BTM (Box Tree Moth) Mesh Exclusion Trial Report

Alex Rimmer[1], Cassie Russell[2], Jeanine West[3], Jamie Aalbers[4], Carla Timm[1], Sandy Smith[1]

[1] University of Toronto, Institute of Forest Conservation
[2]Ontario Ministry of Agriculture, Food and Agribusiness (OMAFA)
[3] Landscape Ontario Horticultural Trades Association (LO)
[4]Canadian Nursery Landscape Association (CNLA)

Introduction

Box tree moth (*Cydalima perspectalis*, BTM) is a significant pest of boxwood (*Buxus* spp.) plants in Ontario. The CFIA (Canadian Food Inspection Agency) has imposed regulations for movement of boxwood outside of regulated areas and therefore boxwood producers must follow a systems approach program to manage the moth and have an approved pest module in place. In the absence of ovicidal control options, farms on pest modules and systems approaches are encouraged to safeguard plants prior to shipping. Safeguarding of plants from a flying pest typically involves a physical barrier to protect the host plants from infestation. Due to box tree moth having multiple generations and asynchronous flight periods in Ontario, some Ontario growers have decided to place a mesh netting over their production cold frames throughout the entire growing season to prevent the pest from flying in and laying eggs on boxwood. Verification of pest free status inside the exclusion area is accomplished through trapping and scouting activities.

In 2023, BTM was observed to have been shipped from facilities in Ontario that did not utilize safeguarding protocols, and there is a demand for information on what screen/mesh options are suitable for boxwood production (allowing sufficient light and ventilation for optimal plant health) while meeting the criteria for excluding the flying moths.

Landscape Ontario collected samples of various mesh options available that could meet the growers' criteria. The trial aimed to evaluate each of the mesh options in experimental insect cages to determine which would allow BTM adults through using a lure on the other side of the mesh. The optimal mesh opening will be the largest possible to prevent the insect from moving through it, while allowing the most light and ventilation. White mesh also allows for increased light transmission, which is another crucial factor for boxwood production.

Materials and Methods

Box tree moth rearing

The box tree moths used for this experiment were a lab population reared at the University of Toronto since April 2022. They were reared at 24°C under 16:8 h L:D photoperiod. Diet consisted of *Buxus x microphylla* c. Green Velvet (Sheridan Nurseries) ad libitum. Larvae were individually reared in 128 mL plastic cups with a moist paper towel. Paper towel and boxwood were replaced every other day.

Pupae were sexed and 6 males (pupating within 72 h) were placed into plastic emergence cages at 24°C under 16:8 h L:D photoperiod. Moths in the emergence cage were given 5% honey water and distilled water.

Mesh exclusion experimental materials

Six mesh exclusion trial cages were constructed using transparent plastic containers (Cambro S-21882 4-Quart, Uline, Milton, Ontario, Canada) and plastic lids (Cambro S-21885, Uline, Milton, Ontario, Canada) with silk screen mesh for ventilation. The trial cages were constructed of two chambers (introduction chamber and attraction chamber) connected by a plastic funnel (H-5214 Uline, Milton, Ontario, Canada) to reduce backward flight of the moth (Figure 1). Each trial cage had one of the 5 treatment meshes (40% shade Gintec black knit, 30% shade Gintec black knit, 60 g ProtekNet Dubois white knit, 25 g Gintec white knit, 30% shade deWitt black knit) that was secured at the mouth of the funnel (Figure 2), and the last cage was a no mesh control. The attraction chamber consisted of a twig of boxwood in water, a yellow sticky card (COSCANA, Mississauga, Ontario, Canada), and a single, 60-day BTM specific pheromone lure (Item #40IPS04, Solida.ca, Saint-Ferréol-les-Neiges, Quebec, Canada).

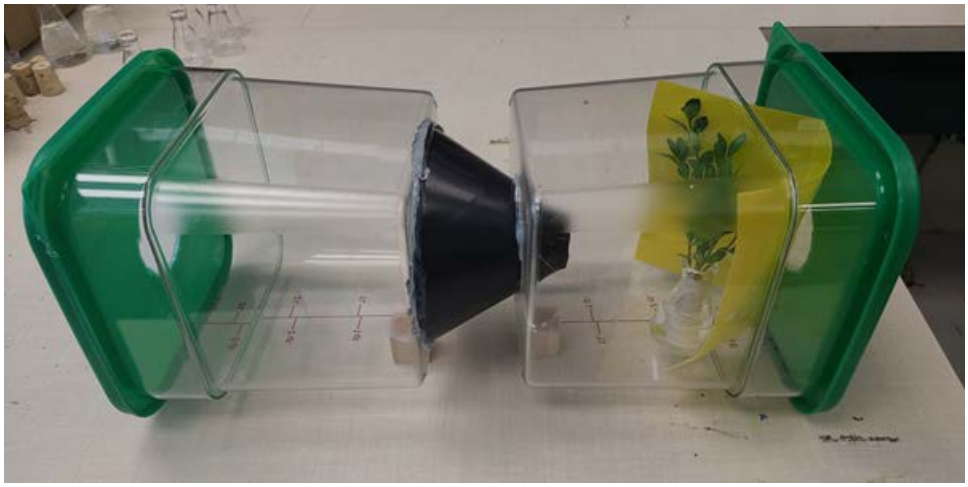

Figure 1. Mesh exclusion experimental set-up with introduction chamber (left) and attraction chamber (right). Picture shown here is the control without mesh at the mouth of the funnel.

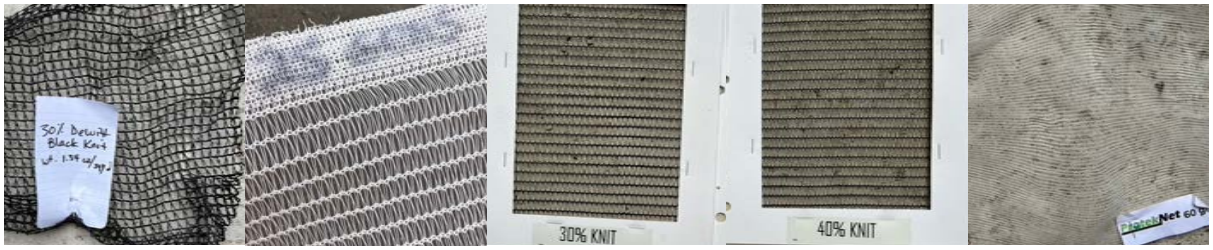

Figure 2. Mesh exclusion materials tested: DeWitt 30% black, Gintec 25g white, Gintec 30% black, Gentec 40% black, and ProtekNet 60g white.

Experiments were carried out within 6 separate growth chambers to prevent pheromone interference between treatments. All growth chambers were set to 25°C and a photoperiod of 16:8h L:D. Treatments were randomly assigned to 1 of the 6 growth chambers at each replication.

Methodology for the mesh exclusion trial

Male moths from each cohort were photographed 24 h post eclosion. 6 females were randomly selected from the same cohort and also photographed at 24 h post eclosion. ImageJ was used to measure thorax and abdomen width of the male and female moths. 7 replications were performed

for a total of 42 male and 42 female moths. A Welch's t-test was performed using R Studio (version 2022.07.1) to determine if there was a difference in male and female thorax width.

Once all 6 males within a cohort emerged more than 72 h prior, they were each randomly assigned to one of the 6 treatments and placed into the introduction chamber. Moths were monitored for up to 7 days to allow time to cross the mesh into the attraction chamber portion of the trial cage. The trial cages were checked daily for the presence of the moth either within the attraction chamber or stuck to the sticky card. Once the moth has been found within the attraction chamber, the moth was removed, the data collected, and the sticky card and boxwood twig were replaced. Once all 6 moths had either crossed into the attraction chamber or 7 days had elapsed, any remaining moths were removed and a new replication began until 7 replications were completed.

Results

Moth size

Male and female thorax and abdominal width measurements are summarized in Table 1. The minimum thorax size for males and females were 2.36 mm and 2.28 mm respectively. The mean thorax size was not significantly different between male and female moths ($t = 0.464$, $p = 0.644$).

Table 1. Moth thorax and abdominal measurements (n=42 for both male and female)

Metric	Male measurements	Female measurements
Minimum thorax width	2.36 mm	2.28 mm
Maximum thorax width	4.17 mm	4.79 mm
Mean thorax width	3.28 mm	3.33 mm
Minimum abdomen width	2.50 mm	2.55 mm
Maximum abdomen width	4.12 mm	5.48 mm
Mean abdomen width	3.17 mm	3.31 mm

Mesh Exclusion Trial

In all but one replication, male moths were able to cross into the attraction chamber in the control with no mesh barrier. In two replicates moths were able to cross through the 30% deWitt mesh barrier to reach the attraction chamber corresponding to a 71% success rate (Figure 3). All other mesh types were 100% effective in preventing the moth from crossing in all 7 replications.

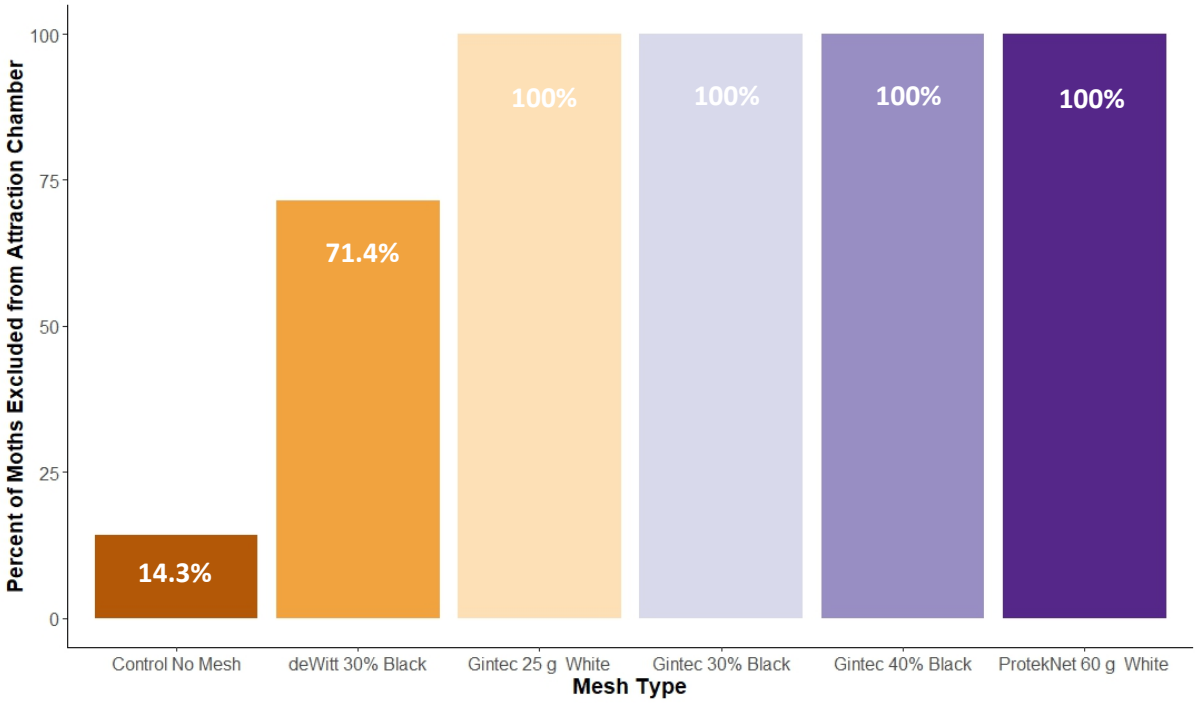

Figure 3. Percent of moths excluded from attraction chamber by the mesh barrier.

Discussion

Both female and male moth thorax and abdominal width varied between approximately 2.3 mm and 4.2 mm with a few larger female outliers. This variation is important to consider when finding an appropriate mesh size to successfully exclude box tree moth adults. Measurements were taken for both male and female moths since although the trials were conducted on male moths, female moths are the target of concern to prevent egg laying on boxwood plants. Male moths were used instead of female moths as no male pheromone lure is produced or available to include in attraction chambers to motivate female moths to cross the mesh barrier. For this reason, female moths were substituted for males in this experiment as there was no statistical difference in thorax width between males and females which was consistent with previous findings on BTM (Hizal et al., 2012). However, this cannot account for differences in behaviour between sexes which may affect moths' motivation to cross obstructions.

Four of the five mesh types that were assessed were able to exclude the moth in all 7 replications. Since the moth was able to cross to the attraction chamber in nearly all of the control replicates and twice in the deWitt 30% shade knit treatment, it is unlikely that this was due to any barriers from the experimental design and can be attributed to the properties of the mesh themselves. The deWitt 30% shade knit had the largest aperture size of all mesh types and also had a more woven squared shape compared to the Gintec (triangular) and ProtekNet (rectangular) which were smaller knits with less open shape. It is unclear if colour had any effect on moth exclusion as this variable was not controlled for in the deWitt 30% shade mesh type.

The deWitt 30% shade knit which has been used previously by some within industry to exclude BTM adults from boxwood (personal communications) is insufficient to prevent all moths from crossing into pest exclusion structures. All three Gintec mesh sizes successfully prevented adult moths from

moving between chambers in laboratory studies. It is unlikely that a smaller mesh size such as the ProtekNet 60 g knit would be required since the smaller aperture size would not exclude adults more effectively and result in less light penetration and ventilation; negatively impacting overall plant health.

Next Steps and Recommendations

Nursery growers who are using pest exclusion netting to protect boxwood plants from box tree moth can have confidence using Gintec 25 g, Gintex 30%, Gintec 40% and ProtekNet 60g mesh types to exclude box tree moth adults from flying into covered production areas to maintain pest free status. Other considerations for mesh selection include light availability for the plants, ventilation to prevent fungal infection, and mesh durability.

Additional trials to evaluate the variability of moth size when grown in a range of temperatures (i.e., < 20C or >20C) would be valuable to clarify if adult size is dependent on environmental conditions. Some lepidoptera respond to colder temperatures by pupating earlier and the result is a smaller adult, which, if true for BTM, could mean that mesh size may have to be smaller to accommodate BTM development in a range of climactic conditions.

It is important to note that this experiment only tested mesh sizes to exclude adult life stages of box tree moth. While larval stages of box tree moth are also mobile, field experience and literature has not observed larvae leaving host plants. Even in large density infestations on a single boxwood shrub, larvae have not left the host plant but will instead feed on the woody bark of boxwood (personal observations). Therefore, it is unlikely a mesh size small enough to prevent movement of boxwood larvae into pest free exclusion structures is necessary. Regardless, it is advised that boxwood not be touching or up against the exclusion mesh, to eliminate the possibility of a continuous path of plant material that could potentially allow the larvae to traverse between unmanaged boxwood outside of mesh and into pest exclusion structures.

Acknowledgements

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