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LIVE OAKS, NEW HOSTS FOR *ODONTOCYNIPS NEBULOSA* KIEFFER (HYMENOPTERA: CYNIPIDAE) IN NORTH AMERICA

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Abstract.—A study of root-feeding insects as potential vectors of the oak wilt fungus *Ceratocystis fagacearum* (T. W. Bretz) J. Hunt in live oaks, revealed root galls induced by the cynipid gall wasp *Odontocynips nebulosa* Kieffer. The incidence of the wasp on roots of four oak species and natural live oak hybrids at 14 root excavation sites in 7 counties throughout the Hill Country of central Texas was surveyed. The study was limited to sites within and adjacent to oak wilt infection centers of the live oak-Ashe juniper ecotype where oak wilt infected live oaks were being uprooted and rogued for disease suppression by the Texas Oak Wilt Suppression Project. This is the first report of this root-galling wasp on live oaks, *Q. fusiformis* Small and *Quercus virginiana* Miller × *Quercus fusiformis* natural hybrids, in North America. The incidence of root-galling by the wasp occurred at relatively low levels among trees examined at excavation sites in each county, indicating a sporadic distribution throughout the region. However, examinations of root-colonization and gall induction by *O. nebulosa* in uprooted live oaks showed relatively high levels of root infestations in some trees, including trees exhibiting symptoms of oak wilt disease. This pattern suggests high population densities in small localized areas. Examinations of individual gall clusters formed by the wasp on live oak roots revealed new details of gall morphology and developmental stages of the insect within galls. The significance of this wasp as a potential vector of the oak wilt fungus is discussed.

Key Words: host-parasite relationships, *Odontocynips nebulosa, Quercus fusiformis, Quercus virginiana,* cynipid gall wasps, *Ceratocystis fagacearum,* oak wilt fungus

The cynipid wasps comprise a large group of phytophagous Hymenoptera (Family Cynipidae) that inhabit angiospermous plants either as gall-makers (subfamily Cynipinae) or as inquinones (subfamily Synerginae). Of the more than 800 Nearctic species recognized in the Cynipidae, 78% induce galls on *Quercus* species (Burks 1979, Dreger-Jauffret and Shorthouse 1992). Approximately 10% of the species from 8 of 37 Nearctic genera listed in the subfamily Cynipinae induce galls on plants from at least 35 additional plant genera (see Burks 1979). Gall induction results from the reactions of host tissues to morphogens secreted by larvae during feeding (Rohfritsch 1992, Shorthouse and Rohfritsch 1992). The Synerginae, accounting for the remaining 12% of recognized species, develop as inquinones within galls induced by

other cynipids, chalcidoids, or dipterous gall-makers in the family Cecidomyiidae (Burks 1979, Ronquist 1994).

Cynipids are not only highly host-specific, but they are remarkably selective in the types of host tissues that they will colonize. Most cynipids that induce galls on oak species selectively infest above-ground tissues of their hosts, including either main stems and branches, twigs, buds, petioles, leaves, flowers, or fruits (e.g., acorns), but rarely several of these tissues on the same host. The greater diversity of somatic, reproductive, and meristematic host tissues serving as niches in above-ground plant tissues relative to root tissues may account for greater numbers of gall-inducing species and more numerous and varied gall morphology types found on above-ground tissues. Although many oak gall wasp species have sexual and parthenogenic generations that develop different gall types on different oak species or plant organs (Askew 1984), this rarely occurs in the same generation.

Relatively little is known about cynipids that cause root galls. Galls produced by most subterranean cynipids form on roots that arise near the crown at or just below the soil surface. Felt (1965) listed only about 40 cynipid species that form galls on plant tissues below ground. More recently, Burks (1979) listed approximately 120 species capable of inducing galls on roots. Fewer than a dozen species have been described as capable of colonizing and forming galls deeper in the soil profile on small fibrous and larger true roots. This small group of subcoronal species includes *Belenocnema treatae* Mayr, *Odontocynips nebulosa,* and *Callirhytis* species.

Odontocynips nebulosa is distinguished from other subterranean gall wasps by its ability to induce the formation of large irregular multilocular galls on the roots of its hosts. It induces single globose galls and larger irregularly-shaped multilocular galls up to 10 cm in diameter on post oak (*Quercus stellata* Wangenh.) roots up to 1.3 cm in diameter (Felt 1965). Weld (1959) found similar multilocular galls induced by this species on post oak and overcup oak (*Quercus lyrata* Walt.). Very little information on the life cycle, host range, host-parasite relationships, and geographical distribution of *O. nebulosa* has been elucidated since the species was described by Kieffer (1910). The genus *Odontocynips* currently is monotypic (Burks 1979).

The majority of cynipids are considered of minor economic importance, although a few species such as the gouty oak gall wasp *Callirhytis quercuspunctata* (Bassett), the horned oak gall wasp *C. cornigera* (Osten Sacken), and the oak rough bulletgall wasp *Disholcaspis quercusmamma* (Walsh) are destructive pests that can cause significant injury and even mortality to landscape oaks (Johnson and Lyon 1988, Eckberg and Cranshaw 1994). A field study was initiated in fall of 1993 to survey populations of root-feeding insects of live oaks that might serve as potential vectors of the oak wilt fungus, *Ceratocystis fagacearum,* in the Texas Hill Country. This fungus is the most serious pathogen causing oak mortality in Texas. During initial stages of this study, root systems of live oak trees that had been pushed over during oak wilt disease suppression activities showed heavy infestations of a root-galling insect. Subsequent investigations showed that the insect responsible for these root galls was *O. nebulosa.* The current research stemmed from the 1993 survey. The objectives were to determine the incidence and severity of *O. nebulosa* infestations of *Quercus* species in and around the perimeter of oak wilt infection centers, elucidate aspects of its biology with respect to host specificity, tissue preferences, and distribution on the host, examine gall morphology and insect development within root galls on *Q. fusiformis* and *Q. virginiana* × *Q. fusiformis* natural hybrids, and document new hosts of the wasp in North America.

Materials and Methods

Field survey and root excavations.—The root excavations and examinations required

to conduct most this study were carried out from July through December 1995 in cooperation with oak wilt suppressions activities of the Texas Oak Wilt Suppression Project, a disease suppression program administered by the Texas Forest Service. The oak wilt fungus commonly moves from tree to tree through root grafts and common root systems formed between adjacent trees. Oak trees around the perimeter of actively expanding oak wilt infection centers are routinely extracted and removed (rogued) using backhoes and bulldozers to create a distance barrier between the advancing front of the infection center and healthy trees in an attempt to prevent root transmission of the oak wilt fungus. Four species of oaks and natural live oak hybrids, all highly susceptible to oak wilt, were pushed over during this roguing process which provided the opportunity to examine and sample the root systems of oaks for root-feeding insects of potential importance as vectors of the oak wilt fungus. The oak species surveyed for *O. nebulosa*-infestations included plateau live oak, *Q. fusiformis* (formerly *Q. virginiana* var. *fusiformis*), *Q. virginiana* × *Q. fusiformis* natural hybrids of plateau live oak and coastal live oak, *Q. virginiana,* Lacey oak, *Quercus glaucoides* Mart. & Gal. (= *Q. laceyi* Small), Spanish oak or Texas red oak, *Q. texana* Buckley (= *Q. buckleyi* Dorr & Nixon), and blackjack oak, *Q. marilandica* Münchh.

The exposed root systems of 1,993 excavated oak trees, uprooted along the perimeter of fourteen oak wilt infection centers, were examined for root galls of *O. nebulosa.* Some trees sampled along the perimeter and within oak wilt centers were infected with the oak wilt fungus. Oak wilt infection centers were selected from seven Texas counties throughout the Hill Country on the Edwards Plateau and Balcones fault zone. In all cases, research trees were located in scattered stands of mixed hardwoods of the live oak-Ashe juniper ecotype bounded on the east side by the central Texas post oak savannah region.

The incidence (frequency of occurrence) of root galling by the wasp was calculated per tree for each oak species sampled within each county. The number of oak wilt infection centers that were present within a 500 m radial distance of root excavation sites also was recorded for each county. The host parameters measured for individual trees included tree species, diameter at breast height (dbh), and distance from the nearest infected tree within an oak wilt infection center. The intensity (severity) and spatial distribution of root colonization on trees with galls was recorded as percent root flare infestation (proportion of major or primary roots arising from root flares that had root galls), total number of galls per primary root and per tree, depth of galled roots, and distance of galls from the main stem or bole. Root galls were collected from individual trees at each excavation site using lopping shears to cut root segments 2–5 cm on each side of individual galls. Measures of gall morphology and root characteristics of galled roots were recorded as unilocular (single-chambered) or multilocular (multichambered) galls with multiple locules, gall dimensions, and corresponding root diameters associated with the galls found on roots from root excavation sites in each county. All data presented are (mean ± 1 SE).

Gall morphology and insect development.—Representative root galls in various stages of development were collected from the root systems of live oaks near Kerrville, TX during root excavations in the fall (18 November) and early spring (11 February). The developmental morphology of root galls (n = 44 for the fall collection, and n = 25 for the spring collection) was examined by exploratory dissections. The outer layers of host tissue forming the wall of the galls were removed in sections to reveal hyperplastic and hypertrophic tissues forming within galls during developmental stages of the wasp from early larval stages until tenerals (callow adults) within gall locules emerged from exit holes chewed through

Table 1. Incidence (frequency of occurrence) of *O. nebulosa* galls on root systems of oaks surveyed around the periphery of oak wilt infection centers from seven counties in central Texas.

Texas County	Oak Wilt Centers[1]	*Q. fusiformis*[2]		*Q. v.* × *Q. f.* Hybrids[3]		*Q. glaucoides*		*Q. texana*		*Q. marilandica*	
		Examined	Galled	Examined	Galled	Examined	Galled	Examined	Galled	Examined	Galled
Bandera	4	293	3 (1.0)	—	—	18	0	35	0	—	—
Bell	2	—	—	20	2 (10.0)	—	—	—	—	—	—
Gillespie	6	1,303	0 (0.0)	—	—	—	—	41	0	25	0
Kendall	2	23	2 (8.7)	—	—	—	—	—	—	—	—
Kerr	1	37	33 (89.2)	—	—	—	—	—	—	—	—
Mills	2	28	3 (10.7)	—	—	—	—	—	—	—	—
Travis	1	—	—	170	2 (1.2)	—	—	—	—	—	—
Total	18	1,684	41 (2.4)	190	4 (2.1)	18	0	76	0	25	0

[1] Numbers of oak wilt infection centers in the vicinity (500 m) of root excavation sites established in each county.

[2] Total number of trees examined and number having root galls of *O. nebulosa.* Values in parentheses indicate the percentage of trees with root galls.

[3] Natural hybrids of coastal live oak and plateau live oak, *Q. virginiana* × *Q. fusiformis.*

the walls. Adults used for examination were reared from additional galls (n = 23) placed in 7–10 × 6.5 cm plastic insect cages with screened lids held at 24 C for 50 days following the fall gall collection. In the last stages of pupation, tenerals were exposed within gall locules by dissection. Representative adult voucher specimens of *O. nebulosa* were deposited 17 February 1994 in the National Museum of Natural History, Smithsonian Institution, Washington, DC (Ref.: TSU Lot no. 94-1040).

Galls (n = 69) examined from the fall and spring collections were placed into three developmental categories including previous-year mature galls, current-year mature galls, and current-year immature galls. Previous-year mature galls were old black weathered galls from which a prior generation had emerged the previous year. Current-year mature galls were light brown and contained locules with larvae, pupae, or tenerals of the next generation to emerge. Current-year immature galls were small, tan-colored galls containing white internal tissue bearing predominantly larval stages. Galls in each developmental category were characterized by measurements of the gall and insect including mean number of chambers per gall, gall chamber size (internal diameter), percentage of insects in larval, pupal, and teneral developmental stages within galls, percent emergence from gall chambers, and percent insect viability and mortality within galls. Means of all measurements were expressed as (mean ± 1 SE).

RESULTS

Field survey and root excavations.—Root galls induced by *O. nebulosa* were found only on live oaks, *Q. fusiformis* and the natural hybrids *Q. virginiana* × *Q. fusiformis,* encountered at the periphery of surveyed oak wilt infection centers (Table 1). Some live oaks (<5%) within the infection center that were infested with the wasp exhibited diagnostic symptoms of oak wilt. Other oak wilt infection centers were found in the vicinity of surveyed areas as well. The infestation rates of individual root systems among all live oak trees at the periphery of oak wilt centers were less than 11% (range 1.0–10.7%) at all but one survey site. At the single site in Kerr County, 89% of surveyed trees exhibiting galled root systems. This high level of incidence was associated with an oak wilt infection center at a rural residence where all of the trees within the center had to be rogued to contain the spread of the disease.

The live oaks rogued and pushed into

Table 2. Host parameters, root infestations, and location of galls on roots of live oaks species in the Texas Hill Country.

Texas County[1]	Tree dbh (cm)	Tree Distance (m) from Infection Center	Root Flare % Infestation[2]	Total No. Galls		Depth (m) of Galled Roots	Gall Distance (m) from Bole
				Per Root	Per Tree		
Bandera	27.9 ± 6.4	4.7 ± 3.7	24.5 ± 2.5	1.3 ± 0.1	9.7 ± 4.1	0.4 ± 0.1	0.8 ± 0.2
Kendall	38.1 ± 2.5	3.6 ± 2.6	18.4 ± 1.7	1.5 ± 0.5	4.5 ± 2.5	0.2 ± 0.1	1.1 ± 0.2
Kerr	33.7 ± 8.9	7.1 ± 3.8	72.3 ± 3.6	1.7 ± 0.3	12.9 ± 5.3	0.3 ± 0.1	1.4 ± 0.3
Travis	18.4 ± 2.7	26.6 ± 5.5	20.9 ± 4.2	1.2 ± 0.1	3.7 ± 1.8	0.4 ± 0.1	1.2 ± 0.2

[1] Live oaks surveyed in Bandera, Kendall, and Kerr counties were *Q. fusiformis,* while those in Travis county were natural hybrids of coastal live oak and plateau live oak, *Q. virginiana* × *Q. fusiformis.* All data represent only trees with root galls of *O. nebulosa.*

[2] Percentage of all primary or major root branches arising from root flares with root galls of *O. nebulosa* on their distal root branchlets (mean ± 1 SE).

piles during this study were small to medium-sized trees up to 40 cm dbh. Most of these trees occurred within a mean distance less than 10 m from an adjacent oak wilt infection center (Table 2). Root infestation rates of live oak primary roots arising from root flares of galled trees ranged from 18–25% at surveyed sites adjacent to infection centers in three counties, but primary roots of live oaks at the Kerr County site exhibited a higher infestation rate. The average number of galls on live oak roots were <2 per individual galled root, although the total number of galls per tree ranged from 4 to 13. Galled roots predominantly were less than 0.5 m from the soil surface. Galls on roots deeper than 1 m were seldom observed even though the bulk of the root mass for most live oaks penetrated down to 2 m below the surface. The root systems of most trees were prevented from deeper soil penetrations due to shallow, rocky soils that are prevalent in this region. Primary and secondary feeder roots of medium-sized live oaks (20–40 cm dbh) typically extended out to 20 m or more from the bole or main stem, however galls usually were located on live oak roots within 2 m of the bole.

Root galls induced by *O. nebulosa* generally occurred on small feeder roots (usually < 1 cm diameter) that arose from primary roots below root flares near the bole. Both unilocular and multilocular galls were observed. Galls presumably formed individually with a single chamber (unilocular) on roots when a single viable larva began feeding following oviposition beneath the root cambium, while multichambered (multilocular) galls resulted when several viable eggs were deposited at a single location in the root. Multilocular galls appeared to form by the growth and fusion of the outer galls (walls) surrounding individual larval chambers. Multilocular galls formed much more frequently than unilocular galls on live oak roots (Table 3). Unilocular galls tend to be globose and isodiametric, ranging in size from 0.3–1.5 cm in diameter. Multilocular galls appeared as broadly-fused aggregates of individual globose galls and at maturity were considerably larger and more irregularly-shaped than unilocular galls. These multilocular galls expanded through cellular proliferations from hypertrophy and hyperplasia to average sizes of 5.4 cm length and 4.0 cm in diameter (n = 178) and contained an average of 25 chambers (cells). Exceptionally large multilocular galls > 10 cm long and >8 cm wide contained 70 or more chambers. Multilocular galls increased in size proportional to the number of chambers fusing to form them. However, the size (internal diameter) of individual chambers was not related to the number of chambers within each gall. Gall chamber size (x) relative to number of chambers (y) was sufficiently variable to prevent a statistically valid linear correlation (n = 442, r^2 = 0.006). Nevertheless,

Table 3. Gall morphology and root diameters associated with *O. nebulosa* galls collected from roots of *Quercus fusiformis* and *Q. virginiana* × *Q. fusiformis* hybrid trees.

Texas County	No. Galls Examined	Gall Morphology (%)[1]		Gall Size (cm)[2]		Root Diameter (cm)
		Unilocular	Multilocular	Length	Width	
Bandera	29	6.9	93.1	4.4 ± 3.1	3.0 ± 2.2	0.5 ± 0.2
Bell	37	8.1	91.9	4.1 ± 2.5	2.2 ± 1.3	0.4 ± 0.1
Kendall	9	22.2	77.8	2.3 ± 1.0	2.1 ± 1.0	0.6 ± 0.1
Kerr	67	4.5	95.5	5.4 ± 2.2	4.0 ± 1.6	0.4 ± 0.2
Mills	36	5.6	94.4	4.0 ± 1.5	2.5 ± 0.9	0.5 ± 0.2

[1] Percent of sampled galls that were unilocular (single chamber) or multilocular (multichambered).
[2] Gall dimensions are for multilocular galls only (mean ± 1 SE).

the linear model and equation ($y = 6.415+0.007x$) describing the relationship was highly significant ($P < 0.001$) and the correlation was positive. Multilocular galls also were proportionally larger as root diameter increased. Galls that formed on roots in the 1–2 cm-diameter range occasionally grew to sizes up to 11 cm long × 10 cm in width. The rate of gall expansion was not measured in this study, but some galls may have attained full size in one growing season since multilocular galls were formed by the growth of individual chambers, and larvae pupated within the locules during the following late fall and winter months. However, this does not preclude the possibility that the insect could either have two generations per year or require 1–2 years for gall development since immature and mature galls were observed in both fall and spring collections.

Gall morphology and insect development.—Ninety-two galls were removed from the root systems of excavated live oaks at the Kerrville survey site during the fall and spring collections. Some of these galls were collected from trees exhibiting the diagnostic veinal necrosis leaf symptom of oak wilt (Fig. 1a). Root segments with galls were taken from feeder roots in the main root ball near the bole (Fig. 1b). Many of the galls close to the soil surface were collected using a hand spade and lopping sheers without extensive excavation (Figs. 1c–d). The apparent disruption of normal plant hormone diffusion down feeder roots caused by gall formation often induced the production of small root sprouts from galls close to the soil surface (Figs. 1c, e). Root segments proximal to the galls toward the root apex were sometimes reduced in diameter or killed by the gall. Mature current-year galls were typically tan to light brown like host roots, while older previous-years galls were dark brown to black and weathered as the root tissue died and decayed (Fig. 1f). The majority of galls collected on 18 November were previous-year galls with exit holes.

All root galls examined in this study contained outer galls that were hard, solid, and woody. Larval chambers were completely surrounded by woody outer root tissue, but were not separated from the outer gall by an internal air space. The gall surfaces were generally smooth, lacking hairs and spines, and were not coated with a sticky resin. Unilocular single-chambered galls had outer walls that were morphologically identical to those of individual chambers within multilocular galls.

The majority of galls collected from root excavations in the fall and spring were dissected immediately to examine concurrent stages of insect and gall development. Others from the fall collection were held at 25°C for seven weeks during which galls were dissected at various stages of development until tenerals began emerging from exit holes in the galls. Gall formation was initiated by early stages of larval feeding beneath root cambial tissue. Host root tissue

Fig. 1. Host-parasite relationships associated with *O. nebulosa*-induced galls collected from roots of live oaks. a, Veinal necrosis leaf symptom of oak wilt-infected tree. b, Location of galled root segments collected on feeder roots in the main root ball near the bole. c, Shallow subterranean root galls with root sprouts, collected before emergence. d, Multilocular (multichambered) gall with exit holes collected after emergence. e, Closeup of unilocular (single-chambered) gall with dead (pruned) distal root section and root sprout. f, Multilocular mature galls on root segments comparing the light-colored, current-year living galls (top segment) with black, necrotic previous-year galls (bottom segment).

surrounding the larva began aberrant cell division and expansion resulting in the formation of localized tissue swellings (Fig. 2a). Dark brown necrotic tissues with oxidized phenolic compounds (presumably lignins and tannins) often formed within the swollen tissues immediately around the larva. The localized aberrant tissue was consumed during feeding by the developing larva to form hollowed-out cells or locular

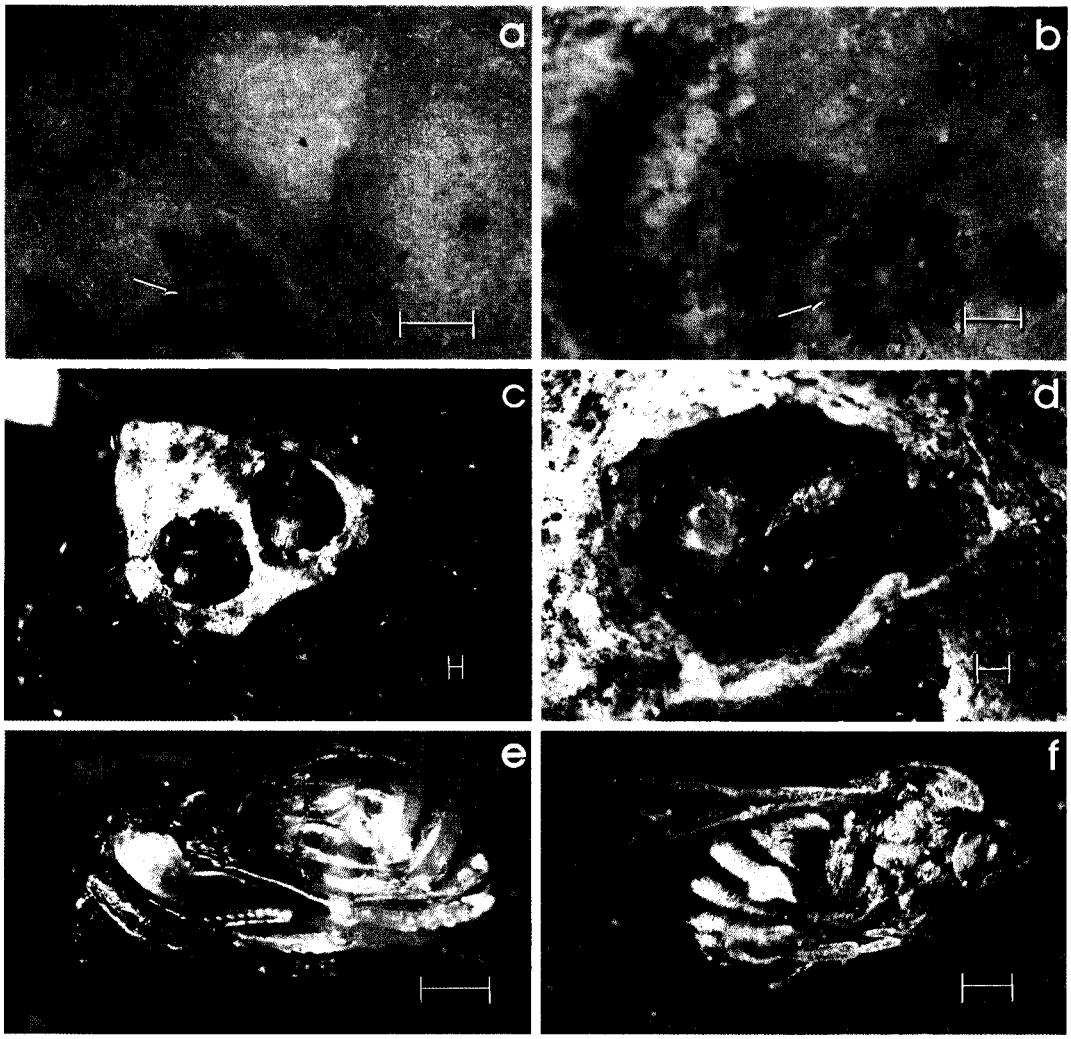

Fig. 2. Developmental morphology of gall formation and insect development in *O. nebulosa*-infested live oak roots. a, Initial stage of gall development with the formation of localized tissue swellings around larva (arrow) surrounded by brown necrotic areas in response to larval feeding. b, Development of locular initial (arrow) in gall tissue resulting from tissue consumption by larva. c, Pupae within two adjacent locules of a multilocular gall. d, Pupa with wing cases during late stages of pupation. e, Opaque pupa with separating exoskeleton, removed from gall chamber immediately prior to molt to teneral (callow adult) stage. f, Adult female with expanded wings ready for flight. Scale bars = 1.0 mm.

initials (Fig. 2b). Larvae continued to feed within developing galls throughout the summer and early fall months, enlarging the locular cavities to form chambers. None of the chambers appeared to contain more than one larva. Larvae began pupating within chambers of the galls in the fall (Fig. 2c). Pupae (Figs. 2d–e) began molting, breaking free from their wing cases to become tenerals while still in the galls. Tenerals emerge from the galls on warm days in late winter to early spring (usually early February in Texas) by chewing through the wall of their chamber and escaping through the exit hole or chewing into the locule of an adjacent chamber with an exit hole to escape. Large numbers of adults were observed emerging from the soil above sub-

Table 4. Gall and insect development, insect viability, and emergence associated with root galls of *O. nebulosa* on infested *Quercus fusiformis* and *Q. virginiana* × *Q. fusiformis* hybrid trees.

	Fall Collection[1]			Spring Collection		
	Previous-year	Current-year		Previous-year	Current-year	
	Mature Galls	Mature Galls	Immature Galls	Mature Galls	Mature Galls	Immature Galls
Gall development[2]						
Galls in category (%)	52.3	38.6	9.1	32.0	52.0	16.0
	(n = 23)	(n = 17)	(n = 4)	(n = 8)	(n = 13)	(n = 4)
No. chambers ($\bar{x}$)	10.3 ± 1.4	9.6 ± 1.5	7.3 ± 1.3	24.8 ± 2.9	21.4 ± 3.3	31.5 ± 6.5
Chamber size ($\bar{x}$)	6.5 ± 0.1	6.7 ± 0.1	3.5 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	4.0 ± 0.1
	(n = 236)	(n = 164)	(n = 29)	(n = 198)	(n = 278)	(n = 126)
Insect development[3]						
Larvae (%)	—	34.9 ± 10.1	96.9 ± 3.1	—	8.8 ± 3.5	90.3 ± 4.0
Pupae (%)	—	27.5 ± 8.4	3.1 ± 3.1	—	0.0 ± 0.0	1.7 ± 1.7
Tenerals (%)	—	0.0 ± 0.0	0.0 ± 0.0	—	23.0 ± 5.7	0.0 ± 0.0
Insect emergence[4]						
Same chamber (%)	57.0 ± 7.6	20.8 ± 9.4	0.0 ± 0.0	53.0 ± 7.4	45.3 ± 7.2	3.7 ± 2.2
Adjacent chamber (%)	19.6 ± 4.6	9.4 ± 5.4	0.0 ± 0.0	31.5 ± 4.3	15.5 ± 3.9	0.0 ± 0.0
Total emergence (%)	77.8 ± 6.7	30.2 ± 10.2	0.0 ± 0.0	79.7 ± 11.3	60.8 ± 8.9	3.7 ± 2.2
Insect viability in galls	77.8 ± 6.7	91.2 ± 3.3	100.0 ± 0.0	85.0 ± 7.1	92.6 ± 3.4	95.7 ± 2.5
Dead or aborted (%)	22.2 ± 6.7	8.8 ± 3.3	0.0 ± 0.0	14.3 ± 6.9	7.4 ± 3.4	4.3 ± 2.5

[1] Root galls examined are representative of a fall (18 November) and early spring (11 February) collection.

[2] Gall developmental categories for each collection include: previous-year mature, current-year mature, and current-year immature galls. Previous-year mature galls refer to those from which adults emerged the previous year. Current-year galls were new galls that formed since emergence of the previous-year generation. Mean values are expressed as (mean ± 1 SE).

[3] The sum of percentages in each column for insect developmental stages, total emergence, and dead or aborted (larvae, pupae, and tenerals) is equal to 100%. Tenerals within sealed galls were distinguished from inactive pupae with wing cases by their activity and partially or fully expanded wings.

[4] Indicates emergence that had occurred prior to gall collection (probably the previous spring for previous-year galls) as indicated by exit holes. Adults emerged through an exit hole either in the same chamber (within which they developed) or an adjacent chamber.

merged galls at the base of live oaks during the late morning of the spring collection. Tenerals tended to emerge from chambers on the sides facing the soil surface. Individuals in chambers facing downward in the soil tended to chew their way through the gall to the top layer of chambers that already had exit holes. Tenerals burrow to the soil surface and emerge as an adult (Fig. 2f). In the laboratory, callow adult females that were artificially freed from gall locules after emerging from pupal cases were capable of flight within 30 min. Tenerals reared from galls without assistance emerged from chambers with partially to fully expanded wings and could fly almost immediately. No parasites were recovered or observed from galls (n = 23) used in rearing the adults. All adults reared from galls collected in the summer and fall months were females indicating that root galls give rise to an asexual generation.

A comparison of the developmental morphology of galls and insects in the fall collection with those in the spring collection yielded different results (Table 4). The spring collection had a higher percentage of galls in the current-year immature and mature developmental categories than in the fall collection. Although gall chamber size (internal diameter) was comparable for galls in all three developmental categories in the fall and spring collections, immature galls were smaller than previous-year and current-year mature galls. Current-year mature galls from the fall collection contained

a higher percentage of larvae and pupae, but had a lower percentage of tenerals and total emergence than current-year mature galls from the spring collection. However, immature galls contained a much higher percentage of larvae than mature galls among current-year galls from both collections. Mature previous-year galls from both collections lacked insect developmental stages since all living tenerals had already emerged prior to collection. Insect emergence was highest in previous-year mature galls and lowest in current-year immature galls for both collections. The majority of tenerals emerging from previous-year and current-year mature galls in both collections came from the same chambers within which they developed while the remaining portion emerged from an adjacent or more distant chamber from which they developed. This latter type of emergence was achieve by tenerals chewing their way into an adjacent chamber from which the occupant had already emerged and created an escape route to the outside of the gall. Insect viability within galls was highest in immature galls and higher in current-year mature galls than in previous-year mature galls.

Discussion

The limited occurrence of *O. nebulosa* on two live oak species and its absence on the red oaks (subgenus *Erythrobalanus*), including blackjack oak, Lacey oak, and Spanish oak in this survey, suggests host specificity to certain oaks in the white oak group (subgenus *Leucobalanus*). Hitherto, *O. nebulosa* has been reported from Georgia and Arkansas only on post oak (*Q. stellata*) and overcup oak (*Q. lyrata*) by Weld (1959), both white oak species. The live oaks are intermediate species having sapwood anatomical characteristics of both the white oak and red oak groups. However, live oaks are generally classified as white oaks based on leaf and acorn characters. *Quercus fusiformis* is well established throughout the Edwards Plateau region of central Texas. The natural continuum of hybrids (*Q. virginiana* × *Q. fusiformis*) that form between coastal and plateau live oaks occurs abundantly in the region between eastern parts of the Edwards Plateau and the Brazos River to the east (Nixon 1984). Consequently, the current study has doubled the host range to include semievergreen oaks and expanded the known habitat of *O. nebulosa* to xeric savannah-woodlands of the southwestern United States. Lyon (1996) recently described seven new cynipid species on leaves and twigs of white oaks from this region. Weld (1960) listed 130 species of phytophagous cynipids from the southwestern United States with an additional 117 gall types that were never associated with a specific cynipid. The specificity with which *O. nebulosa* colonizes and forms galls on the roots of its hosts may be a survival advantage to the species in avoiding dessication in xeric habitats (see Fernandes and Price 1992).

This survey indicates that the incidence of live oak root-galling by *O. nebulosa* around oak wilt infection centers is relatively low (1.0–10.7%) within most of the areas surveyed. However, the small percentage of trees that were infested tended to have relatively high infestations of their root systems based on the percentage of major roots that were galled on individual trees. These data suggest that the occurrence of this wasp is sporadic in the Edwards Plateau region, but that it tends to occasionally form relatively high populations in small localized areas or in individual clumped stands (motts) of live oaks. The occurrence and incidence of the wasp does not appear to be influenced by the infection-status of trees since root-galling did not occur at significantly higher frequency in infected than in healthy live oaks. Therefore, oak wilt infection of live oaks probably does not predispose live oaks to *O. nebulosa*-infestation. Since the wasp occurs in both oak wilt infected and uninfected live oaks, the presence of *C. fagacearum* in the root system does not appear to be a nutritional requirement for larval development.

Thus, any potential ability to vector the oak wilt fungus would likely be passive and not out of necessity in order to complete its life cycle. However, the introduction by the wasp of a highly virulent pathogen such as *C. fagacearum* that causes a fatal disease in live oaks would not necessarily be disadvantageous to the wasp's survival. The above-ground parts of most live oaks that become infected with the oak wilt fungus die within a few months after infection, yet the root systems often survive and are supported by an abundance of root sprouts that quickly develop after the top dies and apical dominance is lost. These new shoots can maintain the living root system indefinitely. Hence, the wasp would not sacrifice its ability to continue colonization of the root system if it were to introduce a lethal pathogen. The abundance of new feeder roots resulting from the growth of many new root sprouts may actually increase the availability of colonizable root mass.

The presence of root galls on oak wilt-infected trees at the advancing front of oak wilt infection centers, the occasional very high root-infestation rates, and the occurrence of nearby oak wilt infection centers indicates the potential opportunity for *O. nebulosa* to acquire inoculum of *C. fagacearum* from oak wilt-infected live oak roots. Larvae feed directly on root tissue (new sapwood) known to serve as a reservoir for *C. fagacearum*-inoculum in infected trees. Root tissue tends to have the highest levels of inoculum because most trees within infection centers become infected through root transmission as a result of root grafting and common root systems often shared by trees within motts (Appel et al. 1995). Root inoculum is particularly important following root transmission since the fungus first enters and accumulates most of its inoculum potential within roots, which is used for subsequent colonization of aerial portions of the tree (Wilson 1995). Furthermore, adult *O. nebulosa* females have the ability to burrow down into the soil and directly penetrate live oak feeder roots with their ovipositor during oviposition. Although we did not examine the vector potential of this wasp here, the opportunities to acquire inoculum and its interaction with live oak roots make it a suitable candidate for further investigation.

The effect of *O. nebulosa*-infestations on root development of live oaks may impact host-pathogen interactions due to feeder-root mortality. The death of feeder roots associated with gall tissue senescence following adult emergence could reduce the vigor of trees making them more susceptible to oak wilt-infection. The majority of root segments collected with galls prior to emergence were alive, suggesting that dead root segments may abscise from infested roots after emergence and decay in the soil. In this way, infested roots may be effectively pruned from the root system following emergence. The death of root segments proximal to the galls toward the root apex appeared to result from the disruption of phloem transport due to the crushing of root phloem by proliferating gall tissue.

The observed emergence of unisexual-generation females of *O. nebulosa* from roots galls in this study raises the question of whether heterogony occurs in this species. Many cynipid gall wasps on oaks commonly have alternation of sexual and asexual generations with the parthenogenic all-female generation usually developing and emerging in the summer and autumn months (Lyon 1963, 1969, 1970; Askew 1984; Rey 1992). Lund et al. (1998) demonstrated heterogony in *B. treatae,* another root-galling cynipid of live oak. In this species, the bisexual generation emerges in the spring from root galls on *Q. fusiformis* and females oviposit into the undersides of leaves, inducing unilocular foliar galls on the same host. Morphologically distinct unisexual-generation females, previously described as *B. kinseyi* Weld (1921), emerge from the leaf galls in the fall and induce multilocular galls on the roots. The cycle of *O. nebulosa* appears to differ in that unisexual-generation females emerge

from root galls in the spring instead of from leaf galls in the fall. It is important in assessing its vector potential to determine whether unisexual-generation females of *O. nebulosa* oviposit into roots, leaves, or some other parts. The ability of this species to vector *C. fagacearum* would be less likely if heterogony occurs with the alternate sexual generation arising from galls on leaves or twigs because these are poor infection courts and poorer sources of inoculum for subsequent infections.

Several important inferences are suggested by the gall morphology and insect development results. The positive correlation of gall chamber size and number of chambers per gall and the corollary increase in overall gall dimensions with increasing chamber number indicate that there is no evidence for chamber dwarfing due to intraspecific competition as galls increase in size. The low mortality of the wasps during development within galls suggests that gall numbers on roots may be used to accurately estimate population density. The occurrence of immature galls in the spring implies that there are either two generations per year or that some galls may take two years to develop. The latter conclusion is more likely since there was no evidence of a fall emergence in galls from the fall collection (no fresh exit holes), the percentage of immature galls was small, prior emergence from immature galls was low, and there was an absence of tenerals within immature galls from the fall and spring collection. The higher percentage of later insect developmental stages within current-year mature galls in the spring than in the fall provides additional support for a single late winter or early spring emergence.

The morphology of the woody multilocular root galls observed in *O. nebulosa* do not fit cleanly into a single structural type as defined by Stone and Cook (1998). The structure of these asexual galls would best be described as a cross (combination) between the S3 and S5 structural stages according to their system for classifying gall structural types. The larval chambers are completely surrounded by, and in direct contact with, woody outer gall tissue. The galls are multichambered, but lack spines on the outer surface. The Stone and Cook system was developed to include the complex and diverse gall types represented in the genus *Andricus* and related oak gallers on above-ground parts of oak species. Perhaps a different or amended system should be devised for oak root gallwasps to account for gall morphological characters resulting from adaptations to roots colonization in soil environments. The morphology of root galls described here may be quite different from asexual galls arising from unisexual-generation females presumably in the summer or fall. Asexual galls could possibly occur on different live oak tissue, on a different oak species, or on roots. We do not currently know whether galls forming on roots can be sexual galls, asexual galls, or both. However, a sexual generation has not yet been confirmed with this species since males have not been observed or described.

Root gall wasps generally are considered to be less common than above-ground gall-makers perhaps because they are rarely observed and their impact on host biology is poorly understood. Root galls no doubt escape observation in most surveys for gall insects. Systematic surveys for root gall wasps of oaks in different habitats should lead to significantly more information on the biology of previously undescribed and unidentified root-galling cynipids. Although recent studies by Shorthouse and Rohfritsch (1992), Askew (1984), Lund et al. (1998), Csóka (1997), and Csóka et al. (1998) have provided new information on the biology of some Nearctic root-galling species, additional work is needed to further elucidate the biology of root gall-makers. Such work may reveal that some root-galling cynipids may have greater significance than is currently attributed to members of this obscure insect group.

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LITERATURE CITED

Appel, D. N., R. S. Cameron, A. D. Wilson, and J. D. Johnson. 1995. How to identify and manage oak wilt in Texas. USDA Forest Service, Southern Research Station, New Orleans, LA. How-To Special Report SR-1.

Askew, R. R. 1984. The biology of gallwasps, pp. 223–271. *In* Ananthakrishpan, T. N., ed., The Biology of Galling Insects. Oxford and IBH Publishing Co., New Delhi.

Burks, B. D. 1979. Superfamily Cynipoidea, pp. 1045–1107. *In* Krombein, K. V., P. D. Hurd, D. R. Smith, and B. D. Burks, eds., Catalog of Hymenoptera in America North of Mexico, Vol. 1. Smithsonian Institution Press, Washington, D. C.

Csóka, G. 1997. Plant galls. Forest Research Institute and Agroinform, Budapest.

Csóka, G., W. Mattson, G. N. Stone, and P. W. Price, eds. 1998. Biology of gall-inducing arthropods. USDA Forest Service, North Central Research Station, St. Paul, MN. General Technical Report.

Dreger-Jauffret, F. and D. J. Shorthouse. 1992. Diversity of gall-inducing insects and their galls, pp. 8–33. *In* Shorthouse, J. D. and O. Rohfritsch, eds., Biology of Insect-induced Galls. Oxford University Press, New York.

Eckberg, T. B. and W. S. Cranshaw. 1994. Occurrence of the oak rough bulletgall wasp, *Disholcaspis quercusmamma* (Walsh) (Hymenoptera: Cynipidae), as a street tree pest in Colorado. Journal of the Kansas Entomological Society 67: 290–293.

Felt, E. P. 1965. Plant gall and gall makers. Hafner Publishing Company, New York, 364 p.

Fernandes, G. W. and P. W. Price. 1992. The adaptive significance of insect gall distribution: Survivorship of species in xeric and mesic habitats. Oecologia 90: 14–20.

Johnson, W. T. and H. H. Lyon. 1988. Insects That Feed on Trees and Shrubs, 2nd ed. Comstock Publishing Associates, Cornell University Press, Ithaca, New York.

Kieffer, J. J. 1910. Description de nouveaux Hyménoptères. Bollettino del Laboratorio di Zoologia Generale e Agraria della Portici 4: 112–113.

Lyon, R. J. 1963. The alternate generation of *Heteroecus pacificus* (Ashmead) (Hymenoptera: Cynipoidea). Proceedings of the Entomological Society of Washington 65: 250–254.

———. 1969. The alternate generation of *Callirhytis quercussuttonii* (Bassett) (Hymenoptera: Cynipidae). Proceedings of the Entomological Society of Washington 71: 61–65.

———. 1970. Heterogony in *Callirhytis serricornis* (Kinsey) (Hymenoptera: Cynipidae). Proceedings of the Entomological Society of Washington 72: 176–178.

———. 1996. New cynipid wasps from the southwestern United States (Hymenoptera: Cynipidae). Pan-Pacific Entomologist 72:181–192.

Lund, J. N., J. R. Ott, and R. J. Lyon. 1998. Heterogony in *Belonocnema treatae* Mayr (Hymenoptera: Cynipidae). Proceedings of the Entomological Society of Washington. 100: 755–763.

Nixon, K. C. 1984. A biosystematic study of *Quercus* series *Virentes* (the Live Oaks) with phylogenetic analysis of Fagales, Fagaceae, and *Quercus*, Ph.D. Dissertation, University of Texas, Austin.

Rey, L. A. 1992. Developmental morphology of two types of hymenopterous galls, pp. 87–101. *In* Shorthouse, J D. and O. Rohfritsch, eds., Biology of Insect-induced Galls. Oxford University Press, New York.

Rohfritsch, O. 1992. Patterns in gall development, pp. 60–87. *In* Shorthouse, J D. and O. Rohfritsch, eds., Biology of Insect-induced Galls. Oxford University Press, New York.

Ronquist, F. 1994. Evolution of parasitism among closely related species: phylogenetic relationships and the origin of inquilinism in gall wasps (Hymenoptera: Cynipidae). Evolution 48: 241–266.

Shorthouse, J. D. and O. Rohfritsch. 1992. Biology of Insect-induced Galls. Oxford University Press, New York.

Stone, G. N. and J. M. Cook. 1998. The structure of cynipid oak galls: Patterns in the evolution of an extended phenotype. Proceedings of the Royal Society of London 265: 979–988.

Weld, L. H. 1921. American gallflies of the family Cynipidae producing subterranean galls on oak. Proceedings of the United States National Museum 59: 238–239, 241–242.

———. 1959. Cynipid Galls of the Eastern United States. Privately published. Ann Arbor.

———. 1960. Cynipid Galls of the Southwest. Edwards Bros., Ann Arbor, Michigan.

Wilson, A. D. 1995. Future direction of USDA Forest Service oak wilt research, pp. 181–186. *In* Appel, D. N. and R. F. Billings, eds., Oak Wilt Perspectives: The Proceedings of the National Oak Wilt Symposium. June 22–25, 1992, Austin, Texas. Information Development, Inc., Houston.