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Short Communication

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Bark Colonization of Kiln-Dried Wood by the Walnut Twig Beetle: Effect of Wood Location and Pheromone Presence

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Abstract

The walnut twig beetle (*Pityophthorus juglandis* Blackman) (Coleoptera: Curculionidae) is a regulated pest in the United States due to its causal role in thousand cankers disease of walnut trees, including the commercially valuable eastern black walnut (*Juglans nigra* L.). Several state quarantines designed to limit spread of *P. juglandis* regulate movement of kiln-dried walnut lumber that contains bark. Previous research demonstrated that *P. juglandis* will enter and re-emerge from bark of kiln-dried, *J. nigra* slabs subjected to extreme beetle pressure (baited with a pheromone lure and hung in infested *J. nigra* trees). This study evaluated *P. juglandis* bark colonization of both kiln-dried and fresh *J. nigra* slabs, varying the presence of aggregation pheromone and relative proximity to a beetle source. Wood treatment, slab location, and pheromone presence all significantly affected *P. juglandis* colonization, as assessed by subsequent beetle emergence. When placed on the ground directly beneath infested trees, kiln-dried slabs were not colonized, and fresh slabs were colonized only when baited with the pheromone lure (6/14 replicates). When placed in crowns of infested trees, kiln-dried slabs were colonized only when baited with pheromone (3/14 replicates), whereas fresh slabs were colonized with and without pheromone (14/14 and 1/13 replicates, respectively). Timing of emergence indicated that beetles did not reproduce in kiln-dried bark. Results suggest that the risk of kiln-dried walnut bark becoming colonized by the *P. juglandis* during movement of commercial wood products is very low. This information may be useful to government agencies that administer quarantines regulating the transport of walnut lumber.

Key words: *Pityophthorus juglandis*, thousand cankers disease, phytosanitary treatment, quarantine, *Juglans nigra*

The walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae: Scolytinae) is a bark beetle native to the southwestern United States and northern Mexico, where it occurs on Arizona walnut, *Juglans major* (Torrey) A. Heller (Bright 1981, Cranshaw 2011). Collections of *P. juglandis* were known from only four southwestern U.S. counties by 1960, but by 2016 the insect had been collected from at least 15 U.S. states and in Italy (Seybold et al. 2012, 2016; Montecchio et al. 2014; Rugman-Jones et al. 2015). In its expanded range, *P. juglandis* and a fungal associate, *Geosmithia morbida* M. Kolařík et al., have been implicated as casual agents of a damaging syndrome known as "thousand cankers disease" (Tisserat et al. 2009, Kolařík et al. 2011). The disease affects several species of walnut and butternut (*Juglans* spp.) as well as wingnut (*Pterocarya* spp.) (Kolařík et al. 2011, Serdani et al. 2013, Hishinuma et al. 2016), but of particular economic concern is its potential impacts on eastern black walnut (*Juglans nigra* L.), a species highly valued for lumber, veneer, and other specialty wood products (Newton and Fowler 2009).

Thousand cankers disease is characterized by progressive crown dieback after multiple attacks by *P. juglandis* populations on host branches and stems (Tisserat et al. 2009, Seybold et al. 2016). Male *P. juglandis* initiate host colonization and produce an aggregation pheromone that is attractive to both males and females (Seybold et al. 2015), and a lure containing this pheromone has been developed for monitoring and research (Seybold et al. 2013). Adult *P. juglandis* females create egg galleries in the phloem where larvae feed and pupate. The fungus *G. morbida* is routinely isolated from beetles and from necrotic bark tissue associated with beetle galleries (Tisserat et al. 2009, Kolařík et al. 2011).

Numerous U.S. states have established quarantines aimed at preventing the spread of *P. juglandis* and *G. morbida*, and regulating the movement of various types of walnut material, including

Table 1. Test statistics for the effects of wood treatment (kiln-dried vs fresh), pheromone presence (baited vs unbaited), and location relative to an infested walnut tree (in the crown vs on the ground) on the emergence of *Pityophthorus juglandis* adults from eastern black walnut (*Juglans nigra*) bark, at four sites in Knox County, TN

Main effect	Presence/absence of *P. juglandis* emergence			Mean number of *P. juglandis* emerged per slab		
	χ^2	df	*P*	*F* value	df	*P*
Wood treatment	20.51	1,13	<0.001	559.7	1,13	<0.001
Pheromone	32.46	1,13	<0.001	559.9	1,13	<0.001
Location	8.64	1,13	0.003	427.9	1,13	<0.001

walnut lumber with bark (Purdue University 2017). In these regulations, kiln-dried lumber is usually exempted from regulation if it is also square-edged and 100% free of bark (North Carolina DACS 2013, Pennsylvania Department of Agriculture 2014, Tennessee Department of Agriculture 2014). Producing completely square-edged, bark-free lumber, however, can be wasteful and expensive as it significantly reduces the yield of the top quality boards (Richards 1973, Regalado et al. 1992) and may be an unnecessary specification if there is a low probability that *P. juglandis* will attack kiln-dried bark during lumber transport.

Audley et al. (2016) demonstrated that *P. juglandis* adults entered and re-emerged from the bark of kiln-dried black walnut slabs that were baited with a pheromone lure and hung in the branches of infested *J. nigra* trees. That experiment represented extreme, artificially created pressure by *P. juglandis* that is unlikely to occur in a typical lumber production and transport stream. This study expands on that of Audley et al. (2016) to further evaluate the risk of *P. juglandis* colonization of kiln-dried walnut, by comparing kiln-dried versus fresh walnut slabs, and varying both the presence of the aggregation pheromone and relative proximity to a beetle source.

Materials and Methods

Uninfested log sections (bolts 20–25 cm in diameter, 30 cm long) were cut on 19 May 2015 from mature *J. nigra* trees in a plantation in McDowell County, NC, outside the known range of *P. juglandis* and lacking symptoms of thousand cankers disease. Each bolt was split through the center on its long axis into eight, equally sized, wedge-shaped slabs with bark retained. Slabs were kiln dried at 77°C (dry bulb, 66°C wet bulb, representing ~60% relative humidity) for 2 wk (21 May to 4 June 2015) in a walk-in kiln (SII Dry Kilns, Lexington, NC) at the University of Tennessee, Knoxville, TN to achieve approximately 8% moisture content (Denig et al. 2000). Fresh walnut bolts of similar size from the same plantation were cut and split into slabs on 15 June 2015 but were not kiln dried. Mean (SE) width of the bark-side edge, bark surface area, and bark thickness were 6.3 (0.09) cm, 195.5 (2.8) cm^2, and 10.7 (0.12) mm, respectively (n = 112 slabs). Kiln-dried and fresh slabs were stored indoors at ambient laboratory conditions (~23°C and 50% humidity) until deployed in the field.

Eight slabs (four kiln-dried, four fresh) were deployed for 2 wk at each of 14 mature *J. nigra* trees located at four suburban properties (five trees at site 1: 36.0795°N, −83.8579°W; four trees at site 2: 35.8216°N, −84.1463°W; four trees at site 3: 35.9719°N, −83.9921°W; one tree at site 4: 36.0945°N, −83.9079°W) in Knox County, TN. Bolts were deployed on 16 June 2015 except for site 3 where they were deployed on 22 June 2015. All trees exhibited branch dieback symptoms characteristic of thousand cankers disease and were known sources of flying *P. juglandis* based on their use in previous experiments (Mayfield et al. 2014, Audley et al. 2016).

At each infested tree, four slabs (two kiln-dried, two fresh) were deployed horizontally at ground level, 2.5–3.0 m from the bole and separated by 90° on cardinal directions, unless obstructions necessitated alternative placement. Slabs were positioned 30 cm above the ground surface by pressing a circular, galvanized steel wire tomato cage into the soil and attaching the slab to the cage with plastic cable ties. One slab of each wood treatment type was baited with a *P. juglandis* pheromone lure (product #300000736, Contech Enterprises Inc., subsequently Scotts Canada, Ltd, Delta, BC, Canada) stapled midway along its length. Slabs representing the four treatment combinations (fresh baited, fresh unbaited, kiln-dried baited, kiln-dried unbaited) were randomly assigned to the four ground positions. A parallel set of these four treatments were deployed simultaneously in the crown of the tree (typically 5–8 m above ground) by inserting an eye screw in the end of each slab, attaching a rope, passing the opposite end of the rope over a branch, and raising the slab into the crown. Crown-deployed slabs were spaced ≥2 m apart and randomly assigned to positions.

After 2 wk, slabs were retrieved from the field and placed indoors in ventilated emergence containers constructed from 19-liter plastic buckets that funneled emerging insects into illuminated collection jars containing propylene glycol antifreeze as a preservative (see Mayfield et al. 2014 for details). One slab from the fresh-unbaited-crown treatment was missing at the time of retrieval from the field, and data from this slab could not be captured. Emerging *P. juglandis* were counted every 2–3 wk for 6 mo (ending 19 Jan 2016). After slabs were removed from containers, the bark was removed from the sapwood and examined for evidence of *P. juglandis* entrance and emergence holes, galleries, and life stages.

Slabs were considered colonized by *P. juglandis* if adult emergence was detected. Logistic regression (the GENMOD procedure) was used to separately evaluate each main effect of wood treatment (kiln dried vs fresh), pheromone presence (baited vs unbaited), and slab location (ground vs crown) on presence or absence of *P. juglandis* emergence, with host tree included in the model as a blocking factor. To analyze the mean number of *P. juglandis* emerged per slab, due to the large number of zero counts, a generalized linear model was used with a Poisson distribution for errors and a log link for the mean. Wood treatment, the presence of *P. juglandis* pheromone, slab location, and tree were included as main effects in addition to the two-way interactions between wood treatment, pheromone presence, and slab location. The P-scale option was added to generate Type 3 F tests for effects in the model. Analyses were performed by using SAS 9.4 (SAS Institute 2012), and P values <0.05 were considered significant.

Results

The main effects of wood treatment, pheromone presence, and slab location significantly affected the presence of *P. juglandis* emergence

Table 2. Emergence of *Pityophthorus juglandis* adults from eastern black walnut (*Juglans nigra*) bark, by wood treatment (kiln-dried vs fresh), pheromone presence (baited vs unbaited), and location relative to an infested walnut tree (in the crown vs on the ground), at four sites in Knox County, TN (*n* = 13 or 14 slabs per location)

		Proportion of slabs with *P. juglandis* emergence		Mean (SE) *P. juglandis* emerged per slab	
Wood treatment	Pheromone	Ground	Crown	Ground	Crown
Kiln-dried	Unbaited	0/14	0/14	0 (0)	0 (0)
Kiln-dried	Baited	0/14	3/14	0 (0)	0.4 (0.2)
Fresh	Unbaited	0/14	1/13	0 (0)	0.1 (0.1)
Fresh	Baited	6/14	14/14	2.3 (1.1)	43.1 (10.3)

and the mean number of beetles emerged per slab (Tables 1 and 2). When placed on the ground directly beneath infested *J. nigra* trees, kiln-dried slabs were not colonized by *P. juglandis*, regardless of whether they were baited or unbaited (Table 2). Fresh slabs placed on the ground were only colonized by *P. juglandis* when they were baited with the pheromone lure; beetles emerged from 6/14 replicates of this treatment with a mean (SE) of 2.3 (1.1) beetles per slab (Table 2).

When placed in the crowns of infested *J. nigra* trees, kiln-dried slabs were not colonized by *P. juglandis* when the pheromone lure was absent. However, a total of five *P. juglandis* adults emerged from 3/14 replicates of the kiln-dried, pheromone-baited treatment (Table 2). These five beetles emerged during the first 5 wk after the slabs were retrieved from the field (Fig. 1). In contrast, all of the fresh, pheromone-baited slabs deployed in the crown were colonized by *P. juglandis*, which emerged at a mean (SE) of 43.1 (10.3) beetles per slab (Table 2). The total number of beetles emerging from this treatment (fresh-baited-crown) peaked at about 5 wk postretrieval (early August), but beetles continued to emerge from these bolts over the next 5 mo, with secondary peaks of emergence in mid-October and early November (Fig. 1). During bark examinations, evidence of *P. juglandis* galleries were found only in slabs from which *P. juglandis* emerged and no larval galleries were found in kiln-dried bark.

Discussion

The results of this study suggest that the probability of *P. juglandis* colonizing the bark of kiln-dried, black walnut lumber is very low.

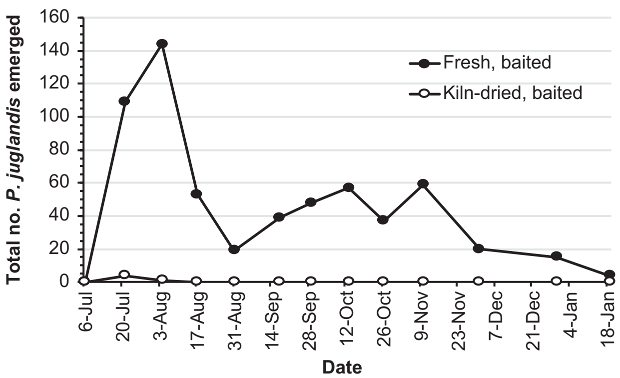

Fig. 1. Total number of *Pityophthorus juglandis* adults emerged from kiln-dried and fresh slabs of eastern black walnut (*Juglans nigra*) after being baited with an aggregation pheromone lure and deployed in the crowns of infested *J. nigra* trees for 2 wk (16 to 30 June 2015) in Knox County, TN (*n* = 14 slabs per treatment).

When kiln-dried slabs were placed on the ground directly beneath infested trees and baited with the *P. juglandis* pheromone, these slabs were not colonized, whereas more than 40% of the fresh slabs baited and placed in the same environment were colonized. Only under the highest pressure scenario, when pheromone-baited slabs were placed in direct proximity to emerging beetles (i.e., in the crowns of infested trees), was there evidence of *P. juglandis* entering and re-emerging from kiln-dried bark. This latter finding is consistent with Audley et al. (2016) who also found that *P. juglandis* would enter and re-emerge from the bark of pheromone-baited, kiln-dried walnut slabs hung in infested trees.

This latter attack scenario (close-range, pheromone-mediated attraction of *P. juglandis* to kiln-dried bark) seems unlikely to occur outside of an experimental setting. Apart from artificial baiting, the *P. juglandis* pheromone should be emitted from kiln-dried material only if one or more *P. juglandis* males (which produce the pheromone) were already present in or on it. However, unbaited kiln-dried material exhibited no evidence of attack in this study, suggesting that it is not attractive to founder male *P. juglandis*. Even fresh *J. nigra* slabs hung in the crown were attacked very infrequently in the absence of a pheromone lure (Table 2). This suggests that the production of the pheromone by a founder male may be relatively important for subsequent colonization of host material. Furthermore, *P. juglandis* colonized 100% and 40% of the fresh, baited slabs placed in the crown and on the ground, respectively, indicating an abundance of flying adults that could potentially have colonized the unbaited kiln-dried slabs, yet did not.

Consistent with the findings of Audley et al. (2016), emergence patterns in this study indicated that kiln-dried bark was unsuitable for *P. juglandis* reproduction. The five adults recovered from kiln-dried, baited, crown-deployed slabs emerged during the first 5 wk postretrieval (Fig. 1). *P. juglandis* larval development times of 4–6 wk and a complete generation within 7 wk at room temperature have been reported (Tisserat et al. 2009, Cranshaw and Tisserat 2012). Although field time (2 wk) plus containerization (5 wk) may have been sufficient for completion of a generation in this study, the absence of emergence from kiln-dried material over the next 5 mo, and no evidence of larval galleries in the bark, indicated that beetles were not reproducing. In contrast, *P. juglandis* emerged continuously for 6 mo from the fresh, baited slabs that had also been placed in the crown (Fig. 2), indicating reproduction and emergence of subsequent generations. This pattern of extended emergence of overlapping *P. juglandis* generations from containerized walnut has been observed in other studies (Mayfield et al. 2014, Audley et al. 2016, Castrillo et al. 2017). Adult emergence was similar in pattern and duration to that reported by Fichtner et al. (2014) from freshly cut *Juglans* sp. firewood in California, but extended later into the calendar year, possibly due to different dates of initial infestation. In contrast, the few beetles emerging from kiln-dried material in this study likely represented adults that were attracted to the pheromone bait, entered the bark, and re-emerged without reproducing.

In conclusion, this study provides evidence that the risk of kiln-dried walnut bark becoming colonized by the *P. juglandis* during the movement of commercial wood products is very low. Material of this type, when not artificially baited with the *P. juglandis* pheromone, was not colonized by *P. juglandis* even when placed in or beneath infested trees. This information may be useful to government agencies that develop or revise quarantines regulating the transport of walnut lumber.

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