N O T E

Harmonia axyridis (Coleoptera: Coccinellidae) Aggregation in Underground Artificial Overwintering Sites[1]

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Originally from Asia, *Harmonia axyridis* (Pallas) was introduced into the United States as a biological control agent. Considered established in Georgia since 1990, laboratory-reared *H. axyridis* were released in Byron from 1978 through 1981 in pecan, *Carya illinoinensis* (Wangenh, K. Koch), orchards to combat the pecan aphids *Monellia caryella* (Fitch) and *Monelliopis pecanis* (Bissell) (Tedders and Schaefer 1994, Ent. News 105: 228–243). While an effective generalist predator, these beetles will migrate away from agriculture fields if they cannot find suitable overwintering sites in the area and are known to overwinter in human dwellings. Although regarded as a beneficial insect, they can become a nuisance in people's houses. When disturbed, reflex bleeding produces an orange secretion that can stain carpets, furniture, clothing, and walls (De Quattro 1995, Agric. Res. J. 43: 4–8). In addition to being a nuisance in dwellings, there are human health concerns with them causing an allergic response in some people (Yarbrough et al. 1999, J. Allergy Clin. Immunol. 104: 704–705). Thus, these negative aspects of the beetles have caused people to consider them as a pest and not just a beneficial insect. Homeowners will often kill these beetles if they enter their home removing the future benefits from predation from those beetles.

It is the lack of suitable over wintering sites around agriculture fields which forces *H. axyridis* to migrate out of agroecosystems and possibly into human residences. It can take time for *H. axyridis* to migrate back from overwintering sites and recolonize cropping systems. Migrating beetles travelling back to agriculture fields from their overwintering sites might lead to a delay in pest suppression early in the growing season. Early presence of predators in cropping systems are known to help suppress pest outbreaks (Costamagna et al. 2015, Ecol. Appl. 25: 1114–1130).

Researchers have proposed building overwintering sites for *H. axyridis,* but either have not tried or failed to attract beetles to overwinter in their structures. Nalepa et al. (2000, J. Entomol. Sci. 35: 150–157) failed to get effective settling in their structures.

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Fig. 1. Artificial overwintering structure placed in ground ready to be buried.

It is possible that the structures were ineffective because the temperature inside them might not have been sufficiently different from temperatures outside to be considered an adequate overwintering site. The buildings where the structures were attached would have had a larger thermal difference inside them than the structures. The buildings also had previously been aggregation sites. Beetles might have left cues from previous years that these were suitable overwintering locations. *Harmonia axyridis* have been found to use substrate marking for indications of aggregation sites (Durieux et al. 2013, PLoS ONE 8(4): e61124. doi:10.1371/journal.pone.0061124). Research from Nalepa et al. (2000) indicated that aggregation behavior might be based on feces and residues that persist from previous years. These markings in the buildings might have attracted the beetles to the buildings as overwintering sites and away from their artificial shelters.

This study tested underground boxes near agricultural fields, with a polyvinyl chloride (PVC) pipe used as a passageway to enter and exit the box, to determine if *H. axyridis* would use them to overwinter. Sides of the artificial overwintering site boxes were constructed of 61 × 61 cm plywood boards of 1.27 cm thickness. Boxes were constructed with no bottom and a 61 × 61 cm 1.27 cm thick plywood board on top with hinges. A 10.16 cm hole was cut into the top of the box. A 10.16 cm wide and 3.05 m tall white PVC pipe with 2.5 cm wide and 15.25-cm-long slits on both side of each pipe at the top and bottom of the pipe was placed in the hole. Pipes were topped with a harrow disc to act as a roof to prevent rain from entering the pipe (Fig. 1). We did not use chemical attractants in this study but instead relied on the white color of the PVC pipe used as the attractant. In China, it has been observed that aggregations of *H. axyridis* favored white walls in villages (Wang et al. 2011, BioControl 56: 193–206).

Inside each box, 4 pieces of 1.2 m rebar were driven 0.6 m into the ground to act as an aggregation substrate in addition to the sides of the boxes.

Boxes were placed at sites and buried on 01 November 2022. Two Kestrel® Drop (Nielsen-Kellerman Company, Boothwyn, PA) temperature and humidity data recorders were placed at each site, one inside and one outside of each of the boxes. Boxes were covered with 15.25 to 20.32 cm of soil to act as a thermal buffer and retain ground heat. Four boxes were constructed in total. Three boxes were placed in Tift Co., GA. Of these structures, one was placed on the Philippi Farm, a U.S. Department of Agriculture (USDA) research farm, along a fence line between an electric power line and research field plots that grow wildflowers, cotton, peanut, millet, maize, and pigeon pea. The second was placed at Jones Farm, a University of Georgia (UGA) research farm. This structure was placed along a hedgerow, between a wooded area and research fields that grow cotton, peanut, and corn. The third was placed along a hedgerow/tree line at a private farm that grows cotton and peanut in rotation. The fourth box was placed at a private farm in Colquitt Co., GA that grows vegetables and grazes cattle. It was placed near a fence line between 2 grazing fields. Boxes were dug up on 29 December 2022 and 30 January 2023 to observe if structures were being used by *H. axyridis.* During those times, presence and numbers of beetles were visually counted and recorded. Dataloggers were recovered and data were downloaded for December and January. On 30 April 2023, boxes were again dug up to verify if the beetles had completely migrated out of the box.

The underground overwintering structures were utilized in 3 of the 4 sites. The greatest number of beetles was recorded at UGA's Jones research farm with a total of 314 and 321 in December and January, respectively. The second highest beetle numbers were recorded at the private cotton/peanut farm which had 187 and 231, respectively. The other private farm only had 27 and 11 beetles during December and January, respectively. The USDA's Philippi research farm had no beetles present at any time checked. None of the beetles were deceased in the boxes in December or January and all of them had migrated out of the boxes by April. Aggregations of beetles were observed within the pipes in the lower section that was in the box, on the walls and top of the boxes, and on the rebar. In December, temperatures recorded from underground at all sites ranged from 10°C to 22.8°C, while temperatures above ground ranged from −6°C to 27.7°C. In January, underground temperatures ranged from 11.8°C to 20.1°C at all sites while temperatures above ground ranged from −2.3°C to 23.9°C.

Now that *H. axyridis* has been found to successfully use some of these underground structures to overwinter, other questions should be addressed to determine how best to utilize them in agroecosystems. Research will also need to be conducted to determine what effect having overwintering sites close to agricultural fields will have on pest suppression in those cropping systems. Beetles were observed entering and leaving the structures during warmer days in the winter (personal observation). Research will need to determine if proximity to the field will lead to earlier colonization of crops. Future research will also need to determine if this earlier colonization will lead to early-season pest suppression compared to fields where beetles must migrate away from and then back to recolonize grower fields. Studies should test if feeding during winter would cause any effect on pest populations in the growing season by lowering pest overwintering populations.

The structures at in the USDA's Philippi research farm and at the private vegetable/cattle farm failed to attract, or attracted few, beetles respectively. These structures were not placed adjacent to tree lines or hedgerows. The other structures that were successful were placed right next to hedgerows and tree lines that remained green all winter long. Nalepa et al. (2005, Environ. Entomol. 3: 425–431) found that color contrast attracted *H. axyridis* to traps without the input of chemical attractants. Was it the color contrast of the white PVC pipe with the green hedgerow which caused the success of those structures? The optimal placement of these structures within agroecosystems needs to be investigated.

Additional tests with different color pipes and/or chemical attractants should also be conducted to see if they attract more beetles than the white PVC pipes used in this study. Kemp and Cottrell (2015, Environ. Entomol. 44: 1395–1406) found yellow traps were better at attracting *H. axyridis* than other colors tested, including white in the growing season, although color preference shifted to white in the winter. Chemical attractants have been found to be effective in some studies at attracting *H. axyridis* (Alhmedi et al. 2010, Eur. J. Entomol. 107: 541–548; Brown et al. 2006, J. Chem. Ecol. 32: 2489–2499; Chun-li et al. 2019, J. Integr. Agric. 18(4): 873–883; Durieux et al. 2012, J. Insect Physiol. 58: 801–807; Leroy et al. 2012, Insect Sci. 19: 372–382 and 498–506; Verheggen et al. 2007, J. Chem. Ecol. 33:2148–2155). While not used in this study, the use of a chemical attractant should be investigated to determine if it might be useful in helping beetles initially aggregate in these novel overwintering sites.

To prevent *H. axyridis* from becoming a pest, researchers have proposed push-pull-trap or push-pull-store strategies where repellents are used to push the beetles away from unwanted dwellings, colored traps or chemical attractants are used to pull them toward traps, then trapped beetles are either killed or stored in cold storage to be released into cropping systems next season (Riddick et al. 2000, Ann. Entomol. Soc. Am. 93: 1314–1321; Riddick and Aldrich 2004, J. Entomol. Sci. 39: 373–386; Sloggett et al. 2011, BioControl 56: 643–661; Verheggen et al. 2007). Killing of trapped beetles might remove the nuisance, but it also removes the benefits of pest predation of the beetles. Push-pull-trap/store would be more desirable since it removes the nuisance and retains the beetles for their beneficial purpose. Research has shown that cold storage of *H. axyridis* is effective, with high fecundity and fertility of the beetles, when stored up to 8 months (Awad et al. 2013, BioControl 58: 657–666). While the push-pull-trap/store strategy might be effective, the drawback is that it requires inputs of time, money, and energy to trap, store, and then release beetles back into agriculture fields. A better option might be a push-pull-overwinter strategy were repellents push the beetles from unwanted structures, attractants pull them toward constructed overwintering sites, and then beetles are just allowed to overwinter naturally in the artificial structures. Once the best way to deploy these structures is determined, a push-pull-overwinter strategy should be investigated to determine if that would be an effective way of keeping these beetles in agroecosystems and away from human dwellings.

A problem with the structures in this study is that they were constructed of wood due to price and time constraints of construction. Wood structures being buried underground will rot eventually. Now that underground structures have been found to be effective, more permanent structures will be constructed for future research of materials that will not rot when buried. At two sites in January, there was water present

due to several days of rain. Fortunately, the beetles in the structures had been able to move further up in the structures to avoid drowning. However, caution should be used when choosing sites for structures to prevent placing them in low points where water could eventually submerge them in heavy rains, thus, drowning the beetles.

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