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Biology and Distribution of Box Tree Moth (*Cydalima per-spectalis*) (Walker, 1859) in Southern Ontario ⁺

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Abstract: Native to Asia, box tree moth (*Cydalima perspectalis*) (Walker, 1859) is an invasive pest first confirmed in Toronto Ontario in November 2018. Present in 36 countries worldwide, the pest is a serious concern to nursery growers and horticulturalists as it causes significant defoliation to boxwood (*Buxus* sp.), its primary host species. Boxwood is a significant nursery crop in Ontario and a popular ornamental landscape plant found in residential and public gardens across the province. In 2019 and 2020, monitoring with pheromone traps and ground surveys of boxwoods helped to delineate the infestation in the Greater Toronto Area. Two generations of the pest, occurring from May to September have been verified in Ontario compared to 3–5 generations observed in its native regions. The larval stage is active between mid-May through mid-June and again from mid-July to late August. Beginning in September, larva enter a state of diapause within a protected webbed hibernaria, a critical aspect of their overwintering success. Results from this study are being used to develop a sustainable pest management program to inform the nursery and landscape industry about proper treatment strategies that can effectively manage this invasive pest.

Keywords: box tree moth, *Buxus* sp., boxwood, flight activity, southern Ontario, pheromone traps, monitoring, citizen science

1. Introduction

The box tree moth (box tree pyralid; BTM), *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae) is a monophagous pest of its primary host species *Buxus* (boxwood), a popular ornamental landscape plant. In November 2018, this pest was confirmed for the first time in North America by the Canadian Food Inspection Agency in Etobicoke, Ontario, a municipality of Toronto, Ontario [1]. Boxwoods are an important nursery crop in the province and a popular ornamental landscape plant found in residential and public gardens across Ontario. Damage caused by BTM can result in complete defoliation of boxwoods if the population is left untreated [2]. Box tree moth poses a serious risk to the economically important nursery industry in Ontario which is responsible for 303 million in farm gate sales in 2020 [3].

Native to East Asian regions of China, Korea, and Japan [4], BTM has rapidly spread around the world over the last 14 years. The original source of the introduction to Ontario is unknown but is suggested to be the result of human induced translocation outside of the nursery sector. To date, there is no scientific information regarding the biology, population ecology, development, or behaviour of BTM in Ontario. Filling this informational gap forms the basis of the present study.

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While alternative host species such as *Euonymus* spp. (*Euonymus japonicus* and *E*. alatus), Japanese pachysandra (Pachysandra terminalis), orange jasmine (Murraya paniculate), and holly (Ilex purpurea) are reported within the pest's native regions in China and Japan [2,5], there is no evidence of the invasive population of BTM feeding on these alternative species in Europe [5,6]. Additionally, there is no indication that BTM prefers a particular cultivar of boxwood or that cultivar type influences BTM development, determined by Leuthardt et al. [7]. All life stages of BTM can be found on boxwood. After emerging from the egg mass, larvae progress through 6-7 instars over a period of 29-33 days [2,8,9]. After a pupal stage lasting 9–10 days, adults are present for *ca.* 14 days [2,9]. Despite below freezing temperatures, the box tree moth overwinters as the third larval instar within a hibernarium. This obligatory diapause is induced by a shortened daylength (< 13.5 h) and decreasing temperature [2,8,10]. Simulated life cycles generated by Suppo et al. [11] indicate a complete generation occurring within 38 days, at 25 °C, compared to the findings of Tabone et al. [9] who found a complete generation required 40-50 days at 25 °C under laboratory conditions. A disparity also exists around the number of generations per year for native versus invasive populations. Within the native regions, 3–5 generations are reported, while 2–3 generations have been recorded in Europe [2].

Dispersal rate within invaded areas is also disputed. Given a suspected yearly dispersal rate of 7–10 km, BTM has the potential for rapid spread throughout invaded areas [12,13]. While host plant (boxwood) presence is an important aspect of BTM dispersal, another key variable is suitable environmental conditions required for development and diapause [10,12]. As well, human-induced dispersion is likely to facilitate accelerated dispersal given that the pest has been found in the highly urbanized landscape of Toronto Ontario. The duration of each life stage, the number of generations occurring per year and tracking where the pest is located within Ontario, all need to be closely monitored for effective management. Monitoring is an integral component of crop or plant protection, particularly for invasive insect pests, as early detection can help initiate control [14]. Santi et al. [15] proved that monitoring with baited pheromone traps in peripheral areas of known infested sites is an effective way to track dispersal and identify BTM populations in new areas. Additionally, Gottig and Hertz [16] demonstrated that trap monitoring of adult flight patterns helped define the number of complete generations per year.

The emphasis of this study was to improve our understanding of BTM's behaviour under Ontario's climatic conditions. Focus was placed on delineating the timing and duration of life stages, number of generations per year and tracking dispersal through multiyear monitoring.

2. Materials and Methods

Monitoring occurred from 15 April to 15 November for two years (2019 and 2020) across southern Ontario primarily in the known infested region of Toronto, Ontario, Canada (43° 42' 0.40" N, –79° 24' 58.68" W). All life stages of BTM, including egg, larva, pupa, and adults, were the subject of monitoring [15]. Data were collected through two survey methods, pheromone traps and ground surveys of host plants (boxwood). Reports of BTM from the general public were also of great value and were verified using submitted photographic evidence [15–17]. In 2020, data loggers were placed at four monitoring sites in Etobicoke, Ontario to collect temperature readings from 24 March to 15 October.

2.1. Pheromone Traps

Modified milk carton traps (Product #2050500; Solida Inc., Quebec), containing a sticky card liner and baited with BTM pheromone lures (Solida Inc., Quebec), were used to attract male BTM moths (Figure 1 (a)) [15,16,18]. Baited traps were installed within 7 m of boxwood plants and at a height of 1.5 m above the ground. Pheromone lures were replaced as needed to provide efficacy throughout the monitoring period. In an effort to

achieve a larger geographical dispersal of traps across southern Ontario, citizens were engaged in a citizen science program to host a pheromone trap during the survey period. A total of 52 traps (2019) and 61 traps (2020) were hosted by citizens through the citizen science program. The number of moths per trap was recorded weekly by members of the research team and by citizen scientists [15,16]. Photographs of the sticky card liner accompanied each citizen scientist report to confirm BTM identification (Figure 1 (b)). Trap captures of BTM outside of the known dispersal zone were confirmed by collections of voucher specimens. Data were recorded from the beginning of May until the end of September in 2019 and 2020.



(a)

(b)

Figure 1. The pheromone trap utilized for box tree moth (BTM) monitoring consisted of: (**a**) a modified milk carton trap (Product #2050500; Solida Inc., Quebec), baited with a BTM pheromone lure; and (**b**) a sticky card inserted in the milk carton trap to capture BTM adult male moths.

2.2. Ground Survey of Host Plants

Ground surveys of boxwood host plants involved physically searching boxwood plant material for all life stages of BTM and evidence of larval feeding, webbing, larval feeal waste, and shed head capsules. For each survey site, the date, address, life stage of BTM found, and measurements of larval body length and head capsule width were recorded [8,10,17]. Ground surveys were conducted from 12 April until 1 September 2019 and from 12 April until 15 November 2020. All occurrences of BTM from both pheromone traps and ground surveys were spatially mapped using Google My Maps [17].

3. Results

3.1. Distribution in Ontario

Occurrences of BTM within Ontario are visually represented in (Figure 2). Box tree moth was first confirmed in Etobicoke, a municipality of Toronto, in the fall of 2018. Monitoring in 2019 yielded a geographical dispersal zone of approximately 390 km², limited to the Toronto area. New positive reports of BTM occurred outside of this zone in 2020 (Figure 2); most notably, approximately 10 km eastward into the city of Scarborough, another municipality of Toronto. Dispersal also occurred northward and westward up to 3 km in each direction in 2020 (Figure 2). Although a larger zone of infestation was observed in 2020 (*ca.* 560 km²) compared to 2019, BTM still appears limited to the Toronto. No other occurrences were confirmed outside of Toronto at the end of 2020.



Figure 2. Spatial distribution map of box tree moth (BTM) within Toronto Ontario (Canada). The first detection site represented by the black marker, BTM occurrences in 2019 in red, and occurrences in 2020 in blue.



Figure 3. Seasonal flight activity of adult male box tree moth (BTM) based on captures in pheromone trapping in 2019 and 2020 within Toronto, Ontario. Pheromone trapping was initiated during calendar week 17 in 2019 and week 20 in 2020. Data are based on 8 traps at the same location each year.

3.2. Seasonal Flight Activity

Moth captures were summarized by calendar week (CW) to allow for comparison between years (Figure 3). Flight activity occurred from late June (CW 26) until mid-September (CW 38). Captures revealed two main flight activity periods. The first beginning in mid-June (CW 26), peaking late June (CW 27) and decreasing until late July (CW 30). The second flight period was much more extended, beginning in early August (CW 32), peaking late August (CW 35) and continuing until mid-September (CW 38). Two seasonal flight activity periods suggest two generations occurring within Ontario. The capture of the first BTM moth differed between 2019 and 2020 with the first capture in 2020 occurred two weeks earlier (CW 26) than in 2019 (CW 28) (Figure 3). Traps for both years were installed in known infested areas, however, applications of *Bacillus thuringiensis* in the vicinity may account for low BTM captures in 2019 during the first flight activity period.



Figure 4. Proposed seasonal life cycle of box tree moth populations in Toronto, Ontario based on pheromone trapping and ground survey data during 2019 and 2020 (Gen = generation).

3.3. Behaviour of Box Tree Moth in Ontario

Ground surveys supported the occurrence of two generations of BTM in Ontario (Figure 4). Overwintering larva were found within protective hibernaria during April and May of each year with larvae observed feeding as early as the first week of May (CW 19) (Figure 5). The first BTM generation remained synchronous, with only marginal overlap of life stages. The first viable pupa was found in the second week of June (CW 24) in 2019 and the first week of June (CW 23) in 2020.

Three general periods of larval feeding were identified between May and September. The first generation of larvae were observed feeding outside their hibernaria between calendar weeks 19-24. Peak feeding activity for the second-generation larvae between calendar weeks 28-33. The second generation was less synchronous with multiple life stages present at the same time (Figure 5). Newly-hatched larvae resulting from the second generation, began feeding in early September (CW 36) but very minimal feeding damage was observed as the larvae were preparing to overwinter. In 2020, hibernaria were first observed in early August (CW 32) and increased in number as the fall approached.

Feeding damage and evidence of webbing presented differently as larva matured through the larval instars. The smaller mandibles of early-instar larvae cause a 'window-pane' damage pattern that involves only one side of the leaf being eaten. Later instar larvae consume complete sections of the leaves, but typically, leaf margins are left intact. All life stages of BTM and larval feeding were observed solely on boxwood and not on any of its previously named alternative hosts. Severe defoliation was rarely observed, but when it occurred, it was found exclusively on boxwood plants where BTM populations had been left unmanaged for multiple generations.



Figure 5. Ground survey observations for life stage development of box tree moth in the Toronto, Ontario region during 2019 and 2020, organized by calendar weeks.

4. Discussion

Our study provides new, essential information about the distribution and biology of BTM in southern Ontario. By the end of 2020 BTM populations were still well within the bounds of the predicted range, given an assumed yearly dispersal of 7–10 km by adult moths [12,13]. Preference for BTM dispersal along the shoreline of Lake Ontario (Figure 2) is indicated by the lateral expansion of the infestation eastward compared to the more limited expansion northward. Moderating climate effects from the lake produce favourable conditions that would contribute to this phenomenon, however, the climate model developed by Canelles et al. [12] clearly shows that both Asian and European BTM populations will spread readily within continental landscapes suggesting that BTM may behave similarly within Ontario in the future.

The accuracy of pheromone traps (Figure 1) to evaluate moth phenology has been called into question for other Crambidae species due to differences in the timing of male and female flight periods [19]. The use of pheromone traps can be disadvantageous because only male BTM moths are attracted to the pheromone (Figure 1 (b)). Since BTM adult moths are nocturnal, the use of light traps may be a more effective way of determining population dynamics. Unfortunately, due to the nature of the trapping sites within the present study, the use of other trapping methods, such as light traps, was not practical. Gottig and Herz [16] demonstrated that there was no significant difference in the ratio of males to females during both flight activity periods of BTM using light traps, concluding that pheromone traps used in our study provide strong support for the phenology of BTM in southern Ontario.

Early diapause induction, as observed in August 2020 (Figure 5), indicates a stronger relationship with decreasing day length (photoperiod) rather than temperature as the diapause signal. This contrasts Poitou et al. [10] who concluded that diapause termination, and thus inversely induction, is primarily influenced by temperature. In their model used to explore BTM phenology within Europe, Suppo et al. [11] included both day length (photoperiod) and temperature as important factors. Unfortunately, hibernaria presence was not consistently recorded during the 2019 ground surveys, and thus it is difficult to discern whether the early diapause induction that occurred during 2020 was an anomaly. Recording hibernaria presence should be a focal point for future monitoring and biological studies. The removal of the traps in mid-September was also premature as moth flight activity may have continued after this date [15,16]. In the future it will be important to leave pheromone traps set up until the end of October to evaluate the possibility of any late season adult flight activity.

Evaluations made through pheromone trap captures and ground surveys have clearly delineated two generations of BTM in southern Ontario (Figure 2 and 3). This reflects observations made by Nacambo et al. [8] in north-western Switzerland and Gottig and Herz [16] in Germany. Comparatively, our findings contradict the majority of reports from Europe where three to five generations occur [2,15,17] suggesting climatic conditions strongly influence BTM life history and phenology. Based on our data to date, it appears that the best time to apply insecticide applications for BTM management in the Toronto area is during the three larval periods, first from mid-May to mid-June, second from mid-July to mid-August, and third at the beginning of September. Precise control measures were adapted from this information, reducing the potential for unnecessary and ineffective insecticide applications in Toronto. Current recommendations involve the use of *Bacillus thuringiensis* (Bioprotec PLUS, Dipel® 2X DF, or Xentari WG) during each larval activity period with a high level of control [16].

5. Conclusion

Box tree moth continues to pose a major threat to boxwood (*Buxus* sp.) in Ontario, with potential for substantial economic and landscape damage in invaded areas across the province. Detailed records of BTM distribution, along with accurate characterization of its biology, will ensure a successful integrated pest management program can be developed.

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