Scolytinae in Nursery and Fruit Crops of Western Kentucky and Seasonal Population Patterns of Four Invasive Ambrosia Beetles[1]

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Abstract Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) inoculate species specific symbiotic fungi into the sapwood of their hosts. Some fungi are innocuous, but others are pathogenic and can kill plants in a short time. The main objectives of this study were to identify ambrosia beetle species found in wholesale and retail nurseries and an apple orchard in western Kentucky and monitor population abundance and phenology of the more common invasive ambrosia beetles. Baker traps baited with ultra-high or standard release ethanol were deployed in late February or March and removed in either fall (2016 and 2017) or August (2018). Sixteen ambrosia beetle species were captured, and eight of them were invasive. The invasive species *Cnestus mutilatus* (Blandford), *Xylosandrus crassiusculus* (Motschulsky), *Xylosandrus germanus* (Blandford), and *Xyleborinus saxesenii* (Ratzeburg) were the most common and abundant species. The highest counts of these invasive species were recorded from April to May. In addition, we are reporting 13 bark beetle species captured in this study; among them, *Phloeotribus dentrifrons* (Blackman) and *Thysanoes fimbricornis* LeConte are reported for the first time for Kentucky, whereas *Scolytus multistriatus* (Marsham) was the only invasive bark beetle collected.

Key Words nursery crops, seasonal population, ethanol trapping, Baker traps

Most ambrosia beetle (Curculionidae: Scolytinae) species are wood-inhabiting insects restricted to dead or recently dead tree tissues; thus, they are inoffensive to living plants in their native habitats or non-native regions (Hulcr and Stelinski 2017). Ambrosia beetles and bark beetles along with other beetle species vector decomposer fungi to fresh dead wood, playing an important role in the forest ecosystems (Jacobsen et al. 2017). Exceptionally, *Platypus quercivorus* (Murayama) kills vigorous oak trees in Japan where it is native (Kamata et al. 2006). Other

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ambrosia beetles, that is, *Megaplatypus mutatus* (Chapuis) (=*Platypus mutatus* Chapuis), *Corthylus columbianus* Hopkins, and *Austroplatypus incompertus* (Schedl), also have been reported to attack healthy angiosperm trees in their native territories (Kuhnholz et al. 2001). Attacks by invasive ambrosia beetles to living plants are conditioned by the physiologic and health status of the plant rather than host susceptibility per se. Healthy plants are not likely to be attacked, whereas weakened plants that release stress-related volatiles (i.e., mainly ethanol) as a consequence of biotic or abiotic stressors, may stimulate the attack by ambrosia beetles (Addesso et al. 2018, Kelsey et al. 2014, 2016, Ranger et al. 2010, 2016, 2019).

In the continental United States, 58 identified exotic ambrosia beetle species were established as of 2010 (Haack and Rabaglia 2013). The geographic colonization by several invasive beetles causes economic losses and ecologic instability to nurseries and native forests, respectively. Among invasive species, *Xylosandrus crassiusculus* (Motschulsky) and *Xylosandrus germanus* (Blandford) have been identified as the most destructive ambrosia beetles to nursery crops in the United States. They have a wide host range and can attack trees, bushes, and vines in ornamental nurseries, orchards, plantations, and landscapes (Ranger et al. 2016). According to field and container nursery growers from the southeastern United States, *X. crassiusculus* was ranked third as a key pest, and 18% of nursery growers identified it as prevalent and difficult to control (Fulcher et al. 2009). *Cnestus mutilatus* (Blandford) is another emerging pest in nurseries. In fact, Oliver et al. (2012) found *C. mutilatus* widely distributed in Tennessee and considered a new pest for nursery crops with unknown magnitude of damage. *Cnestus mutilatus* was first reported for Kentucky in 2013; although a single specimen was found in Whitley County, it was believed it could be widely distributed because of its widespread dispersal in the neighboring states (Leavengood 2013). Preventive pyrethroid sprays applied directly on the trunk are the preferred management practice to control ambrosia beetles before they enter the sapwood. However, chemical control efficiency relies on the time when the chemical is applied (Reding et al. 2011). Thus, monitoring ambrosia beetles during early spring provides information on the peak flight emergence, when females are looking for hosts to initiate new colonies.

In Kentucky, ambrosia beetles occasionally attack container-grown nursery crops, apple trees and saplings, and landscape plants causing substantial losses. Indeed, all production systems included in this study, except for a field nursery crop production located in Todd County, have reported ambrosia beetle attacks in recent years. Nonetheless, identity of damaging species was unknown. The main goal of this study was to provide information on the species composition of ambrosia and bark beetles in western Kentucky. In addition, seasonal population patterns of *C. mutilatus*, *X. crassiusculus*, *X. germanus*, and *X. saxesenii* (Ratzeburg) are reported for the first time for Kentucky.

Materials and Methods

Field sites. In 2016, traps were deployed in two wholesale tree nurseries (one each in Todd and Calloway Counties), and experimental plots of apple and peach

(Caldwell County) in the University of Kentucky's Research and Education Center at Princeton in western Kentucky. A retail nursery (Graves County) was added to the site list in 2017 and 2018.

Trapping and monitoring. Modified Baker traps were chosen to capture ambrosia beetles (Ranger et al. 2010). Traps were built using clear plastic 2-L soda bottles on the top of 500-mL water bottles, which were joined using a double hose connector. Four rectangular holes (5×10 cm^2) were made on the periphery of the soda bottle. A plastic clear planter saucer (36-cm diameter) was attached to the top of the soda bottle to protect the trapping solution from rain. The hanger was an eye bolt inserted at the center of the bottom of the soda bottle and saucer that were held tightly together using a nut and a locknut. An ultra-high release, heat-sealed pouch filled with 95% ethanol (260 mg/d at 20°C, Contech Enterprises Inc., Burnaby, BC, Canada) or a heat-sealed, permeable membrane pouch containing 95% ethanol (65 mg/d at 25°C; AgBio Inc., Westminster, CO) was suspended inside the soda bottle. The latter lure was replaced after 3 mo. About 150 mL commercial antifreeze was poured in the lower bottle to catch and kill insects attracted to the traps.

Traps were hung from tree branches about 1 m above the ground and placed on the edge of woods that surrounded nurseries and orchards and inside the orchards and nursery stocks. In 2016, four traps were deployed in the Calloway wholesale nursery and three in each Caldwell orchard and Todd wholesale nursery during March through November. In 2017 and 2018, four traps per location were set, which were monitored during February through October and March through August, respectively. Catching bottles were replaced biweekly in 2016. For the next 2 yr, populations were monitored weekly from March to May (period of overwintering ambrosia beetle emergence) and biweekly thereafter. Catching bottles were brought to the laboratory for examination. After filtering and rinsing each sample, ambrosia beetles were grouped and tallied using a dissecting stereoscope. Populations were recorded for *C. mutilatus*, *X. crassiusculus*, *X. germanus*, and *X. saxesenii* as mean number beetles per trap per week (B/T/W). Ambrosia beetle species were surveyed for each location and identified to species by specialists. An ambrosia beetle collection is available at the Insect Museum in the Department of Entomology at the University of Kentucky, Lexington, KY.

Results

Detection of ambrosia beetles and bark beetle species. In a period of 3 yr, 29 ambrosia and bark beetle species were identified in nursery crop production and an experimental orchard located in Caldwell, Todd, Graves, and Calloway Counties in western Kentucky (Table 1). Invasive ambrosia beetles such as *C. mutilatus, X. saxesenii, X. crassiusculus*, and *X. germanus* were captured at each location; therefore, they were chosen to study their seasonal population patterns. *Monarthrum fasciathum* (Say) and *Xyleborus ferrugineus* (F.) were the only native ambrosia beetle species found in all surveyed counties. Thirteen bark beetles were identified, with *Scolytus multistriatus* (Marsham) being the only invasive species captured. Among the native bark beetles, the capture of *Phloeotribus dentrifrons* (Blackman) and *Thysanoes fimbricornis* LeConte signifies, to the best of our knowledge, first time reports in Kentucky. All nine invasive species recorded in this study (eight ambrosia beetles and one bark beetle), except *Scolytus multistriatus* (Marsham), were captured in Graves County.

Seasonal population patterns. By the fourth week of April 2016, *X. crassiusculus* reached 146.04 ± 5.86 (mean ± SEM) B/T/W (Fig. 1A), which decreased sharply by the end of May (5.89 ± 0.60 B/T/W). Thereafter, few specimens were captured until they were no longer detected in early November. Spring emergence of ambrosia beetles began earlier in western Kentucky in 2016 (Fig. 1A), because the initial and progressive population increases were not recorded. Therefore, in 2017, the traps were set in mid-February, and monitoring was performed weekly during March and April. *Xylosandrus crassiusculus* started to emerge in mid-March 2017 and reached the highest peak of 73.50 ± 35.96 B/T/W by the third week of April, then the number of beetles declined, and low numbers of 1.94 ± 7.73 B/T/W were found after the first week of July. The 2016 and 2017 population patterns coincided in the drastic decline of beetle captures in late April. In 2018, *X. crassiusculus* emergence, in all counties, experienced a delay and reduction in the population in comparison with 2016 and 2017, reaching 15.06 ± 6.01 B/T/W the second week of May. Only in the summer of 2018, a rising trend of *X. crassiusculus* was recorded from a trap located in the woods near a creek in Todd County, where the maximum population (195 B/T/W) was recorded in August. This phenomenon was not observed in previous years.

In 2016, *C. mutilatus* was captured in high numbers (69.28 ± 57.98 B/T/W) by the third week of April and then it declined to zero captures in October (Fig. 1B). In 2017, *C. mutilatus* began emerging in early April and reached high populations the third week of April at 84.94 ± 24.71 B/T/W. Thereafter, the population remained low for the rest of the season. In 2018, the number of *C. mutilatus* captures were very low in all counties (Fig. 1B) at all times; the maximum count was 0.69 ± 0.41 B/T/W in early May.

Overall, *X. germanus* was captured in low numbers in western Kentucky. The highest count in 2016 was 9.75 ± 0.18 B/T/W in late April. In 2017, this species was initially detected the second week of March at low numbers, reaching a maximum of 2.38 ± 0.65 B/T/W by the second week of April. In 2018, the initial captures began in April in all locations, the numbers remained low until the end of the growing season. The highest count of 2.44 ± 1.12 B/T/W was recorded the last week of May (Fig. 1C). No *X. germanus* specimens were captured after August in 2016 and 2017; however, a few individuals were captured in September and October 2018.

Xyleborinus saxesenii was widely spread and active during the monitoring period, although in low numbers. It was captured as early as late February in 2017 and reached the highest captures of 4.06 ± 1.52 B/T/W at the end of March. In 2018, no beetles were captured until the second week of April; however, the counts were very low at 0.31 ± 0.14 B/T/W.

Discussion

Detection of ambrosia beetles and bark beetle species. A diverse and large group of bark and ambrosia beetles was identified in western Kentucky; invasive ambrosia beetle species were more numerous and widely distributed than native species. Natural hardwood lots surrounded the locations where these surveys were

Table 1. Species of ambrosia and bark beetles captured in three nurseries and an orchard in western Kentucky in 2016, 2017, and 2018.

		Orchard	Nurseries		
Species	**Origin**	**Caldwell**	**Calloway**	**Graves**	**Todd**
Ambrosia beetles					
Ambrosiodmus rubricollis (Eichhoff)	Invasive		∆	∆	∆
Cnestus mutilatus (Blandford)	Invasive	∆	∆	∆	∆
Cyclorhipidion bodoanum (Reitter)	Invasive	∆		∆	
Dryoxylon onoharaense (Murayama)	Invasive			∆	∆
Euwallacea validus (Eichhoff)	Invasive			∆	
Xyleborinus saxesenii (Ratzeburg)	Invasive	∆	∆	∆	∆
Xylosandrus crassiusculus (Motschulsky)	Invasive	∆	∆	∆	∆
Xylosandrus germanus (Blandford)	Invasive	∆	∆	∆	∆
Corthylus columbianus Hopkins	Native		∆		
Corthylus punctatissimus (Zimmermann)	Native			∆	
Monarthrum fasciathum (Say)	Native	∆	∆	∆	∆
Mornathrum mali (Fitch)	Native		∆		∆
Xyleborus ferrugineus (Fabricius)	Native	∆	∆	∆	∆
Xyleborus intrusus or pubescens	Native	∆			
Xyleborus pubescens Zimmermann	Native			∆	
Xyloterinus politus (Say)	Native		∆		
Bark beetles					
Ambrosiodmus tachygraphus (Zimmermann)	Native				∆
Hylocurus langstoni (Blackman)	Native	∆			
Hylocurus rudis (LeConte)	Native		∆		∆

Table 1. Continued.

		Orchard	Nurseries		
Species	Origin	Caldwell	Calloway	Graves	Todd
Hypothenemus dissimilis (Zimmermann)	Native	Δ	Δ	Δ	Δ
Hypothenemus eruditus Westwood	Native			Δ	
Hypothenemus interstitialis (Hopkins)	Native		Δ		
Hypothenemus seriatus (Eichhoff)	Native	Δ			Δ
Hypothenemus sp	Native		Δ		Δ
Phloeotribus dentifrons (Blackman)	Native	Δ			
Phloeotribus frontalis (Olivier)	Native	Δ			
Pityophthorus sp.	Native				Δ
Scolytus multistriatus (Marsham)	Invasive	Δ			
Thysanoes fimbricornis LeConte	Native	Δ	Δ		

Detection of species within a county is noted with a triangle symbol.

conducted in Caldwell, Todd, and Calloway Counties, whereas the location in Graves County was located within a wildlife refuge with a dense hardwood forest. Forest composition and climate influence diversity and activity density of native ambrosia beetle species in deciduous temperate forests, and higher forest richness favor the establishment of exotic beetles (Rassati et al. 2016). Furthermore, forest stand characteristics such as age and structure determined ambrosia beetle diversity, abundance, and density (individuals/stem/ha). These parameters slightly impacted ambrosia beetle diversity and the high exotic vs. native species proportion in an oak-hickory forest stand in Missouri (Reed and Muzika 2010).

In this study, native bark beetles consisted of 12 species; all these species have been previously reported for Kentucky (Gomez et al. 2018, Rabaglia et al. 2006), except *P. dentifrons* and *T. fimbricornis*. Among these native species, *Ambrosiodmus tachygraphus* (Zimmermann), *Xyleborus ferrugineus* (F.), and *Monarthrum fasciathum* (Say) have been reported attacking unhealthy peach trees (Kovach and Gorsuch 1985). *Corthylus columbianus* (Zimmermann) attacks healthy trees (Kühnholz at al. 2001) and affects timber quality.

Four invasive ambrosia beetle species, *Xylosandrus crassiusculus* (Motschulsky), *Xylosandrus germanus* (Blandford), *Cnestus mutilatus* (Blandford), and *Xyleborinus saxesenii* (Ratzeburg) were collected from the four western Kentucky counties. Furthermore, *X. crassiusculus* and *C. mutilatus* were the most abundant

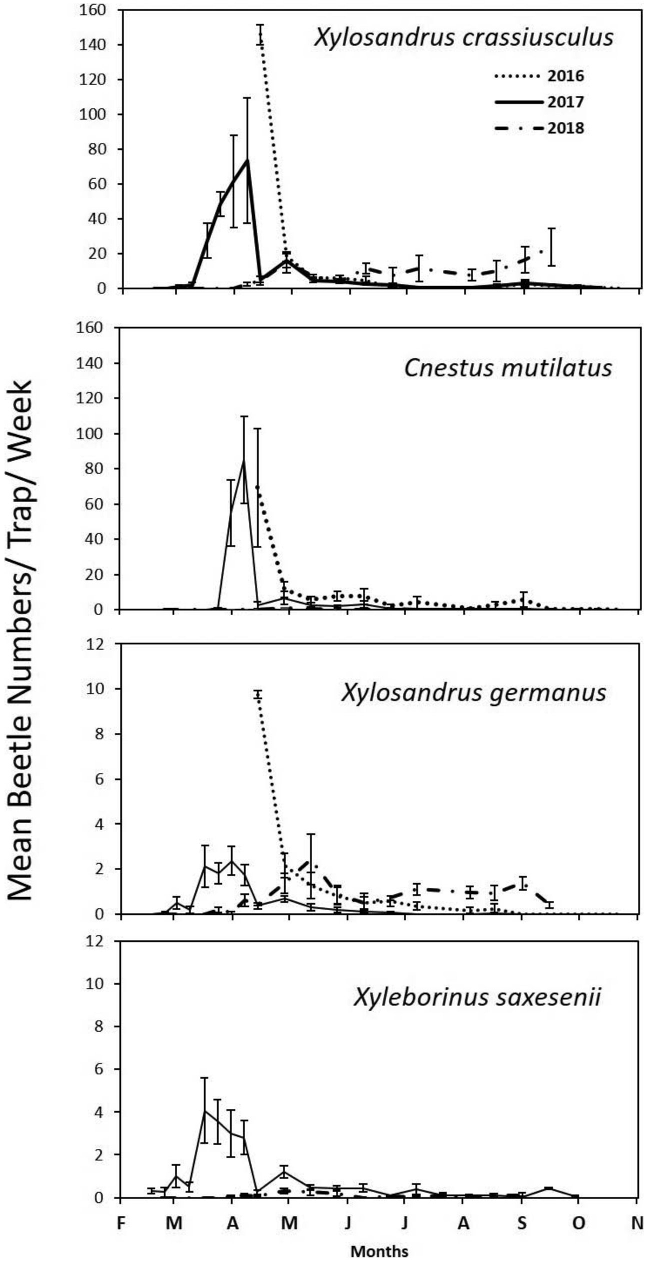

Fig. 1. Seasonal population patterns of (A) *Xylosandrus crassiusculus,* (B) *Cnestus mutilatus,* (C) *Xylosandrus germanus*, and (D) *Xyleborinus saxesenii* in nurseries and an experimental orchard western Kentucky in 2016–2018.

species in all locations. Some invasive ambrosia beetles, once established in new habitats, typically outnumber the populations of native species (Gandhi et al. 2010, Helm and Molano-Flores 2015, Miller and Rabaglia 2009, Miller et al. 2015, Reed and Muzika 2010). Similarly, in a deciduous temperate forest, populations of non-native ambrosia beetle species were higher and were present for longer periods during the growing season compared with native species (Rassati et al. 2016). In western Kentucky, *X. germanus* was captured in low numbers in spring and absent in early summer in 2017 and 2018. In Ohio, *X. germanus* dominated over *X. crassiusculus* population and showed seasonal flight patterns that differed in 2 successive years. Perhaps the former species is adapted to lower temperatures (Reding et al. 2011) compared with the most moderate temperatures of Kentucky. This species was absent in a survey conducted in southern Mississippi in 2010, which might be a consequence of warmer temperatures in the southern latitude (Werle et al. 2012). *Xylosandrus crassiusculus* and *X. germanus* are considered the most aggressive species to the nursery industry and landscape in the United States (Frank and Sadof 2011, Oliver and Mannion 2001, Ranger et al. 2016).

Cnestus mutilatus demands close attention, because it has been reported as a new pest of nursery and landscape in Tennessee (Oliver et al. 2012). In a hardwood forest of Mississippi, *C. mutilatus*, like other non-native ambrosia beetle species, showed low host specificity, attacking 16 species in 12 plant families. However, noticeable preference for sweetgum (*Liquidambar styraciflua* L.) was noticed. In addition, *C. mutilatus* only attacked unhealthy trees, specifically branches with dieback or dead trees (Stone et al. 2007). In this study, *C. mutilatus* was observed attacking eastern redbud (*Cercis canadensis* L.) twigs, whose caliper (about 6 mm) was barely larger than their body length.

One to two specimens of European elm bark beetle, *Scolytus multistriatus* (Marsham), were found in Caldwell and Todd Counties every year during the duration of this survey. These very low counts are probably related to the low efficiency of ethanol-baited traps compared with pheromone-baited traps (Blight et al. 1983, Cuthbert and Peacock 1978). This species is among the most devastating insect pest worldwide that carries Dutch elm disease, a fungal disease caused by *Ophiostoma ulmi* (Buisman) Nannfelt and *Ophiostoma novo-ulmi* Brasier, responsible for native elm trees decline (Brasier 1991).

Ambrosiodmus rubricollis is a non-native species that was first reported in the United States in 1942 from Maryland (Bright 1968); thereafter, it has been frequently captured in the mid-Atlantic and southeastern United States (Rabaglia et al. 2006). This species has been found attacking old peach trees (*Prunus persica* L.) with a 15-cm-diameter tree trunk (Kovach and Gorsuch 1985).

During this study, *X. saxesenii* was found in low numbers at all sites; nonetheless, it attacked, along with *X. crassiusculus* and *X germanus*, container-grown tulip poplar *(Liriodendron tulipifera* L.) and willow oak *(Quercus phellos* L.) that had root rot in the 2018 spring in Graves County. Kelsey and Joseph (1998) reported that Douglas fir trees infected with black stain-root disease [*Leptographium wageneri* (Kendrick) Wingfield] contained 19 times more ethanol in the sapwood than healthy roots, which could explain why some beetles are attracted to diseased trees. Preventive fungicide application to control root rot caused by *Phytophthora cinnamomi* Rands in redbuds and tulip poplar reduced ambrosia beetle attacks (Adesso et al. 2018). In Tennessee, *X. saxesenii* was common and the most abundant species in a chestnut (*Castanea* spp.) nursery; nonetheless, the tree attack was low (Oliver and Mannion 2001). However, it has been reported causing severe damage in peach (Kovach and Gorsuch 1985).

Dryoxylon onoharaense (Murayama) was first collected in Mississippi in 1981 (Schiefer 2015). Thus far, few specimens were detected in artificially stressed black walnut trees (*Juglans nigra* L.) in Indiana (Reed et al. 2015) and live cottonwood (*Populus deltoidess* W. Bartram ex Marshall) stems (Coyle et al. 2005). *Cyclorhipidion bodoanum* (=*Xyleborus californicus* Wood) was reported from the eastern United States and Arkansas in 2000 (Vandenberg et al. 2000). Gomez et al. (2018) reported this non-native species from several southeastern states of the United States, except Kentucky.

Ambrosiodmus rubricollis is commonly found in southeastern states and the mid-Atlantic (Gomez et al. 2018). This Asian species attacks many broadleaf tree species and occasional damage has been reported for conifers (Faccoli et al. 2009).

Seasonal population patterns. Ambrosia beetle monitoring started the third week of April in 2016; however, it was determined that *X. crassiusculus* and *C. mutilatus* can be active out of their galleries as late as October in western Kentucky. On the contrary, *X. germanus* was active until August. Because April and May are the critical months when ambrosia beetles emerge in large populations in western Kentucky, it is important to deploy traps by the fourth week of March. Seasonal flight patterns were different for all ambrosia beetle species in 2017 and 2018. Here, two main factors, namely lure type and temperature, might have influenced the differences of the activities of ambrosia beetles. A release rate for ethanol of 65 mg/d at 25°C was used in 2018, whereas an ultra-high release rate of 260 mg/d at 20°C was used in 2016 and 2017. It has been shown that the amount of ethanol determined the attractiveness of ambrosia beetles to the traps; more beetles were caught when ethanol lure amount increased (Ranger et al. 2011, Reding et al. 2011). The daily maximum temperatures preceding the beetle emergence might have played an important role in the first spring flight out of the galleries of overwintered beetles, delaying the emergence and reducing the number of ambrosia beetles caught in 2018 (Fig. 1). Reding et al. (2013) found a consistent association between *X. germanus* emergence and plant phenological stages combined with a temperature of 20°C for 3 successive days in northern Ohio. This threshold has been a useful tool to predict activity during early to mid-spring for some other ambrosia beetle species as well. In western Kentucky, the maximum daily temperatures above 20°C were more frequent in 2016 and 2017 compared with the temperatures of the early spring in 2018 (Fig. 2). Thus, the lower spring temperatures in 2018 could have caused a delay of beetle emergence and high mortality of beetles because of either food scarcity or low temperatures.

Only one trap located in Todd County showed a high population increase, which occurred in the summer of 2018. This event was notably different from the 2 previous years. It is likely that the host resources and water available near the trap location may have determined these outcomes; water can attenuate changes in temperature in surrounding areas. Also, Iidzuka et al. (2016) reported that host resource availability varies throughout the time affecting ambrosia beetle community structures and population dynamics year after year.

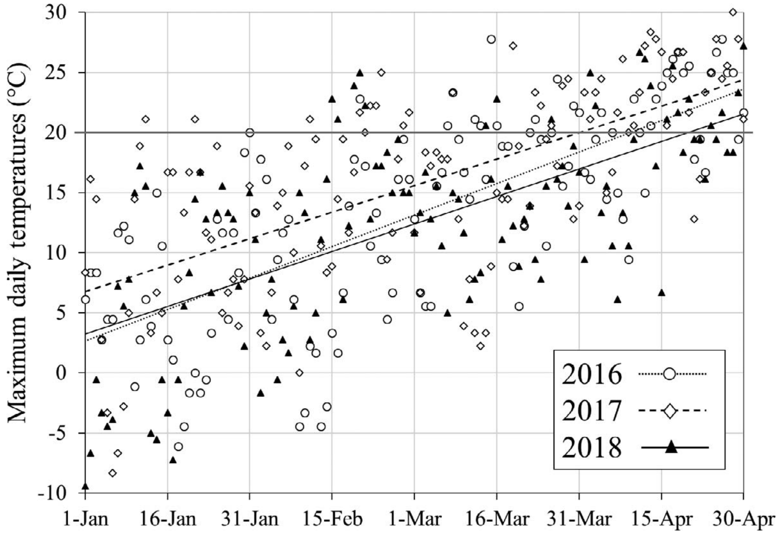

Fig. 2. Maximum daily temperatures (°C) in the University of Kentucky's Research and Education Center at Princeton between 1 January and 30 April 2016, 2017, and 2018. Attack and flight activity of *X. germanus* occurred when maximum daily temperatures are >20°C. Maximum daily temperature rarely surpassed the 20°C threshold in 2018; thus, these temperatures might have affected ambrosia beetle populations shown in the low trap captures compared with 2016 and 2017.

In this 3-yr study, 16 ambrosia beetles and 13 bark beetle species were identified; among them, the invasive species encompasses 8 and 1, respectively. *Xylosandrus crassiusculus* and *C. mutilatus* were the most abundant invasive beetle species; the former has been the main species responsible of all attacks in container nursery crops and landscape plants in this region (Z. Viloria and R.T. Villanueva, Univ. of Kentucky, unpubl. data). Based on the information obtained in this study, ambrosia beetle monitoring in western Kentucky should start in late March when spring temperatures start to increase (>21°C) to determine ambrosia beetle emergence timing and population trends. April and May are critical months for ambrosia beetle management in western Kentucky because of the large number of individuals that emerge from their nests in early spring and the vulnerability of plants after winter.

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