OXFORD

Plant-Insect Interactions

Seasonal and Regional Distributions, Degree-Day Models, and Phoresy Rates of the Major Sap Beetle (Coleoptera: Nitidulidae) Vectors of the Oak Wilt Fungus, *Bretziella fagacearum*, in Wisconsin

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Subject Editor: Joe Louis

Abstract

Oak wilt is a lethal disease caused by the invasive fungus *Bretziella fagacearum*, which is transmitted belowground via root grafts and aboveground by sap beetles (Nitidulidae). Attempts to limit spread and impact of *B. fagacearum* emphasize limiting harvesting and pruning to periods of vector inactivity. However, there is limited information on sap beetle activity periods, responses to temperature, and phoresy frequencies of fungi. We sampled two major vectors in Wisconsin, *Colopterus truncatus* and *Carpophilus sayi*, for 2 yr to quantify their seasonal and geographic abundances. Trapping was performed in 12 oak stands, and beetles were assayed for *B. fagacearum*. *C. truncatus* was captured from March until November, peaking during April and May. *C. sayi* was captured from April until November, peaking in May and July. Relative abundances (*N* = 15,980) were 59.3% *C. truncatus* and 40.7% *C. sayi*. *C. sayi* was more abundant in southern Wisconsin, whereas *C. truncatus* was more evenly distributed. Both species were present at asymptomatic sites. All sites with oak wilt centers yielded beetles with viable fungal propagules, with the frequency of association ranging from 1 to 50%. Sites asymptomatic for oak wilt contained both beetle species, but no vector-borne viable pathogen. Degree-day models were constructed to improve the generality of these results and estimate cumulative emergences across a latitudinal range over the previous 10-yr means and extremes. Because activity by *C. truncatus* and *C. sayi* spans the seasonal activities of oak wilt vectors, these results can help guide oak management practices.

Key words: *Colopterus truncatus*, *Carpophilus sayi*, *Quercus*, spatial distribution, temperature modeling

Oak wilt is a lethal disease of native oak trees caused by the fungus *Bretziella fagacearum* (Bretz) (Microascales: Certocystidaceae) Z. W. De Beer, Marinc., T. A. Duong, and M. J. Wingf (syn. *Ceratocystis fagacearum*) (De Beer et al. 2017). Oak wilt was first discovered in the United States in the Upper Mississippi River Valley, and historical records suggest that *B. fagacearum* could have been in the Upper Midwest as early as the 1890s (Henry et al. 1944, True et al. 1960, Gibbs and French 1980). It was first identified in Wisconsin in 1942 (Henry et al. 1944) and was recognized as a significant problem by the 1950s (Bretz 1953). It currently affects many midwestern, northeastern, and southern states (Cervenka et al. 2001). This fungus is also of major concern as a potential introduced species into Eurasia (Gibbs 2003).

Results of recent phylogenetic analyses led to the establishment of a monotypic genus for this pathogen species, thus moving it from *Ceratocystis* to *Bretziella* (De Beer et al. 2017). The general biology of *B. fagacearum*, however, resembles that of native *Ceratocysis*

species in being a wound-colonizing plant pathogen (Engelbrecht et al. 2004, Juzwik et al 2008). Further support for the re-classification includes the type of disease caused and the manner of its sporulation. No native *Ceratocystis* causes a true vascular wit disease as does *B. fagacearum*, and pathogens coevolved with their host(s) rarely cause high levels of mortality (Kile 1993). Furthermore, most *Ceratocystis* species form sporulation mats on exposed wood (Fergus and Stambaugh 1957), whereas *B. fagacearum* produces enclosed sporulation mats between the inner bark and outer sapwood with opposing, dense, sterile tissue (pressure pad) that pushes the bark away from the wood (Gibbs and French 1980). The latter often results in vertical cracks in the bark, which give insects access to the fungal mat in the resulting cavity.

The phylogenetic and population genetics studies, historical incidence and spread data, and lack of coevolved host resistance in the red oak species support the view that *B. fagacearum* is a nonnative

invasive species (Kile 1993, Engelbrecht et al. 2004, Juzwik et al. 2008, Harrington 2009). The population structure of *B. fagacearum* shows evidence of a genetic bottleneck consistent with the hypothesis that the fungus was introduced to the United States (Juzwik et al. 2008).

All oak species (Fagaceae: *Quercus*) are at least partially susceptible, but *B. fagacearum* is most damaging on species of the red oak group (Lobatae) (Juzwik 2011). Infection causes xylem vessels to clog, leading to host wilting or death. The fungus can spread in two ways, belowground via root grafts between neighboring trees (Blaedow and Juzwik 2010) and over longer distances by insect vectors (McMullen et al. 1955). Root grafts can connect oaks that are 15 m or greater apart (Bruhn et al. 1991; O'Brien et al. 2003), making it very difficult and expensive to control this pathogen once established. Root disruption can halt transmission, but repeated treatments may be required to ensure no new root grafts form (Juzwik et al. 2010). When root disruption is not possible, felling of wilted and neighboring trees followed by stump removal has been used in some forest stands (Juzwik et al. 2011). Because stopping belowground transmission is expensive and laborious, natural resource managers and urban foresters emphasize disease prevention. Guidelines for pruning or harvesting oak trees and removing potential spore-producing trees are aimed largely at preventing insect-borne transmission.

The primary vectors of *B. fagacearum* are considered to be sap beetles (Coleoptera: Nitidulidae). The different species vary in their degree of association with *B. fagacearum* (Norris 1956, McMullen et al. 1960). Sap beetles are attracted to decaying fruit, fermenting or live plant saps, and fungi (Downie and Arnett 1996). Volatiles produced by *B. fagacearum* attract several nitidulid species (Lin and Phelan 1992), which can externally acquire spores as they visit mats. SEM photographs of nitidulid beetles collected from oak wilt mats document the occurrence of conidia and ascospores of the fungus on all body parts (Juzwik and French 1983). When a contaminated beetle flies to a new location and lands on a freshly wounded oak tree, it can lead to transmission. All nearby root-grafted trees can subsequently become infected.

Six nitidulid species are the most common insects on *B. fagacearum* mats in Minnesota (Cease and Juzwik 2001), Wisconsin (McMullen et al. 1960), and Illinois (Himelick and Curl 1958), with other nitidulids detected less frequently. The relative abundances and apparent roles of each beetle species appear to vary among states (Cease and Juzwik 2001). *Colopterus truncatus* and *Carpophilus sayi* were described as the most common species dispersing from areas in close proximity to fungal mats in Minnesota, with *C. truncatus* adults more common on newly forming mats and *C. sayi* more common on mature to old mats (Cease and Juzwik 2001, Ambourn et al. 2005). In Minnesota, *C. truncatus* and *C. sayi* accounted for >95% of nitidulids collected from fresh wounds (≤5 d old) during April–June on asymptomatic trees in locations containing oak wilt and often carried viable *B. fagacearum* (Juzwik et al. 2004). Three other species (*Cryptarcha ampla*, *Lobiopa* spp., and *Glischrochilus quadrisignatus*) accounted for >79% of nitidulids visiting fresh wounds on healthy oaks from early July to mid-October, but only 2 of 387 yielded viable *B. fagacearum* (Juzwik et al. 2006). Thus, Ambourn et al. (2005) proposed that *C. truncatus* and *C. sayi* were the principal vectors there. These species are particularly pertinent to management because their early (*C. truncatus*) and late (*C. sayi*) activity periods span the seasonality of nitidulids known to carry *B. fagacaerum*.

The life histories of *C. truncatus* and *C. sayi* are poorly understood. Kyhl (2006) found one *C. truncatus* adult in the bark furrow of a mat-producing tree during winter, and several emerged from litter at the base of mat-producing trees in spring. McMullen (1955) reported that *C. sayi* could oviposit on *B. fagacearum* cultures in the laboratory and recorded mean development times of 3 d for eggs, 19 d for larvae, and 7 d for pupae. Kyhl (2006) found larvae of both *C. truncatus* and *C. sayi* on *B. fagacearum* mats (59% combined) in Minnesota and western Wisconsin. These species are widely distributed: *C. truncatus* ranges from Nova Scotia to the Yukon in Canada, and Florida to California in the United States, south to Brazil and Chile; *C. sayi* ranges from Nova Scotia to Saskatchewan down to Georgia through New Mexico (Parsons 1943, Price 2003, Hayslett et al. 2008, Bousquet et al. 2013, Evans 2014). Both species show a wide range of host plant associations (Price 2003). For example, *C. truncatus* contributes to pollination of *Calycanthus occidentalis* (Calycanthaceae) in the southern United States (Nicely 1965).

Guidelines for managing oak wilt are based largely on preventing the incidence of new infection courts caused by pruning or cutting, during periods when potential vectors are active and red oaks are most susceptible to infection (Juzwik et al. 1985). For example, in Minnesota, the period between November and March is considered a safe time to prune, April to mid-July is considered a high risk period, and mid-July through the end of October is considered a low hazard period (Juzwik et al. 2015). Oak harvesting guidelines for oak timberlands in Wisconsin are based on a similar approach (WI DNR 2016).

Implementing sustainable management programs to prevent insect transmission of *B. fagacearum* requires accurate quantification of seasonal flight periods, quantification of phoresy rates and their seasonal variation, and degree-day models to address annual and regional variability and changing climate. Such data and models are largely lacking, and only general information upon which to establish no-cut and no-pruning guidelines is available. Setting these restrictions too broadly hinders timber and arborist operations, while setting them too narrowly risks further spread. In Wisconsin, the current Oak Harvesting Guidelines (WI DNR 2016) distinguish between counties north and south of a long-recognized natural tension zone that separates regions of starkly different geological history (West 1961), climate (Kucharik et al. 2010), soil (Bockheim and Hartemink 2017), and flora (Curtis 1959). Counties north of the tension zone have cutting restrictions from 15 April to 15 July, and counties south of this zone have restrictions from 1 April to 15 July (WI DNR 2016). A team of representatives from various government agencies and private sectors periodically review these guidelines to update them based on the latest scientific findings.

In this study, we sought to 1) characterize the phenology, abundance and distribution of two major vectors, *C. truncatus* and *C. sayi*, in Wisconsin; 2) quantify the frequencies of association of *C. truncatus* and *C. sayi* with *B. fagacearum*; and 3) develop degree-day models of *C. truncatus* and *C. sayi* to guide decisions about the timing of pruning and silvicultural practices.

Materials and Methods

Relative Abundance, Spatial distribution, and Seasonality of *C. truncatus* and *C. sayi*

Study Sites

Twelve forested sites throughout Wisconsin were selected for sampling during 2015 and 2016 (Fig. 1). Sites were chosen based on Wisconsin DNR records of the incidence and distribution of oak wilt. All sites were state parks, except for two state and county forests, and each contained at least one predominantly oak stand. Site locations, oak components, and oak wilt histories are shown in
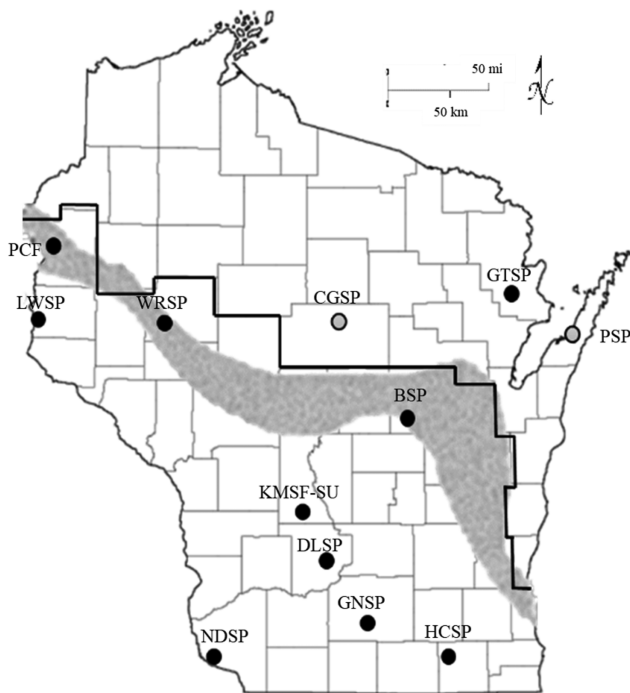
Fig. 1. Locations of oak sites sampled consecutively in 2015 and 2016 for *Colopterus truncatus* and *Carpophilus sayi*. Black circles: sites with known oak wilt. Gray circles: sites without known oak wilt. Bold black line shows the division of northern and southern zones used in Oak Harvesting Guidelines (WI DNR 2016). The Wisconsin tension zone (Anderson 2005, Curtis and McIntosh 1951) is shown in gray.

Table 1. Ten sites contained at least one known oak wilt center, and two sites were outside the known distribution of oak wilt.

Sampling

Six wind-oriented funnel traps (Dowd et al. 1992), as modified by Jagemann 2017, were placed at each site. In sites positive for oak wilt, traps were arranged around one or more infected oak trees and placed ~10 m apart. Recently wilted oak trees were examined for the presence of *B. fagacearum* mats between the inner bark and outer sapwood (Fergus and Stambaugh 1957). At the two asymptomatic sites, traps were placed within the selected oak stand. Traps were hung from limbs of trees with white rope 1–1.5 m above the ground. Rope connected the trap to the tree through a fishing hook clip swivel, which allowed the trap to spin freely in the wind.

Six traps per site were baited with a combination of 10 ml fermenting flour dough and a synthetic pheromone lure. Traps were sampled, emptied, and replenished with fresh dough weekly, and pheromone lures were changed every 4 wk, as per vendor recommendation. The dough bait consisted of 360-g whole wheat flour, 200-g sugar, and 1.5 tablespoons yeast mixed with equal parts water (Kyhl et al. 2002, Bartelt et al. 2004). In 2015, three traps were baited with a pheromone developed for *C. sayi*, (*E,E,E,E*)-3,5,7-trimethyl-2,4,6,8-decatetraene and (*E,E,E,E*)-7-ethyl-3,5-dimethyl-2,4,6,8-undecatetraene (product ID: IPM-CAR SAYI), and three were baited with one developed for *C. truncatus*, (*E,E,E,E*)-3,5,7-trimethyl-2,4,6,8-decatetraene (product ID: IPM-COL TRUN). Pheromone lures were obtained from Great Lakes IPM (Vestaburg, MI). In 2016, we used (*E,E,E,E*)-3,5,7-trimethyl-2,4,6,8-decatetraene in all six traps due to the lack of interspecific specificity, higher overall trap catch with this compound in 2015 (see Results), and the unavailability of the other lure.

Collection vials contained shredded paper to allow the beetles to initially separate themselves to prevent contamination among beetles, and a small section of pest strip (NUVAN Prostrip, AMVAC Chemical Corporation, Axis, AL) to rapidly kill beetles. Personal observation (J. Juzwik) has found that collection vials with shredded paper and an insecticide strip kept adult insects separated from each other.

Each site was equipped with one HOBO Tidbit Data Logger (Onset Computer Corporation, Bourne, MA) that recorded daily maximum and minimum temperatures; data loggers were placed on the north side of a tree containing a wind-oriented funnel trap and on the northern side of the site.

In 2015, traps were deployed from 19 to 29 May. Eight sites were sampled weekly until 29 August–11 September, for a total of 16 wk. Sampling continued at the other four sites until no *C. truncatus* or *C. sayi* were captured for two consecutive weeks (Governor Nelson State Park [GNSP], Devil's Lake State Park [DLSP], Polk County Forest [PCF], and Willow River State Park [WRSP]: All abbreviations are as in Table 1). Sampling at these sites continued until 23 November for a total of 28 wk of trapping.

In 2016, traps were deployed from 7 to 28 March. The sampling effort was modified to focus on the beginning and end of current cutting guidelines. Traps were first sampled weekly until both species were captured at each site, then once every 3 wk starting 18 April–3 June, then weekly from 7 to 29 July, and then every 3 wk for the remainder of the season. Trapping was performed for all sites until 8 September (18 wk of sampling total), at which time eight sites were discontinued. Trapping at the same four sites as in 2015 continued until neither beetle species was captured for two consecutive weeks. This extended sampling continued for another 6 wk, ending with 24 wk of sampling on 6 December across a 40-wk period.

Insect Identification

C. truncatus and *C. sayi* were identified using keys and photographs from Price (2003). Identified insects were counted and placed individually in sterile 1.5-ml micro-centrifuge tubes (Celltreat, Pepperell, MA) and stored at –20°C. Other nitidulid species were identified, and the dates of first and last capture in 2016 were recorded. Two sampling periods between first and last capture of these other nitidulid species were also counted and recorded to provide a coarse estimate of abundance. Unidentified insects were placed in sterile 1.5-ml micro-centrifuge tubes labeled with week and location of capture, and stored at –20°C.

Voucher specimens were submitted to the University of Wisconsin Insect Research Collection (Madison, WI). For each year of collection, two of each study species from each of four sections of Wisconsin (northeast, northwest, southeast, and southwest) were taken from the beginning and end of each season, for 32 specimens each of *C. truncatus* and *C. sayi*.

Association of *B. fagacearum* with *C. truncatus* and *C. sayi*

The presence of viable *B. fagacearum* propagules on *C. truncatus* and *C. sayi* was determined by serial dilution plating using previously published methods (Ambourn et al. 2005). All assayed beetles were processed within 6–10 mo of collection in 2015 and 3–6 mo of collection in 2016. Each beetle was placed into 0.5-ml sterile distilled water and agitated with a vortex mixer. The fungal propagules were then dislodged from the insect using a tip sonicator for 10 s. The resulting suspension of distilled water and fungal propagules was serially diluted (10^{-1} and 10^{-2}), and 0.5-ml aliquots of each dilution

Table 1. Oak sites in Wisconsin, listed from north to south, used to sample *Colopterus truncatus* and *Carpophilus sayi* in 2015 and 2016

Site	Abbreviation	Latitude	Longitude	Hectares	Political boundary	Natural boundary	Year oak wilt detected	No. of oak wilt pockets	Hectares affected by oak wilt	Oak component (%)
Polk County Forest	PCF	45°46′27″ N	92°46′45″ W	6,947	S	N	1944	>100	3,000–5,000	44.2
Governor Thompson State Park	GTSP	45°20′04″ N	88°14′52″ W	1,214	N	N	Prior to 2002	11	28	31
Council Grounds State Park	CGSP	45°11′07″ N	89°44′35″ W	204	N	N	Not detected	0	0	7.5
Peninsula State Park	PSP	45°09′04″ N	87°13′03″ W	1,528	N	N	Not detected	0	0	9
Willow River State Park	WRSP	45°01′10″ N	92°41′05″ W	1,178	S	N	Prior to 2000	Not available	400–500	50
Lake Wissota State Park	LWSP	44°58′12″ N	91°17′52″ W	430	S	N	1990	14	145	23
Hartman Creek State Park	HCSP	44°19′24″ N	89°13′02″ W	607	S	N	1970	10	120	32
Buckhorn State Park	BSP	43°56′21″ N	90°00′14″ W	2,315	S	S	Prior to 1977	>30	2,000	59
Devil's Lake State Park	DLSP	43°24′53″ N	89°42′47″ W	4,047	S	S	2013	<5	<5	80
Governor Nelson State Park	GNSP	43°08′06″ N	89°26′11″ W	171	S	S	2002	3	4	14
Kettle Moraine State Forest—Southern Unit	KMSF-SU	42°52′40″ N	88°31′45″ W	8,620	S	S	1970	Not available	Not available	43
Nelson Dewey State Park	NDSP	42°43′51″ N	91°01′10″ W	Unknown	S	S	Not available	Not available	Not available	Not available

Sites listed as N (north) or S (south) under the political boundary heading follow the Wisconsin Oak Harvesting Guidelines (WI DNR 2016). Sites N or S under the natural boundary heading follow the Wisconsin tension zone. GPS coordinates from WGS84 data. Incidence and extent of oak wilt, and oak component in the management units, provided by Wisconsin DNR.

were spread onto lactic acid-amended potato dextrose agar plates. Each dilution was spread onto two plates and incubated for 14 d in darkness at 24°C.

For each week of fungal sampling, three individuals each of *C. truncatus* and *C. sayi* were randomly selected from each of the 12 sites. In 2015, a subsample of beetles was tested for fungal presence weekly from 19 May to 7 August, every other week from 7 August to 31 September, and every 3 wk from October onward, for a total of 18 of the 28 sampling weeks tested. In 2016, all collection weeks were tested.

Colony appearance and asexual spore characteristics were used to identify *B. fagacearum* (Henry 1944, McCartney et al. 2003). Molecular methods were used to confirm identification. In 2015 and 2016, four random isolates (for a total of eight) of *B. fagacearum* were identified by the Wisconsin Plant Disease Diagnostic Clinic (Madison, WI). DNA extraction was performed using Qiagen Plant DNeasy Mini Kits (Hilden, Germany). PCR was completed using the general ITS1F and ITS4 fungal primers. Sanger sequencing of PCR products was performed by Functional Biosciences, Inc., Madison, WI.

Phenological Modeling of *C. truncatus* and *C. sayi* Flight

For each year, site-specific daily maximum and minimum temperatures were used to estimate daily degree-day accumulations from 1 January using a modified sine wave method (Allen 1976). Degree-day accumulation was then linked to the number of *C. truncatus* and *C. sayi* trapped at each combination of site, sampling interval, and year. Because base minimum temperature thresholds for these species are unknown, we estimated degree-day accumulation using base thresholds of 0, 5, and 10°C. We used these three thresholds to determine whether they yielded different patterns of adult beetle flight over the course of a season. Because the general pattern of flight was similar across the three thresholds (see Results), we chose 5°C as a minimum base threshold for subsequent degree-day modeling.

After linking trap catch by sampling interval, site, and year to the corresponding degree-day accumulation, we calculated the proportion of trap catch by degree-day accumulation pooled over site and year. We pooled over all sites and years to develop a broadly applicable model of cumulative emergence as a function of degree-days, enabling us to estimate different percentiles of flight including initial, peak, and last flight. Unweighted models consist of trap catch data that are not adjusted for site-specific, season-long numbers. We developed a relationship between the proportion of *C. truncatus* and *C. sayi* adults and accumulated degree-days, which was then modeled using locally weighted (adjusted for site-specific, season-long numbers) nonparametric regression in R (R Development Core Team 2016). This model was then used to estimate the degree-days at which 5, 10, 25, 50, 75, 90, and 95% of adult beetles for each species were captured.

Our final step was to link model predictions to calendar dates to inform management guidelines. We selected three weather stations that span the current latitudinal range of oak wilt in Wisconsin: Arlington University Farm (43.3008°N, −89.3269°W, 314 MSL), Hancock Experimental Farm (44.1186°N, −89.5358°W, 294 MSL), and Merrill (45.1786°N, −89.6616°W, 408 MSL). For each weather station, we obtained daily maximum and minimum temperatures from 1 January 2007 to 31 December 2016 (National Centers for Environmental Information 2017). We used a modified sine wave method (Allen 1976) to estimate daily degree-day accumulation for each year (minimum base threshold = 5°C), from which we estimated a 10-yr average degree-day accumulation for each calendar

day. Additionally, we selected the coldest (2009) and hottest (2012) years from this period based upon yearly degree-day accumulation. We used the 10-yr average, the cold year example, and the hot year example to estimate the calendar day at which the required degree-days for 5, 10, 25, 50, 75, 90, and 95% of adult beetle flight were obtained.

Data Analyses

Data were analyzed using R statistical software v3.2.2, R Studio, and R lme4 (Bates et al. 2014, R Development Core Team 2016, R Studio 2016). Numbers of *C. truncatus* and *C. sayi* captured in 2015 and 2016 were summarized by species, date, location, and temperature (°C) at the location and date of capture. Two methods were used when separately evaluating northern and southern field sites. The first used the Wisconsin tension zone; sites within or above this diagonal zone were considered northern, and those below it were considered southern (Fig. 1, gray area). When using this definition, six sites were northern and six were southern. The second method used a political division, the Wisconsin DNR Oak Harvesting Guidelines (Fig. 1, solid black line) dividing northern and southern counties. When using this definition, three sites were northern and nine were southern.

To assess potential differences in trap catch based on the synthetic lures used in 2015, likelihood and significance of predictors were determined using a linear mixed effects model fit with restricted maximum likelihood, using a type III ANOVA with a Satterthwaite approximation for degrees of freedom (Bolker 2009). We analyzed the main fixed effects of lure and species, and their interaction, on the numbers of beetles trapped, with site was a random effect. Differences in trap catch were based on both study species. We also evaluated whether the relative ranking of abundances among sites varied between years. Separate tests were performed for *C. truncatus* and *C. sayi* using a Spearman rank correlation test.

Sources of variation in trap catch between northern and southern regions of Wisconsin were assessed separately for each species, using linear mixed effects models fit with restricted maximum likelihoods, and analyzed using a type III ANOVA with a Satterthwaite approximation for degrees of freedom. Year was a fixed effect, and site was a random effect. Northern versus southern trap catches were compared based on the Wisconsin tension zone (Curtis and McIntosh 1951, Anderson 2005). Sites located within and north of the tension zone were analyzed as north, and sites below the Wisconsin tension zone were analyzed as south (Table 1).

Potential sources of variation in phoresy rates of viable *B. fagacearum* propagules were analyzed using a generalized linear mixed model and a logit link function. The response variable was the presence or absence of viable propagules, the fixed effects were beetle species and region, and the random effects were dilution and trapping site.

Results

Relative Abundance, Spatial Distribution, and Seasonality of *C. truncatus* and *C. sayi*

In 2015, 65.3% of both beetle species combined (N = 5,276) were captured with the *C. truncatus* lure, consisting of 1,986 *C. truncatus* and 3,290 *C. sayi* (Supp. Fig. 1 [online only]). In total, 2,801 beetles of both species (34.7%) were captured with the *C. sayi* lure, consisting of 1,233 *C. truncatus* and 1,568 *C. sayi*.

The two synthetic pheromone lures, when deployed in combination with dough bait, resulted in very high cross-attraction, as indicated by an insignificant lure by species interaction (F = 0.62; df = 1,

33.003; P = 0.44; Supp. Fig. 1 [online only]). Overall, 62% of all *C. truncatus* and 67% of all *C. sayi* were caught in traps baited with the lure intended for *C. truncatus*, (E,E,E,E)-3,5,7-trimethyl-2,4,6,8-decatetraene. There was a marginally significant effect of lure type on total trap catch (F = 4.01; df = 1, 33.003; P = 0.05), with slightly more attracted to (E,E,E,E)-3,5,7-trimethyl-2,4,6,8-decatetraene. In 2015, 8,075 individuals of the two target species were trapped, including 3,217 *C. truncatus* (39.8%) and 4,858 *C. sayi* (60.2%). In 2016, 7,905 individuals were trapped, including 6,258 *C. truncatus* (79.2%) and 1,647 *C. sayi* (20.8%). Over both study years, more *C. truncatus* (9,475: 59.3%) than *C. sayi* (6,505: 40.7%) were captured. Total capture for both years was 15,980 individuals of the two study species.

The spatial distributions of *C. truncatus* and *C. sayi* across the 12 sites are shown from northernmost to southernmost in Fig. 2. Both species were caught at each of the sites each year, including the sites without known oak wilt, CGSP and PSP. *C. truncatus* were captured in high numbers at both the northern and southern sites, and showed no difference between sites north versus south of the tension zone (F = 1.062; df = 1, 10.184; P = 0.32). In contrast, *C. sayi* was only captured in high numbers in sites south of the tension zone, where it was more abundant overall (F = 11.1349; df = 1, 11.028; P = 0.0066).

There was a positive association between the relative number of *C. sayi* caught in a site in 2015 and the relative number caught at the same site in 2016 (Spearman's ρ = 0.93; df = 10; P < 0.0001). In contrast, there was no relationship between *C. truncatus* abundance among various sites between years (ρ = 0.38; df = 10; P = 0.22).

In 2015, when trapping began in May, *C. truncatus* was captured from 25 May until 9 November, and *C. sayi* was captured from 25 May until 16 November (Table 2). In 2016, when trapping began in March, *C. truncatus* was captured from 14 April until 9 September, and *C. sayi* was captured from 18 April until 22 November.

Weekly trap catch data are shown in Fig. 3. In 2015, *C. truncatus* peak capture was in May (but note the later start date) and had elevated numbers in July. In 2016, *C. truncatus* peak capture occurred from April to May and declined rapidly throughout. Overall, 98% of *C. truncatus* were captured from 1 April to 15 July in 2016. In 2015, *C. sayi* peak capture was in May and again from July to August, tapering off slowly throughout the remainder of the sampling season (Fig. 3). In 2016, *C. sayi* peaked from April to May in 2016 and declined throughout the remainder of the season. Overall, 86% of *C. sayi* were captured from 18 April to 15 July in 2016.

Seasonal trap catch patterns of *C. truncatus* and *C. sayi* are related to the current Wisconsin Oak Harvesting Guidelines in (Table 3). In the year of full sampling, 2016, no *C. truncatus* were captured before the beginning of the no-cut guidelines in the northern zone (15 April), and 0.57% were caught before the no-cut interval in the southern zone (1 April; Table 3). No *C. sayi* were caught in either zone before these dates. Overall, 4.8% of 481 *C. truncatus* were caught after the no-cut guideline in the north (15 July), and 0.5% of 5777 were caught after the no-cut interval in the south. Overall, 64.4% of 59 *C. sayi* were caught after the no-cut interval in the north and 26.7% of 1588 were caught after the no-cut interval in the south. Combining both species, no beetles were captured before the no-cut interval in the north and 0.45% were captured before the no-cut interval in the south. After the no-cut interval, 11.3% of beetles were captured in the north, and 6.15% were captured in the south (Table 3). Vector species activity in relation to current Wisconsin oak harvesting guidelines is illustrated in Supp. Fig. 2 (online only), in which each point represents capture at a site on the calendar day the beetles were collected.
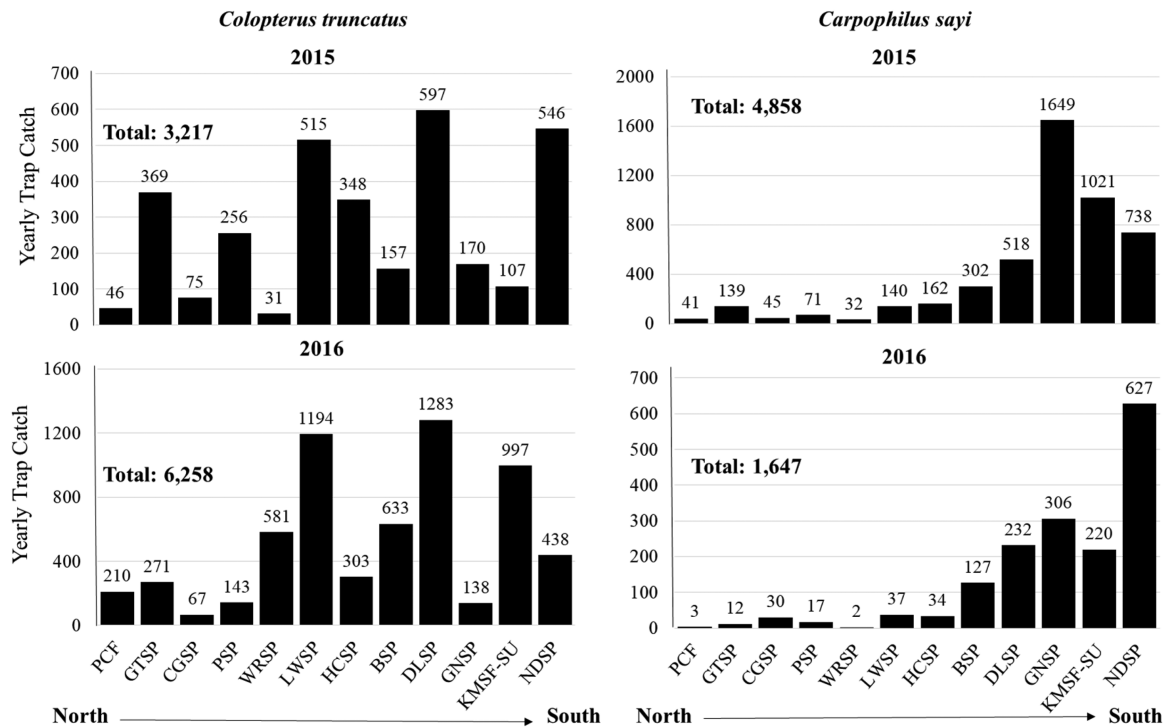
Fig. 2. Trap catch of adult *Colopterus truncatus* and *Carpophilus sayi* at 12 oak sites in Wisconsin during 2015 and 2016. Sites are arranged on x-axis from north to south. In 2015, sampling occurred from 19 May to 30 November. Sampling at eight sites ended in mid-September and four sites (GNSP, DLSP, PCF, and WRSP) were sampled through November. In 2016, sampling occurred from 7 March to 24 November. Sampling at eight sites ended in mid-September, and the same four sites as in 2015 were sampled through November.

Table 2. First and last capture dates of *Colopterus truncatus* and *Carpophilus sayi* at 12 Wisconsin sites in 2015 and 2016

Site	2015								2016							
	Sampling period		*C. truncatus*		*C. sayi*				Sampling Period		*C. truncatus*		*C. sayi*			
	Start date	End date	First capture	Last capture	First capture	Last capture			Start date	End date	First capture	Last capture	First capture	Last capture		
PCF	22 May	6 Nov.	29 May	2 Oct.	29 May	25 Sept.			19 Mar.	24 Nov.	15 April	19 Aug.	3 June	13 June		
GTSP	20 May	11 Sept.	26 May	11 Sept.	26 May	11 Sept.			11 Mar.	9 Sept.	22 April	19 Aug.	13 May	22 July		
CGSP	20 May	11 Sept.	26 May	11 Aug.	26 May	18 Aug.			11 Mar.	9 Sept.	15 April	19 Aug.	6 May	19 Aug.		
PSP	20 May	9 Sept.	26 May	18 Aug.	26 May	18 Aug.			28 Mar.	6 Sept.	18 April	26 July	9 May	6 Sept.		
WRSP	22 May	6 Nov.	29 May	30 Oct.	29 May	7 Aug.			19 Mar.	17 Nov.	1 April	20 May	13 May	14 July		
LWSP	20 May	9 Sept.	25 May	9 Sept.	25 May	9 Sept.			11 Mar.	9 Sept.	15 April	9 Sept.	6 May	27 July		
HCSP	19 May	7 Sept.	26 May	18 Aug.	26 May	7 Sept.			28 Mar.	6 Sept.	22 April	9 Sept.	13 May	9 Sept.		
BSP	19 May	29 Nov.	25 May	31 Aug.	25 May	7 Sept.			7 Mar.	6 Dec.	11 April	16 Aug.	25 April	6 Sept.		
DLSP	19 May	30 Nov.	25 May	9 Nov.	25 May	16 Nov.			7 Mar.	15 Nov.	21 Mar.	5 Sept.	18 April	15 Nov.		
GNSP	19 May	7 Sept.	25 May	24 Aug.	25 May	24 Aug.			7 Mar.	5 Sept.	18 April	12 July	18 April	5 Sept.		
KMSF-SU	19 May	7 Sept.	25 May	17 Aug.	25 May	7 Sept.			7 Mar.	8 Sept.	7 April	8 Sept.	18 April	8 Sept.		
NDSP	19 May	7 Sept.	25 May	7 Sept.	25 May	7 Sept.			1 April	9 Sept.	7 April	8 Sept.	21 April	8 Sept.		

Sites are listed from north to south, in the Wisconsin Oak Harvesting Guidelines (WI DNR 2016). Council Grounds State Park and Peninsula State Park have no known oak wilt.

Association of *B. fagacearum* with *C. truncatus* and *C. sayi*

In 2015, both *C. truncatus* and *C. sayi* were carrying viable fungal propagules during the first week of sampling, 19–25 May (Fig. 4). Of the 341 *C. truncatus* tested, 16 were positive for the fungus. Beetles with positive tests occurred from 19 May to 14 July. In 2015, no *C. truncatus* tested positive for viable fungal propagules after the Wisconsin DNR Oak Harvesting Guidelines. Of the 415 *C. sayi*

tested in 2015, 31 were positive for viable fungal propagules. Beetles that tested positive occurred from 19 May to 31 August. In 2015, three beetles tested positive for viable fungal propagules after the Wisconsin DNR Oak Harvesting Guidelines no-cut period: on 21 July at GTSP, 31 August at DLSP, and 31 August at KMSF-SU.

Of the 283 *C. truncatus* collected and assayed in 2016 when trapping began on 7 March, 20 were positive for *B. fagacearum*, and 1 tested positive outside of the current no-cut period of the DNR
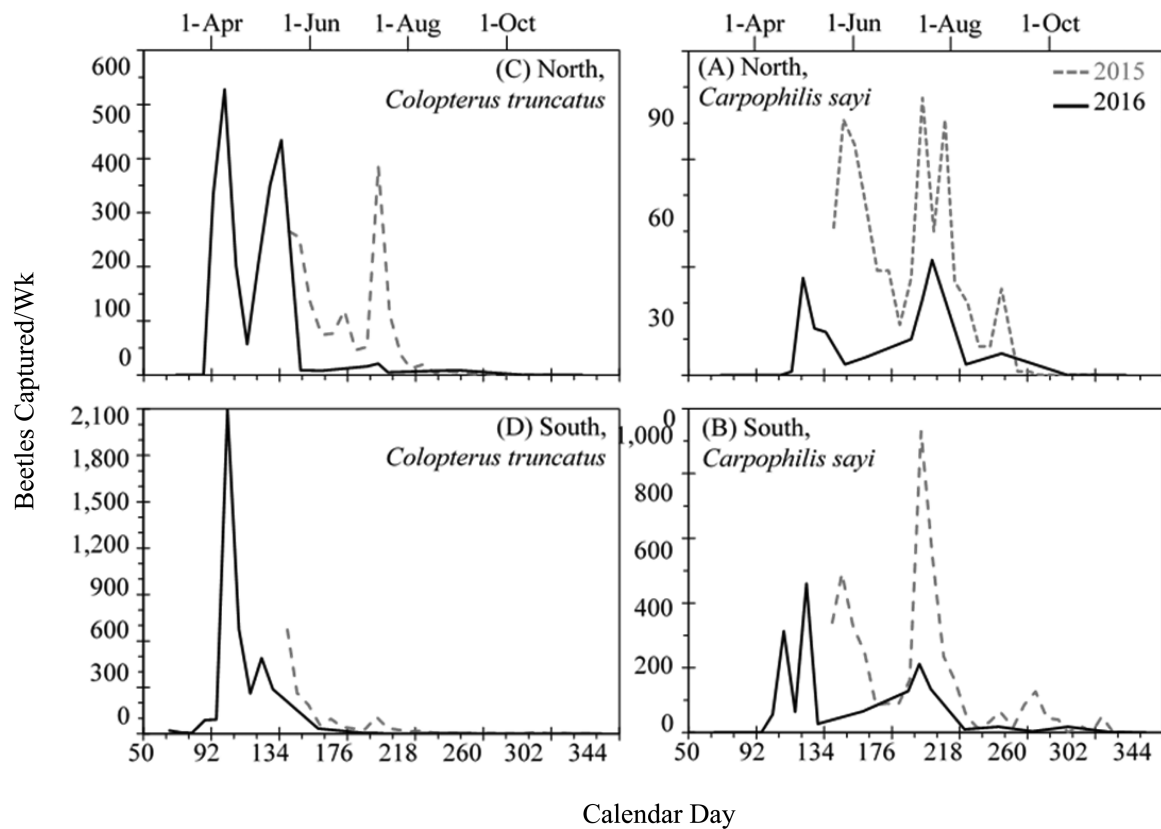
Fig. 3. Weekly trap catch of adult *Colopterus truncatus* and *Carpophilus sayi* in 12 oak sites in Wisconsin during 2015 and 2016. Sites within or above the Wisconsin tension zone (*N* = 6) are considered northern, and those below are considered southern (*N* = 6). Data are arranged in bins, based on all beetles caught within each week, to highlight seasonal trends. Graphs include zeros. In 2015, sampling occurred from 19 May to 30 November. Sampling at eight sites ended in mid-September, and four sites were sampled through November. In 2016, sampling occurred from 7 March to 24 November. Sampling at eight sites ended in mid-September, and the same four sites as in 2015 were sampled through November.

Table 3. Relationship of seasonal trap catches of *Colopterus truncatus* and *Carpophilus sayi* in 2016 to Wisconsin DNR Oak Harvesting Guidelines

	Study species	Percent of beetles captured before no-cut guidelines	Percent of beetles captured during no-cut guidelines	Percent of beetles captured after no-cut guidelines	Number of beetles
North	*C. truncatus* + *C. sayi*	0	88.7	11.3	540
	C. truncatus	0	95.22	4.78	481
	C. sayi	0	35.59	64.41	59
South	*C. truncatus* + *C. sayi*	0.45	93.4	6.15	7,365
	C. truncatus	0.57	98.93	0.5	5,777
	C. sayi	0	73.3	26.7	1,588

Data based on Wisconsin DNR division of northern and southern zones, for which current no-cut guidelines are 15 April–15 July for northern counties, and 1 April–15 July for southern counties (WI DNR 2016).

oak harvesting guidelines. Beetles that tested positive had been collected between 11 April and 14 July. The single beetle captured and tested from 11 April was caught at DLSP, south of the Wisconsin tension zone. No *C. truncatus* were captured before 8 April. Of the 216 *C. sayi* tested for viable fungal propagules in 2016, 18 were positive. Beetles that tested positive occurred from 18 April to 26 July. One *C. sayi* collected after the no-cut time period in 2016 tested positive for the fungus. This beetle was collected on 26 July from DLSP (Fig. 4).

The proportions of *C. truncatus* and *C. sayi* combined carrying viable *B. fagacearum* propagules did not differ between study years

(Z = 0.64; *P* = 0.52). There was likewise no difference in the proportions of combined study beetles carrying viable fungal propagules between northern and southern sites (Z = 0.67; *P* = 0.50).

Phenological Modeling of *C. truncatus* and *C. sayi* Flight

In 2016, the earliest captures of *C. truncatus* adults occurred from 14 March to 22 April, depending on site. The earliest captures of *C. sayi* occurred from 18 April to 3 June. For *C. truncatus*, the mean temperature for the week (i.e., the mean of the week-long maximum and minimum temperatures) at a site when beetles were
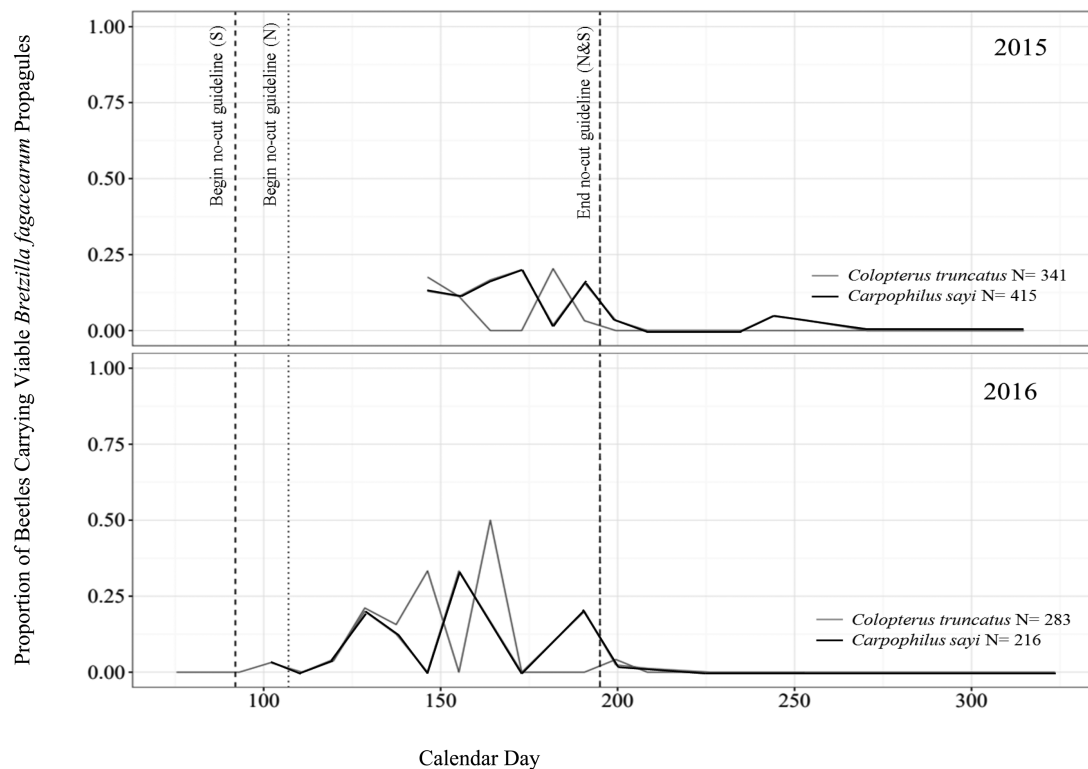
Fig. 4. Proportions of adult *Colopterus truncatus* and *Carpophilus sayi* beetles carrying viable *B. fagacearum* propagules in Wisconsin. Solid vertical lines represent beginning of no-cut period for current Wisconsin DNR Oak Harvesting guidelines in northern (15 April) and southern (1 April) counties. Dotted vertical lines represent end of no-cut period for all counties (15 July; WI DNR 2016).

first captured was 20.3 ± 2.3°C, the median was 21.2°C, and the maximum was 33.2°C. For *C. sayi*, the mean temperature for the week at a site when beetles were first captured was 26.0 ± 2.2°C, the median was 26.1°C, and the maximum was 38.8°C. There were two and four sites at which *C. truncatus* and *C. sayi*, respectively, were first obtained at temperatures >30°C, but this likely reflects unusually warm weather during the period between weekly sampling intervals. The average temperature recoded at the last capture of *C. truncatus* was 19°C in 2015 and 25.6°C in 2016. For *C. sayi*, the average temperature at last capture was 24.6°C in 2015 and 25.9°C in 2016.

Total accumulated degree-days at the study sites are shown for base thresholds of 0, 5, and 10°C in Supp. Table 1 (online only). Unweighted degree-day models showed only a small lag for *C. truncatus* (~50 accumulated degree-days) before first flight (Supp. Fig. 3 [online only]) at the base threshold of 10°C (at 5°C base threshold, ~100 degree-days; at 0°C, ~250 degree-days). In contrast, unweighted degree-day models for *C. sayi* indicated an accumulation of ~200 degree-days before first flight at the base thresholds of 5 and 10°C, and ~100 degree-days at the 0°C base threshold. The first 1,000 degree-days of the 5°C base temperature models for *C. truncatus* and *C. sayi* are shown in Supp. Fig. 4 (online only) to better illustrate the accumulated degree-days estimated for the 25th and 50th percentiles of initial flight. The temperatures at which initial adult capture occurred for *C. truncatus* (8.4°C) and *C. sayi* (11.1°C) suggest using a base temperature of 5°C for adults of *C. truncatus* and 10°C for adults of *C. sayi*. First derivative graphs of both species, which indicate potential changes in slope in the relationship between beetle capture and degree-day accumulation, are presented in Supp. Fig. 4 [online only]. *C. truncatus* showed one large peak very early in the season, and a very small second peak late in the

season. *C. sayi* exhibited two relatively distinct peaks; one early in the season and one after 1,000 degree-days.

Weighted degree-day models, which are adjusted for the season-long population density of *C. truncatus* and *C. sayi* adults at each site, are shown in Fig. 5. These models likewise showed a similar fit irrespective of the base temperature threshold. Flight activity appears to begin at ~50 accumulated degree-days (base temperature 10°C) for adult *C. truncatus* and ~200 degree-days (base temperature 10°C) for *C. sayi*.

Selected percentages of cumulative emergence of *C. truncatus* and *C. sayi* that correspond to a given number of accumulated degree-days are shown in Table 4. For example, 5% of *C. truncatus* are active by 128 degree-days (base 5°C), and 95% have been captured by 1,207 degree-days. In contrast, 5% of *C. sayi* are not active until 340 degree-days have accumulated, and 95% have not been captured until 2,344 degree-days. These results are translated into calendar dates and further generalized in Fig. 6 and Supp. Table 2 (online only). The percentages of cumulative flight are also shown for various weather stations that span the latitudinal distribution of oak wilt in Wisconsin. For each location and percentage of cumulative flight, three dates are shown: the mean based on the previous 10 yr, the coldest year during that period, and the warmest year during the same period. Note that these calendar dates do not show a strict north–south direction (Supp. Table 2 [online only]), presumably owing to microsite features.

Additional Nitidulidae Captured

In addition to *C. truncatus* and *C. sayi*, 10 other species of Nitidulidae were captured (Table 5). These species have variable degrees of reported association with *B. fagacearum*, oak wilt, and/or healthy oak trees. For example, *Fabogethes nigrescens* (Stephens) has
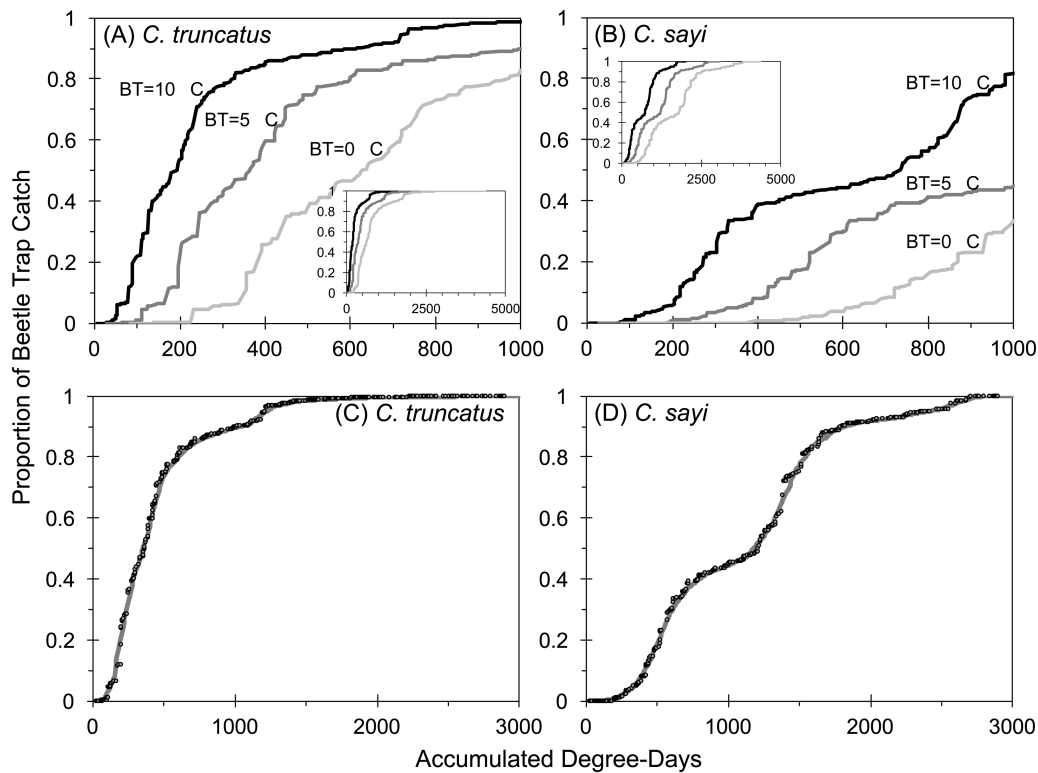
Fig. 5. Observed proportion of adult catch for *Colopterus truncatus* (A) and *Carpophilus sayi* (B) over accumulated degree-days (beginning on 1 January) when using a minimum base temperature threshold (BT) of 0, 5, and 10°C. The insert graphs show the trap catch over the entire season, whereas the primary graphs show the initial flight. A season-long model of adult beetle catch is shown for *C. truncatus* (C) and *C. sayi* (D) when using a minimum base temperature threshold of 5°C; open circles represent observed data when pooled across years and sampling locations, and the gray line represents a locally weighted nonparametric fit.

Table 4. Degree-day estimates for specific percentages of adult beetle flight for *Colopterus truncatus* and *Carpophilus sayi* (minimum base temperature threshold = 5°C; degree-days counted from 1 Jan)

Percent of flight	*C. truncatus*	*C. sayi*
5	128.3	339.6
10	157.4	412.5
25	215.8	550.9
50	354.2	1,170.3
75	507.2	1,461.8
90	995.4	1,789.7
95	1,206.7	2,343.5

not been related to oak wilt; *C. ampla* Erichson was not observed carrying *B. fagacearum* by Juzwik et al. (2004) and was likewise uncommon on fungal mats (Cease and Juzwik 2001); and in some cases the genus but not necessarily the species given in Table 5 (e.g., *Carpophilus corticinus* Erichson) have well-documented associations with *B. fagacaerum* and/or oak wilt (Ambourn 2004, Bartelt et al. 2004).

Discussion

Adult *C. truncatus* and *C. sayi* exhibit distinct seasonal patterns, largely driven by temperature. *C. truncatus* and *C. sayi* began flight at 8.4 and 11.1°C, respectively, and were active from approximately 21 March to 9 September and 18 April to 15 November, respectively,

over the complete-season sampling period. When these results are generalized using degree-day models averaged over the last 10 yr, 90% of *C. truncatus* fly between April 23 and July 22 in southern Wisconsin, and May 1 and July 27 in northern Wisconsin. Ninety percent of *C. sayi* fly between May 23 and November 14 in southern Wisconsin, and May 27 and mid-November in northern Wisconsin (Fig. 6). The patterns of trap catch suggest one and possibly two generations per year by *C. truncatus*, versus two or overlapping generations by *C. sayi*, based on its initial emergence peak and equally sized second peak after 1,000 degree-days (base temperature 5°C).

Differences in observed degree-day accumulations prior to initial flight provide some insight into the life histories of these two vector species. Specifically, the lack of a substantial lag prior to flight suggests that *C. truncatus* most likely overwinters in the adult stage and merely requires sufficient warming to initiate flight. This agrees with the finding of an adult during winter by Kyhl (2006). In contrast, *C. sayi* more likely overwinters in a juvenile or immature adult stage that still requires degree-day accumulation to complete development. These results appear to be robust, in that degree-day models showed similar trends whether a base threshold of 0, 5, or 10°C was used.

There were also differences in spatial trends between these vectors. *C. sayi* show a latitudinal gradient, with more beetles occurring in southern than in northern Wisconsin. *C. truncatus* appears to be relatively evenly distributed throughout the state. Populations of *C. sayi* exhibit relatively consistent spatial trends, with the same sites yielding the same relative numbers of beetles despite inter-year variation in absolute numbers. In contrast, spatial patterns of *C. truncatus* appear more variable.
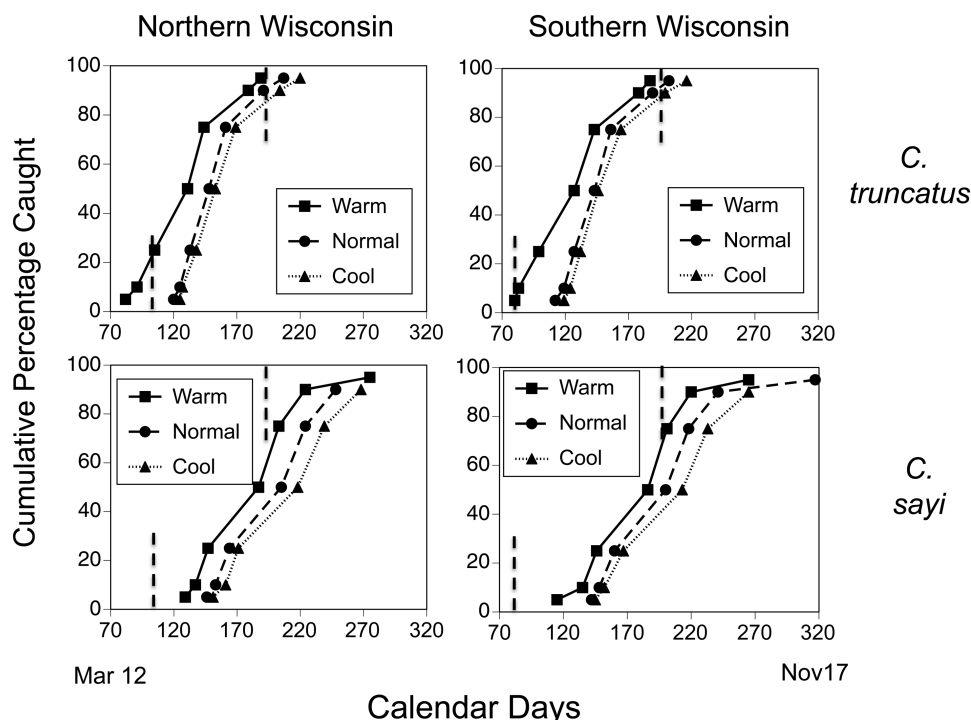
Fig. 6. Estimated percentages of cumulative flight by *C. truncatus* and *C. sayi* at specific dates using daily temperature data from two representative weather stations in southern (Arlington University Farm) and northern (Merrill) Wisconsin. Mean temperatures are based upon 10-yr averages (2007–2016), and warm and cool predictions used the warmest (2012) and coolest (2009) year over the last 10 yr. Vertical dotted lines represent the beginning and end of the current Wisconsin no-cut guidelines. Raw data, and an additional site in central Wisconsin not included in the figure to avoid clutter, are in Supp. Table 2 (online only).

Table 5. Other sap beetle species (Coleoptera: Nitidulidae) captured in traps baited with dough bait and (*E,E,E,E*)-3,5,7-trimethyl-2,4,6,8-decatetraene lures in wind-oriented funnel traps

Species	First capture	Last capture	Association with oak/oak wilt	Reference
Fabogethes nigrescens (Stephens)	12 Mar. 2016	2 Sept. 2016	Unknown	N/A
Carpophilus corticinus Erichson	12 Mar. 2016	12 Oct. 2016	Dispersing in oak wilt stands	Hayslett et al. (2008)
Glischrochilus quadrisignatus (Say)	26 Mar. 2016	8 Nov. 2016	Dispersing in oak wilt stands; occurs on oak wilt mats	Juzwik and French (1985)
Glischrochilus fasciatus (Olivier)	26 Mar. 2016	10 Nov. 2016	Dispersing in oak wilt stands; occurs on oak wilt mats	Juzwik and French (1985); Cease and Juzwik (2001)
Glischrochilus sanguinolentus (Olivier)	2 April 2016	8 Nov. 2016	Dispersing in oak wilt stands; occurs on oak wilt mats	Juzwik and French (1985)
Cryptarcha ampla Erichson	30 April 2016	21 Sept. 2016	Occurs on fresh wounds of healthy oaks in/near oak wilt centers; occurs on oak wilt mats	Cease and Juzwik (2001); Juzwik et al. (2004)
Colopterus maculatus (Erichson)	30 April 2016	12 Oct. 2016	Occurs on fresh wounds of healthy oaks in/near oak wilt centers	Hayslett et al. (2008)
Carpophilus hemipterus (Linnaeus)	30 April 2016	8 Nov. 2016	Occurs on fresh wounds of healthy oaks in/near oak wilt centers	Juzwik et al. (2004)
Cryptarcha strigulata Parsons	30 April 2016	30 Aug. 2016	Unknown	N/A

The extent to which there are documented degrees of association between various species and *B. fagacearum* and/or oak wilt varies, including no association (e.g., *F. nigrescens*), infrequent association, documented vectoring by another member of the same genus but not this species (e.g., *C. corticinus*), etc. See text for references.

The capture of both *C. truncatus* and *C. sayi* in sites without known oak wilt in Wisconsin and Minnesota (Ambourn et al. 2005) provides further evidence that the distribution of this disease is not currently limited by the geographic ranges of these beetles. Thus, transmission and infection could probably occur if the pathogen were introduced. These captures also indicate that *C. truncatus* and *C. sayi* are not dependent on *B. fagacearum* and that *B. fagacearum* has not invaded the full range of these beetles. Both beetle species have been associated with fungi or wounds on a wide variety of hosts from multiple plant families, and with a wide range of *Ceratocystis* species and other fungal groups, across a wide range of habitat types (Price 2003). However, populations of *C. truncatus* and *C. sayi* may be higher in sites containing oak wilt during some years (Ambourn et al. 2005).

Overall phoresy rates in sites containing oak wilt were relatively consistent between years, averaging 7.0–8.2% for adult *C. truncatus* and 8.9–11.8% for *C. sayi*, respectively. However, there were strong seasonal trends. The incidence of viable fungal propagules peaked from May to June among *C. truncatus*, and June to July among *C. sayi*. Viable fungal propagules were not found on either beetle species after 31 August. Likewise, no viable fungal propagules were found on either species from the two sites without known oak wilt. These results are lower than phoresy rates estimated in Minnesota using similar methods for *C. truncatus* (21.8–26.0%), but similar to those for *C. sayi* (6.9–9.3%) in oak wilt-affected stands in 2002–2003 (Ambourn et al. 2005). These phoresy rates appear to reflect relatively diffuse associations, compared with rates commonly found in more tightly connected symbiotic associations. For example, 51% of spruce beetles, *Dendroctonous rufipennis* Kirby (Coleoptera: Curculionidae), captured in flight traps in Alaska carry *Leptographium abietinum* Peck, with which it coevolved (Aukema et al. 2005).

Although this study was not designed to compare recreational with production forests, it is noteworthy that the only forest actively undergoing logging (PCF; Table 1) yielded the highest phoresy rate (35% in 2015), with 1 wk in June yielding 100% positive results (*N* = 3). However, this site also had oak wilt longer than the others. It is also noteworthy that after this forest was selectively logged during the following winter, only 17% of beetles tested positive in 2016. Future studies should explicitly test the role of management regime on phoresy rates of oak wilt vectors, and the potential of remedial measures such as selective, well-timed cutting of oak wilt-killed trees with sporulation mats to reduce those rates.

These results can be used to refine existing guidelines for managing oak wilt in urban and timberland forests. The trapping data indicate that less than 1% of *C. truncatus* and *C. sayi* were captured before the no-cut period specified in the WI DNR oak harvest guidelines (political boundary; WI DNR 2016). Further, no beetles tested positive for *B. fagacearum* before the no-cut period. Less than 5% of *C. truncatus* were captured after the no-cut period in their region. Of concern, 64 and 27% of *C. sayi* were captured after the no-cut period in the northern and southern zones, respectively. However, only four *C. sayi* tested positive for viable *B. fagacearum* propagules after the no-cut period. Based on these data, the beginning of the current Wisconsin DNR guidelines seems well constructed, but the end date of 15 July should be more carefully examined, especially in relation to phoresy. These results follow a similar trend to that reported in Minnesota by Ambourn et al. (2005), in which both *C. truncatus* and *C. sayi*, and particularly the latter, were captured in late summer of at least 1 yr, including infested individuals.

The ability of the existing guidelines to prevent the spread of oak wilt will be challenged as temperatures warm. For example, within the last 10 yr, there has already been at least one season during which between 10 and 25% of *C. truncatus* likely flew prior to the no-cut period in both southern and northern Wisconsin, with substantially more in some specific sites (Fig. 6). During this same year, between 25 and 50% of *C. sayi* flew after the no-cut guideline in both southern and northern Wisconsin.

The overall risk of insect transmission can be modeled as an interaction of beetle numbers, the proportion carrying viable spores, and the proportion of viable spore-carrying beetles whose contact with hosts results in infection. Both the number and proportion of beetles carrying viable fungi decline later in the season. We currently lack data on vector transmission efficiency (Daugherty et al. 2010), but it likely declines later in the season as well. Specifically, the ability of *B. fagacearum* to infect trees increases when large-diameter xylem vessels are common in the tissue proximal to the vascular cambium, as in ring-porous red

oak species during spring (Sachs et al. 1970, Tainter and Baker 1996, Blaedow 2009, Nixon 2009). Smaller-diameter vessels, which are less conducive to within-tree establishment and spread of *B. fagacearum*, become predominant in red oaks during summer (Wheeler and Thomas 1981). Earlywood begins to form in red oaks about 5 wk before the first leaves unfold and continues for another 5 wk (Zasada and Zahner 1969). Latewood begins to form in July in the Great Lakes region (Wheeler and Thomas 1981, Nixon 2009). Overall, the interaction of vector numbers, phoresy rates, and host susceptibility appears to maximize risk of insect-transmitted *B. fagacearum* during the spring and early summer, and reduce risk thereafter.

Our results also indicate that a single pheromone can be used to sample the two most important oak wilt vectors in Wisconsin, due to a high degree of cross-attraction. This could facilitate future research by enabling larger sample sizes using only a single compound and reducing cost. The pheromone (*E,E,E,E*)-3,5,7-trimethyl-2,4,6,8-decatetraene captured more of each beetle species than did (*E,E,E,E*)-3,5,7-trimethyl-2,4,6,8-undecatetraene and (*E,E,E,E*)-7-ethyl-3,5-dimethyl-2,4,6,8-undecatetraene. This agrees with Kyhl et al. (2002) that decatetraene is the crucial pheromone component in all tested blends. Cross-attraction may be relatively common among nitidulids, as Williams et al. (1995) and Bartelt et al. (2004) found significant cross-attraction among various *Carpophilus* species and pheromones.

These results suggest several avenues for additional research. First, assaying beetle phoresy rates at multiple time points across a full season dilutes the sample size at any particular time. Increased sampling is needed near the end of the current no-cut period, because our data show some adults, especially *C. sayi*, are still active then. If additional sampling validates that phoresy is low by mid-July, the risk posed by late-flying beetles is accordingly relatively low. Second, PCR and culturing methods should be calibrated by applying both to the same beetles. PCR assays can generate false positives because they do not distinguish between DNA of living versus dead fungi (Wu et al. 2007, Yang and Juzwik 2017), but, conversely, using serial dilution plating to obtain counts of living fungus propagules may generate false negatives. Once a correction factor is quantified, PCR could be applied more widely, as it is less laborious. Third, we lack critical information on the basic life histories, behavior, and chemical ecology of the major beetle vectors. In particular, larval feeding substrates, overwintering dynamics, cues by which beetles orient to wounds and infected trees, and their full host plant and microbial associations are poorly understood. Fourth, the vector transmission efficiencies of the major nitidulid associates of *B. fagacearum* need to be quantified, particularly in relation to the phenology and morphology of vessel formation.

The temperature windows and phoresy rates we quantified for the major, season-encompassing vectors of *B. fagacearum* in Wisconsin can be applied across years and regions with similar species compositions. Flight periods of nitidulids can vary markedly between years (Dowd and Nelsen 1994), so degree-day models can provide generality. Moreover, our degree-day models could be useful in efforts to predict flight periods of these nitidulid vectors of oak wilt under future climate conditions. Lastly, these results can inform management decisions, but policy makers and resource managers will need to incorporate a variety of socioeconomic and operational factors as they weigh the risks and costs associated with implementing guidelines.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Acknowledgments

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