

# *Xylosandrus germanus* (Coleoptera: Curculionidae: Scolytinae) Occurrence, Fungal Associations, and Management Trials in New York Apple Orchards

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## Abstract

*Xylosandrus germanus* (Blandford) has caused increasing damage in high-density New York apple orchards since 2013, resulting in tree decline and death. We documented their occurrence and timing in > 50 orchards using ethanol-baited traps from 2014 to 2016. First captures ranged from 48 to 83 degree days (base 10 °C) from 1 January. Captures were numerically higher at the orchard-woods interface than within the orchard interior, but differences were not significant in locations with lower populations. Control using insecticide trunk sprays was tested in potted, waterlogged apple trees placed in orchards and nurseries, and inside wooded areas adjacent to orchards. A verbenone repellent was used in combination with trunk sprays to improve control. Overall, insecticide sprays were inconsistent and marginal in preventing new infestations. Chlorpyrifos significantly reduced infestations versus lambda-cyhalothrin and untreated trees at one location in the 2015 orchard trials, and versus untreated trees at one location in the 2016 nursery trials, but otherwise performed no better than other treatments. The addition of verbenone to either the check or permethrin treatments resulted in significantly fewer attack sites containing brood at one orchard site in 2016. Chlorpyrifos, lambda-cyhalothrin, and permethrin significantly reduced the number of attack sites containing adults compared with untreated trees at one nursery trial location in 2016, but were otherwise ineffective in reducing numbers of trees in other locations and infestation categories. We found several fungal and bacterial species associated with *X. germanus* and its infestation of apples. These microbes likely play a minimal role in apple decline.

**Key words:** scolytine beetles, black stem borer, physiological stress, verbenone, *Fusarium solani*

*Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae: Scolytinae), also known as the black stem borer, is a serious pest in ornamental tree nurseries and landscapes in North America (Oliver and Mannion 2001, Rabaglia et al. 2006, Reding et al. 2010, Ranger et al. 2016a). A native of Asia, it now occurs in central Europe and the United States, having been first documented in New York in greenhouse-grown grape stems (Felt 1932, Weber and

McPherson 1983). Since then, it has become established in much of the United States (Wood 1982, Rabaglia et al. 2006). It has previously been noted as a pest in ornamental nurseries, with a wide host range including oak (*Quercus* spp.), elm (*Ulmus* spp.), maple (*Acer* spp.), beech (*Fagus* spp.), redbud (*Cercis* spp.), hickory (*Carya* spp.), chestnut (*Castanea* spp.), magnolia (*Magnolia* spp.), dogwood (*Cornus* spp.), and black walnut (*Juglans nigra* L.) (Hoffmann 1941,

Weber and McPherson 1983, Ranger et al. 2010). Infestations in apple (*Malus domestica* Borkh.) orchards were first reported in Ohio in 1982 (Hall et al. 1982).

The adult female, which is ~2 mm in length, attacks and bores 1-mm-diameter holes to form galleries in the heartwood of trunks or limbs of stressed, dying, or recently dead trees, including stressed trees that are apparently healthy (Hoffmann 1941, Grégoire et al. 2001, Kühnholz et al. 2001, Oliver and Mannion 2001, Reding et al. 2010). Galleries are excavated by the foundress, and comprise entrance tunnels, brood chambers containing eggs and immatures, and branch tunnels where the young develop; this arrangement accommodates all life stages and developmental processes of the insect's life history. Larvae pass through three instars, and development from egg to the adult stage takes ~30 d (Hoffmann 1941, Schneider and Farrier 1969, Ranger et al. 2016a). The species is bivoltine in New York and overwinters as adults, primarily females, in galleries of its host plants. The galleries are frequently located at the base of the trunk, and can contain dozens of beetles (Weber and McPherson 1983).

*X. germanus* derives nourishment during the larval and adult stages from a mutualistic fungus carried by the adult female in a mycangium, a specialized internal pouch structure located between the pronotum and mesothorax (Francke-Grossman 1967), and introduced into host plants during gallery excavation. The ambrosia fungus associated with *X. germanus* is *Ambrosiella grosmanniae* C. Mayers, McNew & T.C. Harr. (Mayers et al. 2015), visible in the galleries as an abundant grayish-white mycelial growth. The beetles feed directly on this fungal growth, rather than the host plant tissue (Baker and Norris 1968, Weber and McPherson 1984). However, their presence signals the tree that it is under attack, and as the tree walls off its vascular system in response, infestation symptoms develop that include wilting, dieback, tree decline, and death (Buchanan 1941, Weber and McPherson 1983, Oliver and Mannion 2001, Gilrein 2011, Reding et al. 2013a, Ranger et al. 2016a).

Current studies suggest that this species invades from nearby wooded areas to attack stressed trees (Ranger et al. 2013b), but there is relatively little research on movement of *X. germanus* from wooded areas into nurseries or orchards. A variety of stressors, including flooding, drought, and very low temperature exposure have been identified as potential causes of physiological stresses that preferentially attract these beetles (Hoffmann 1941, Kühnholz et al. 2001, Ranger et al. 2013a). Trees under this type of stress produce several types of volatiles, among them ethanol, which has been documented to be a strong attractant to the beetles (Moeck 1970, Montgomery and Wargo 1983, Kühnholz et al. 2001, Ranger et al. 2013a). Ethanol-baited traps have been shown to be useful for monitoring the flight activity of *X. germanus* in ornamental nurseries (Oliver and Mannion 2001, Reding et al. 2010). Reding et al. (2013b) detected no attack or flight activity for *X. germanus* unless temperatures reached at least 20 °C for 1–2 d, with a mean occurrence of first capture in ethanol-baited bottle traps of 76 degree days (DD) (base 10 °C) from 1 January. Ranger et al. (2013a) demonstrated that *X. germanus* preferentially lands on and attacks physiologically stressed hosts, and that ethanol plays a primary role in mediating this interaction.

In commercial ornamental tree nurseries, growers routinely rely on insecticide trunk sprays to prevent new infestation and colonization of trees by *Xylosandrus* spp. adults, but successful control is difficult (Hudson and Mizell 1999, Frank and Sadof 2011, Ranger et al. 2016b). Because of the beetles' small size and cryptic nature, early attack often occurs before the realization that they are active, which results in damage occurring before protective sprays can be

applied. For effective protection against these insects, pesticide applications must be closely timed with insect attack, applied repeatedly, and have long residual activity. Pesticide sprays that are closely timed with beetle activity have been proposed as being the most efficacious, economical, and environmentally sustainable approach in ornamentals plantings (Oliver and Mannion 2001, Mizell and Riddle 2004), although the suitability of this tactic in apple orchards has not previously been shown. A reliable monitoring system would give growers the ability to coordinate any needed control treatments with beetle activity.

Few studies have specifically addressed control of *X. germanus*, but successful control of a related species, *Xylosandrus compactus* (Eichhoff), was achieved in flowering dogwood (*Cornus florida* L.) trees sprayed with chlorpyrifos early in the spring (Mangold et al. 1977). In vitro tests showed high toxicity of chlorpyrifos, bifenthrin, and permethrin against *Xylosandrus crassiusculus* (Motschulsky) (Hale and Oliver 1999). However, Mizell and Riddle (2004) found chlorpyrifos to be less effective against *X. crassiusculus* in dipped hardwood bolts than either bifenthrin or cypermethrin, although no insecticide was completely effective in preventing attacks. Frank and Bambara (2009) describe chlorpyrifos as being largely ineffective against this species. Castrillo et al. (2011) evaluated the entomopathogenic fungus *Metarhizium brunneum* against *X. germanus* adult females, and found that inocula carried into the gallery or produced by the infected parent can significantly impact the developing brood. No insecticide efficacy trials have previously been conducted against *X. germanus* in apple orchards.

In addition to the impact that infestations have on tree health, there is added concern related to the potential for the beetles' ability to vector pathogens. Some affected trees exhibited sap production and, in some instances, fire blight ooze was found issuing from beetle entry holes. Such oozing was found to occur at a height of >2 m on the trunk, indicating the possibility that these insects could be contributing to the spread of this disease, which is caused by the enterobacterial pathogen *Erwinia amylovora* (Burrill). Previous research has identified a symbiosis between *Xylosandrus* spp. beetles and canker-causing *Fusarium solani* (Mart.) Sacc., initiated by a buildup of beetle populations on prunings or dead trees and branches that are allowed to remain in plantings (Baker and Norris 1968, Kessler 1974). Other fungi, including *Cephalosporium* sp. and *Graphium* sp., have also been isolated from these beetles (Baker and Norris 1968, Kühnholz et al. 2001), which could also potentially reduce overall tree health if found in association with beetle attacks. *X. germanus* infestations in apples have previously been reported in association with *E. amylovora*, although the beetles were apparently attracted secondarily to trees weakened by an initial fire blight infection (Hall et al. 1982). Indeed, we found several instances where *E. amylovora* was isolated from surface-sterilized *X. germanus* in orchards where fire blight was active (Tancos et al. 2016).

In 2013, infestations of *X. germanus* were seen for the first time in commercial apple trees in multiple western New York sites (Agnello et al. 2015); some affected trees additionally exhibited symptoms of fire blight. Trunk tissue samples taken that year from one of six orchard sites exhibiting trunk infestation symptoms plus oozing sap confirmed the presence of fire blight at the site of wounding. Moreover, we found one instance where *E. amylovora* was isolated from surface-sterilized *X. germanus* (Tancos et al. 2016). In addition, *Nectria haematococca* [anamorph: *Fusarium solani* (Mart.) Sacc.] was routinely recovered from heartwood around surrounding entry holes and from several beetles in 2014 (K.D.C., unpublished data). By the end of 2013, hundreds of infested trees in

high-density apple plantings were removed during the growing season. To date, numerous additional infestation sites have been documented, extending as far east as Long Island, and it appears that this species of scolytine beetle may have been present in New York apple-growing regions for some years before first being detected, as it now can be found in nearly every orchard showing these types of tree decline symptoms.

In 2014, we initiated trapping and inspection programs in the apple-growing region along western Lake Ontario in New York, and expanded into additional growing areas around the state in 2015–2016, to document the insect's occurrence, distribution, and timing of flight activity. Concurrently, infested trees and beetle specimens were sampled and assessed for the presence of fungal and bacterial pathogens occurring in association with active infestations. In 2015 and 2016, we evaluated a series of preventive trunk treatments for their efficacy in protecting trees in apple orchards and nurseries from attack by *X. germanus*. Here we report the findings from each of these studies.

## Materials and Methods

### Trapping Trials

In 2014, traps were placed in seven commercial apple orchards in Wayne Co. and one orchard in Orleans Co., NY, where trees were showing symptoms of infestation, and from which *X. germanus* adults had previously been collected. These high-density plantings of dwarf trees were bordered by hedgerows and woods, which were assumed to be a source of immigrating beetles. Traps consisted of inverted 1.75-liter plastic juice bottles (Simply Orange Juice Co., Apopka, FL), which had 6- by 10-cm rectangles cut out of each of the sides (Schultz and Doughty 2013), and were baited in the upper portion of the traps with pouch-style dispensers loaded with 10 ml of 95% ethanol having a release rate of 65 mg/d at a constant 30 °C (Standard Release ethanol lures, AgBio, Westminster, CO); ~50 ml of propylene glycol placed in the cap was used as a capture medium. The traps were suspended from 1.2-m-tall metal garden hangers at a height of 1 m; at each site, two traps were placed on an edge of the planting adjacent to a wooded area, and two additional traps were located in the orchard interiors, ~20–30 m from the orchard edge and in proximity to previously attacked trees, to verify their attractiveness and ability to catch adults inside the orchards. Traps were checked one to two times per week starting in mid-April, before maximum temperatures of 20 °C began to occur, and through the summer until 23 September. Beetles trapped were collected, sorted, and identified.

In 2015, traps were placed similarly at a total of 48 orchards around the state, grouped into three general regions by county: Lake Region-West (Lake-W) (Niagara Co., 5; Orleans Co., 11, set out on 27 April); Lake Region-East (Lake-E) (Wayne Co., 14, set out 14–30 April); and Eastern NY (ENY) (Clinton Co., 5; Saratoga Co., 3; Washington Co., 1, set out on 25 May; and Columbia Co., 2; Dutchess Co., 2; Ulster Co., 5, set out on 12 June). Traps used in the Lake-E and ENY regions in 2015 and 2016 were similar in design to the 2014 traps, but instead used 1.9-liter plastic decanter-style bottles (Freund Container & Supply, Chicago, IL), and water plus a few drops of dish soap instead of propylene glycol as a capture medium. Traps were checked weekly until 14 September (Lake-W), 10 September (Lake-E), and 31 August (ENY), and captures recorded as previously described.

In 2016, traps were placed at a total of 43 orchards: Lake-W (Niagara Co., 4; Orleans Co., 12, set out on 18 April); Lake-E

(Wayne Co., 8; Ontario Co., 1, set out on 13–19 April); and ENY (Clinton Co., 5; Essex Co., 1; Saratoga Co., 1; Washington Co., 1, set out on 28 April; and Columbia Co., 2; Dutchess Co., 2; Ulster Co., 5, set out on 19 April). Traps were checked weekly until 30 August (Lake-W), 20 September (Lake-E), and 30 September (ENY), and captures recorded as previously described.

### Characterization of Bacterial and Fungal Pathogens Associated With Infested Heartwood, Blistered Bark, and *X. germanus* Adults

To better understand involvement of pathogenic fungi and bacteria in this tree decline, and the ability of *X. germanus* to vector pathogenic bacteria and fungi, 51 samples of infested apple wood from declining trees were obtained from orchards in Orleans Co. and Wayne Co., NY, in the summer of 2015. Samples consisted of trunks of older (>5 yr) trees and sections of the central leaders from younger (<5 yr) trees. All samples were trimmed to include entry holes and were stored at 4 °C until isolation. Immediately before isolation, the bark from apple samples was disinfested by wiping with 70% ethanol for 3 min. The bark was then removed, and a 5-mm-diameter brass cork borer was used to remove ~3- by 5-mm sections of the woody tissue around the entry holes. These pieces were further surface-sterilized in 70% ethanol for 1 min and then rinsed twice in sterile distilled water. Samples were then plated before plating onto BD Difco Potato Dextrose Agar (PDA; Becton, Dickinson, and Co., Franklin Lakes, NJ) amended with streptomycin and chloramphenicol at 100 ppm to isolate fungi. Samples were also plated on Crosse Goodman medium (CG) (Crosse and Goodman 1973) and King's B medium (KB) (King et al. 1954) to isolate *E. amylovora* and *Pseudomonas* spp., which can be pathogenic to apple.

In addition to the wood tissue around entry holes, isolations were made from 35 samples that had blistered bark filled with white undifferentiated callus tissue around entry holes (Fig. 1). Isolations were made directly from the deposits of callus cells underneath the bark around the entry holes. For these isolations, the surface of the bark was also disinfested by wiping with 70% ethanol for 3 min. After disinfection, the bark was then peeled back and ~1-mm-diameter spheres of the unexposed callus cells were plated on PDA amended with streptomycin and chloramphenicol at 100 ppm, CG, and KB to isolate fungi and bacteria. All isolations were incubated in the dark at 25 °C for fungal medium (PDA) and 28 °C for bacterial medium (CG and KB). In addition to the infested areas, healthy wood near the entry holes was also extracted and plated as a negative control after being surface-sterilized.

For each isolated single fungal colony obtained, two 5-mm plugs were transferred to fresh PDA medium and incubated in the dark at 25 °C. After 1 wk of incubation, isolates were subjected to rDNA-based identification for fungi as previously described (White et al. 1990). Briefly, 200 mg of mycelium was ground in liquid nitrogen for DNA extraction using Omega E. Z. N. A. Plant DNA DS (Omega Bio-tek Inc, Norcross, GA) according to manufacturer's instructions. Extracted DNA was then subjected to polymerase chain reaction (PCR) using primers ITS1 and ITS4 (Table 1). PCR reactions were 25 µl in volume and consisted of 8 µl of H<sub>2</sub>O, 12.5 µl of EmeraldAmp GT PCR Master Mix (Takara, Mountain View, CA), 1 µl each of forward and reverse primer, and 2.5 µl of extracted fungal DNA. Cycling parameters consisted of an initial denaturation of 5 min at 94 °C, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s; this was followed by a final extension at 72 °C for 7 min.



**Fig. 1.** Apple tree samples displaying blistered bark with proliferations of white callus tissue. (A) Section of a central leader of a 1-yr-old apple tree declining from *X. germanus* infestation. (B) Portion of the trunk of a 4-yr-old tree with *X. germanus* entry holes and deposits of callus tissue underneath the epidermis in the young green bark. (C) Microscopic view of the enlarged undifferentiated apple callus cells. Scale bar in (A) and (B) represents 5 cm; the scale bar in (C) represents 150  $\mu\text{m}$ .

PCR products were separated by gel electrophoresis using 1% agarose gels in 1 $\times$  Tris-borate-EDTA buffer (44.5mM Tris-borate and 1mM EDTA, pH 8.0) at 90 V for 60 min. Single-band PCR products were purified for sequencing using a Zymo DNA Clean & Concentrator kit (Zymo Research, Irvine, CA) according to

**Table 1.** Primers used in PCR bacterial and fungal identifications in this study

Primer Name	Sequence 5'-3'	Reference
ITS1	TCCGTAGGTGAACCTGCGG	White et al. 1990
ITS4	TCCTCCGCTTATTGATATGC	White et al. 1990
PsF	GGTCTGAGAGGATGATCAGT	Widmer et al. 1998
PsR	TTACCTCCACCTCGCGGG	Widmer et al. 1998
AJ75	CGCATTACGGGCTTCGCAGAT	McManus and Jones 1994
AJ76	AACCGCCAGGATAGTCGCATA	McManus and Jones 1994

manufacturer's instructions and sequenced at the Cornell Biotechnical Resource Center in Ithaca, NY, using an ABI 3730xl capillary electrophoresis sequencer (Applied Biosystems, Waltham, MA). Identification of rDNA sequence data analysis was accomplished using Basic Local Alignment Search Tool (BLAST) on NCBI GenBank databases.

Single isolated bacterial colonies were subjected to growth on diagnostic medium and to molecular diagnostic tests to identify presence of *E. amylovora* and *Pseudomonas* spp., two common bacterial pathogens of apple, as previously described by Tancos and Cox (2017). In brief, colonies displaying characteristic cratering morphology on CG were considered putative *E. amylovora*, whereas colonies fluorescing on KB were considered putative *Pseudomonas* spp. Following morphological characterization, bacterial samples were prepared for subsequent molecular identification by PCR (Tancos and Cox 2017). Putative *Pseudomonas* spp. and *E. amylovora* colonies were amplified with species-specific primers PsF/R for 16S rRNA and AJ75/76 for the presence of pEA29, respectively (Table 1). Single colonies of bacteria were suspended in 500  $\mu\text{l}$  of H<sub>2</sub>O, and 2.5  $\mu\text{l}$  of the bacterial suspension was used in a 25  $\mu\text{l}$  of PCR reaction identical to that described for fungi above. Cycling parameters were identical to those used for fungi, except the annealing temperature for *Pseudomonas*- and *E. amylovora*-specific primer pairs were 60  $^{\circ}\text{C}$  and 52  $^{\circ}\text{C}$ , respectively. PCR products were separated using gel electrophoresis on 1% agarose gels in 1 $\times$  Tris-borate-EDTA buffer (44.5mM Tris-borate and 1mM EDTA, pH 8.0) at 90 V for 60 min. The presence of a single band in the agarose gel confirmed the identity of putative *Pseudomonas* or *E. amylovora* colonies.

From the 51 apple wood samples, 34 of the wood samples contained *X. germanus* adults. These mature adults were extracted from the same entry holes examined for wood samples described above. To characterize the microflora of *X. germanus*, isolations were made directly from the outer surface of the beetles and from the internal contents following surface sterilization. Single adults of *X. germanus* were vortexed for 20 s at 100 rpm in 100  $\mu\text{l}$  of sterile distilled water. The beetle was then removed from the rinsate, which was plated onto PDA, KB, and CG media as described above. The beetle was surface-sterilized in a 15% bleach solution for 45 s, and washed twice with sterile distilled water. Following sterilizations, the beetle was cut into four pieces and placed gut-side down onto PDA and CG medium with two beetle sections on each medium. Fungal and bacterial colonies that developed on the media were isolated to single colonies. Bacteria isolates were transferred again to KB, and both fungal and bacterial colonies were identified as described above for wood isolations.

**Table 2.** Preventive trunk treatments tested against *X. germanus* attack in apple orchard and nursery trials, 2015–2016.

Trial/Appl. date	Treatment a.i. (trade name)	Rate (milliliter formulated product per liter water)			
Orchard 2015					
–	check	–			
7–8 May	chlorpyrifos (Lorsban 4EC)	3.75			
7–8 May	lambda-cyhalothrin (Warrior II 2.08CS)	0.20			
7–8 May	gamma-cyhalothrin (Declare 1.25EC)	0.16			
Orchard 2016					
–	check	–			
10 May	chlorpyrifos (Lorsban 4EC)	3.75			
10 May	lambda-cyhalothrin+chlorpyrifos (Cobalt 2.5/0.129EC)	3.24			
10 May	permethrin (Perm-Up 3.2EC)	0.78			
10 May	fenprothrin (Danitol 2.4EC)	1.25			
Trial/Appl. date 1	Treatment a.i. (trade name)	Rate (milliliter formulated product per liter water)	Appl. date 2	Treatment	Rate (milliliter formulated product per liter water)
Nursery 2015					
–	check/not flooded				
–	check/flooded				
14 May	chlorpyrifos (Lorsban 4EC)	5.0			
14 May	chlorpyrifos (Lorsban 4EC)	5.0	29 May	permethrin (Perm-Up 3.2EC)	10.0 <sup>a</sup>
14 May	permethrin (Perm-Up 3.2EC)	10.0 <sup>a</sup>	29 May	permethrin (Perm-Up 3.2EC)	10.0 <sup>a</sup>
14 May	lambda-cyhalothrin (Grizzly Z 1EC)	1.58 <sup>a</sup>	29 May	lambda-cyhalothrin (Grizzly Z 1EC) <sup>a</sup>	1.5
14 May	lambda-cyhalothrin (Grizzly Z 1EC)	1.58 <sup>a</sup>	29 May	Keyplex	2.5
14 May	<i>Metarhizium</i> (Met 52EC)	100.0	29 May	<i>Metarhizium</i> (Met 52EC)	100.0
Nursery 2016					
–	check/flooded				
18–19 May	chlorpyrifos (Lorsban 4EC)	3.79			
18–19 May	chlorpyrifos (Lorsban 4EC)	3.79	1–3 Jun	fenprothrin (Danitol 2.4EC)	1.26
18–19 May	chlorpyrifos (Lorsban 4EC)	3.79	1–3 Jun	lambda-cyhalothrin (Grizzly Z 1EC)	0.2
Trial/ Appl. date 1	Treatment	Rate (milliliter formulated product per liter water)	Appl. date 2	Treatment	Rate (milliliter formulated product per liter water)
18–19 May	permethrin (Perm-Up 3.2EC)	10.0 <sup>a</sup>	1–3 Jun	permethrin (Perm-Up 3.2EC)	10.0 <sup>a</sup>
18–19 May	lambda-cyhalothrin (Grizzly Z 1EC)	0.2	1–3 Jun	lambda-cyhalothrin (Grizzly Z 1EC)	0.2
18–19 May	permethrin (Perm-Up 3.2EC)	0.79	1–3 Jun	permethrin (Perm-Up 3.2EC)	0.79
18–19 May	<i>Metarhizium</i> (Met 52EC)	50.0	1–3 Jun	<i>Metarhizium</i> (Met 52EC)	50.0

Appl., Application; a.i., active ingredient.

<sup>a</sup> Based on label rates against bark beetles for ornamentals (Perm-Up) or non-agricultural deciduous nursery trees (Grizzly Z).

## Management Trials

Field trials were conducted in commercial apple orchards and apple nurseries in 2015 and 2016 to evaluate the effectiveness of insecticide sprays in preventing *X. germanus* attacks.

### Orchards

In 2015, the efficacy of insecticide trunk sprays was evaluated against infestations of *X. germanus* in two commercial orchards having documented infestations; the apple varieties in the trial sites were ‘Ginger Gold’ (Sodus, NY) and ‘Paula Red’ (Medina, NY). All treatments were replicated in randomized complete block plots consisting of 10–12 consecutive trees in the 2–3 edge rows adjacent to wooded areas located at each of the trial locations. Potted 2-yr old ‘Mutsu’ trees (height, 2.0 m) on B.118 rootstock (Wafler Nurseries, Wolcott, NY), planted in a pine bark mulch mixture in 11-liter pots, were in turn placed into 19-liter pots that were lined with a plastic contractor bag of 2-mil (0.051 mm) in thickness, which were then flooded to induce stress and promote ethanol production (Ranger et al. 2013a). These pot-in-pot units were placed within the rows between the orchard trees, with five pots per replicate, and four replicates per treatment in each orchard. The trunks of the potted trees plus the orchard trees were sprayed using a Nifty Pul-Tank handgun sprayer (Rears Manufacturing Co., Eugene, OR) on 7–8 May, before the start of significant *X. germanus* flight activity. The treatments, which corresponded to labeled field rates (Table 2), were: chlorpyrifos (Lorsban Advanced, Dow AgroSciences, Indianapolis, IN); lambda-cyhalothrin (Warrior II, Syngenta Crop Protection, Greensboro, NC); gamma-cyhalothrin (Declare, Cheminova, Research Triangle Park, NC); and an untreated check (potted trees only; orchard trees in check plots were sprayed with chlorpyrifos, as above).

Water levels in the pots were checked periodically during the summer, and bags were replaced in any pots where water had been lost from leaks. Treatment efficacy was assessed for evidence of new infestations by a preliminary visual trunk inspection for fresh holes or sawdust on the trees on 8–9 July, after termination of the first flight. A final evaluation of the potted trees was conducted on 12–14 August, at which time trees were destructively sampled by scraping off the bark and dissection in the lab to document total numbers per tree of attack sites (holes), sites with empty galleries, galleries containing live adults, dead adults, and brood.

In 2016, trials again consisted of potted apple trees, which were placed inside wooded areas directly adjacent to orchards with known *X. germanus* infestations rather than within the orchard plantings themselves; the orchards, both located in Wayne Co., were at the same Sodus location as in 2015, and in Wolcott, NY. The same pot-in-pot system was used as in 2015, this time using 2-yr-old ‘Rome Beauty’ trees on M.111 rootstock (Wafler Nurseries) that were flooded to induce stress. In addition, to increase their attractiveness to the beetles and ensure sufficient attacks to adequately evaluate the treatments, individual ethanol lures were attached with a twist tie at a height of ~1 m to each tree trunk. Each lure consisted of a 12-cm length of 2.5-cm (W), 2-mil (0.051 mm) thickness lay-flat low-density polyethylene tubing (Item No. PT20, International Plastics, Greenville, SC), filled with 10 ml of 95% ethanol and sealed at both ends using an impulse sealer (H-190, ULINE, Pleasant Prairie, WI); the average release rate was 87 mg/d at room temperature. On 10 May, the tree trunks were sprayed with the following insecticides (Table 2) using a battery-powered backpack sprayer (416-LI, Solo, Newport News, VA) with a flat fan nozzle (TJ-60, Tee-Jet, Wheaton, IL): chlorpyrifos (Lorsban Advanced, Dow

AgroSciences); chlorpyrifos + lambda-cyhalothrin (Cobalt, Dow AgroSciences); permethrin (Perm-Up, UPI, King of Prussia, PA); fenprothrin (Valent BioSciences, Walnut Creek, CA); plus an untreated check.

Pots were arranged in circular five-pot groups containing one tree of each insecticide treatment, and replicated 10 times in each wooded site. Another identical 10-replicate set of pot groups was also deployed at each site, with a permeable membrane pouch containing 92% verbenone (BeetleBlock-Verbenone; 50 mg/d at 25 °C; AgBio) hung at a height of ~1 m on a garden stake placed in the center of each five-pot group. Each insecticide-only group of pots was paired with an insecticide + verbenone group, with a minimum of 8 m between groups within a pair and between neighboring pairs of pot groups. Water levels in the pots were checked periodically during the summer, similarly to 2015. Half of the 10 replicate pairs of insecticide-only and insecticide + verbenone pot groups were evaluated on 6 July, after the completion of the first adult flight, by dissection in the lab to determine numbers of attack sites and infestation levels, as in 2015, and the remaining trees were similarly evaluated on 19 August.

### Nurseries

In 2015, a trial was conducted using potted “sleeping eye” apple trees (30 cm in height) budded onto Budagovsky 9 rootstock (Wafler Nurseries). Sleeping eye trees were produced by budding and growing the rootstock in the nursery for 1 yr, and then cutting them off above the bud before being shipped for use. The trees were allowed to grow after potting for 2–3 wk, and then moved to two test sites and sprayed with candidate insecticide treatments. The two locations were in Wolcott and Medina, NY. There were four replicates of eight randomly assigned treatments, with four trees per treatment per replicate. Insecticides were applied at 276 kPa (40 psi) with a CO<sub>2</sub> sprayer (R&D Sprayers, Opelousas, LA), using a hand wand with a full cone nozzle to apply 25 ml per tree to cover all the bark surfaces of the nursery trees. A pot-in-pot system was used to induce stress by flooding, as in the orchard trials. The nursery container was placed in a 27-liter pot lined with a plastic bag, which was flooded to induce ethanol production to attract *X. germanus* adults.

The potted trees were placed along a wooded edge west of a nursery planting at Wolcott, and on the southern wooded edge of an established orchard with a known *X. germanus* infestation at Medina. Insecticide treatments consisted of either one (14 May, early during the adult flight period) or two (14 and 29 May) applications, and consisted of the following treatments (Table 2): untreated/not flooded (pots were checked weekly and watered enough to keep the soil moist); untreated/flooded; chlorpyrifos (Lorsban Advanced), permethrin (Perm-Up), lambda-cyhalothrin (Grizzly Z, Winfield Solutions, St. Paul, MN), KeyPlex micronutrient mixture (350DP, KeyPlex, Winter Park, FL), *Metarhizium anisopliae* Strain F52 (Met52 EC, Novozymes Biologicals, Salem, VA). Trees were evaluated for infestations on 23 July (Wolcott) and 29 July (Medina), in the same manner as the orchard trials, by dissecting each tree in the laboratory to document total numbers per tree of attack sites (holes); sites with empty galleries; and galleries containing live adults, dead adults, and brood.

In 2016, the nursery trials used ‘NY-1’ trees grafted onto M9/337 rootstock that were grown in the nursery for 1 yr (1 m in height, 0.5- to 1.0-cm caliper), dug in the fall and put into cold storage. In the spring (28 March–11 April), trees were planted into pine bark mulch mix in tall 15-liter plastic nursery containers. The trees were held in a barnyard at each farm trial site (Albion, NY, and the same

Wolcott nursery site as in 2015), and watered regularly to promote foliar growth. On 18–19 May, trees were transported to the respective test locations, inside the woods on the eastern edge of a woodlot adjacent to a nursery planting at each site. Pots were placed into 27-liter squat nursery containers lined with a plastic contractor bag and flooded to promote stress, as described above. An ethanol lure similar to those used in the 2016 orchard trials was also attached with a twist tie to each tree.

Pots were set in a double row on a heavy plastic strip to prevent weed growth. Eight insecticide treatments, randomly assigned to six-tree plots per treatment and replicated four times, were applied (18–19 May) using a CO<sub>2</sub> sprayer as in 2015. Treatments consisted of: untreated/flooded; chlorpyrifos (Lorsban Advanced); fenprothrin (Danitol); lambda-cyhalothrin (Warrior II); permethrin (Perm-Up); and *Metarhizium* (Met52 EC) (Table 2). Follow-up applications in appropriate treatments followed on 1 and 3 June. Half of the trees were removed from the field on each of two dates, 13 July and 2 August, and dissected in the laboratory to evaluate levels of infestation, as described above.

### Statistical Analysis

Data were analyzed separately by year, trial, and site. Trapping data were fit to a generalized linear model with a Poisson distribution and a log-link, with fixed effects of placement (edge or interior) and trap site (farm) for each sample date. Overdispersion was assessed by looking at the ratio of the residual deviance and degrees of freedom. If this ratio was >1.5 (Zuur et al. 2009), it was corrected using a quasi-GLM (quasi-Poisson) model; all models were fit using R statistical software (R Core Team 2016). A *P*-value of 0.05 was used to test whether there were differences in the edge mean and interior mean using a *z*-statistic for Poisson distributions and a *t*-statistic if a quasi-Poisson model was used; *P* values were corrected using Holm's correction (Holm 1979), which controls the familywise error-rate for multiple comparisons.

For the orchard trials, the 2015 preliminary evaluation data, which were proportions of trees showing new infestations, were modeled as a continuous variable, and means were separated using Tukey's test. For the 2015 final evaluation data and all the 2016 evaluation data, a generalized linear model with a Poisson distribution and a log-link was fit; fixed effects were treatment and evaluation date (both years), and presence of verbenone (2016 only). Overdispersion was corrected as mentioned before using a quasi-GLM model and fit using R. The linear model tested for overall significant treatment differences and pairwise comparisons of least-squares means were analyzed and adjusted for using Tukey's multiple comparison procedure (Lenth 2016).

For the nursery trials, a generalized linear model with a Poisson distribution and a log-link was fit; fixed effects used in the model were treatment (both years) and evaluation date (2016 only), and overdispersion was corrected as above. Pairwise comparisons between all treatments were conducted; where a significant difference was found between the check and another treatment, Dunnett's *P*-value adjustment was applied. A significance level of  $\alpha = 0.05$  was used for all analyses.

## Results

### Trapping Trials

Adult captures in 2014 indicated that the ethanol-baited bottle traps were effective in detecting and monitoring the presence of *X. germanus*, with first sustained flight activity recorded in western New York on 13 May (Fig. 2), corresponding to an accumulation of

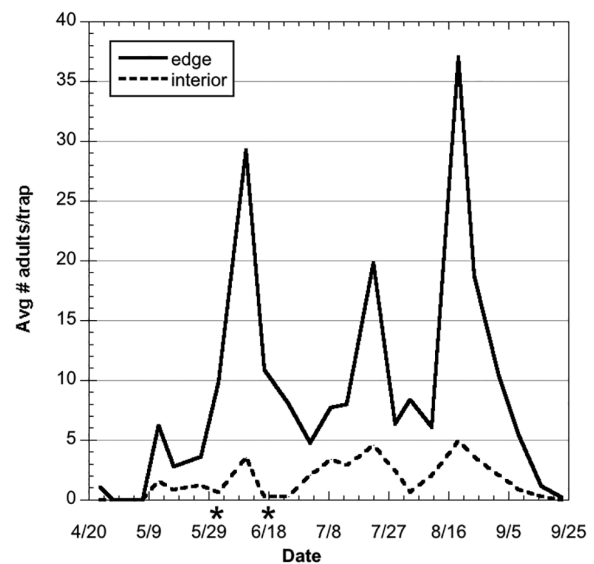


Fig. 2. Captures of *X. germanus* adults in ethanol-baited bottle traps in western New York, 2014; asterisks designate dates on which captures at the orchard edge were significantly higher than those in the interior (generalized linear model,  $P < 0.05$ ).

83–100 DD<sub>10°C</sub> from 1 January among the different trap sites (NYS IPM Program 2009–2017). Peak capture of adults occurred on 11 June, for beetles emerging from overwintering sites in search of new host trees, with the first flight period extending through June. The second flight began in early July, reaching a peak on 20 August, and continued at diminishing levels until mid-September. Mean captures of 2–32X more beetles were captured in block edges adjacent to woods than in the orchard interiors; after correcting for multiple comparisons, differences were found to be significant on 2 June ( $z = -4.696$ ,  $P < 0.001$ ) and 17 Jun ( $z = -4.849$ ,  $P < 0.001$ ) (Fig. 2).

Flight activity in the Lake Ontario Region started somewhat earlier in 2015, with sustained captures seen by 5 May (corresponding to 64–106 DD<sub>10°C</sub> among trap sites). Peak trap numbers were reached on 19 May and 2 June in the Lake-W and Lake-E counties, respectively, with the first flight subsiding regionally by 10 June (Fig. 3). Traps in ENY were not deployed early enough to detect the first adult emergence, but did document the presence of *X. germanus* in a number of orchard locations, including some not exhibiting signs of infestation (Fig. 4). Beetle captures there were much lower overall, with first flight captures subsiding to their lowest levels between 8–23 June. In the Lake Ontario region, traps located on orchard edges caught an average of 1.2–16.2X more adults than those in the orchard interior, with significant differences found in Lake-W on 5 May ( $z = -4.501$ ,  $P < 0.001$ ) and 26 May ( $z = -8.627$ ,  $P = 0.016$ ) plus 17 August ( $z = -4.333$ ,  $P < 0.001$ ); and in Lake-E on 12 May ( $t = -4.896$ ,  $P = 0.015$ ), 19 May ( $t = -7.271$ ,  $P = 0.002$ ), 26 May ( $t = -10.273$ ,  $P = 0.024$ ), 2 June ( $t = -14.216$ ,  $P = 0.017$ ), 10 June ( $z = -4.459$ ,  $P < 0.001$ ), 16 June ( $z = -4.554$ ,  $P < 0.001$ ), and 22 June ( $z = -4.389$ ,  $P < 0.001$ ), and 4 August ( $t = -6.207$ ,  $P = 0.012$ ), 17 August ( $z = -4.079$ ,  $P = 0.001$ ), and 24 August ( $z = -2.821$ ,  $P = 0.043$ ). The same trend was seen in ENY on most, but not all, sample dates, as the ratio of edge:interior trap numbers ranged from 0.8–12; differences were significant on 1 June ( $z = -3.376$ ,  $P = 0.013$ ) and 6 July ( $z = -3.125$ ,  $P = 0.030$ ).

In 2016, beetle activity began at a continuous but low level on 19 April statewide, although sustained captures did not occur until 15–17 May (corresponding to 50–65 and 48–109 DD<sub>10°C</sub> among

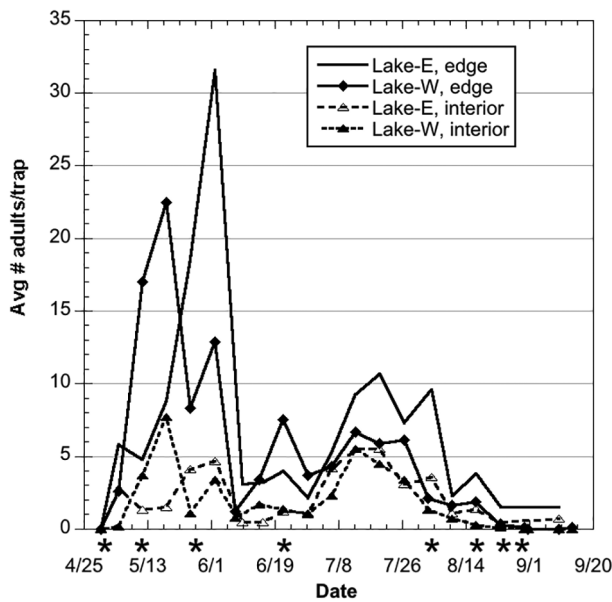


Fig. 3. Captures of *X. germanus* adults in ethanol-baited bottle traps in western New York, 2015; asterisks designate dates on which captures at the orchard edge were significantly higher than those in the interior (generalized linear model,  $P < 0.05$ ).

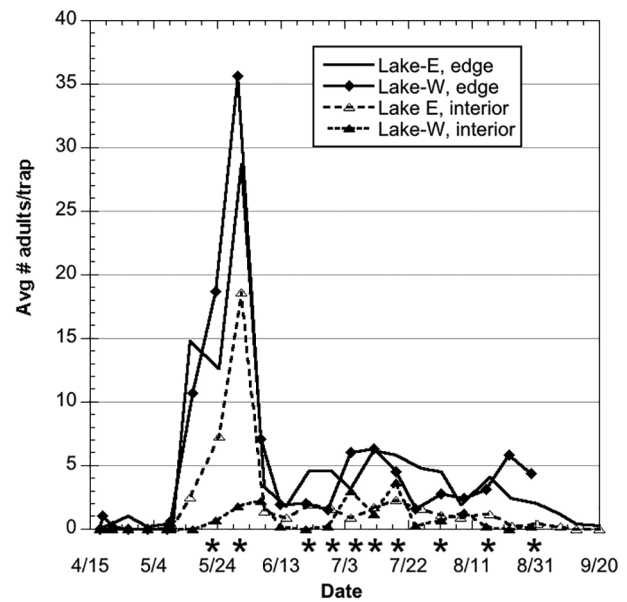


Fig. 5. Captures of *X. germanus* adults in ethanol-baited bottle traps in western New York, 2016; asterisks designate dates on which captures at the orchard edge were significantly higher than those in the interior (generalized linear model,  $P < 0.05$ ).

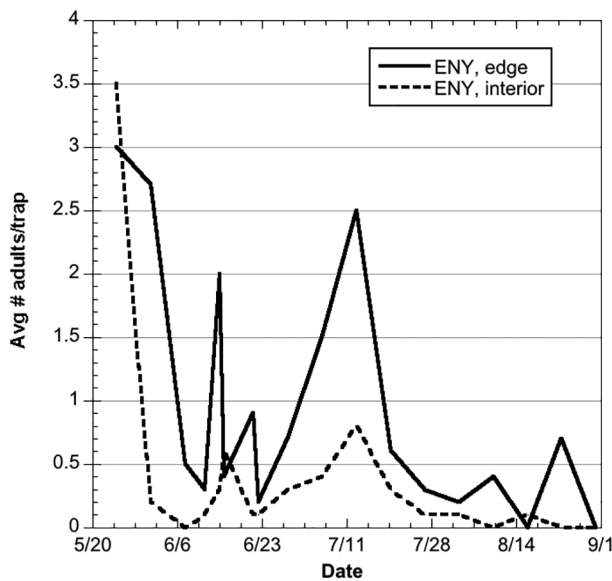


Fig. 4. Captures of *X. germanus* adults in ethanol-baited bottle traps in eastern New York, 2015.

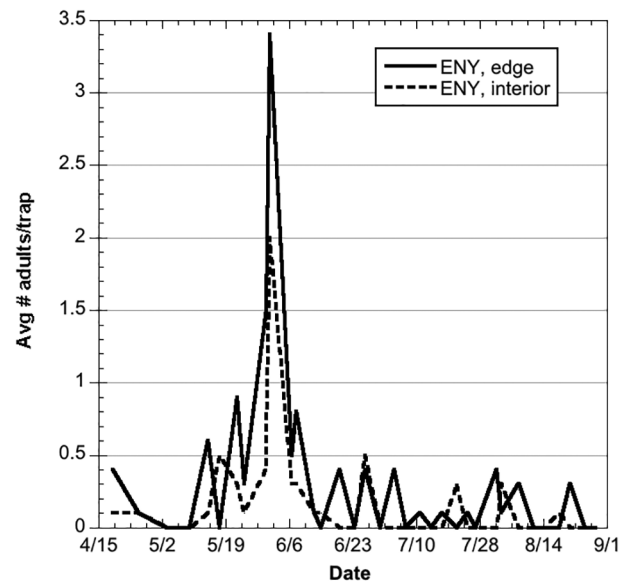


Fig. 6. Captures of *X. germanus* adults in ethanol-baited bottle traps in eastern New York, 2016.

trap sites in the Lake Ontario and ENY regions, respectively) (Figs. 5–6). The first flight, which peaked in the Lake Ontario counties on 31 May and 1 Jun in ENY, subsided by 13–15 June statewide. Trends similar to 2015 in the ratios of orchard edge to interior adult captures were seen in the respective regions, with a range of 1.5–28X as many edge captures in the Lake Ontario counties; significant differences were found in Lake-W on 24 May ( $z = -6.874$ ,  $P < 0.001$ ) and 31 May ( $t = -9.895$ ,  $P = 0.023$ ), 16 August ( $z = -2.975$ ,  $P = 0.044$ ) and 30 August ( $z = -3.023$ ,  $P = 0.040$ ); and in Lake-E on 22 June ( $z = -3.192$ ,  $P = 0.028$ ), 29 June ( $z = -3.956$ ,  $P = 0.002$ ), 5 July ( $z = -3.125$ ,  $P = 0.034$ ), 19 July ( $z = -3.678$ ,  $P = 0.005$ ), 27 July ( $z = -3.779$ ,  $P = 0.003$ ,

24 August ( $z = -3.023$ ,  $P = 0.044$ ), and 1 September ( $z = -3.028$ ,  $P = 0.044$ )). Ratios of 0.5–4.0 edge:interior captures in ENY were obtained, but differences were not significantly different on any date.

#### Bacterial and Fungal Associations

From the 51 apple samples, eight fungal species were consistently isolated from infested wood surrounding the entry hole. These included *Ambrosiella xylebori*, *Nectria haematococca*, *Alternaria alternata*, *Fusarium acuminatum*, *Trichoderma gamsii*, *Ophiostoma quercii*, *Cytospora sophorae*, and *Paraconiothyrium brasiliense*.



Of the eight fungal species recovered, *A. xylebori* and *N. haematococca* were the most abundant (Fig. 7). No fungi were recovered from any of the 51 samples from the healthy wood adjacent to the entry holes. No bacteria were recovered from any of the wood samples. By comparison, bacterial colonies were recovered from the callus deposits of 31 of the 35 samples with blistered bark (Fig. 1). Fluorescent *Pseudomonas* spp. were obtained from 16 of the blistered bark samples, whereas miscellaneous bacteria species of no known pathogenic consequence in apple were recovered from the remaining 15 samples. Interestingly, *E. amylovora* was not recovered in any of the callus deposits underneath blistered bark. Fungi were isolated from only four blistered samples. Of the fungi recovered, *Al. alternata*, *Cytospora sophorae*, and *Paraconiothyrium brasiliense* were found in two, one, and one blisters, respectively.

From the surfaces of *X. germanus* adults, fungi were isolated from 11 and bacteria were isolated from 17 beetles. No fungal or bacterial colonies were isolated from the surface of the six remaining beetles. Of the bacteria recovered, none of the isolates were *Pseudomonas* spp., *E. amylovora*, or any other species potentially pathogenic to apple. From the 11 adults from which fungi were recovered, a total of five fungal species were isolated. These included *A. xylebori*, *N. haematococca*, *A. alternata*, *F. acuminatum*, and *O. querci*. Many of the fungi isolated from the beetle surfaces were the same as those isolated from the wood at the entry holes. Of the fungi isolated, *N. haematococca* (*Fusarium solani*) was the most frequently recovered, while *A. xylebori* was isolated from the surface of only one adult (Fig. 7B).

From the internal contents of *X. germanus* adults, fungi were recovered from 11 beetles and bacterial colonies from 14 beetles. The internal contents of the remaining 11 beetles yielded no fungal or bacterial colonies. As with the surface isolations from *X. germanus*, none of the bacterial colonies were *Pseudomonas* spp., *E. amylovora*, or any other species potentially pathogenic to apple. Of the 11 beetles where fungal colonies were obtained from internal contents, only *N. haematococca* and *A. xylebori* were recovered. Of the two species, *N. haematococca* was the most frequently recovered, with *A. xylebori* only being recovered from the same beetle where the fungus was also recovered from the surface (Fig. 7C).

## Management Trials Orchards, 2015

In the preliminary evaluations, conducted 8–9 July, the efficacy of the trunk sprays in the potted trees was not consistent between the two locations, with no statistical difference in percent infested trees among treatments at the Sodus farm, but significantly fewer infested potted trees treated with chlorpyrifos than in the check plots ( $F=4.955$ ,  $df=3, 9$ ;  $P=0.0267$ ) at the Medina farm (Table 3). There were no significant treatment differences in percent infested trees in the established orchard trees at either farm; no untreated check was included in the orchard tree treatments because of grower concerns over leaving any trees without a preventive trunk spray. Results of the final evaluations on 12–14 August also varied between farms (Table 3). At Sodus, no significant differences among treatments were seen in any of the infestation categories. At Medina, trees in the chlorpyrifos plots had fewer attack sites (chi-square = 9.5864,  $df=3$ ;  $P=0.0224$ ) and occurrences of empty galleries (chi-square = 18.794,  $df=3$ ;  $P<0.001$ ) than those treated with lambda-cyhalothrin, but neither differed significantly from the untreated check.

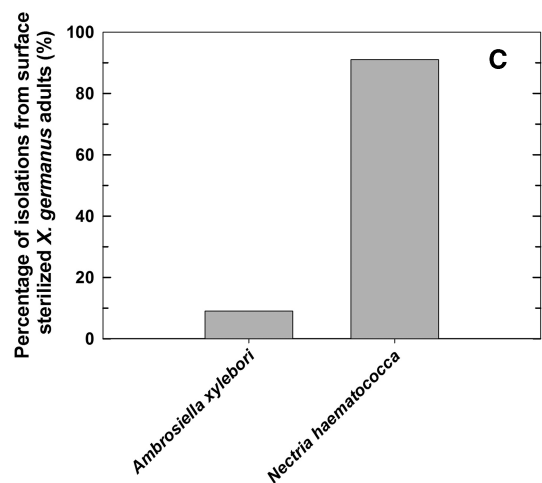
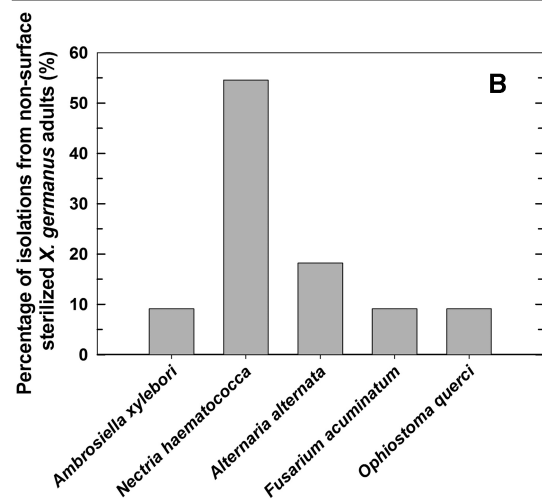
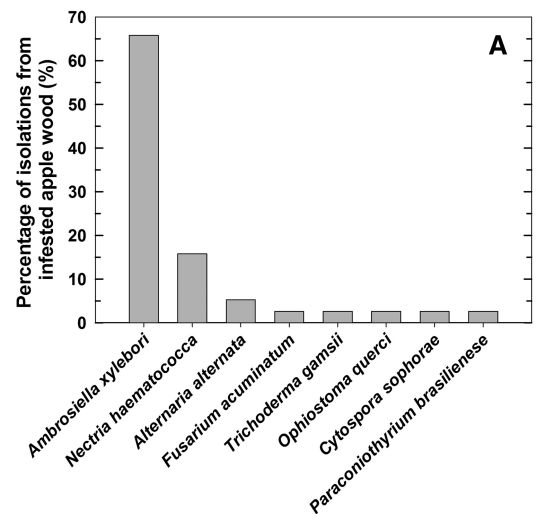


Fig. 7. Bar charts displaying the relative abundance of the fungal species isolated from infested wood or *X. germanus*. (A) The relative abundance the fungal species recovered from infested wood of the 51 apple samples. The relative abundance the fungal species recovered from the surface (B) and the internal contents (C) of the 34 *X. germanus* adults recovered from apple samples.

**Table 3.** Mean ( $\pm$  SE) infestation levels by *X. germanus* in apple trees at two locations, on two evaluation dates, after trunk application of a preventive insecticide, 2015.

Treatment	8–9 July evaluation <sup>a</sup>		12–14 Aug. evaluation <sup>b</sup>				
	% infested trees		Mean no. attack sites/tree	Mean no. sites/tree containing			
	Potted	Orchard		Empty galleries	Brood	Live adults	Dead adults
<b>Sodus</b>							
Check	35.0 $\pm$ 12.5	–	2.3 $\pm$ 0.8	1.3 $\pm$ 0.5	0.3 $\pm$ 0.2	0.5 $\pm$ 0.2	0.1 $\pm$ 0.1
Lambda-cyhalothrin	15.0 $\pm$ 9.6	75.0 $\pm$ 9.6	1.0 $\pm$ 0.6	0.8 $\pm$ 0.4	0.1 $\pm$ 0.1	0.1 $\pm$ 0.2	0.1 $\pm$ 0.1
Gamma-cyhalothrin	30.0 $\pm$ 5.8	75.0 $\pm$ 9.6	1.0 $\pm$ 0.3	0.9 $\pm$ 0.3	0.3 $\pm$ 0.2	0.4 $\pm$ 0.2	0.1 $\pm$ 0.1
Chlorpyrifos	40.0 $\pm$ 11.5	87.5 $\pm$ 5.0	1.3 $\pm$ 0.6	0.9 $\pm$ 0.5	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1	0.1 $\pm$ 0.1
<b>Medina</b>							
Check	45.0 $\pm$ 9.6a	–	1.7 $\pm$ 0.6ab	1.0 $\pm$ 0.3ab	0.3 $\pm$ 0.1	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0
Lambda-cyhalothrin	35.0 $\pm$ 9.6ab	50.0 $\pm$ 12.9	2.3 $\pm$ 0.3a	1.5 $\pm$ 0.2a	0.3 $\pm$ 0.1	0.1 $\pm$ 0.0	0.2 $\pm$ 0.1
Gamma-cyhalothrin	20.0 $\pm$ 8.2ab	45.0 $\pm$ 12.6	1.0 $\pm$ 0.4ab	0.5 $\pm$ 0.3ab	0.1 $\pm$ 0.1	0.0	0.0
Chlorpyrifos	5.0 $\pm$ 5.0b	27.5 $\pm$ 9.6	0.4 $\pm$ 0.6b	0.2 $\pm$ 0.3b	0.1 $\pm$ 0.2	0.0 $\pm$ 0.1	0.0

<sup>a</sup> Within a location, means in a column followed by the same letter are not significantly different (Tukey's test,  $P < 0.05$ ).

<sup>b</sup> potted trees only; within a location, means in a column followed by the same letter are not significantly different (chi-square,  $P < 0.05$ ).

### Orchards, 2016

Results on both evaluation dates at Wolcott (Table 4) showed no statistical differences between the insecticide-alone or insecticide + verbenone treatments in any of the infestation categories. There was a significant effect of evaluation date for the number of attack sites (higher on 19 August, chi-square = 37.898,  $df = 1$ ;  $P = 0.0246$ ) and the number of sites containing empty galleries (higher on 19 August, chi-square = 49.524,  $df = 1$ ;  $P < 0.001$ ) and brood (higher on 6 July, chi-square = 25.116,  $df = 1$ ;  $P < 0.001$ ). At Sodus (Table 5), results on 6 July showed no statistical differences between the insecticide-alone or insecticide + verbenone treatments in any of the infestation categories. However, on the 19 August evaluation date, the addition of verbenone to either the check or permethrin treatments resulted in significantly fewer attack sites containing brood ( $F = 8.4809$ ,  $df = 4, 6$ ;  $P = 0.0120$ ). In addition, there was a significant effect of evaluation date for the number of attack sites (higher on 19 August, chi-square = 37.898,  $df = 1$ ;  $P = 0.0246$ ), number of sites containing empty galleries (higher on 19 August, chi-square = 115.05,  $df = 1$ ;  $P < 0.001$ ), brood (lower on 19 August, chi-square = 13.617,  $df = 1$ ;  $P = 0.0190$ ), and live adults (higher on 19 August, chi-square = 65.619,  $df = 1$ ;  $P < 0.001$ ).

### Nurseries, 2015

No significant differences at either nursery location were found among any of the treatments in the different infestation categories: number of attack sites (holes), or sites containing empty galleries, brood, dead adults, or live adults (Table 6). The *Metarhizium*-treated trees had few attacks overall, but the trees suffered severe phytotoxicity in the form of burned leaf tissue, from either the formulation or concentration of the product as applied (1:10 v/v dilution, Table 2), making this an unacceptable treatment option.

### Nurseries, 2016

No significant differences among treatments were found at Albion on 13 July, but on the 2 August evaluation date, the single-application chlorpyrifos ( $z = 2.677$ ;  $P = 0.0438$ ) and two-application permethrin-high rate ( $z = 2.677$ ;  $P = 0.0438$ ) treatments had significantly fewer attack sites per tree than the flooded check treatment (Table 7). In addition, the number of attack sites

containing adults on 2 August was lower in the treatments receiving a single application of chlorpyrifos ( $z = 3.156$ ;  $P = 0.0102$ ), sequential applications of chlorpyrifos followed by lambda-cyhalothrin ( $z = 3.403$ ;  $P = 0.0044$ ), or two applications of lambda-cyhalothrin ( $z = 3.156$ ;  $P = 0.0102$ ) or the low rate of permethrin ( $z = 3.437$ ;  $P = 0.0039$ ), than in the flooded check (Table 7). At Wolcott, there were no significant differences among treatments on either evaluation date (Table 8). No phytotoxicity was noted in the *Metarhizium* treatments, which was applied using a lower (1:20 v/v) dilution rate this year (Table 2).

## Discussion

The start of *X. germanus* flight activity in New York apple plantings each season appeared to be similar across the state's different climatic regions, according to our trapping results, and comparable to seasonal timings reported in other states such as Ohio (Reding et al. 2013b) and Tennessee (Oliver and Mannion 2001). First captures of adults in ethanol-baited bottle traps ranged from 48–83 DD<sub>10°C</sub> from 1 January over the course of our study, which is consistent with the 76 DD reported by Reding et al. (2013b), who noted that no flight activity was detected during a monitoring period unless there had been 1–2 d with temperatures of at least 20°C. In New York, the date of first sustained capture ranged from 5–17 May across the state during this period. Although captures of adults were routinely numerically higher in traps placed at the orchard-woods interface than those located within the orchard interior, differences tended not to be significant in locations with lower populations, such as in the ENY orchards, where peak numbers never exceeded three to four per trap on a weekly basis (Figs. 4, 6). Werle et al. (2015) noted that trap position could play an important role in trapping efficacy, finding that numbers of scolytine beetles captured were greatest 13 m inside the forest habitat adjacent to tree nurseries, and progressively decreased proceeding to the nursery edge and interior. They identified the nursery-forest interface as an optimal trap location, where a ring of traps so placed might be capable of protecting tree crops, by intercepting dispersing females from reaching the nursery interior.

We trapped greater numbers of *X. germanus* adults during the first flight in the spring, and therefore concentrated our management

**Table 4.** Mean ( $\pm$  SE) infestation levels by *X. germanus* in potted apple trees on two evaluation dates, after a trunk application of a preventive insecticide, with and without a verbenone repellent (Wolcott, 2016).

Evaluation date <sup>a</sup> /Treatment	No. attack sites/tree	No. sites/tree containing			
		Empty galleries	Brood	Live adults	Dead adults
<b>6 July</b>					
Check, no verbenone	6.2 $\pm$ 2.1	1.8 $\pm$ 0.9	0.4 $\pm$ 0.4	1.0 $\pm$ 0.4	0.4 $\pm$ 0.2
Check + verbenone	4.6 $\pm$ 2.7	1.2 $\pm$ 0.6	1.0 $\pm$ 0.8	0.8 $\pm$ 0.8	0.6 $\pm$ 0.6
Chlorpyrifos/lambda-cyhalothrin, no verbenone	6.2 $\pm$ 2.7	0.2 $\pm$ 0.2	1.2 $\pm$ 0.8	1.0 $\pm$ 0.5	1.8 $\pm$ 1.1
Chlorpyrifos/lambda-cyhalothrin + verbenone	6.6 $\pm$ 2.9	1.6 $\pm$ 1.0	1.4 $\pm$ 0.7	1.0 $\pm$ 0.8	0.8 $\pm$ 0.5
Fenprothrin, no verbenone	9.6 $\pm$ 2.9	3.0 $\pm$ 2.3	2.0 $\pm$ 1.3	1.0 $\pm$ 0.4	1.2 $\pm$ 0.7
Fenprothrin + verbenone	5.4 $\pm$ 1.4	1.4 $\pm$ 0.9	0.6 $\pm$ 0.4	0.2 $\pm$ 0.2	1.2 $\pm$ 0.6
Chlorpyrifos, no verbenone	9.6 $\pm$ 5.8	0.4 $\pm$ 0.4	1.6 $\pm$ 1.1	0.8 $\pm$ 0.6	2.2 $\pm$ 1.4
Chlorpyrifos + verbenone	11.6 $\pm$ 4.1	1.0 $\pm$ 1.0	2.4 $\pm$ 1.0	1.2 $\pm$ 1.2	2.8 $\pm$ 0.6
Permethrin, no verbenone	10.8 $\pm$ 3.9	2.0 $\pm$ 2.0	2.4 $\pm$ 1.1	1.4 $\pm$ 1.0	1.8 $\pm$ 0.9
Permethrin + verbenone	11.8 $\pm$ 4.8	0.0	1.4 $\pm$ 0.5	2.6 $\pm$ 1.0	1.2 $\pm$ 0.7
<b>19 Aug.</b>					
Check, no verbenone	18.0 $\pm$ 4.9	2.4 $\pm$ 0.8	1.2 $\pm$ 0.6	4.4 $\pm$ 2.1	2.6 $\pm$ 0.9
Check + verbenone	11.4 $\pm$ 5.4	2.2 $\pm$ 1.0	0.0	0.2 $\pm$ 0.2	2.6 $\pm$ 1.4
Chlorpyrifos/lambda-cyhalothrin, no verbenone	10.8 $\pm$ 1.9	4.2 $\pm$ 0.9	0.4 $\pm$ 0.2	0.4 $\pm$ 0.2	2.0 $\pm$ 0.8
Chlorpyrifos/lambda-cyhalothrin + verbenone	6.4 $\pm$ 3.2	2.0 $\pm$ 0.8	0.4 $\pm$ 0.2	1.0 $\pm$ 0.6	0.8 $\pm$ 0.5
Fenprothrin, no verbenone	14.8 $\pm$ 5.0	3.4 $\pm$ 1.7	1.0 $\pm$ 0.6	1.6 $\pm$ 0.7	1.4 $\pm$ 0.5
Fenprothrin + verbenone	11.8 $\pm$ 4.5	3.6 $\pm$ 1.0	0.2 $\pm$ 0.2	1.4 $\pm$ 1.2	1.0 $\pm$ 0.4
Chlorpyrifos, no verbenone	17.0 $\pm$ 6.5	6.0 $\pm$ 1.9	0.4 $\pm$ 0.4	1.6 $\pm$ 1.4	3.4 $\pm$ 1.9
Chlorpyrifos + verbenone	7.2 $\pm$ 4.7	2.0 $\pm$ 0.8	0.4 $\pm$ 0.4	1.4 $\pm$ 1.2	0.2 $\pm$ 0.2
Permethrin, no verbenone	7.2 $\pm$ 4.3	3.0 $\pm$ 2.8	0.2 $\pm$ 0.2	0.8 $\pm$ 0.6	1.0 $\pm$ 0.6
Permethrin + verbenone	17.0 $\pm$ 3.6	4.8 $\pm$ 1.6	0.6 $\pm$ 0.2	1.2 $\pm$ 0.4	3.2 $\pm$ 0.6

<sup>a</sup> Significant main effect of evaluation date (chi-square,  $P < 0.05$ ): higher on 6 July for no. sites containing brood, and higher on 19 Aug. for no. attack sites and no. sites containing empty galleries.

**Table 5.** Mean ( $\pm$  SE) infestation levels by *X. germanus* in potted apple trees on two evaluation dates, after a trunk application of a preventive insecticide, with and without a verbenone repellent (Sodus, 2016).

Evaluation date <sup>a</sup> /Treatment	No. attack sites/tree	no. sites/tree containing			
		Empty galleries	Brood <sup>b</sup>	Live adults	Dead adults
<b>6 Jul</b>					
Check, no verbenone	13.0 $\pm$ 4.9	1.6 $\pm$ 0.8	2.4 $\pm$ 1.3	0.0	4.0 $\pm$ 1.4
Check + verbenone	11.6 $\pm$ 6.3	0.6 $\pm$ 0.6	2.0 $\pm$ 1.1	0.4 $\pm$ 0.2	4.4 $\pm$ 2.8
Chlorpyrifos/lambda-cyhalothrin, no verbenone	7.0 $\pm$ 3.4	1.0 $\pm$ 0.5	1.2 $\pm$ 0.6	0.2 $\pm$ 0.2	1.6 $\pm$ 0.7
Chlorpyrifos/lambda-cyhalothrin + verbenone	7.6 $\pm$ 2.7	1.4 $\pm$ 0.7	2.4 $\pm$ 1.2	0.0	2.8 $\pm$ 1.4
Fenprothrin, no verbenone	9.0 $\pm$ 2.7	0.0	1.2 $\pm$ 0.6	0.0	3.6 $\pm$ 1.2
Fenprothrin + verbenone	5.4 $\pm$ 1.2	0.0	0.6 $\pm$ 0.2	0.4 $\pm$ 0.2	1.0 $\pm$ 0.8
Chlorpyrifos, no verbenone	8.6 $\pm$ 4.1	0.6 $\pm$ 0.6	1.2 $\pm$ 0.7	0.6 $\pm$ 0.6	3.4 $\pm$ 2.1
Chlorpyrifos + verbenone	11.4 $\pm$ 3.5	0.4 $\pm$ 0.4	4.6 $\pm$ 2.0	0.0	4.2 $\pm$ 2.0
Permethrin, no verbenone	14.4 $\pm$ 5.0	1.6 $\pm$ 0.7	1.8 $\pm$ 0.5	0.2 $\pm$ 0.2	3.4 $\pm$ 1.4
Permethrin + verbenone	11.0 $\pm$ 1.9	0.8 $\pm$ 0.4	2.2 $\pm$ 0.2	0.4 $\pm$ 0.4	2.4 $\pm$ 1.3
<b>19 Aug</b>					
Check, no verbenone	17.0 $\pm$ 7.5	4.4 $\pm$ 1.7	1.4 $\pm$ 1.4	2.6 $\pm$ 1.5	1.8 $\pm$ 1.1
Check + verbenone	11.0 $\pm$ 3.9	3.8 $\pm$ 7.2	1.2 $\pm$ 0.5*	1.8 $\pm$ 0.9	1.2 $\pm$ 0.4
Chlorpyrifos/lambda-cyhalothrin, no verbenone	13.4 $\pm$ 2.2	4.8 $\pm$ 1.9	1.0 $\pm$ 0.8	1.8 $\pm$ 0.5	1.6 $\pm$ 0.2
Chlorpyrifos/lambda-cyhalothrin + verbenone	8.6 $\pm$ 2.6	2.4 $\pm$ 0.9	0.6 $\pm$ 0.6	1.0 $\pm$ 0.8	0.6 $\pm$ 0.4
Fenprothrin, no verbenone	5.8 $\pm$ 1.7	1.0 $\pm$ 0.6	0.2 $\pm$ 0.2	1.0 $\pm$ 0.6	2.2 $\pm$ 1.0
Fenprothrin + verbenone	17.4 $\pm$ 1.1	6.0 $\pm$ 1.5	1.4 $\pm$ 0.6	2.8 $\pm$ 1.2	1.4 $\pm$ 0.5
Chlorpyrifos, no verbenone	13.6 $\pm$ 6.6	2.4 $\pm$ 1.7	1.6 $\pm$ 0.8	2.8 $\pm$ 1.4	4.6 $\pm$ 2.3
Chlorpyrifos + verbenone	10.6 $\pm$ 4.6	3.8 $\pm$ 1.8	0.2 $\pm$ 0.2	1.6 $\pm$ 0.9	0.8 $\pm$ 0.5
Permethrin, no verbenone	17.2 $\pm$ 9.0	3.4 $\pm$ 1.7	2.4 $\pm$ 1.5	1.6 $\pm$ 1.1	3.0 $\pm$ 1.7
Permethrin + verbenone	22.8 $\pm$ 5.6	7.2 $\pm$ 3.4	0.6 $\pm$ 0.2*	3.0 $\pm$ 0.9	3.8 $\pm$ 1.0

<sup>a</sup> Significant main effect of evaluation date (chi-square,  $p < 0.05$ ): on 6 July, higher no. sites containing brood; on 19 Aug., higher no. attack sites and sites containing empty galleries and live adults.

<sup>b</sup> Asterisks denote treatments with significantly fewer sites containing brood from addition of verbenone (Tukey's test,  $P < 0.05$ ).

**Table 6.** Mean ( $\pm$  SE) infestation levels by *X. germanus* in potted apple trees at two nurseries after one or two trunk applications of preventive insecticides, 2015.

Treatment/Location	No. attack sites/tree	No. sites/tree containing			
		Empty galleries	Brood	Live adults	Dead adults
Wolcott					
1 Check-not flooded	0.0	0.0	0.0	0.0	0.0
2 Check-flooded	0.9 $\pm$ 0.5	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1
3 Chlorpyrifos	0.1 $\pm$ 0.1	0.0	0.0	0.0	0.0
4 Chlorpyrifos/permethrin	0.1 $\pm$ 0.1	0.0	0.0	0.0	0.0
5 Permethrin (2 apps)	0.6 $\pm$ 0.3	0.0	0.0	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1
6 Lambda-cyhalothrin (2 apps)	1.0 $\pm$ 0.3	0.1 $\pm$ 0.1	0.0	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1
7 Lambda-cyhalothrin/Keyplex	0.4 $\pm$ 0.2	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.4 $\pm$ 0.3	0.2 $\pm$ 0.1
8 <i>Metarhizium</i> (2 apps)	0.1 $\pm$ 0.1	0.0	0.0	0.0	0.0
Medina					
1 Check-not flooded	0.0	0.0	0.0	0.0	0.0
2 Check-flooded	0.0	0.0	0.0	0.0	0.0
3 Chlorpyrifos	0.3 $\pm$ 0.2	0.0	0.0	0.0	0.0
4 Chlorpyrifos/permethrin	0.0	0.0	0.0	0.0	0.0
5 Permethrin (2 apps)	0.4 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.0
6 Lambda-cyhalothrin (2 apps)	0.1 $\pm$ 0.1	0.0	0.0	0.0	0.0
7 Lambda-cyhalothrin/Keyplex	0.3 $\pm$ 0.3	0.3 $\pm$ 0.2	0.3 $\pm$ 0.2	0.1 $\pm$ 0.1	0.2 $\pm$ 0.2
8 <i>Metarhizium</i> (2 apps)	0.0	0.0	0.0	0.0	0.0

apps, applications.

**Table 7.** Mean ( $\pm$  SE) infestation levels by *X. germanus* in potted apple trees at a nursery on two evaluation dates, after one or two applications of preventive insecticides (Albion, 2016).

Treatment/Evaluation date	No. attack sites/tree <sup>a</sup>	No. sites/tree containing		
		Empty galleries	Brood	Adults <sup>a</sup>
13 Jul				
1 Check, flooded	0.5 $\pm$ 0.2	0.5 $\pm$ 0.2	0.1 $\pm$ 0.1	0.8 $\pm$ 0.5
2 Chlorpyrifos	0.3 $\pm$ 0.1	0.1 $\pm$ 0.1	0.0	0.3 $\pm$ 0.3
3 Chlorpyrifos/fenprothrin	0.1 $\pm$ 0.1	0.0	0.0	0.0
4 Chlorpyrifos/lambda-cyhalothrin	0.1 $\pm$ 0.1	0.0	0.0	0.0
5 Permethrin, high rate (2 apps)	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.0	0.0
6 Lambda-cyhalothrin (2 apps)	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	0.0	0.0
7 Permethrin, low rate (2 apps)	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	0.1 $\pm$ 0.1	1.4 $\pm$ 1.1
8 <i>Metarhizium</i> (2 apps)	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.0	0.1 $\pm$ 0.1
2 Aug				
1 Check, flooded	1.3 $\pm$ 0.6	0.8 $\pm$ 0.3	0.3 $\pm$ 0.1	2.1 $\pm$ 1.4
2 Chlorpyrifos	0.2 $\pm$ 0.2*	0.2 $\pm$ 0.2	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1*
3 Chlorpyrifos/fenprothrin	0.0	0.0	0.0	0.0
4 Chlorpyrifos/lambda-cyhalothrin	0.8 $\pm$ 0.3	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.3 $\pm$ 0.3*
5 Permethrin, high rate (2 apps)	0.2 $\pm$ 0.1*	0.0	0.0	0.0
6 Lambda-cyhalothrin (2 apps)	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	< 0.1 $\pm$ 0.1*
7 Permethrin, low rate (2 apps)	0.3 $\pm$ 0.2	0.2 $\pm$ 0.2	0.1 $\pm$ 0.1	0.2 $\pm$ 0.2*
8 <i>Metarhizium</i> (2 apps)	0.0	0.0	0.0	0.0

apps, applications.

<sup>a</sup> Asterisks denote treatments significantly different from Check on 2 Aug. (Dunnnett's adjustment,  $P < 0.05$ ).

trials against this generation. Timing of tree attacks by *X. germanus* and *X. crassiusculus* has been reported to be closely related to spring trap collections (Oliver and Mannion 2001, Mizell and Riddle 2004), and first-generation *X. germanus* has been found to be more damaging than the second generation in previous studies (Heidenreich 1960, Mizell et al. 1998), so this has been proposed as the preferred timing for chemical control efforts (Mizell and Riddle 2004, Frank and Sadof 2011).

The results of our investigations of microbial associations indicated that several fungal species are associated with *X. germanus* and its infestations of apple wood. However, fungal species like *Al. alternata*, *T. gamsii*, and *P. brasiliense* have not been reported as pathogens of apple or woody perennials in general. Although both *A. xylebori* and *N. haematococca* were primarily isolated from wood galleries, the consequences of cultivation or association with either of these fungi in regards to tree health and decline is still

**Table 8.** Mean ( $\pm$  SE) infestation levels by *X. germanus* in potted apple trees at a nursery on two evaluation dates, after one or two applications of preventive insecticides (Wolcott, 2016).

Treatment/Evaluation date	No. attack sites/tree	No. sites/tree containing		
		Empty galleries	Brood	Adults
12 Jul				
1 Check, flooded	0.3 $\pm$ 0.2	0.1 $\pm$ 0.1	0.0	0.0
2 Chlorpyrifos	0.1 $\pm$ 0.1	0.0	0.0	0.0
3 Chlorpyrifos/fenprothrin	0.4 $\pm$ 0.3	0.4 $\pm$ 0.3	0.2 $\pm$ 0.2	1.5 $\pm$ 1.5
4 Chlorpyrifos/lambda-cyhalothrin	0.3 $\pm$ 0.2	0.1 $\pm$ 0.1	0.0	0.1 $\pm$ 0.1
5 Permethrin, high rate (2 apps)	0.3 $\pm$ 0.1	0.1 $\pm$ 0.1	0.0	0.0
6 Lambda-cyhalothrin (2 apps)	0.3 $\pm$ 0.2	0.0	0.0	0.0
7 Permethrin, low rate (2 apps)	0.3 $\pm$ 0.2	0.1 $\pm$ 0.1	0.0	0.1 $\pm$ 0.1
8 <i>Metarhizium</i> (2 apps)	0.2 $\pm$ 0.1	0.0	0.0	0.0
27 Jul				
1 Check, flooded	0.3 $\pm$ 0.1	0.1 $\pm$ 0.1	0.0	0.0
2 Chlorpyrifos	0.1 $\pm$ 0.1	0.8 $\pm$ 0.1	0.0	0.1 $\pm$ 0.1
3 Chlorpyrifos/fenprothrin	0.2 $\pm$ 0.1	0.0	0.0	0.0
4 Chlorpyrifos/lambda-cyhalothrin	0.3 $\pm$ 0.2	0.0	0.1 $\pm$ 0.1	0.0
5 Permethrin, high rate (2 apps)	0.0	0.0	0.0	0.0
6 Lambda-cyhalothrin (2 apps)	0.0	0.0	0.0	0.0
7 Permethrin, low rate (2 apps)	0.8 $\pm$ 0.5	0.1 $\pm$ 0.1	0.0	0.0
8 <i>Metarhizium</i> (2 apps)	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.0

apps, applications.

unknown. The presence of the other fungi in the genera *Ophiostoma* and *Cytospora*, known to be pathogenic in other woody perennials, is somewhat alarming. However, the species we recovered are not reported apple pathogens and they could simply be opportunists that are colonizing the wood damaged by *X. germanus* tunneling. At the same time, *X. germanus* cultivation of wilt pathogens in the *Fusarium* genus could exacerbate the decline of the tree, and tunneling by the insect could promote the introduction of known limb and canker pathogens such as *Botryosphaeria dothidea*.

Although we recovered *E. amylovora* from *X. germanus* in 2013 (Tancos et al. 2016), bacterial pathogens were not recovered from the entry holes or the beetles in 2015. The bacteria recovered from adults and infested wood tissue are likely just undocumented endophytes present through chance encounters. By comparison, the consistent recovery of fluorescent *Pseudomonas* spp. from produced callus tissue deposits under blistered bark around entry holes is more indicative of a pathogenic response. Although it has not been reported in North America, *Pseudomonas* spp. are common epiphytes in apple (Tancos and Cox 2017) and have been implicated in bark blister disease in South Africa (Mansvelt and Hattingh 1986), and may be responsible for the blistering symptoms observed in western New York apple trees. Mansvelt and Hattingh did not report the presence of any borer injury in the trees, but *X. germanus* entry holes could easily be missed if the blistering was as spectacular as reported in the current study. The weakening of trees, combined with tissue injury from *X. germanus* tunneling, could have favored the entry of *Pseudomonas* spp. and subsequent blistering of bark and callus production. The blistering of the bark is likely a symptom of the problem and not a cause of decline. Such symptoms could lead to increased risk of winter injury, but would not lead to overall tree decline. Regardless, the precise cause of tree decline and the importance of microbial involvement remains uncertain. Given the weakly pathogenic nature of *N. haematococca*, the most prevalent microbe involved in the complex, we believe that wilting symptoms observed on apple trees are most likely the result of tunneling, as

opposed to fungal and bacterial pathogens. However, this cursory survey can say little about the impact such fungi may have on the decline overall. Further controlled studies would be needed to better understand the role fungi and bacteria play in this decline complex.

In both our orchard and nursery management trials, we found applications of trunk sprays to be inconsistent and marginal in preventing new infestations of this insect. Total numbers of attacks in all of the 2015 trials were relatively low, possibly owing to insufficient ethanol production by the small potted trees during the first half of the season, which may have hampered our ability to adequately evaluate the efficacy of the insecticide treatments. We attempted to address this limitation in 2016 by affixing an individual ethanol lure to each tree. In this study, chlorpyrifos significantly reduced infestations compared with untreated trees and those treated with lambda-cyhalothrin at one location in our 2015 orchard trials, and in comparison with untreated trees at one location in our 2016 nursery trials, but otherwise performed no better than any other treatment, including the untreated checks. Similarly, in trees treated using several products (chlorpyrifos, lambda-cyhalothrin, and permethrin) the number of attack sites containing adults was significantly reduced compared with untreated trees at one nursery trial location in 2016, but the products were otherwise ineffective in reducing numbers in other locations and infestation categories, such as number of attacks, and numbers of attack sites containing brood or adults.

In the 2015 nursery trial, the check trees that were not flooded were not attacked by the adults and grew normally into the season, indicating that nonflooded potted trees were not stressed into producing ethanol that would be attractive to the beetles, consistent with the findings of Ranger et al. (2013a). Population pressure was lower at Medina, and no infestations were seen at this location in the flooded check trees, which were either not producing enough ethanol to attract the adults or were less attractive than the larger orchard trees under natural flood stress located adjacent to the pots.

Similarly variable results in chemical control of scolytine beetles have been reported by Hale and Oliver (1999), Mizell and Riddle (2004), Frank and Sadof (2011), Reding et al. (2013a), and Ranger et al. (2016a). Somewhat better control has been obtained with multiple applications (Frank and Sadof 2011), but no insecticide completely prevented attacks from occurring (Hale and Oliver 1999, Mizell and Riddle 2004, Ranger et al. 2016b). We assessed only single applications in our orchard trials, and treatments consisting of two applications were effective only in certain instances (for permethrin and lambda-cyhalothrin) in our 2016 nursery trial (Table 7). Previous studies conducted on chemical control of scolytine beetles have shown some success using other pyrethroids such as bifenthrin and cypermethrin (Mizell and Riddle 2004, Frank and Bambara 2009, Frank and Sadof 2011, Reding et al. 2013a, Ranger et al. 2016a,b), but we elected to confine our orchard trials to products registered in New York for this use in apples. The micronutrient mixture (KeyPlex), a crop amendment intended to prevent nutrient deficiency, was included in the 2015 nursery trials as a possible means of diminishing the impact of beetle attacks on tree conductive tissues, but no treatment effect was seen in our results.

The entomopathogenic fungus *Metarhizium anisopliae* Strain F52 has shown some potential efficacy against *X. germanus* (Castrillo et al. 2011), and a two-application treatment did reduce numbers of attacks and associated infestations in our nursery trials, but these results did not separate out statistically. Other microbial insecticides such as *Beauveria bassiana* (Bals.) have also been found to be virulent to *X. germanus* and *X. crassiusculus*, not only when taken up by the foundresses but also when inocula on the cadavers are spread to progeny (Castrillo et al. 2011, 2013). The success of this approach would depend on the ability to sufficiently infect the adults before they bore into the host tree, to allow eventual establishment of the fungus in the gallery, and enable its spread to other adults and brood present in the chambers.

We incorporated the repellent verbenone into our 2016 orchard trial treatments in an effort to discourage beetle attacks on the potted trees, as suggested by Ranger et al. (2013b), who reported a higher density of attacks from several scolytine beetle species including *X. germanus* on trap trees farthest away from a verbenone dispenser. This compound, an oxidation product of the monoterpene  $\alpha$ -pinene (Birgersson and Leufvén 1988), is an anti-aggregation pheromone component of several *Dendroctonus* bark beetles (Borden 1985, Lindgren and Miller 2002), shown to inhibit the attraction of *X. germanus* to artificially damaged red pine (*Pinus resinosa* Aiton) trap trees and baited traps (Dodds and Miller 2010). It has been found to deter both *X. germanus* and *X. crassiusculus* from ethanol-baited traps, and to reduce beetle attacks on ethanol-injected trap trees (Dodds and Miller 2010, Burbano et al. 2012, Ranger et al. 2013b, VanDerLaan and Ginzler 2013). Verbenone reduced trap catch of *Dendroctonus brevicornis* LeConte near an attractant source in the field (Bedard et al. 1980), as well as landings and attacks on attractive host trees (Bertram and Paine 1994).

Although combining verbenone dispensers with insecticide sprays resulted in some numerical reduction of infestation numbers and categories in our trials, effects were significant only at the Sodus location, and only for numbers of sites containing brood in the permethrin and check treatments (Table 5). It is possible that verbenone volatilizing from a pouch dispenser in proximity to the trees was insufficiently active against the beetles searching for a host to colonize. Gillette et al. (2006) reported that lodgepole pines (*Pinus contorta* Douglas ex Loudon) sprayed directly with verbenone-releasing flakes had significantly lower attack density by *Dendroctonus ponderosae* Hopkins than untreated control trees for up to 8 wk after

application, and none of the treated trees was attacked by red turpentine beetle *Dendroctonus valens* LeConte, whereas control trees averaged nearly two attacks per tree. Ten months after application, treated trees showed significantly lower mortality than control trees. In tests of repellents for the redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff, dollops of SPLAT (ISCA Technologies, Riverside, CA) wax-based matrix containing verbenone alone and combined with methyl salicylate directly applied to bolts of redbay, *Persea borbonia* [L.], significantly reduced beetle captures and boring holes compared with untreated bolts (Hughes et al. 2017). Treatment effects persisted over a 10-wk period.

Kühnholz et al. (2001) have proposed several factors as potential contributors to an increased incidence of scolytine beetle infestations in living trees, including 1) flight activity early in the spring, when the tree is unable to withstand attacks, a phenomenon that could be related to climate change; 2) ability to survive on symbiotic fungi without relying on the host tree tissue; 3) presence of potentially pathogenic fungi that can predispose the host to beetle infestation; 4) cryptic beetle behavior that promotes movement over long distances and establishment in new habitats; and 5) a complex chemical ecology that enables the adults to perceive stress-related volatiles produced by otherwise apparently healthy trees.

Control of this pest is a challenge, as it is not possible to determine whether or when an orchard might be susceptible to *X. germanus* attack or to identify which trees will be targeted, although infestations were more likely to be found in sections of the orchard where trees had been subjected to extended periods of standing water, particularly near orchard edges adjacent to woodlots or hedgerows. This insect has been present in and around apple-growing regions in New York for a number of years (Felt 1932), but has only recently begun attacking commercial apple trees, and the reasons for this shift in behavior are unknown. Similar infestations in apples have been recognized recently in neighboring states, including New Jersey (Nielsen 2014) and Michigan (Haas et al. 2016), although in the former case, the species responsible is the granulate ambrosia beetle, *X. crassiusculus*. A succession of growing seasons featuring stresses such as low winter temperatures and drought conditions has been suggested as one possible factor. LaSpina et al. (2013) have proposed that numerous occurrences of *Xylosandrus* spp. attacks observed in European stands of beech (*Fagus sylvatica* L.) were due to ethanol production by freeze-damaged necrotic tissues. Impact of summer droughts on tree carbon reserves can create a carbon deficit that reduces or delays cold hardening, representing another potential source of stress to the tree (Thomas and Ahlers 1999, Bréda et al. 2006). Kühnholz et al. (2001) note that, in a warming climate, trees that do not enter complete overwintering dormancy would undergo a longer than normal period of anaerobic metabolism in the spring, accompanied by a greater buildup of ethanol that could predispose them to attack by secondary scolytine beetles. The phenological state of tree growth is evidently an important determinant of the tree's susceptibility to beetle attack, which was found to cease in ornamental nursery plantings after leafing out was complete (Hudson and Mizell 1999).

Preventive trunk sprays of insecticides are not necessarily practical or even effective enough to be economically warranted in all cases. Possible alternative approaches under consideration include microbial controls (e.g., *Trichoderma harzianum* Rifai, *Metarhizium* spp., *Beauveria bassiana*) targeted against either the beetles or the symbiotic fungus (Castrillo et al. 2011, 2013), but are not yet sufficiently developed for practical use. Current recommendations generally advise removal and burning of infested trees, but with the suggestion to leave them in place for some time after

attacks are initiated, where they can act as attractants for other adults, before being removed (Mizell and Riddle 2004, Ranger et al. 2016a). In our observations, infested trees tend to occur in batches, with dozens in one part of the orchard showing die-off or decline, whereas adjacent and presumably healthy trees are unaffected; this is consistent with reports by others (Mizell and Riddle 2004, Ranger et al. 2016a). Inspections of infested orchards in western New York show a tree loss of up to 30% in some cases; however, not all trees that are attacked die, and the occurrence of callus tissue underneath old attack sites in some trees provides an indication that a certain proportion can apparently recover. The results of these studies support the utility of using ethanol-baited traps for detecting adult flight activity in the spring, and timing trunk sprays of insecticides that may offer some control of colonizing beetles; however, there are still many questions needing to be addressed before effective solutions and recommendations are available. It is clear that maintenance of tree health and avoidance of potential sources of stress will be an important component of any effective management program of this pest in commercial apple orchards.

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