COOPERATIVE AGREEMENT ACCOMPLISHMENT REPORT
Biological Control Program
Plant Protection and Quarantine
and
The Maine Department of Agriculture, Conservation and Forestry

Period of Performance: September 1, 2021, to November 30, 2022 (extension).

I. Introduction

This report summarizes progress towards reaching the objectives, milestones, and accomplishments outlined in cooperative agreement AP21PPQFO000C311 between The Maine Department of Agriculture, Conservation and Forestry and the Animal and Plant Health Inspection Service's (APHIS) Plant Protection and Quarantine (PPQ) program. The agreement covers a joint approach to implementing a biological control program that targets black swallow-wort (dog-strangling vine), *Vincetoxicum nigrum, in Maine.*

Introducing non-native species can cause significant changes in habitat, making that habitat unfit for the species that are native to it. These habitats become unfit because native herbivores, especially arthropod herbivores, cannot consume the invasive plant leaving the plant to spread without any natural control. Black swallow-wort (*Vincetoxicum nigrum*) is an herbaceous plant originating from Europe that nativized in the Northeastern United States and eastern Canada during the mid-19th century (DiTommaso et al., 2005). In its native habitat, *V. nigrum* grows on forested slopes; however, in North America, it can grow freely in both open fields and the understory of forested areas (Weed et al., 2010). *Vincetoxicum spp.* can produce up to 250 wind-dispersed seeds per plant, which can travel 18 meters or up to 80 if the seeds are released from an elevated point such as a vine that has climbed up a tree or other structure (Alred 2021). Since *V. nigrum's* naturalization, its range has expanded from eastern North America to central North America.

The expanding range of *V. nigrum* is concerning because *Vincetoxicum spp.* is disruptive to the local ecology. These plants create monocultures that outcompete native vegetation (Ernst & Cappuccino, 2005). These monocultures support less arthropod biodiversity. The decline of arthropod diversity presents a series of consequences on restoration and land management and the animals that use these arthropods for food (Ernst & Cappuccino 2005). Monarch butterflies (*Danaus plexippus L.*) are especially threatened by *Vincetoxicum spp.* When given a choice between *Vincetoxicum spp. a*nd common milkweed (*Asclepias syriaca L.*), monarch butterflies have been found to lay 10-20% of their eggs on *Vincetoxicum spp.* even though the eggs laid on these plants do not survive (Casagrande & Dacey, 2007). In addition to threatening local ecology, *Vincetoxicum spp.* may also be a forestry issue. Small trees at a restoration site in Ontario were smothered by *Vincetoxicum* vines, and New York state Christmas tree growers have reported *V. rossicum* — a similar species known as pale swallow-wort — vines posing increased competition to their trees over the past ten years (DiTommaso et al., 2005).

It is difficult to combat the spread of *Vincetoxicum* spp. with herbicides or mechanical means alone. Herbicides can reduce the biomass and regeneration of *Vincetoxicum* spp., but requires multiple applications to control the population. For example, single applications of glyphosate have been demonstrated not to be satisfactory (DiTommaso et al., 2005), however a combination of mechanical means and herbicides has shown promise for pale swallow-wort (*V. rossicum*) (DiTommaso et al., 2013). While this is encouraging, these results were determined to be habitat specific and would only work effectively in some scenarios.

An alternative option to suppress invasive plants is to use biological control. A major concern when using an organism for biological control is damage to non-target species. Studies have demonstrated that the moth species *H. opulenta* is a specialist that only feeds on *Vincetoxicum spp.* (Weed and Casagrande, 2010). *H. opulenta* has the potential to act as a defoliant as it lays an average of 600 eggs each generation and can produce up to two generations in a year (Weed and Casagrande, 2010). The removal of leaves can suppress the growth of *Vincetoxicum spp.* This suppression may allow other native species to regenerate (Weed and Casagrande, 2010). A previous experiment at the Central Experimental Farm, Ottawa, Ontario, successfully established a population of *H. opulenta* in a release effort that began in 2014. In 2015 2 adult *H. opulenta* moths emerged from overwintering in a field cage (Bourchier et al., 2019). Blacklighting in 2016 re-confirmed that *H. opulenta* had successfully overwintered when three adults were observed; later that year, naturally occurring larva were confirmed after finding 14 occurrences of leaf feeding damage (Bourchier et al., 2019). In 2017, swallow-wort herbivory was observed up to 2 km away from the initial release site, and systematic yellowing of vines was observed at sites with concentrated herbivory from *H. opulenta* (Bourchier et al., 2019). This study has found increased damage to *V. nigrum* vines consistently from 2014 to 2018, however, the density of *H. opulenta* is not currently high enough to impede growth (Bourchier et al., 2019).

One of the challenges to establishing *H. opulenta* in Maine could be summer daylength is not long enough. *H. opulenta* enter diapause when daylength drops from 16 hours to 15 hours with 50% of them entering diapause with a daylength of 15 hours and 35 minutes (Jones et al., 2020). Ottawa, Ontario experiences a 15-hour and 40 minute daylength at its longest and 69 days with over 15 hours of light while *H. opulenta*'s home region in Ukraine experiences a 16-hour 2 minuet longest day and 81 days that are light for at least 15-hours (Jones et al., 2020). Many parts of Maine that could benefit from this biological control are farther south than Ottawa, Ontario meaning they would experience fewer days with daylength of 15 hours or more and would have shorter daylengths on the summer solstice (June 21^{st}). This is a concern because if the released *H. opulenta* enter diapause after the first generation they will not be an effective biocontrol. This is because only one generation would lead to much slower population growth as well as a long period of time that would allow *V. nigrum* vines to recover from the damage inflicted earlier in the season (Jones et al., 2020).

An attempt to control *V. nigrum* through a limited releases of *H. opulenta* in Maine has already occurred along the ocean in Ogunquit, Maine*.* The release sites were located on a public trail (Marginal Way) where *V. nigrum* has densely invaded the trail-side vegetation, and volunteer groups annually work to manually remove seed pods with a goal of re-introduction of native plants to the area (Joan Griswold, *personal communication)*. The first release was conducted in 2018, at one location along the Marginal Way. This release consisted of a total of 350 larvae being released into a cage at two time points for the 2018 releases resulted in minimal *H. opulenta* defoliation. It was thought that this low success was due to high temperatures and inclement weather. The project was paused until 2021, when 20 male and 20 female adult *H. opulenta* moths were released with the intent that they would lay eggs on swallow-wort. These moths were released in two cages along Marginal Way, with a protective shade cloth added above the cages to protect the *H. opulenta* from inclement weather. Defoliation was observed within these two cages, which were removed in July of 2021.

II. Objectives

The objectives of this study were to continue release trials of *H. opulenta* in Maine (Biocontrol Release) and evaluate if populations became established at previous release locations (Establishment Study). Specifically, our objectives were to:

Biocontrol Release:

(1) Measure reproductive success and survival rate of *H. opulenta* at several developmental stages and relate reproduction and survival to environmental conditions, daylength and vegetation characteristics at new release locations;

(2) Measure the impact of *H. opulenta* herbivory on *V. nigrum* growth in relation to phenological, ecological and environmental conditions at new release locations.

Establishment Study:

(3) Relate overwintering survival of *H. opulenta* to seasonal and overwintering environmental conditions and vegetation characteristics at past release locations;

(4) Monitor for adult *H. opulenta* that may have established at past release locations.

III. Milestones

Methods

Biocontrol Release

Study Locations: Release (N = 2) and control locations (N = 2) were selected in the early spring (Table 1). All four sites were selected to have sufficient *V. nigrum* (approximately 70% leaf cover) for *H. opulenta* larvae development. Sites were also selected to have sufficient shade as it has been found that *H. opulenta* larvae do not survive optimally in high-light conditions (Weed and Casagrande, 2010; Joan Griswold *personal communication*). A cage (Bioquip, 1406A Outdoor Cage, 6 x 6 x 6', 18 x 14 Screen Mesh) was built in each location and marked with a sign to educate the public about the purpose of the cage (Figure 1). Within each cage, a tempo disc (Tempo Disc 4 in 1 Bluetooth Wireless Thermometer, SKU: S2-SJNQ-V022, Blue Maestro Inc.) was affixed with a plastic dish arranged above the monitor to protect it from rain. Environmental data collection included hourly temperature, humidity, dewpoint, and pressure for the duration of the sampling period.

Obtaining and Rearing *H. opulenta*: Pupa (N = 132; 60F, 72M) were obtained from the Tewksbury Lab rearing facility via mail on June 14, 2022 and were inspected for movement to confirm that the pupa were alive (Figure 2A, 2B) When pupa were unpacked it was discovered that seven of the adults had already emerged; all were male. Pupa were reared in the lab in four bug dorm cages of varying sizes (Figure 2C). All four cages had a layer of vermiculite covering the bottom and 2-3 flowering *V. nigrum* vines sitting in a beaker of water in the cage. The beakers of water were covered with *V. nigrum* leaves to prevent moths from drowning. The cages were left in an empty conference room facing away from the window so the pupa would not experience direct sunlight. The room was kept at 23°C. After three days, pupa were transported to the field in the same bug dorms that they had stayed in as pupa and were released in the cages set up in the two treatment sites. No additional food was placed in the cage as the *V. nigrum* inside had already begun to bloom.

Field Release and Cage Removal: Bug dorms were placed in treatment cages and opened on June 17, 2022. There was heavy rain that cleared up before putting out the bug dorms. On June 23, 2022 all bug dorms were removed. A total of 66 *H. opulenta* were released per treatment site, either released as adults or pupa needing to finalize development (4 pupa at Treatment 1, 14 pupa at Treatment 2). Following the Tewksbury release protocol, cages were removed when foliage inside the cage was completely depleted. The Treatment 2 cage was taken down on August 10, 2022, and the Treatment 1 cage was taken down on September 29, 2022. After the first frost on October 4, 2022, the control cages and all tempo discs were removed.

Sampling Method: Sampling methods closely followed protocols developed by Lisa Tewksbury. Sites were sampled approximately once per week, starting on July 7, 2022, and ending on October 4, 2022. Site visits included a 5-minute visual survey of *V. nigrum* plants in a one-meter square outside of the cage, and a second survey within the cage. During the visual survey, any *H. opulenta* were recorded along with life stage (instars of larva, pupa, adults, and eggs). A damage rating was assigned to the area (none = no damage on leaves; low = holes in a few scattered leaves; medium = holes in many leaves; high = extensive damage on most leaves). Damage was also quantified through counting the number of leaves with holes from a randomized survey of 20 leaves in the cage. To further classify development of *H. opulenta,* holes were categorized as windowpane or full holes, which correspond to development of the caterpillars. In addition to *V. nigrum* damage assessment methods, at each site visit when *V. nigrum* growth within cages was visually assessed, a photo was also taken in the same spot within cages to serve as a visual marker of the impact of *H. opulenta* herbivory on *V. nigrum* growth.

Establishment Study

To determine if the previous releases were successful in establishing populations of *H. opulenta,* the locations of previous release sites were investigated July 13, 2022 for evidence of immature activity, including feeding holes in *V. nigrum* leaves and frass. Six locations were chosen along an approximately one-mile path (Marginal Way, Ogunquit Maine) surrounding the 2018 and 2021 release sites. Three of these sites were the previous cage locations. Sites were visually surveyed for 5 minutes for damage on *V. nigrum* plants to determine if any caterpillar activity had taken place. All visual sampling took place on July 13, 2022. A black light (120-volt Eco smart LED) was used to monitor for adults. This blacklight was powered using a 550 to 685 amp battery (DieHard brand RV/Boat) connected to a heat lamp (300 Watt/120 Volt Brooder). These two devices were connected using an inverter (Duracell high power) with the lamp plugged into one of the power inverter's AC outlets. The light was shone on a white sheet approximately 6 ft x 6 ft hung on a 50 ft rope being strung between two branches of a large tree on-site (Figure 3). The location was chosen on an access path onto Marginal Way from Israel Head Road, a location centralized between the 2021 release and the 2018 release sites. Monitoring took place at dusk and continued until 11:00 PM, as a previous study never found *H. opulenta* adults after 10:00 PM (Bourchier et al., 2019). The sheet was continuously monitored for adult moths that resembled *H. opulenta.* Photos were taken of each potential *H. opulenta.* One moth was collected into a jar with Ethyl acetate for identification in the laboratory.

Results

Biocontrol Release

Measurements were successfully taken at all four locations from July 7 through October 4 (Tables 3 – 6). After releasing adults and remaining pupae into cages on June 17, 2022, larva were first observed at Treatment 1 on July 13 (Table 4) and on July 7 at Treatment 2 (Table 5).

At the Treatment 1 location, there were two generations of *H. opulenta* during the sampling period (Table 4). Pupa and adults were observed on August 10. At this date, the daylength was 14:15, and the average temperature was 20.98 °C (Table 4). A second generation of early instar larva were observed (including new windowpane damage) on August 18, which was characterized by a daylength of 13:53 and average temperature of 17.78 °C (Table 4). The second generation of pupa was observed on September 15, which was characterized by a daylength of 12:32 and average temperature of 18.69 °C (Table 4). *V. nigrum* damage almost immediately reached a "Medium" damage rating (July 13), received a "High" rating by September 1, and the cage had to be removed due to 100% defoliation on September 15 (Table 4, Figure 3). At the Treatment 1 location, damage was observed to *V. nigrum* plants outside of the cage from July 29 through October 4, indicating that *H. opulenta* was able to complete development outside of the cage.

At the Treatment 2 location, there were two generations of *H. opulenta* during the sampling period (Table 5). A second generation of early instar larva or pupa were not observed, as the cage had to be removed on July 29 due to 100% consumption of *V. nigrum* leaves within the cage (Table 5). However, adult *H. opulenta* were observed on August 10 at the location even with the cage removed. At this date, the daylength was 14:15, and the average temperature was 17.61 °C (Table 5). *V. nigrum* damage almost immediately reached a "Medium" damage rating (July 7), received a "High" rating by July 22, and the cage had to be removed due to 100% defoliation on July 29 (Table 5, Figure 3). At the Treatment 2 location, damage was observed to *V. nigrum* plants outside of the cage from July 13 through October 4, indicating that *H. opulenta* was able to complete development outside of the cage.

At both control locations, no *H. opulenta* or damage to *V. nigrum* plants were observed inside or outside of the cages (Table 2, Table 3). Environmental metric (temperature, relative humidity, dew point, and vapor pressure) averages and standard deviations across the entire sampling period were compared across the four locations at four time stamps (6:00 AM, 12:00 PM, 6:00 AM, 12:00 AM) to represent day and evening differences at locations.

In the morning (6:00 AM), the temperature was relatively similar across all four locations, with a slightly higher mean at Control 1, and a wider range at Control 2 (Fig. 4A). The relative humidity and vapor pressure were both also relatively similar across all four locations (Fig. 4B, 4D). Finally, the dew point had a similar mean at all locations except for Control 1, which was higher (Fig. 4C). In the afternoon (12:00 PM), the temperatures at Treatment 1 and Treatment 2 were similar, with a slightly higher average temperature at Treatment 1 (Fig. 5A). Both control locations were hotter than treatment locations, but with a similar average (Fig. 5A). The opposite trend was seen for relative humidity, with a similar average at Treatment 1 and 2, both higher than Control 1 and 2 (Fig. 5B). Dew point and vapor pressure were similar across all four sites (Fig. 5C, 5D). In the evening (6:00PM) and at midnight (12:00 AM), all four sites were relatively similar across all metrics (Fig. 6, Fig. 7), except for a slightly higher temperature and dew point at Control 1 at 6:00 PM (Fig. 6A, 6C).

Establishment Study

No *H. opulenta* larva or evidence of larva in the form of windowpane or hole damage were observed during the visual surveys. No *H. opulenta* adults were observed during the black lighting sampling event, however, the setup did attract several other species of moths and other taxa, indicating that it was attractive.

Discussion

Biocontrol Release

The release at two new locations in Harpswell, Maine, were both successful with a second generation of *H. opulenta* developing, providing useful life-history information for Maine that can be used for determining establishment sampling dates in future years of this study. Generally, *H. opulenta* were more successful at the Treatment 2 location, with an overall higher number of early season caterpillars observed, which fed more rapidly on the plants in the cage. The Treatment 2 location was slightly warmer than the Treatment 1 location in the early season, which could lead to faster development of the larva. It is difficult to compare the success of *H. opulenta* at these locations once the cages were removed, however, it is encouraging that adults were observed at both locations.

Establishment Study

While it is discouraging that we did not find evidence of reestablishment, it is not surprising given the history of releases at this location, which were relatively low in number and only one year included shaded cages. It is possible that the extreme environmental conditions alongside the ocean may have caused caterpillars not to survive. It is also possible that we surveyed too early to find adults (July 13), as the second generation of moths at our treatment locations were not present until August 10. On a positive note, however, during our survey, there were fewer *V. nigrum* plants than anticipated. This was likely due to a labor-intensive 9-year ongoing project in the area where volunteers pick *V. nigrum* pods once per year. The PI of that project, Joan Griswold, estimates that from 2012 to 2021, approximately 3.5 tons of *V. nigrum* pods (~10.8 million seeds) have been removed.

IV. Other

We were not necessarily expecting a second generation of *H. opulenta* during the sampling period and were pleasantly surprised to find one. We also found that the cages did not contain all of the larva which hatched, as damage was found outside of the cages, however, Otherwise the cages worked as intended. The COVID-19 pandemic pushed the originally planned dates for the project forward, and pieces of the planning process for the 2022 season were interrupted due to the early arrival of PI Peterson's child. Some planned items for purchase (including a mercury vapor light in lieu of a black light) were unavailable due to Bioquip going out of business. The plan had also been to have two black light sampling events for *H. opulenta* adults. However, heavy rainstorms canceled the second planned event.

References

Alred, B., Haan, N., Landis, D. A., & Szűcs, M. (2021). Does the presence of the Biological Control Agent, *hypena opulenta* (lepidoptera: Erebidae) on swallow-worts deter monarch oviposition? *Environmental Entomology*, *51*(1), 77–82. https://doi.org/10.1093/ee/nvab121

Bourchier, R. S., Cappuccino, N., Rochette, A., des Rivières, J., Smith, S. M., Tewksbury, L., & Casagrande, R. (2019). Establishment *of Hypena opulenta* (lepidoptera: Erebidae) on vincetoxicum rossicum in Ontario, Canada. Biocontrol Science and Technology, 29(9), 917–923. https://doi.org/10.1080/09583157.2019.1608511

Casagrande, R. A., & Dacey, J. E. (2007). Monarch butterfly oviposition on swallow-worts (vincetoxicum spp..). Environmental Entomology, 36(3), 631–636. https://doi.org/10.1603/0046-225x(2007)36[631:mboosv]2.0.co;2

DiTommaso, A., Lawlor, F. M., & Darbyshire, S. J. (2005). The biology of invasive alien plants in Canada. 2. *cynanchum rossicum* (Kleopow) borhidi *vincetoxicum rossicum* (Kleopow) barbar.] and *cynanchum louiseae* (L.) Kartesz & Gandhi *vincetoxicum nigrum* (L.) moench]. *Canadian Journal of Plant Science*, *85*(1), 243–263. https://doi.org/10.4141/p03-056

DiTommaso, A., Milbrath, L. R., Bittner, T., & Wesley, F. R. (2013). Pale swallow-wort (*vincetoxicum rossicum*) response to cutting and herbicides. *Invasive Plant Science and Management*, *6*(3), 381–390. https://doi.org/10.1614/ipsm-d-12-00078.1

Ernst, C. M., & Cappuccino, N. (2005). The effect of an invasive alien vine, Vincetoxicum Rossicum (Asclepiadaceae), on arthropod populations in Ontario Old Fields. *Biological Invasions*, *7*(3), 417–425. https://doi.org/10.1007/s10530-004-4062-4

Jones, I. M., Seehausen, M. L., Bourchier, R. S., & Smith, S. M. (2020). The effects of photoperiod on diapause induction in hypena opulenta (Lepidoptera: Erebidae), a biological control agent against invasive swallow-worts in North America. Environmental Entomology, 49(3), 580–585. https://doi.org/10.1093/ee/nvaa030

Weed, A. S., & Casagrande, R. A. (2010). Biology and larval feeding impact of Hypena opulenta (Christoph) (Lepidoptera: Noctuidae): A potential biological control agent for Vincetoxicum nigrum and V. rossicum. Biological Control, 53(2), 214–222. https://doi.org/10.1016/j.biocontrol.2009.12.004

Table 1. Sample site locations for the study.

Site Name	Latitude	Longitude	Town	County	State
Control 1	43.7477708	-70.0092545	Harpswell	Cumberland	ME
Control 2	44.30356	-69.771223	Augusta	Kennebec	ME
Treatment 1	43.718603	-70.0021201	Harpswell	Cumberland	ME
Treatment 2	43.74741	-70.0069129	Harpswell	Cumberland	ME

Figure 1. Cages (Bioquip, 1406A Outdoor Cage, 6 x 6 x 6', 18 x 14 Screen Mesh) in each study location, marked with a sign to allow for members of the public to learn about the project and contact the PI with questions.

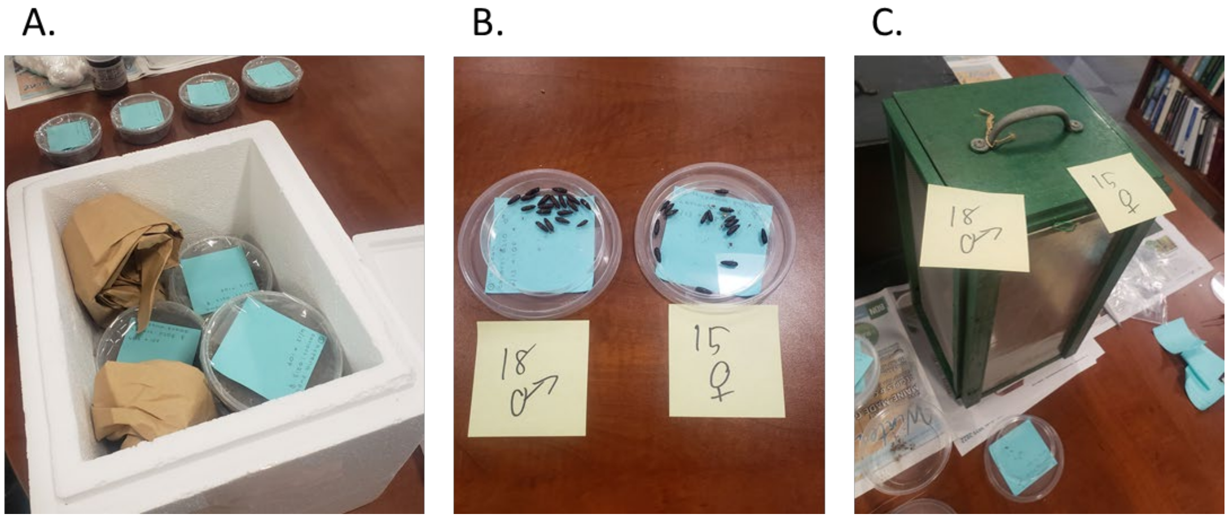

Figure 2. Arrival of pupa in the mail (A), which were sexed by the Tewksbury lab (B), and reared to adults in bugdorm enclosures in the laboratory in Augusta, Maine (C).

Figure 3. Blacklight setup during the reestablishment study in Ogunquit, Maine.

Table 2. Control 1 environmental conditions, observed *H. opulenta,* evidence of *H. opulenta* within cages (WP = windowpane damage, H = full holes) and damage rating (#/20 leaves with damage) throughout the entire sampling period. Damage rating to plants is rated within and outside of the cage (none = no damage on leaves; low = holes in a few scattered leaves; medium = holes in many leaves; high = extensive damage on most leaves).

	Environmental Conditions				**Observed *H. opulenta***					***H. opulenta* Evidence**		***V. nigrum* Damage**		
Date	**Sunrise**	**Sunset**	**Daylight Length**	**Avg. Temp (°C)**	**Total**	**Instar 1-3**	**Instar 4-5**	**Pupa**	**Adults**	**WP**	**H**	**% Leaves Damaged**	**Cage Damage Rating**	**Outside Damage Rating**
7-Jul	5:03	20:25	15:22	20.0	0	0	0	0	0	0	0	0	None	None
13-Jul	5:08	20:22	15:14	22.3	0	0	0	0	0	0	0	0	None	None
22-Jul	5:16	20:15	14:59	21.7	0	0	0	0	0	0	0	0	None	None
29-Jul	5:23	20:07	14:44	23.6	0	0	0	0	0	0	0	0	None	None
10-Aug	5:37	19:52	14:15	19.2	0	0	0	0	0	0	0	0	None	None
18-Aug	5:46	19:39	13:53	17.2	0	0	0	0	0	0	0	0	None	None
26-Aug	5:55	19:26	13:31	20.3	0	0	0	0	0	0	0	0	None	None
1-Sep	6:02	19:15	13:13	14.7	0	0	0	0	0	0	0	0	None	None
15-Sep	6:18	18:50	12:32	NA*	0	0	0	0	0	0	0	0	None	None
29-Sep	6:35	18:23	11:48	NA*	0	0	0	0	0	0	0	0	None	None
4-Oct	7:20	17:25	10:05	NA*	0	0	0	0	0	0	0	0	None	None

**Weather monitor failed on September 15, September 29, and October 4 at this location.*

Table 3. Control 2 environmental conditions, observed *H. opulenta,* evidence of *H. opulenta* within cages (WP = windowpane damage, H = full holes) and damage rating (#/20 leaves with damage) throughout the entire sampling period. Damage rating to plants is rated within and outside of the cage (none = no damage on leaves; low = holes in a few scattered leaves; medium = holes in many leaves; high = extensive damage on most leaves).

Environmental Conditions					**Observed *H. opulenta***					***H. opulenta* Evidence**		***V. nigrum* Damage**		
Date	**Sunrise**	**Sunset**	**Daylight Length**	**Avg. Temp (°C)**	**Total**	**Instar 1-3**	**Instar 4-5**	**Pupa**	**Adults**	**WP**	**H**	**% Leaves Damaged**	**Cage Damage Rating**	**Outside Damage Rating**
7-Jul	5:03	20:25	15:22	18.96	0	0	0	0	0	0	0	0	None	None
13-Jul	5:08	20:22	15:14	20.98	0	0	0	0	0	0	0	0	None	None
22-Jul	5:16	20:15	14:59	24.06	0	0	0	0	0	0	0	0	None	None
29-Jul	5:23	20:07	14:44	20.65	0	0	0	0	0	0	0	0	None	None
10-Aug	5:37	19:52	14:15	19.58	0	0	0	0	0	0	0	0	None	None
18-Aug	5:46	19:39	13:53	16.77	0	0	0	0	0	0	0	0	None	None
26-Aug	5:55	19:26	13:31	19.03	0	0	0	0	0	0	0	0	None	None
1-Sep	6:02	19:15	13:13	16.58	0	0	0	0	0	0	0	0	None	None
15-Sep	6:18	18:50	12:32	20.13	0	0	0	0	0	0	0	0	None	None
29-Sep	6:35	18:23	11:48	12.63	0	0	0	0	0	0	0	0	None	None
4-Oct	7:20	17:25	10:05	7.25	0	0	0	0	0	0	0	0	None	None

Table 4. Treatment 1 environmental conditions, observed *H. opulenta,* evidence of *H. opulenta* within cages (WP = windowpane damage, H = full holes) and damage rating (#/20 leaves with damage) throughout the entire sampling period. Damage rating to plants is rated within and outside of the cage (none = no damage on leaves; low = holes in a few scattered leaves; medium = holes in many leaves; high = extensive damage on most leaves).

Environmental Conditions					**Observed *H. opulenta***					***H. opulenta* Evidence**		***V. nigrum* Damage**		
Date	**Sunrise**	**Sunset**	**Daylight Length**	**Avg. Temp (°C)**	**Total**	**Instar 1-3**	**Instar 4-5**	**Pupa**	**Adults**	**WP**	**H**	**% Leaves Damaged**	**Cage Damage Rating**	**Outside Damage Rating**
7-Jul	5:03	20:25	15:22	16.94	1	0	0	0	1	8	2	50%	Low	None
13-Jul	5:08	20:22	15:14	18.08	2	2	0	0	0	5	6	55%	Medium	None
22-Jul	5:16	20:15	14:59	20.51	1	1	0	0	0	2	10	60%	Medium	None
29-Jul	5:23	20:07	14:44	19.73	0	0	0	0	0	1	17	90%	Medium	Low
10-Aug	5:37	19:52	14:15	20.98	14	0	0	2	12	0	19	95%	Medium	Low
18-Aug	5:46	19:39	13:53	17.78	2	0	0	0	2	2	18	100%	Medium	Low
26-Aug	5:55	19:26	13:31	19.12	4	4	0	0	0	0	19	95%	Medium	Low
1-Sep	6:02	19:15	13:13	20.00	5	4	1	0	0	0	20	100%	High	Medium
15-Sep	6:18	18:50	12:32	18.69	1	0	0	1	0	*No Leaves Remain*		100%	High	Medium
29-Sep	6:35	18:23	11:48	20.00	0	0	0	0	0	*Cage Removed*		100%	High	High
4-Oct	7:20	17:25	10:05	10.25	0	0	0	0	0	*Cage Removed*		100%	High	High

Table 5. Treatment 2 environmental conditions, observed *H. opulenta,* evidence of *H. opulenta* within cages (WP = windowpane damage, H = full holes) and damage rating (#/20 leaves with damage) throughout the entire sampling period. Damage rating to plants is rated within and outside of the cage (none = no damage on leaves; low = holes in a few scattered leaves; medium = holes in many leaves; high = extensive damage on most leaves).

	Environmental Conditions				**Observed *H. opulenta***					***H. opulenta* Evidence**		***V. nigrum* Damage**		
Date	**Sunrise**	**Sunset**	**Daylight Length**	**Avg. Temp (°C)**	**Total**	**Instar 1-3**	**Instar 4-5**	**Pupa**	**Adults**	**WP**	**H**	**% Leaves Damaged**	**Cage Damage Rating**	**Outside Damage Rating**
7-Jul	5:03	20:25	15:22	20.68	10	9	0	0	1	13	1	70%	Medium	None
13-Jul	5:08	20:22	15:14	20.96	13	13	0	0	0	13	6	95%	Medium	Low
22-Jul	5:16	20:15	14:59	22.60	10	7	3	0	0	0	20	100%	High	Low
29-Jul	5:23	20:07	14:44	20.18	2	0	2	0	0	*Cage Removed*		100%	High	Medium
10-Aug	5:37	19:52	14:15	17.61	2	0	0	0	2	*Cage Removed*		100%	High	Medium
18-Aug	5:46	19:39	13:53	16.84	0	0	0	0	0	*Cage Removed*		100%	High	Medium
26-Aug	5:55	19:26	13:31	18.85	0	0	0	0	0	*Cage Removed*		100%	High	Medium
1-Sep	6:02	19:15	13:13	18.28	0	0	0	0	0	*Cage Removed*		100%	High	Low
15-Sep	6:18	18:50	12:32	14.77	0	0	0	0	0	*Cage Removed*		100%	High	Medium
29-Sep	6:35	18:23	11:48	12.58	0	0	0	0	0	*Cage Removed*		100%	High	Medium
4-Oct	7:20	17:25	10:05	10.04	0	0	0	0	0	*Cage Removed*		100%	High	Low

Figure 3. Comparison photos within cages at each location.

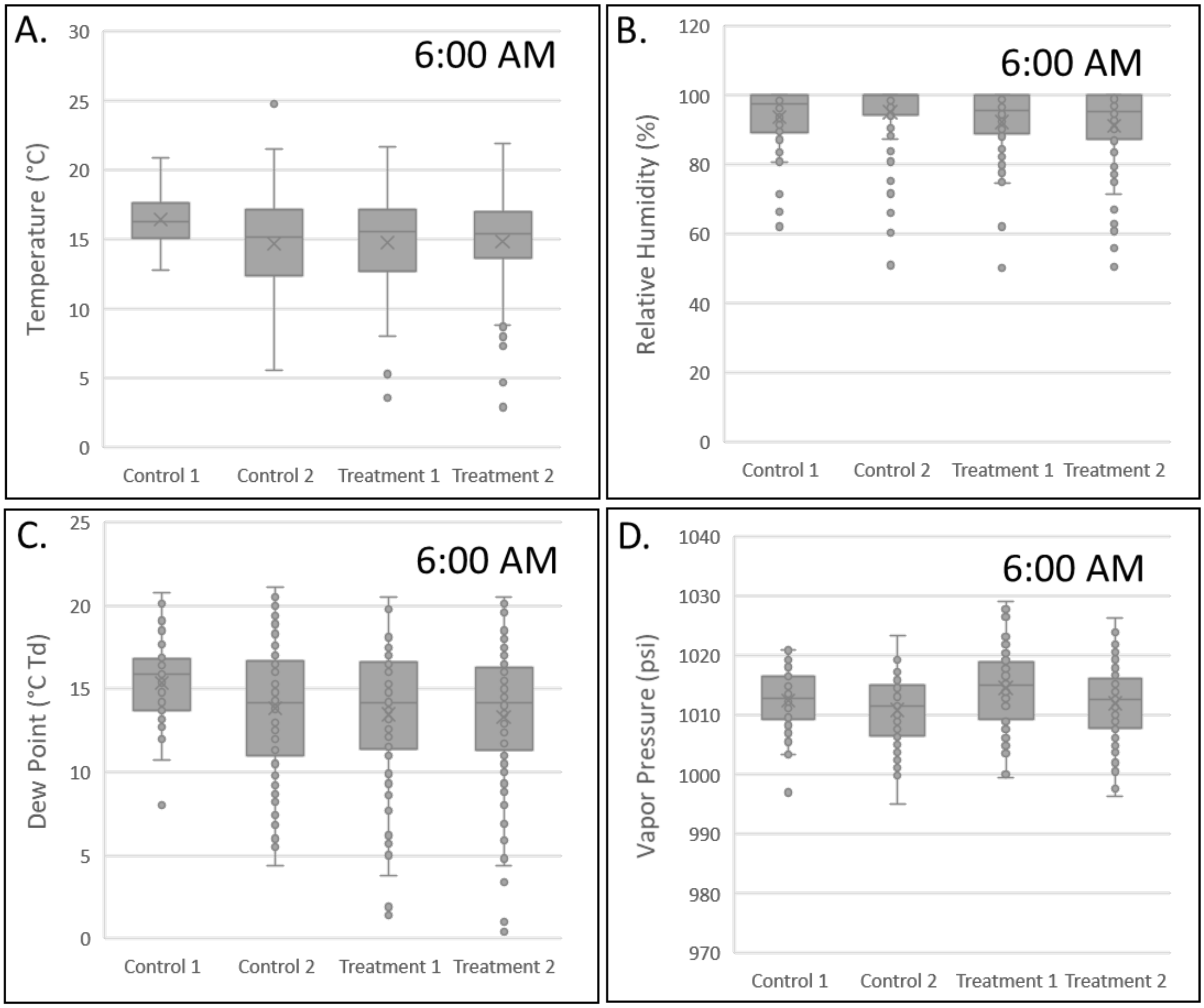

Figure 4. Comparison of the mean and standard deviation of environmental metrics at 6:00 AM across the four locations from July 7, 2022 through October 4, 2022 including Temperature (A), Relative Humidity (B), Dew Point (C), and Vapor Pressure (D).

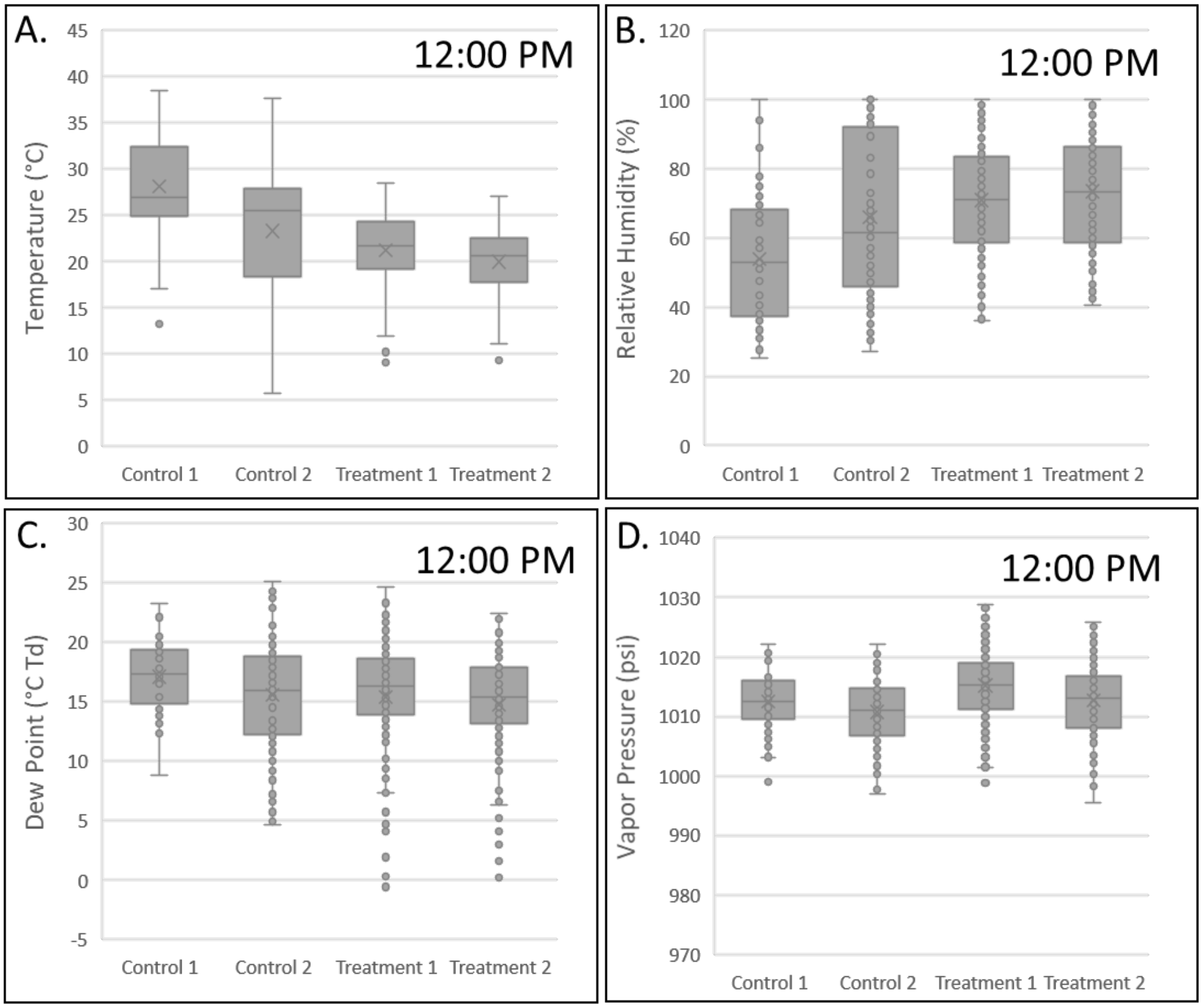

Figure 5. Comparison of the mean and standard deviation of environmental metrics at 12:00 PM across the four locations from July 7, 2022 through October 4, 2022 including Temperature (A), Relative Humidity (B), Dew Point (C), and Vapor Pressure (D).

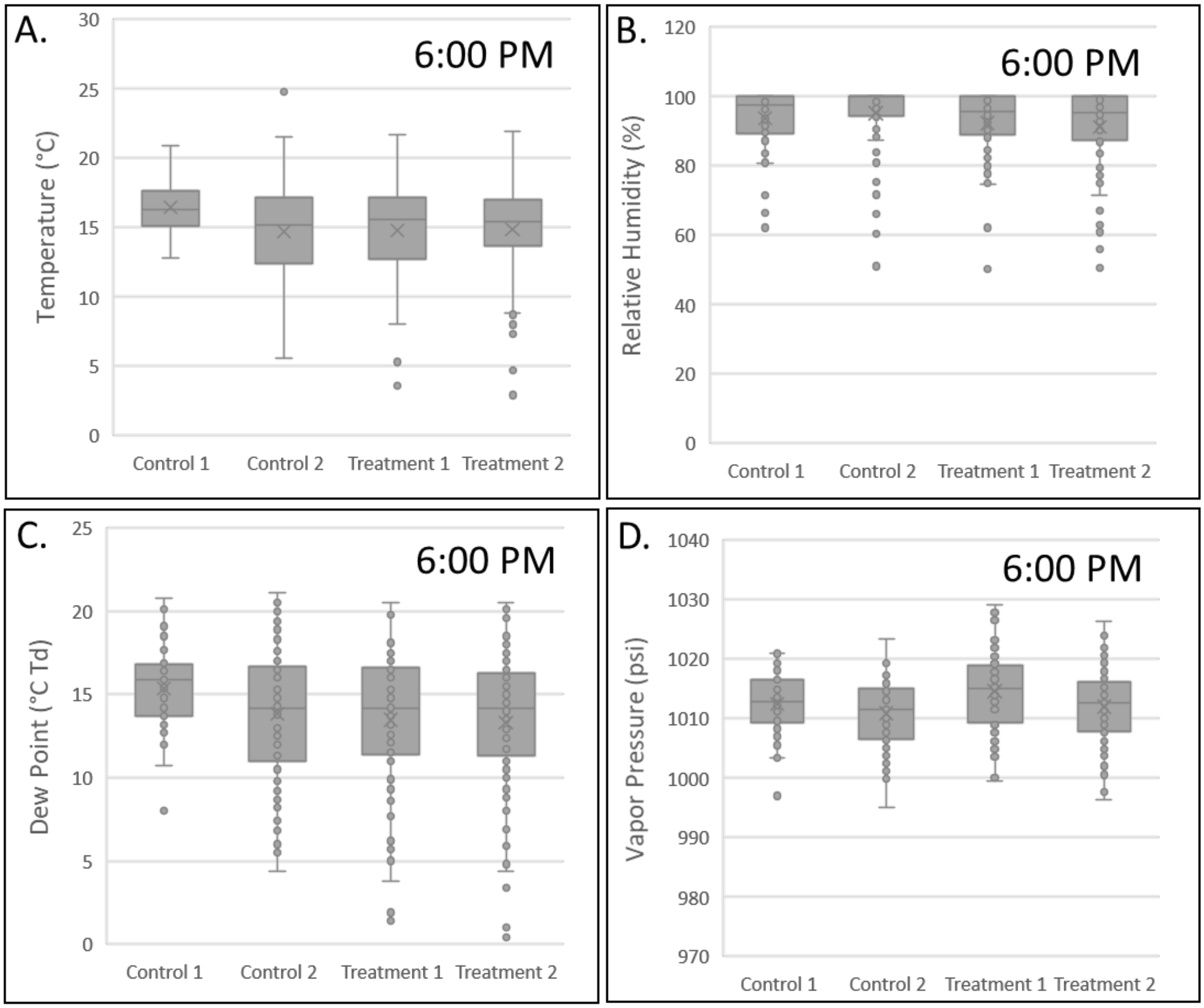

Figure 6. Comparison of the mean and standard deviation of environmental metrics at 6:00 PM across the four locations from July 7, 2022 through October 4, 2022 including Temperature (A), Relative Humidity (B), Dew Point (C), and Vapor Pressure (D).

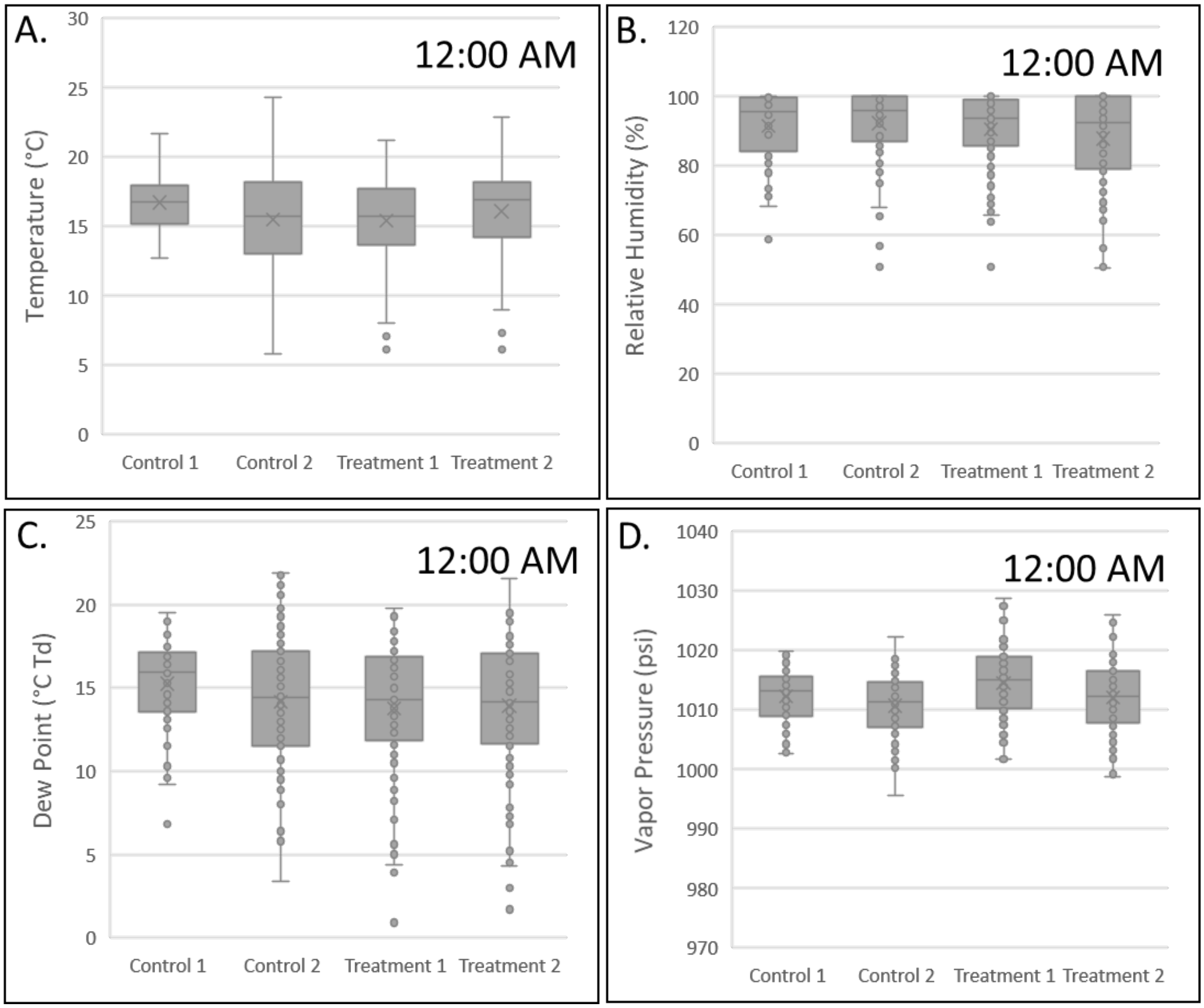

Figure 7. Comparison of the mean and standard deviation of environmental metrics at 12:00 AM across the four locations from July 7, 2022 through October 4, 2022 including Temperature (A), Relative Humidity (B), Dew Point (C), and Vapor Pressure (D).