0
Replica 2	S	Pruned	1031	28	237
	S	Unpruned	1203	13	122
	N	Pruned	833	1	1
	N	Unpruned	792	0	0

* may not reflect actual numbers as some leaves completely consumed

Discussion

The results indicate that different cultivars had significantly different levels of infestation by *C. perspectalis*, with *B. sempervirens* plants having significantly more infestation than the *B. microphylla* 'Faulkner' plants, and the highest levels on average of any plants in the box plant trial. The 'Faulkner' plants also had significantly fewer infestation than *B. suffruticosa* plants. However, there was no significant difference between *B. suffruticosa* and *B. sempervirens* plants. This suggests that planting 'Faulkner' cultivars may be a viable option for reducing *C. perspectalis* infestation. 'Faulkner' has previously been shown to be less popular for oviposition, but it has been suggested that this may be due to the reduced leaf area of this cultivar, which belongs to the species *Buxus microphylla* (Leuthardt and Baur 2012). Additionally, Leuthardt suggests that *B. sempervirens* was not selected preferentially for oviposition by *C. perspectalis*. However, Leuthardt did not take plant damage into account when studying cultivar preference; more studies on selection by cultivar are needed in order to conclude if oviposition is preferential.

In this study the different box plant architectures had no significant results for any measures taken during surveying, this was also the case for pruning severity and pruning timing, suggesting that the shape or level/timing of pruning does not affect oviposition.

It appears that at least 12 pairs of moths are required to achieve mating success and oviposition under these experimental conditions and that there was no mating success in the chambers that faced Northwards, this is likely because these chambers were more exposed to sunshine and heated up more during the day. In future work shelter should be erected to prevent this. For the second trial there was a difference between pruned and unpruned plants that may warrant further investigation.

Conclusions

This study suggests that, of the parameters trialled, only cultivar type has an affect on *C. perspectalis* oviposition, and the other factors investigated in this study do not affect oviposition for this species. For gardeners and commercial growers this suggests that the best way to reduce *C. perspectalis* infestation is to grow the 'Faulkner' cultivar instead of other cultivars no other significant methods for oviposition limitation were found in this study. However, this suggests that for gardeners that are caring for already planted box plants there is little to be done to control infestation efficiently. The only other method available is removing *C. perspectalis* by hand, which is labour-intensive and time-consuming. In future, in addition to insecticides, effective biological control agents may become available to non-professional growers, allowing for easier control.

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