

NATIONAL  
 OAK WILT  
SYMPOSIUM

JUNE 4-7, 2007 • AUSTIN, TEXAS

**The Proceedings of the 2<sup>nd</sup> National Oak Wilt Symposium**

**Edited by:**

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**Sponsored by**

**International Society of Arboriculture – Texas Chapter**

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USDA Forest Service, Forest Health Protection**

**2009**

## INSECT VECTORS OF THE OAK WILT FUNGUS IN MISSOURI AND TEXAS

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### **ABSTRACT**

The oak wilt fungus, *Ceratocystis fagacearum*, is transmitted overland by beetles in the family Nitidulidae and oak bark beetles in the genus *Pseudopityophthorus*. Studies were performed in 2005 and 2006 to determine the beetle species involved in transmission in Missouri and Texas. From this data, we hypothesize that *Colopterus truncatus*, *Co. niger* and *Co. semitectus* are vector species during the spring in Missouri and that the period of greatest risk for transmission is April and May with less risk in June. *Co. truncatus* was identified as a vector in Texas although other species may be involved. *Pseudopityophthorus pruinosus*, while present, could not be conclusively determined to be a vector in Texas. *Co. truncatus* appears to be a vector across the range of the disease while the involvement of other nitidulid beetle species varies with location. The contribution of oak bark beetles to the spread of the disease may also vary with location.

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**Key words:** *Ceratocystis fagacearum*, *Colopterus*, Nitidulidae, *Pseudopityophthorus*

Beetles in the family Nitidulidae (Coleoptera) and oak bark beetles (Coleoptera: Curculionidae: Scolytinae) in the genus *Psuedopityophthorus* are considered the principal insect vectors of the oak wilt fungus, *Ceratocystis fagacearum* (Bretz) Hunt, in the U.S. (Gibbs and French 1980). Nitidulid beetles transmit the fungus when they visit fresh wounds on healthy oak (*Quercus* spp.) following visitation of fungal mats on oak wilt killed trees (Gibbs and French 1980). Oak bark beetles oviposit (lay eggs) in oak wilt-killed trees (Griswold and Bart 1954, Buchanan 1956). The larvae develop in the branches and when the new adults emerge they may carry the fungus on their bodies (Stambaugh et al. 1955, Rexrode, Kulman and Dorsey 1965, Berry and Bretz 1966). These adults can then transmit the disease when they feed on healthy oak trees (Buchanan 1958). Insect transmission, called above ground or overland transmission, can be prevented by removing wilted trees, avoiding the creation of wounds during periods of high risk, and painting unavoidable wounds made during this time period (French and Juzwik 1999, O'Brien et al. 2000, Juzwik et al. 2004).

Although the whole family of Nitidulidae has been implicated in transmission, research done in Minnesota suggests that only two species are the principal vectors in that area of the state (Juzwik, Skalbeck and Newman 2004b). In research done on nitidulid beetle transmission in Iowa, Norris (1956) concluded again that only a few species were likely to be important in transmission. Interestingly, while one key species (*Colopterus truncatus*) was the same between these two studies, the other species implicated in transmission were different. Also, while oak bark beetles are considered principal vectors in Missouri (Berry and Bretz 1966) and West Virginia (Rexrode and Jones 1971), research done in Minnesota (Ambourn, Juzwik and Eggers 2006) and Ohio (Rexrode 1967) does not support their role as principal vectors in the areas where the research was done. This leads to questions about which species are really involved in transmission and if the species involved are the same across the range of the oak wilt disease.

Studies were conducted in 2005 and 2006 in Missouri and Texas to determine the species responsible for transmission of the oak wilt fungus in each location. An effort was also made to identify periods of time during which there is a high risk of overland transmission. Information about the species involved and when they are capable of transmission can be used to find periods of high and low risk for transmission, thereby allowing for better disease management. Specifically, wounding studies were conducted in the spring of 2005 and 2006 in Missouri to collect nitidulid beetle species visiting fresh wounds on oaks in the red oak group (*Quercus* section *Lobatae*) and carrying the pathogen. One wounding study was also completed in Texas in February of 2005 by wounding red oak and live oak trees (*Quercus virginiana* and *Q. fusiformis*). In 2006, studies were done in Texas red oak wilt centers to trap for dispersing nitidulid beetles carrying the fungus in the late winter and spring. Also in Texas, trapping was done in the late winter and spring of 2005 and 2006 to collect dispersing oak bark beetles that may carry the fungus.

## MATERIALS AND METHODS

### Study Sites

All study sites in all years consisted of active oak wilt centers which were defined as areas with oak trees recently killed by oak wilt. Recently-killed red oaks producing mats served as sources of the fungus for wounding- and dispersing nitidulid beetle studies. These recently-killed oaks were also a possible source of collection for oak bark beetles.

### **Wounding and Beetle Collection**

Wounds were created on healthy oaks to provide an infection court and a nitidulid beetle attractant. Each tree was wounded twice, once on the east-facing side and once on the west-facing side. A 5 cm hole saw was used to remove a round piece of bark to the outer xylem. The removed bark plug was re-inserted and held in place with a nail to create an attractive niche for the nitidulid beetles. Rain flaps were attached above the wound to prevent rain water from washing the insects out of the wounds below. Nitidulid beetles were collected from wounds once each day for six to nine days after wounding. Beetles were placed individually in sterilized 1.5 ml microcentrifuge tubes and the tubes were stored on ice during transport to the lab where they were stored at -2° C until they could be shipped on ice to the University of Minnesota where they were again stored at -2° C until processed.

Wounding studies were conducted using red oak trees in Missouri at two sites each year in 2005 and 2006 with a total of three sites, using one site both years. Wounding events occurred once a month in mid April, mid May and mid June. In Texas, there was one wounding event, using both red oak and live oak trees, at one site in late February of 2005.

### **Trapping of Dispersing Nitidulid Beetles**

Dispersing nitidulid beetles were collected from four red oak sites in central Texas. At each site, wind-oriented funnel traps with fermenting flour dough and either *Colopterus truncatus* or *Carpophilus sayi* aggregation pheromone were placed in trees or bushes (Kyhl et al. 2002, Bartelt et. al 2004). Beetles were collected from traps and the baits were changed once a week for 14 weeks from early February through mid May, 2006. The contents of the traps were then shipped on ice to the University of Minnesota for processing. Beetles were placed individually in sterile micro centrifuge tubes and stored at -2° C until processed.

### **Trapping of Dispersing Oak Bark Beetles**

Dispersing oak bark beetles were collected using window flight traps (without bait) from four red oak sites and four live oak sites in 2005 and from four red oak sites in 2006. Two traps were installed in the mid crown of each oak wilt killed tree on a rope and pulley system. Contents of the traps were collected once a week from early February to mid May. The contents of each trap were placed in a plastic bag and shipped on ice to the University of Minnesota for processing. Beetles were placed individually in sterile microcentrifuge tubes and stored at -2° C until processed.

### **Beetle Processing**

All beetles were identified to species and the number of each species recorded. Beetles from all studies were assayed for pathogen presence following the same procedure. Beetles in their individual microcentrifuge tubes were macerated in 0.5 ml of sterile water with a tip sonicator to dislodge and expose fungal material. The macerated beetle in water was then used to create three ten-fold dilutions; 0.5 ml of suspension was then plated on each of three lactic acid amended potato dextrose agar plates. The plates were incubated in the dark at 24° C and examined after ten days for presence of the fungus (Cease and Juzwik 2001). The fungal colonies were identified by morphology and presence of endoconidia (Barnett 1953). The numbers of colonies on each plate for one selected dilution were counted and the colony-forming units per beetle were calculated.

## RESULTS AND CONCLUSIONS

### Missouri

**Beetle Species Abundance.** Fourteen species of nitidulid beetles were collected over the two years (Table 1). *Colopterus truncatus*, *Co. semitectus*, and *Co. niger* represented 64% of all beetles captured (Table 1). *Cryptarcha ampla* was the fourth most abundant species overall but still less than half as abundant as the third most abundant species (Table 1). Most beetles were collected from one of the three sites. This site, Little Lost Creek, was used in both years of the study. Fewer *Colopterus truncatus*, *Co. semitectus* and *Co. niger* individuals were captured at Little Lost Creek in June as compared to April and May (Fig. 1).

**Beetle Species with *C. fagacearum*.** Of 230 individual nitidulid beetles assayed, 23 yielded the oak wilt fungus. *Colopterus truncatus*, *Co. niger*, and *Co. semitectus* were the only beetle species contaminated with the fungus. Furthermore, only beetles captured in April and May yielded the pathogen (Fig. 1). A higher contamination frequency was found in 2006 with the majority of contaminated beetles captured in April (31% of all April beetles assayed) (Fig. 1).

**Conclusions.** We hypothesize that *Co. niger*, *Co. truncates*, and *Co. semitectus* (Fig. 2) are vectors of *Ceratocystis fagacearum* in east central Missouri based on their abundance in fresh wounds during spring months while contaminated with the pathogen. Ten percent of the nitidulid beetles captured that were assayed for the pathogen yielded the fungus. Although this shows that these nitidulid beetle species are capable of transmission, this is much lower than contamination rates of beetles from wounds in spring in Minnesota (Juzwik et al. 2004b) and in late February in Texas (Hayslett et al. 2005).

Contaminated beetles were found in greatest numbers in April with some in May and none in June. Current control measures for oak wilt in Missouri include avoidance of wounding from April through June to prevent infection. Our results suggest that April is a period of higher risk and that risk is lower in May and lowest in June. Additional data is needed to confirm this. If oaks could be wounded in June without risk of infection, this would give home owners, tree care professionals, and forest managers additional time to prune or harvest oaks.

### Texas

**Wound-inhabiting Nitidulid Beetles.** One species, *Colopterus truncatus*, accounted for all (n=184) nitidulid beetles collected during late February from fresh wounds on oaks in the red oak and live oak stands at the Langford Ranch in 2005. High numbers of *Co. truncatus* collected from either red or live oaks yielded *C. fagacearum*. Overall, 83% of all collected beetles were carrying the fungus with contamination frequencies ranging from 71 to 100% depending on the tree species and wound age (Table 2).

**Dispersing Nitidulid Beetles.** Six species of nitidulid beetle were captured with the wind-oriented funnel traps, baited with *Colopterus* or *Carpophilus* pheromone and with dough; two *Colopterus* spp. (*Co. truncatus* and *Co. maculatus*), *Cryptarcha concinna*, and three *Carpophilus* spp. (*Ca. freemani*, *Ca. mutilates*, and *Ca. marginellus*). *Colopterus truncatus* accounted for 47% of nitidulid beetle counts at the Langford site and 6% at the Solana site (Table 3). *Carpophilus* spp. comprised 49% of nitidulid beetle counts at the Langford site and 74% of those

at the Solana site (Table 3). Peaks in beetle abundance over time were difficult to distinguish with the low beetle counts. Of 110 nitidulid beetles processed from this study, only three yielded *C. fagacearum* in culture; these were *Co. truncatus* collected from Langford Ranch between 7 - 14 March and 27 March - 4 April.

**Dispersing Oak Bark Beetles.** Few oak bark beetles were captured in the non-baited, window flight traps. Only two *Psuedopityophthorus pruinosis* individuals were collected from two traps in one tree out of the 24 traps placed in 12 live oak trees at three sites between 1 March and 15 May, 2005. Larger numbers of *P. pruinosis* were captured in traps located in red oaks. Over the same time period, 16 oak bark beetles were obtained from 5 trees of the 24 traps placed in 12 trees at 4 sites; similarly, 23 beetles were captured from 6 trees out of the 22 traps placed in 11 trees at 4 sites between 6 February and 12 May, 2006 (Fig. 3). Peaks in beetle abundance by month could not be determined from the low beetle counts. Of 36 *P. pruinosis* assayed, none yielded *C. fagacearum* in culture.

**Conclusions.** The abundance of *Co. truncatus* contaminated with *C. fagacearum* in fresh oak wounds is evidence of its role as vector of the oak wilt fungus in central Texas. Although this data was collected only for February in one site and one year, *Co. truncatus* have been collected in other studies in Texas. In our dispersing nitidulid beetle study, we collected *Co. truncatus* from the same site the next year and a few individuals carried the fungus. Dispersing *Co. truncatus* were collected from other sites in 1984, 1985, and 2006, although relatively few were captured and none carried the fungus (Appel et al. 1986, 1990). None of the other species collected in our study in 2006 carried the fungus. In 1984 and 1985, a few individuals from two species, *Colopterus maculatus* and *Cryptarcha concinna*, collected dispersing in oak wilt centers, were contaminated with *C. fagacearum* (Appel et al. 1990). However, it is unknown if these species also visit fresh oak wounds. Based on this data, we suggest that *Co. truncatus* is a vector of *C. fagacearum* in central Texas and that other species are likely involved as well.

In 2006, only a few dispersing nitidulid beetles (0-15) were collected each week and so determination of any time when beetles are most active is difficult. However, the results of this study are consistent with those of Appel and colleagues (1990), except for the addition of late February (Texas Forest Service), for time periods during which nitidulid beetles are active and carry the oak wilt fungus (March through July).

As compared to a previous study done in Minnesota (Ambourn et al. 2006) using similar methods, oak bark beetle captures were overall very low. Although *P. pruinosis* was present in oak wilt centers and there was some evidence of colonization in oak wilt-killed red oaks, dispersing beetle numbers were so low that it seems unlikely that this species is a common vector of the fungus in central Texas. In terms of disease management, this data supports the current guidelines of avoiding wounds in late winter and spring to prevent spread by nitidulid beetles.

## DISCUSSION

*Colopterus truncatus* was identified as a vector of the oak wilt fungus in both Missouri and Texas. This is in accordance with previous vector studies in Minnesota (Cease and Juzwik 2001, Juzwik et al. 2004b) and Iowa (Norris 1956) where it is also considered a vector based on mat surveys and wounding studies. Indeed, *Co. truncatus* is present throughout the United States (Parsons 1943) and has been identified either as a vector or as a possible vector in every location where nitidulid beetle transmission has been investigated. This species is found throughout the

central U.S. in a close association with the oak wilt disease cycle. Specifically, *Co. truncatus* is found both in fresh oak tree wounds (Dorsey and Leach 1956, Norris 1956, McMullen, Shenefelt and Kunts 1960, Juzwik, Skalbeck and Neuman 2004, Hayslett et al. 2005, Hayslett, Juzwik and Moltzan 2006) and on fungal mats (Curl 1955, McMullen et al. 1955, Norris 1956, Stambaugh and Fergus 1956, Cease and Juzwik 2001) where it is known to feed, oviposit and rear broods (Dorsey and Leach 1956, Norris 1956).

In a related Minnesota study, more *Co. truncatus* were found dispersing in active oak wilt centers than in oak stands without an active oak wilt center (Ambourn, Juzwik and Moon 2005). In an Iowa study, the timing of visitation by *Co. truncatus* adults to oak wilt fungal mats was viewed as favorable both for acquiring the fungus and for successful development of eggs deposited in the mats (Norris 1956). Specifically, *Co. truncatus* was found to visit mats very early when ascospores are sticky and viability is highest. These data support the theory of an ecological relationship between the insect and *C. fagacearum*.

The *Colopterus* species *Co. niger* and *Co. semitectus*, identified by this study as vectors in east central Missouri, have been recognized as potential vectors by studies done in a few other states (Craighead, Morris and Nelson 1953, Curl 1955, Norris 1956, True et al. 1960). Interestingly, *Carpophilus sayi* and *Epuraea* were not indicated as possible vectors in Missouri or Texas but have been considered possible vectors in studies done in other states (Craighead, Morris and Nelson 1953, Curl 1955, Norris 1956, Stambaugh and Fergus 1956, McMullen, Shenefelt and Kuntz 1960, Juzwik, Skalbeck and Newman 2004). These data point to the involvement of only a few species in transmission of the fungus and that the suite of species may vary by location.

Research done in Missouri (Rexrode and Jones 1972), Ohio (Rexrode 1969), and West Virginia (Rexrode and Frame 1973) has provided evidence that two species of oak bark beetle, *Psuedopityophthorus minutissimus* and *P. pruinosis*, are capable of transmitting the oak wilt fungus. *P. minutissimus* is considered a vector in Missouri based on the high numbers of beetles emerging from oak wilt-killed trees and the high frequency with which they are contaminated with the oak wilt fungus (Berry and Bretz 1966). Although *P. minutissimus* has been implicated as a vector in Minnesota and Ohio, it does not appear to be a common vector in these locations. In Ohio, only 11% of oak wilt-killed trees were found to be colonized by *P. minutissimus* or *P. pruinosis* (Rexrode 1967). In Minnesota, only 0.4 to 1.3% of several hundred *P. minutissimus* individuals dispersing in oak wilt-killed tree crowns were found to carry the pathogen (Ambourn, Juzwik and Eggers 2006).

*Psuedopityophthorus pruinosis* has been implicated as a vector in West Virginia, again based on high contamination frequencies (Rexrode and Jones 1971). In our Texas study, only a few *P. pruinosis* individuals were found dispersing in the crowns of the oak wilt-killed trees examined. This suggests that *P. pruinosis* is infrequently involved in transmission of the oak wilt fungus in central Texas. Again, this data indicates that the common insect vector species may vary with location.

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Table 1. Nitidulid beetle captures by species from fresh wounds on red oaks in spring at three sites in Missouri.

	Number of Beetles				
	Perry 2005	Lost Creek 2005	Prairie 2006	Lost Creek 2006	All sites
<i>Colopterus truncatus</i>	0	4	4	66	74
<i>Colopterus semitectus</i>	0	11	5	48	64
<i>Colopterus niger</i>	0	17	3	24	44
<i>Colopterus maculatus</i>	1	9	1	1	12
<i>Carpophilus sayi</i>	1	1	0	19	21
<i>Carpophilus corticinus</i>	0	1	1	8	10
<i>Cryptarcha ampla</i>	5	14	0	0	19
<i>Cryptarcha concinna</i>	0	7	1	0	8
<i>Lobiopa Undulata</i>	0	14	2	1	17
<i>Glischerochilus obtusus</i>	0	5	0	2	7
<i>Aphicrosis ciliatus</i>	0	5	0	0	5
<i>Prometopia sexmaculata</i>	0	3	0	1	4
<i>Epurea</i> spp.	0	0	0	1	1
Total	7	91	17	171	286

Table 2. Numbers of *Colopterus truncatus* collected from fresh wounds on 13 healthy red and 10 healthy live oak trees near an oak wilt center at a ranch in central Texas in February 2005 and assayed for the oak wilt fungus.

Tree stand type	Days after wounding	Number of beetles	
		Assayed	with Cf <sup>a</sup>
Live oak	1	-- <sup>b</sup>	--
	2	43	30
	3	0	0
	4	0	0
	5	0	0
	6	5	4
Red oak	1	109	97
	2	6	6
	3	2	2
	4	0	0
	5	17	12
	6	2	2

<sup>a</sup> Number of beetles contaminated with *Ceratocystis fagacearum*.

<sup>b</sup> Not applicable, no collections were made in live oak on day one.

Table 3. Nitidulid beetle captures by location collected with baited funnel traps placed in red oak wilt centers at four central Texas locations during late winter and spring, 2006.

Species	Number of beetles captured at:			
	Langford	Solana	Johnson	TNLA
<i>Colopterus truncatus</i>	27	3	0	2
<i>Colopterus maculatus</i>	0	4	4	3
<i>Cryptarcha concinnus</i>	2	7	0	2
<i>Carpophilus spp.</i>	28	40	0	6

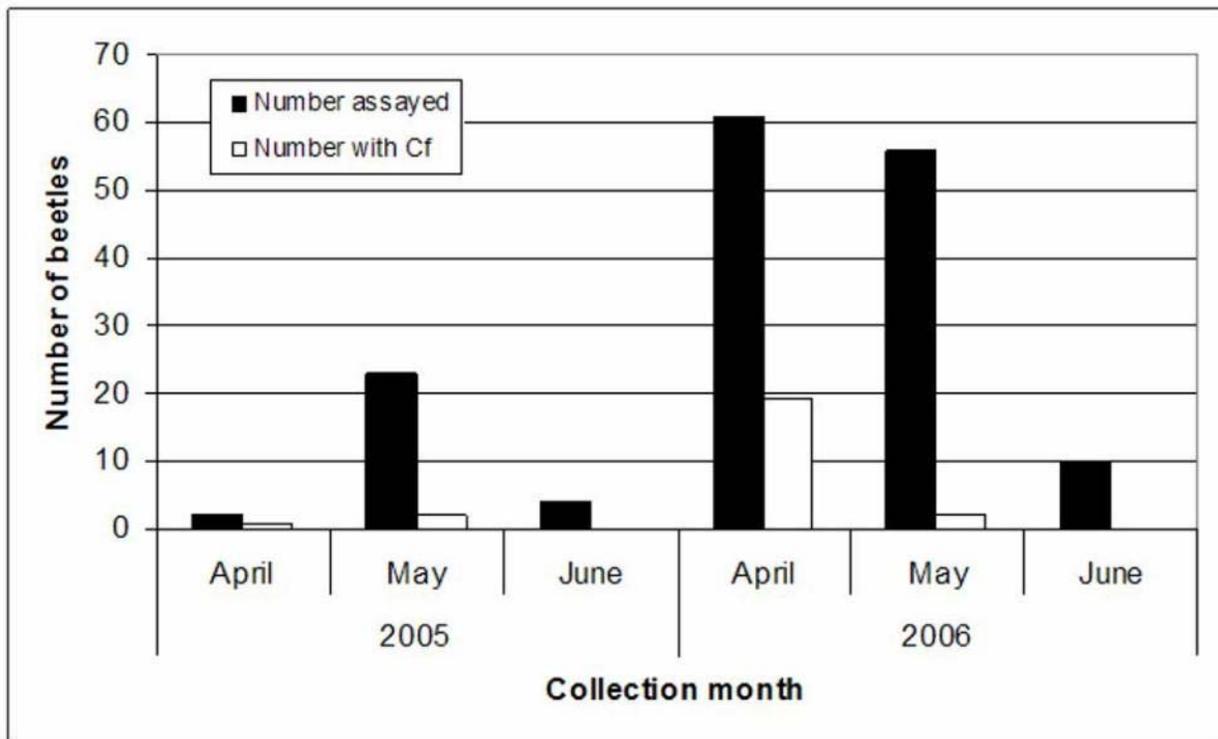


Figure 1. Number collected and frequency of *Ceratocystis fagacearum* (Cf) isolated from three *Colopterus* spp. (*Co. truncatus*, *Co. niger*, and *Co. semitectus*) collected from fresh wounds on 12 healthy red oak trees at Little Lost Creek Conservation Area in Missouri.

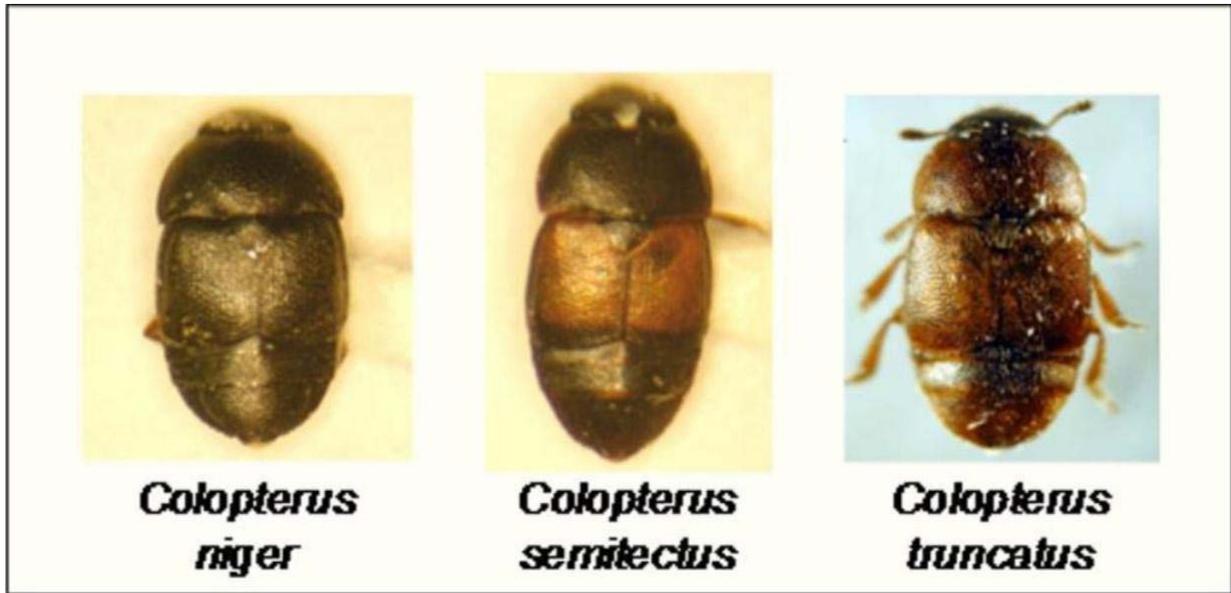


Figure 2. Photographs of the three *Colopterus* spp. identified as vectors of the oak wilt fungus in east central Missouri. Photographs taken by Maya Hayslett and Angie Ambourn.

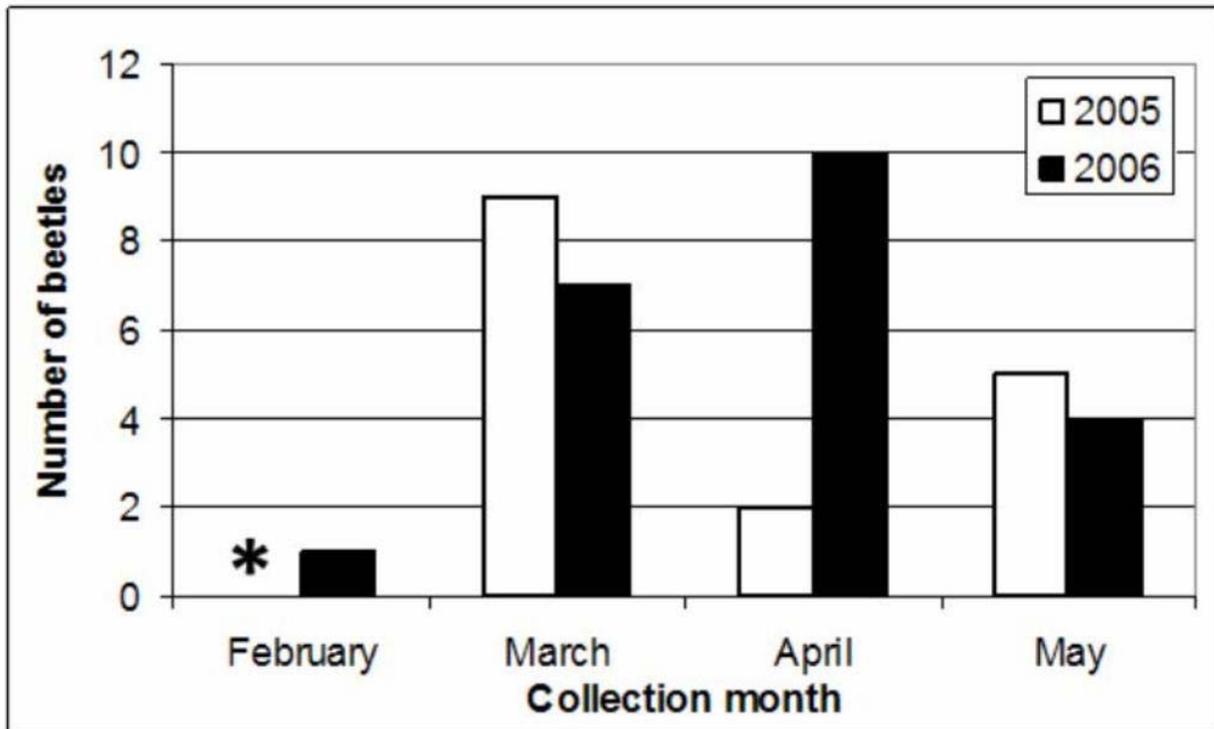


Figure 3. Number of *Psuedopityophthorus pruinosus* collected using non-baited flight traps in the crowns of oak wilt killed red oaks in central Texas. \*No collections were made in February, 2005.