

Interception Strategies for Managing Exotic Ambrosia Beetles (Coleoptera: Curculionidae) in Nurseries¹

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Exotic ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) cause considerable damage in ornamental tree nurseries and other tree crops in North America. Ambrosia beetles bore into the xylem of trees to establish gardens of symbiotic fungi, which are the source of nutrition for adults and larvae (Wood 1982, Great Basin Nat. Memoirs. 6: 1–1359). Establishment of fungal gardens is crucial as some species of ambrosia beetles delay oviposition until their symbiotic fungi are growing (French and Roeper 1972, Can. Entomol. 104: 1635–1641; Weber and McPherson 1983, Ann. Entomol. Soc. Amer. 76: 455–462; Peer and Taborsky 2005, Evolution 59: 317–323). Colonization of nursery trees by ambrosia beetles often leads to wilting, stem dieback, or death (Ranger et al. 2016, J. Int. Pest Manag. 7: 1–23). Nursery growers rely on preventive trunk sprays of insecticides to protect trees from ambrosia beetles. However, insecticide sprays were inconsistent at preventing colonization attempts by ambrosia beetles in previous research (Frank and Sadoff 2011, J. Econ. Entomol. 104: 1960–1968; Reding et al. 2013, J. Econ. Entomol. 106: 289–298).

Xylosandrus crassiusculus (Motschulsky) and *Xylosandrus germanus* (Blandford) are two of the most damaging ambrosia beetles in ornamental tree nurseries in North America (Oliver and Mannion 2001, Environ. Entomol. 30: 909–918; Fulcher et al. 2012, J. Int. Pest Manag. 3: 1–8; Reding et al. 2013; Ranger et al. 2016). Both species overwinter in galleries in host trees, then in the spring, emerging females search for new hosts to colonize. *Xylosandrus crassiusculus* and *X. germanus* preferentially colonize physiologically stressed trees emitting ethanol (Ranger et al.

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2021, Can. Entomol. 153: 103–120). Ethanol, which benefits the establishment of fungal gardens and, thus, beetle colonization (Ranger et al. 2018, Proc. Natl. Acad. Sci. USA 115: 4447–4452), is a primary attractant for *X. crassiusculus* and *X. germanus* (Oliver and Mannion 2001; Ranger et al. 2021). Woodland habitats adjacent to nurseries are primary sources of *X. crassiusculus*, *X. germanus*, and other ambrosia beetles invading nurseries each season (Reding et al. 2015, J. Econ. Entomol. 108: 1947–1953; Werle et al. 2015, Florida Entomol. 98: 884–891). Thus, ethanol-baited traps or ethanol-injected trap trees positioned at the woodland/nursery interface could be an effective tactic for intercepting beetles as they emigrate into nurseries. If not effective as a stand-alone management tool, then the interception tactic could mitigate ambrosia beetle pressure and improve the efficacy of insecticidal trunk sprays.

Ethanol-baited traps captured hundreds of *X. crassiusculus* and *X. germanus* per trap in previous research (Oliver and Mannion 2001; Reding et al. 2010, J. Environ. Hort. 28: 85–90; Reding et al. 2011, J. Econ. Entomol. 104: 2017–2024). Similarly, *X. crassiusculus* and *X. germanus* readily burrowed into trees injected with ethanol, whereas no burrowing was evident in adjacent noninjected trees (Ranger et al. 2010, Agric. For. Entomol. 12: 177–185; Reding et al. 2013). Injecting trees with ethanol to create highly attractive trap-trees might be useful for attracting ambrosia beetles away from crop trees. Every year ornamental tree growers have trees to cull, which could be injected with ethanol to use as trap-trees. Concentrating beetles on injected trap trees, which can later be removed and destroyed, may reduce colonization pressure on saleable trees and improve the efficacy of insecticide treatments.

The objectives of the current research were to (a) determine whether using traps to intercept *X. germanus* and other ambrosia beetles emigrating from adjacent habitats reduces the activity of beetles within nurseries and (b) determine whether ethanol-injected trap-trees reduce colonization attempts by ambrosia beetles on insecticide-treated trees.

Interception research was conducted in commercial nurseries in 2015 and 2016. Bottle traps (Reding et al. 2015) baited with ethanol were used for intercepting *X. germanus* and other ambrosia beetles emigrating into nurseries from adjacent woodlands and for sentinel traps within nurseries. Interception would be considered a successful tactic for managing *X. germanus* in nurseries if captures in sentinel traps were reduced in nursery plots guarded by interception traps. Traps were placed in nurseries in the spring before the emergence of *X. germanus*. Interception traps were positioned inside nurseries parallel to and within 1 m of the interface with an adjacent woodland. Sentinel traps were placed within nurseries at various distances from the woodland/nursery interface including 13, 25, 50, and 100 m and within adjacent woodlands at 13 m from the interface (hereafter referred to as the woodland trap). Sentinel traps were positioned at various distances from the woodland/nursery interface to determine whether captures would be influenced by the proximity to intercept traps. Lateral distance between sentinel traps was 25 m. Experiments were set up as randomized complete block designs with nursery as the blocking factor. There were two adjacent plots (100-m width) at each nursery with a sentinel trap of each distance per plot and plots 25 m apart. One plot was guarded by intercept traps, whereas the other plot was unguarded. Intercept traps were rotated to the

opposite plot each time traps were checked. Traps were checked at 6- to 15-d intervals, and all Scolytinae individuals were identified to the species level using available keys (Wood 1982; Rabaglia et al. 2006, Ann. Entomol. Soc. Am. 99: 1034–1056).

In 2015, the experiment was conducted in four commercial nurseries in northern Ohio, with three nurseries in Lake Co. and one in Wayne Co. Five intercept traps were used for each protected plot. Each intercept trap was positioned to align with a sentinel trap and were 25 m apart for a total of 5 traps guarding a plot. Traps were placed in nurseries on 15 April 2015, and the experiment continued through 23 June 2015. In 2016, the experiment was conducted in five commercial nurseries in Lake Co. In this experiment, intercept traps were positioned 10 m apart with 11 traps guarding each protected plot. Traps were placed in nurseries on 30 March and the experiment ran through 28 June 2016.

Captures of *X. germanus* were $\log(X+1)$ transformed for analysis. Transformed data were analyzed by repeated measures analysis of variance using Proc Mixed in SAS (SAS Institute 2013, SAS Institute Inc. Version 9.4. SAS Institute, Cary, NC). For an analysis of interception data, captures in sentinel traps within nurseries (13 m, 25 m, 50 m, and 100 m) were combined (woodland trap data excluded) and captures at each sentinel position including in woodlands were analyzed separately. The presence of intercept traps (present or absent) was the between-subjects factor with captures of *X. germanus* over time the repeated measure. If significant effects of intercept or intercept \times time were detected, captures of *X. germanus* were compared for each sample date using least square means (lsmeans) with the pdiff option in Proc MIXED (SAS Institute 2013).

Captures of *X. germanus* were compared among the various sentinel trap positions (distance from woodland/nursery interface) using repeated measures analysis of variance (Proc MIXED). In this analysis, the distance from the woodland/nursery interface was the between-subjects factor and captures of *X. germanus* over time the repeated measure. If significant effects of distance or distance \times time were detected, captures of *X. germanus* were compared for each sample date using lsmeans with the Tukey adjustment and pdiff options in Proc MIXED (SAS Institute 2013).

Another experiment was designed to determine whether ethanol-injected trap trees reduced colonization attempts by ambrosia beetles on insecticide-treated trees and improved the efficacy of preventive treatments. The experiment was conducted during spring 2015 and was set up as a completely randomized design in a field adjacent to a woodland. There were eight replications of treatments positioned in a single row parallel to and within 1 m of the woodland, with replications placed 10 m apart. Treatments were paired trees, which included an unsprayed trap-tree and a tree sprayed with bifenthrin (OnyxPro, FMC Corporation, Philadelphia, PA). The trap trees were either high-attraction injected with 50% ethanol or equivalent-attraction injected with 5% ethanol. Sprayed trees in both treatments were injected with 5% ethanol to ensure the attraction of ambrosia beetles. Equivalent attraction trap-trees were included as a nonsprayed control for trees injected with 5% ethanol. Trees within treatments were spaced 0.5 m apart. Experimental trees were injected with 75 ml of ethanol (50% or 5%) using the Arborjet® Tree I.V. Delivery System (Woburn, MA) (Ranger et al. 2010, Reding et al

2013). Experimental trees were red maple (*Acer rubrum* L.) obtained as a bare root and potted in a soilless substrate in 57-L (#15) containers. Trees were injected with ethanol on 19 May and set up in the field 20 May. Attacks were counted at 3- to 9-d intervals through 18 June. During evaluation, a wax pencil was used to mark tunnel entrances to prevent recounting attacks. A different color pencil was used for each count so excavated beetles could be associated with a specific date. Trees were transported to the lab for the final evaluation where beetles were excavated for identification. Scolytinae individuals were identified to the species level using available keys.

Data on total ambrosia beetle colonization attempts (tunnel entrances) and attempts by *X. germanus* (tunnels with beetles present) were $\log(X+1)$ transformed for analysis. Analyses compared total colonization attempts and attempts by *X. germanus* on bifenthrin-treated trees in relation to the paired trap tree (high versus equivalent attraction). Transformed data ($\log(X+1)$) were analyzed by repeated measures analysis of variance with trap-tree (high or equivalent attraction) as the between-subjects factor and total attempts or attempts by *X. germanus* on bifenthrin-treated trees through time as the repeated measures (Proc MIXED, SAS) (SAS Institute 2013). When significant trap-tree or trap-tree \times time effects were detected for total colonization attempts or *X. germanus* attempts, data were compared for each sample date using lsmeans and the pdiff option in Proc MIXED (SAS Institute 2013).

During experiments conducted in 2015 and 2016, 22 and 24 species of Scolytinae were captured, respectively. *Xylosandrus germanus* was the most abundant species accounting for 87% and 85% of the 2,936 and 14,064 Scolytinae captured in 2015 and 2016, respectively. Intercept traps captured 2,522 and 11,914 *X. germanus* in 2015 (5 traps per plot) and 2016 (11 traps per plot), respectively. However, the presence of intercept traps did not reduce captures of *X. germanus* in sentinel traps when captures within nurseries were combined or sentinel trap positions analyzed separately (Tables 1, 2). Although not significant, cumulative captures of *X. germanus* tended to be slightly higher in plots without intercept traps each year. Captures of *X. germanus* were highest in the woodland traps and decreased as the distance from the nursery/woodland interface increased ($F=4.98$; $df=4, 15$; $P=0.009$, in 2015; $F=28.34$; $df=4, 20$; $P<0.001$, in 2016).

Trees sprayed with bifenthrin and paired with high- or equivalent-attraction trap trees had no differences in total colonization attempts (2.6 ± 2.3 versus 1.0 ± 0.9 per tree, respectively) ($F=0.37$; $df=1, 63$; $P=0.55$) or attempts by *X. germanus* (1.9 ± 1.7 versus 0.4 ± 0.4 per tree, respectively) ($F=0.64$; $df=1, 63$; $P=0.43$). Total colonization attempts were about 7.1 times greater in high- versus equivalent-attraction trap trees (82.8 ± 14.4 versus 11.7 ± 5.9 per tree, respectively), whereas attempts with *X. germanus* were about 5.7 times greater in high versus equivalent attraction trees (43.9 ± 9.8 versus 7.6 ± 4.0 per tree, respectively).

In the current research, interception traps positioned at the woodland/nursery interface captured thousands of ambrosia beetles. However, intercepting large numbers of emigrating *X. germanus* did not reduce captures within nurseries regardless of trap proximity to the woodland/nursery interface. Addesso et al. (2019, J. Econ. Entomol. 112: 753–762) had similar results with *X. crassiusculus*; they tried to protect sentinel trees injected with ethanol and ethanol-baited sentinel traps. In

Table 1. Statistics for repeated measures analysis of *X. germanus* captures in plots protected by interception traps versus those without, in 2015.

Trap Position	Effects	df Numerator	df Denominator	F	P
Combined* in nursery	Interception	1	6	0.07	0.807
	Time	6	36	13.38	<0.0001
	Time × interception	6	36	0.25	0.956
13 m	Interception	1	6	0.00	0.992
	Time	6	36	8.42	<0.0001
	Time × interception	6	36	0.12	0.993
25 m	Interception	1	6	0.00	0.954
	Time	6	36	10.94	0.0001
	Time × interception	6	36	1.56	0.187
50 m	Interception	1	6	0.14	0.722
	Time	6	36	6.15	0.0002
	Time × interception	6	36	0.56	0.756
100 m	Interception	1	6	0.29	0.608
	Time	6	36	21.91	<0.0001
	Time × interception	6	36	0.55	0.764
Woodland (-13 m)	Interception	1	6	0.20	0.672
	Time	6	35	7.68	<0.0001
	Time × interception	6	35	0.19	0.977

Trapping data were log(X+1) transformed for analysis by Proc MIXED.
* Woodland trap data were not included.

their research, sentinel trees and traps were positioned 15 m from a woodland/field interface with various densities of interception traps 5 m from the interface. Werle et al. (2019, J. Appl. Entomol. 21: 168–179) tested a push-pull tactic wherein intercept traps and a repellent were used to protect flood-stressed trees. The traps and repellent did not reduce colonization attempts by *X. crassiusculus* or *X. germanus* on flood-stressed trees. In 2015, interception traps were positioned 25 m apart, and a slight numerical reduction in captures of *X. germanus* occurred in plots guarded by those traps. Consequently, in 2016, more interception traps were added and positioned 10 m apart. We hypothesized that placing traps at 10-m intervals would further reduce captures within guarded plots. We also presumed that placing traps at 10-m intervals around the perimeter of their nurseries would be acceptable to growers. The increased trapping did not lead to a further reduction in captures within the guarded nursery plots. McLean and Borden (1979, J. Econ. Entomol. 72:

Table 2. Statistics for repeated measures analysis of *Xylosandrus germanus* captures in plots protected by interception traps versus those without, in 2016.

Trap Position	Effects	df Numerator	df Denominator	F	P
Combined* in nursery	Interception	1	8	0.04	0.852
	Time	7	53	42.3	<0.0001
	Time × interception	7	53	0.46	0.862
13 m	Interception	1	8	0.04	0.855
	Time	7	53	15.72	<0.0001
	Time × interception	7	53	0.46	0.857
25 m	Interception	1	8	0.03	0.861
	Time	7	53	33.27	<0.0001
	Time × interception	7	53	0.62	0.741
50 m	Interception	1	8	0.11	0.749
	Time	7	53	19.20	<0.0001
	Time × interception	7	53	0.40	0.900
100 m	Interception	1	8	0.09	0.766
	Time	7	53	27.47	<0.0001
	Time × interception	7	53	0.66	0.702
Woodland (–13 m)	Interception	1	8	1.00	0.346
	Time	7	53	16.91	<0.0001
	Time × interception	7	53	0.69	0.676

Trapping data were log(X+1) transformed for analysis by Proc MIXED.
* Woodland trap data were not included in combined nursery data.

165–172) concluded that successful pest suppression by mass trapping is related to pest pressure. *Xylosandrus germanus* populations may be too high in our area to significantly reduce emigration into nurseries by trapping.

In the current research, trap-trees in close proximity (0.5 m) to test trees did not reduce colonization pressure on the test trees. Our results were similar to those of Adesso et al. (2019) who found that trap-trees injected with 50% ethanol did not prevent colonization attempts by ambrosia beetles on unsprayed trees injected with 1% ethanol. Colonization attempts on the high-attraction trap-trees were seven times greater than on equivalent-attraction trap-trees. However, there were no differences in colonization attempts on sprayed trees guarded by high- or equivalent-attraction trap-trees. It is possible there was some “spillover” from the high-attraction trees to the sprayed trees. In previous research, spillover did not

occur when injected trees were paired with noninjected trees (Ranger et al. 2010, Reding et al. 2013).

In summary, ethanol-baited traps positioned between emergence sites and nurseries were not effective at reducing activity of *X. germanus* within nurseries. Trap trees injected with high concentrations of ethanol did not reduce pressure from ambrosia beetles on adjacent trees injected with low concentrations of ethanol and sprayed with insecticides.