# Trapping the Walnut Twig Beetle[1] to Determine Flight Patterns in Colorado

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**Abstract.** The walnut twig beetle, *Pityophthorus juglandis* Blackman, and its fungal associate *Geosmithia morbida* Kolařík et al., produce thousand cankers disease, which has devastated black walnut (*Juglans nigra* L.) in several states of the western U.S.A. To better understand beetle flight dynamics and life history, flight times were documented in the urban corridor along the Colorado Front Range during 2 years -- in 2008 with yellow sticky card traps and in 2012 with baited multiple funnel traps. Walnut twig beetles were determined to be active throughout the trapping season from March through November, exhibited overlapping generations, and potentially a bivoltine life cycle. Most beetles were collected from traps between July and September, and the greatest trap density frequently occurred in August. The total number of beetles collected throughout the trapping season differed at each location. In 2008 the number of beetles collected ranged from 12 to 145, and from 60 to 12,909 in 2012. More beetles were found in municipalities experiencing early disease development when compared to cities with chronic disease.

## Introduction

Thousand cankers disease of walnut trees (*Juglans* spp.) is caused by the combined activity of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae), and the filamentous ascomycete *Geosmithia morbida* Kolařík et al. (Ascomycota: Hypocreales: Bionectriaceae) (Tisserat et al. 2009, Kolařík et al. 2011). Historically, walnut twig beetle caused minor damage to Arizona walnut (*Juglans major* (Torr.) Heller) in its native range of New Mexico and Arizona (Blackman 1928, Cranshaw 2011). However, beetles vectoring *G. morbida* cause considerable damage to several species of walnut native to North America (Utley et al. 2013). One of the most susceptible species is valuable black walnut (*J. nigra* L.) (Harlow and Harrar 1969, Newton et al. 2009, Tisserat et al. 2009, Utley et al. 2013).

The walnut twig beetle creates galleries in the phloem and carries *G. morbida*, a canker-inducing fungus. Cankers form near wounds that beetles make in trees and develop from numerous point introductions of the fungus, which cumulatively compromise the phloem (Tisserat et al. 2011). Initial symptoms are ultimately followed by death of the tree 1 to 2 years after introduction of disease. Therefore, disease usually is detected after the tree is severely colonized by both

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causal agents (Tisserat et al. 2009). Eventual symptoms of affected trees include yellowing and thinning of leaves in the upper canopy, and dieback of the upper canopy. Early detection and monitoring are needed to manage the disease.

Traps often have been used to monitor bark beetles (Schlyter et al. 1987, Barclay et al. 1998, Seybold et al. 2012b). Multiple funnel traps visually attract and catch bark beetles in numbers comparable to those by sticky traps (Lindgren 1983). They also can be used in combination with semiochemicals to increase attraction by beetles (Hansen 1996, Bentz 2006). Peak flight times are ascertained by beetle abundance in the traps (Safranyik and Linton 1993). Therefore, monitoring traps not only can document the presence of species but elucidate the efficiency of cultural and chemical controls through more targeted application (Peck et al. 1997).

Information is available on dispersal behavior of walnut twig beetles. Tisserat et al. (2009) tracked beetle flight times with sticky card traps from April through October 2006 at Boulder, CO, but peak flight times and flight patterns of walnut twig beetle in Colorado were not indicated. Similar to other bark beetles (Gray 1972, Rasmussen 1974), activity of walnut twig beetle adults is associated with air temperature. Trapping studies in California inferred that flights of walnut twig beetle peaked when temperatures were between 23°C and 27°C and in the absence of direct sunlight (Seybold et al. 2012b, Chen and Seybold 2014). Flight activity in California and Italy decreased when temperatures cooled to less than 18°C (Seybold et al. 2012b, Faccoli et al. 2016).

Specific flight activity data available on walnut twig beetles in California and Italy might not correlate with adult flight activity patterns in Colorado where air temperature patterns are different. Many factors, including trap site (e.g. topography, elevation, density of the tree stand, trap sampling range), environmental conditions (e.g. wind speed, wind direction, temperature) and beetle dynamics (e.g. size of the beetle population, distance to the trap) affect trapping results (Safranyik and Linton 1993, Schmid et al. 1992, Hansen 1996, Barclay et al. 1998, Bentz 2006). Genetic diversity of walnut twig beetles also is different in the Colorado Front Range (Rugman-Jones et al. 2015), which might contribute to differences in life history. In this study, nonbaited sticky traps and baited multiple funnel traps were used to better understand flight behavior of walnut twig beetles in Colorado. The objectives of the study were to (1) provide information on site-specific abundance of walnut twig beetle in four Colorado municipalities, (2) use flight patterns to interpret the life history of walnut twig beetle in Colorado, and (3) apply peak flight times to management recommendations.

## Materials and Methods

Flight patterns of adult walnut twig beetles were tracked with yellow sticky card traps in 2008 and with eight-unit funnel traps in 2012. This enabled sampling two phenomena: (1) random flights by the beetle with sticky card traps, and (2) attraction of the beetles to a specific lure in multiple funnel traps. Locations along the Colorado Front Range (approximately 1,550 m in elevation) (Marr 1961) were selected based on the visible presence or known recent introduction of thousand cankers disease.

From June through December 2008, yellow sticky card traps measuring 28 x 23.5 cm (Trece, Inc., Adair, OK) were placed in three randomly selected diseased black walnut trees in Prospect Park at Wheat Ridge, Jefferson County, CO. The trees were referred to as “east” (39°77’79.4’’ N, -105°12’49.5’’ W), “northwest”

(39°77'80.1'' N, -105°12'52.4'' W), and "southwest" (39°77'77.9'' N, -105°12'52.5'' W) based on their location in the park. A trap was tied at a height of approximately 3.5 m in a branch in each tree. Traps were removed and replaced at biweekly intervals. Walnut twig beetle adults were identified and quantified with the aid of a stereomicroscope at 20X magnification. The daily total number of beetles was determined by averaging the number of beetles caught in the trap over the amount of time between collections of samples. Voucher specimens were deposited in the C. P. Gillette Museum of Arthropod Diversity at Colorado State University, Fort Collins.

During 2012, abundance of walnut twig beetles was tracked on one black walnut tree confirmed to have thousand cankers disease at four locations: Boulder northwest (40°03'60.7'' N, -105°26'30.7'' W) (Boulder County), Boulder northeast (40°03'42.8'' N, -105°17'75.1'' W) (Boulder County), Fort Collins (40°33'02.1'' N, -105°06'38.8'' W) (Larimer County), and Denver (39°72'55.6'' N, -104°90'84.5''W) (Denver County), CO. An eight-unit multiple funnel trap (Contech Enterprises Inc., Victoria, British Columbia, Canada) was hung close to the trunk of each tree, on a branch at a height of approximately 3 m. Each trap was baited with a lure developed by Seybold et al. (2011) containing a male-produced aggregation pheromone (Contech Enterprises Inc., Victoria, BC, Canada). Lures were changed and beetles were collected from traps at approximately 2-week intervals. Walnut twig beetles were identified and counted as described previously. Trapping data were collected from March through November or as long as possible before the diseased trees were removed.

## Results and Discussion

During 2008, at Wheat Ridge, peak flight activity of walnut twig beetles occurred in late July through September, although there were slight differences in flight patterns between trap locations. Most walnut twig beetle adults were documented in July at the "east" location (Fig. 1A) and in August at the "northwest" location (Fig. 1B). Most adult beetles were caught during August and September at the "southwest" location (Fig. 1C). Flight patterns in 2008 varied slightly by location, but numbers of flying beetles caught in the traps consistently peaked in late summer. Although the traps were in close proximity to each other, the number of beetles collected in the traps varied considerably among trees (Table 1).

In 2012, the multiple funnel trap at Denver collected the most walnut twig beetle adults in September although a smaller peak occurred in July (Fig. 2A). Most beetles at both Boulder locations were caught in August (Fig. 2BC). At Fort Collins, peak flight activity occurred in July (Fig. 2D), but this early peak probably was because the diseased tree with the multiple funnel trap was removed in July. Because of risk of spreading disease, the trees at Fort Collins and Boulder northeast were removed on 20 July and 6 November 2012, respectively. Multiple small walnut trees, ≤15 cm in diameter, remained at the sites, so traps were relocated and monitored until mid-November at both locations.

Although the tree at Fort Collins had minimal symptoms of thousand cankers disease when removed, it sustained tens of thousands of walnut twig beetle larvae as determined by adult emergence studies from felled logs (Sitz 2013). Results demonstrated that the causal agents were typically well established before thousand cankers disease was detected, as documented by Tisserat et al. (2009).

The results also emphasized the importance of monitoring trees to aid in early detection of disease (MDA 2014).

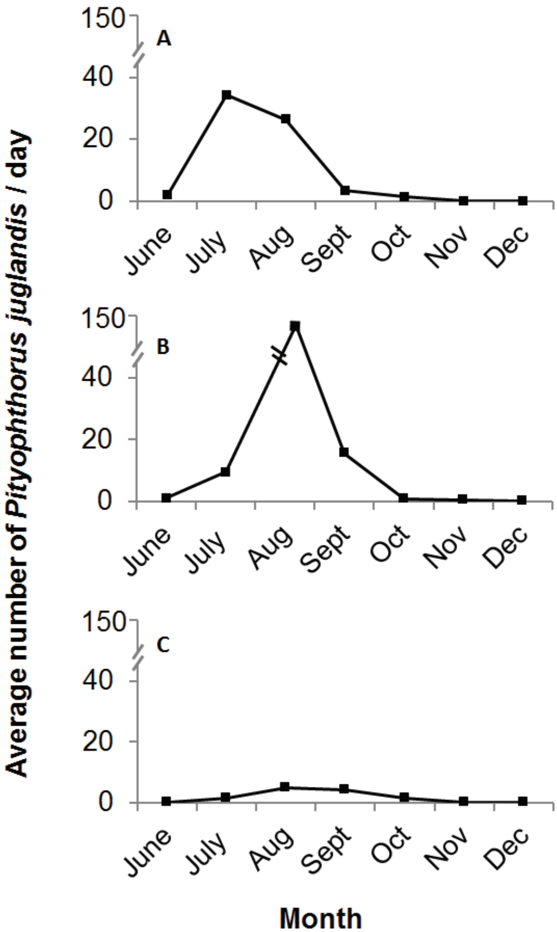


Fig. 1A-C. Trap catches of walnut twig beetle, *Pityophthorus juglandis*, are shown as the average number of beetles per day from yellow sticky card traps fastened to black walnut trees during the specified month of 2008. Sites monitored at Wheat Ridge, CO, were (A) east, (B) northwest, and (C) southwest.

Table 1. Total Number of Walnut Twig Beetles, *Pityophthorus juglandis*, Collected from Sticky Card Traps during the Sampling Period in 2008

| Trap location | Year | *n* |
|---|---|---|
| Wheat Ridge east | 2008 | 67 |
| Wheat Ridge southwest | 2008 | 12 |
| Wheat Ridge northwest | 2008 | 145 |

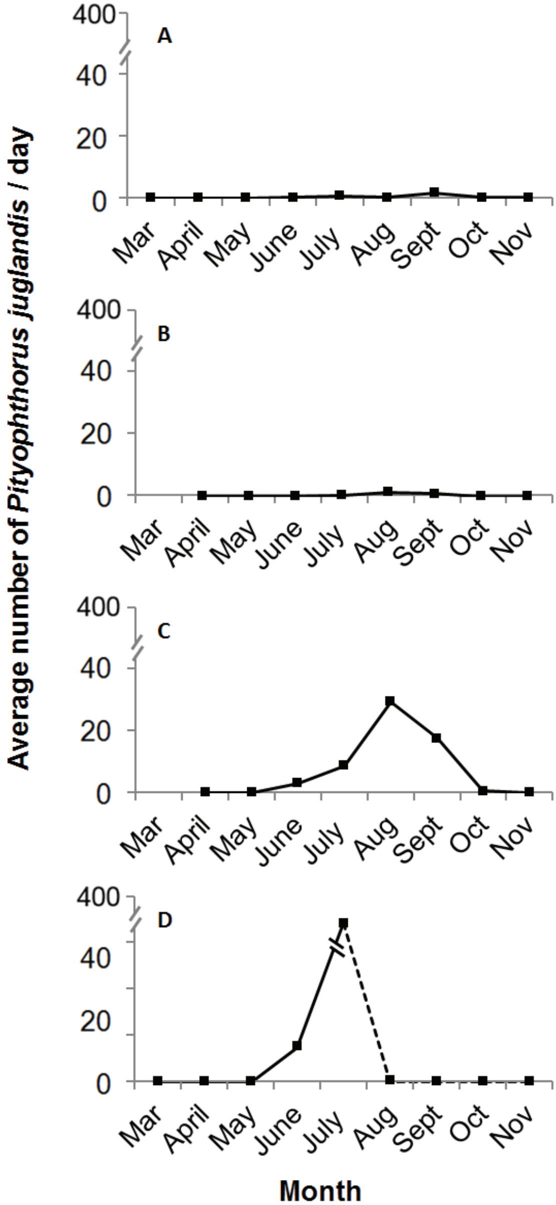


Fig. 2A-D. Trap catches of walnut twig beetle, *Pityophthorus juglandis*, are shown as the average number of beetles caught each day in multiple funnel traps fastened to black walnut trees during the specified month of 2012. The sites monitored along the Colorado Front Range were (A) Denver, (B) Boulder northwest, (C) Boulder northeast, and (D) Fort Collins, CO. The dotted line represents data collected after the original trap tree was removed but the trap was relocated several yards away.

Numbers trapped indicated peak flight activity of walnut twig beetle adults between mid-July and mid-September. At all but the Denver site, a distinct month-long peak of flight activity occurred in late summer. This supports overlapping generations, as previously documented for walnut twig beetle (Luna et al. 2013, Nix 2013, Faccoli et al. 2016). Trap data at Denver indicated two peak adult flight periods 1 month apart, in July and in September, inferring two emergence periods. Contrary to univoltine life history with overlapping generations reported in Italy (Faccoli et al. 2016), Denver trap data indicated at least a potential bivoltine life cycle. Many bark and twig beetles develop into adults 4 to 8 weeks after eggs are deposited (Trimble 1924, Amman et al. 1974, Stark 1982). Nix (2013) reported a generation of walnut twig beetles could be completed in 4 to 5 weeks. Therefore, multiple generations most likely occurred along the Front Range of the Colorado Rockies.

It is not unusual to find species of *Pityophthorus* emerging during several months. For example, Peck et al. (1997) found *Pityophthorus confinis* LeConte flying throughout the season, with peaks in May, June, and July. Hedlin and Ruth (1970) reported emergence of *P. orarius* Bright from mid-July to mid-August. Similar to findings by Tisserat et al. (2009), flights of walnut twig beetles occurred during the entire trapping period in Colorado. This study extended the documented period of time when trap catches occurred in Colorado, from April through October (Tisserat et al. 2009), to March through November (Fig. 2B). Flights probably occurred on warm days throughout the year. This phenomenon was documented in California and Tennessee where a few flying walnut twig beetle adults were collected during winter months (Seybold et al. 2012a, Nix 2013).

Our data showed a trend where more beetles were found at locations where disease was beginning to develop compared to areas previously infected by thousand cankers disease. By 2012, thousand cankers disease had been at west Boulder and Denver for several years whereas it was the first record of walnut twig beetles and thousand cankers disease at Fort Collins (Tisserat et al. 2009, Sitz 2013). The number of beetles collected throughout the season in the trap at Fort Collins exceeded 10,000 individuals, more than collected in any other trap (Table 2). Tree diseases can shift from epidemics to endemics (Webber 2000), and many factors could play a role in progression of thousand cankers disease in Colorado. The dramatic differences in abundance of walnut twig beetles at sites along the Front Range might be caused by varying degrees of resistance by black walnut, different complexes of insect natural enemies, or dynamic insect-pathogen associations (Gupta et al. 1972, Bonello et al. 2001, Awmack and Leather 2002, Sitz et al. 2017).

Walnut twig beetles fly during the spring, summer, and fall, although the most consistent peak occurs in late summer. Beetles probably also fly on warm days in

Table 2. Total Number of Walnut Twig Beetles, *Pityophthorus juglandis*, Collected Using Multiple Funnel Traps with a Male Aggregation Pheromone during the 2012 Sampling Period

| Trap location | Year | *n* |
|---|---|---|
| Denver | 2012 | 95 |
| Boulder northwest | 2012 | 60 |
| Boulder northeast | 2012 | 2,729 |
| Fort Collins | 2012 | 12,909 |

winter. In Colorado, walnut twig beetle clearly has overlapping generations, but it might have a bivoltine life cycle. The number of beetles trapped varied considerably among the trees. In Colorado, a large number of adult beetles were active during the initial phase, but numbers decreased during the chronic phase of disease development. Thousand cankers disease should be monitored by trapping beetles, because the number of beetles trapped is an early measure of disease presence. If walnut twig beetles are identified, control should be used while the area is experiencing the initial phase of disease development and before late summer when peak flight occurs.

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