Conspecific pollen loads on insects visiting female flowers on parasitic *Phoradendron californicum* (Viscaceae)

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Phoradendron (Viscaceae) is a large genus of perennial plants called mistletoe that parasitize a wide variety of woody plants (Trelease 1916, Kuijt 2003). The genus includes 234 species found in the Americas from the northern United States to northern Argentina and from eastern Pacific islands to throughout the Caribbean (Kuijt 2003). Phoradendron plants penetrate trunks or branches with their haustorium, a root-like structure that taps into host-plant xylem. Plants obtain water and other inorganic nutrients from their hosts and produce...
organic nutrients from photosynthesis in leaves and stems (Kuijt 1969, 2003). Insects and other invertebrates may feed and develop on mistletoe as observed in *Phoradendron tomentosum* Engelmann (Whittaker 1984).

Flowers on *Phoradendron* (Kuijt 2003) are very small, typically around 1 mm in diameter, and unisexual. Male flowers are spherical with 3 or 4 petals that enclose an open space, and each petal supports a minute sessile anther. Female flowers are similar but contain a short style with an indistinct rounded or faintly bilobed stigma atop an inferior ovary. Both sexes of *Phoradendron* flowers contain a vestigial nectar gland described by Trelease (1916) as “apparently adapted to pollination by such short-tongued insects as flies and small bees.”

*Phoradendron californicum* Nuttall, or desert mistletoe, is a dioecious species with scale-like leaves found in the Mojave and Sonoran Deserts of the southwestern United States and northwestern Mexico (Trelease 1916, Kuijt 2003). The species mainly parasitizes trees and shrubs in Fabaceae including *Acacia*, *Cercidium*, *Olneya*, and *Prosopis* (Hawksworth and Wiens 2002). Desert mistletoe grows to 2 m in diameter and contains hanging clusters of branching 0.4–1.0-m-long, grayish or reddish green stems (Hawksworth and Wiens 2002, Kuijt 2003). Flowers are borne on inflorescences, 5–25 mm long on male plants and 5–10 mm long on female plants, that branch oppositely along stems. Scanning electron micrographs of *P. californicum* pollen show tricolporate grains with a rounded-triangular shape in polar view and a subprolate shape in equatorial view (Kuijt 2003). Grains measure 31 μm across the polar axis and 24 μm across the equatorial axis. Flowering by *P. californicum* occurs during January–March (Hawksworth and Wiens 2002).

Desert mistletoe is an important food source for vertebrates. Fruits of *P. californicum* are eaten by a variety of birds, and one species, the Phainopepla (*Phainopepla nitens* [Passeriformes: Ptilogonatidae]), is dependent on desert mistletoe fruits. Defecation of fruits on host plants disperses the plant, resulting in a mutualistic symbiosis between *P. californicum* and *P. nitens*. Preferential landings by birds on host plants that are already parasitized likely cause desert mistletoe plants to become aggregated (Larson 1996).

The unisexual flowers in *Phoradendron* require insects to transport pollen from male to female flowers. Dioecious flowering in *P. californicum* and many other species of mistletoe further requires insects to carry pollen from male to female plants. Studies on pollination in *Phoradendron* are lacking despite the genus’s parasitic habit, unusually small flowers, myriad species, wide distribution, and importance to wildlife. Insect pollination in *P. californicum* is even more obscure due to its winter flowering. The only observation of the species’ pollination has been by Kuijt (2003), who writes that flowers are, “very fragrant, on warm days attracting numerous insects in a season when few other local plants are in flower.”

Pollination of *P. californicum* within the Mojave Desert in southern Nevada was investigated by examining pollen loads on insects visiting female flowers. I considered the following questions: (1) What species of insects carry *P. californicum* pollen to female flowers? (2) Do individuals of these species carry different amounts of *P. californicum* pollen? (3) How specific are insects to desert mistletoe flowers based on pollen loads? (4) Which taxa of insects are more likely to pollinate *P. californicum* female flowers?

**METHODS**

The study was conducted in the Eldorado Mountains along State Highway 165 near Nelson, Clark County, Nevada. Insects were collected from *P. californicum* plants parasitizing *Acacia greggii* A. Gray (Fabaceae) shrubs that grew within sand and gravel washes at 3 sites at different elevations. The lowest site (35.706° N, 114.786° W) was at an elevation of 643 m and supported mostly *A. greggii*, *Larrea tridentata* (DC) Coville (Zygophyllaceae), *Hymenoclea salsola* A. Gray (Asteraceae), and *Encelia farinosa* Torrey & A. Gray (Asteraceae). The middle site (35.702° N, 114.820° W) was 2.8 km west at an elevation of 871 m and supported mostly *A. greggii*, *L. tridentata*, *H. sal-sola*, *Chlopis linearis* (Cavanilles) (Bignoniaceae), and *Crysothamnus* sp. (Asteraceae). The highest site (35.740° N, 114.839° W) was 4.4 km north of the middle site at an elevation of 1037 m and supported mostly *A. greggii*, *L. tridentata*, *Ephedra nevadensis* S. Watson (Ephedraceae), and *Prunus fasciculata* A. Gray (Rosaceae). Maximum air temperatures...
averaged 12 °C in January and 36 °C in July, and yearly rainfall averaged 196 mm, 27 km south at Searchlight during 1913–2013 (DRI 2015). Rainfall near Nelson totaled 115 mm during 2014 and 67 mm during December 2014 and January 2015 (CCRFCD 2015), the 2 months preceding the study.

Insects were collected from 2 female-flowering *Phoradendron californicum* plants (or clumps of plants) around 1 m in diameter on different *Acacia greggii* shrubs at each site. Each *A. greggii* shrub also supported at least one male-flowering *P. californicum* plant that was next to, or separate from, the sampled female plant. I collected insects from the same plants on 6 dates during 2–12 February 2015 at the lower site, 8 dates during 2–18 February 2015 at the middle site, and 7 dates during 9–20 February 2015 at the upper site. Male flowers (Fig. 1a) were open and producing pollen during collections. Insects on open, female flowers (Fig. 1c) were individually aspirated into a 125-mL plastic screw-capped flask containing a 4-dram glass vial with 4 mL of 70% EtOH. I aspirated each insect through a 4–13-cm-long tube with a 3-, 7-, or 10-mm-diameter opening (dependent on the size of the insect) to the top of the flask where it dropped into the alcohol. Insects were aspirated from each plant for 20–85 min on each date, for a total 31.5 h,

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**Fig. 1.** *Phoradendron californicum* on *Acacia greggii* near Nelson, southern Nevada, 5–9 February 2015: a, male flowers; b, pollen from male flowers in equatorial view (top) and polar view (bottom); c, female flowers, dark structures at centers are red styles; d–e, lateral views of dried *Eupeodes volucris* female aspirated from female flower on 20 February 2015; d, head and proboscis; e, labellum at end of proboscis with pollen or other material attached to setae at lower left. Scale bars in panels a, c, and d are 1 mm; scale bar in panel b is 10 μm; scale bar in panel e is 0.1 mm.
during 09:40–15:55 Pacific Standard Time. Air temperature during collections was 15–28 °C, relative humidity 24%–29%, cloud cover 0%–90%, and wind speed <10 km · h⁻¹. Plants other than *P. californicum* were not observed flowering except for a few distant *E. farinosa* plants and one nearby *Bebbia juncea* (Bentham) E. Greene (Asteraceae) plant at the lower site. A pressing (UNLV 64206) of a host shrub, a sampled female plant, and an adjacent male plant were deposited at the Wesley E. Niles Herbarium, University of Nevada, Las Vegas.

Desert mistletoe pollen was examined after mounting in polyvinyl alcohol (Dafni 1992). I collected male inflorescences at the middle site into 70% EtOH on 9 February 2015 and shook pollen from flowers. Alcohol containing pollen was centrifuged at 3400 revolutions · min⁻¹ for 3 min, and the alcohol was drawn off and replaced with 3.5 mL of water. I poured the water and suspended pollen into a Teflon evaporating dish with a 5.5-cm-diameter bottom. Hydrolyzed polyvinyl alcohol (1 mL of 12% solution) was added to the water, vortexed, and dried 2 h at 55 °C. Pollen grains embedded in the resulting circular clear-plastic film were viewed in brightfield microscopy, measured with an eyepiece reticle at 40×, photographed through a 100× oil-immersion objective lowered onto the film, and described following Faegri et al. (1989).

Pollen on insects aspirated from female flowers was extracted, mounted, and examined. I added 4 mL of water to each collection vial and vortexed the vial for 30 s. All of the alcohol solution was drawn from the vial and its suspended pollen embedded in polyvinyl alcohol as described above. I mounted smaller insects such as fruit flies (Tephritidae) on points after drying in hexamethyldisilazane (HMDS) to prevent shrinkage (Brown 1993). Larger insects were pinned. One-quarter of each circular film was sampled for pollen. I pressured the film and multiplied the number of pollen grains embedded in polyvinyl alcohol (Dafni 1992). I mounted smaller insects with a mechanical stage and recognized pollen grains by their yellow color and symmetrical shape. I counted pollen grains and categorized each as *P. californicum* or as differing from *P. californicum*. Pollen that could not be distinguished as *P. californicum* at 100× was viewed at 200×. High pollen loads on 3 of the 5 naturalized honey bees (*Apis mellifera* L. [Apidae]) I collected required pollen to be sampled differently. I scanned once across the film and multiplied the number of pollen grains by 14 (the typical number of scans) to estimate the pollen count in one-quarter of the film. Predominant pollen other than *P. californicum* on insects was also described following Faegri et al. (1989).

Insects collected on *P. californicum* were identified to the lowest taxonomic rank possible. I only identified insects with >1 specimen in a species. Diptera in Syrphidae, Anthomyiidae, Muscidae, Calliphoridae, and Sarcophagidae were keyed to genus with Vockeroth and Thompson (1987), Hackett (1987), Hackett and Vockeroth (1987), and Shewell (1987a), Shewell (1987b), respectively. Flies in Syrphidae, Muscidae, and Calliphoridae were further identified to species by comparing specimens with those of the genus at the Entomology Research Museum, University of California, Riverside. Species of Tephritidae were keyed with Foote et al. (1993) and checked against specimens at the museum. Species descriptions in Cole (1969) and Miranda et al. (2013) were also used to identify Diptera. For specimens of Hymenoptera, I keyed a species of Halictidae to subgenus with Michener (2000), and a species of Pteromalidae was identified to genus by R. Burks. Vouchers of insects (UCRC ENT 456-366–377, 456-432, 456-485–488, 456-501, 456-820, 456-893–894, 456-992) other than *A. mellifera* were deposited at the museum.

Desert mistletoe pollen loads were compared among insect species excluding *A. mellifera*. The first quartile (containing the lower 25% of observations), median, and third quartile (containing the lower 75% of observations) of the pollen counts from each species were calculated with Systat (version 10.2, Chicago, IL). I compared pollen counts among insect families with a one-way nonparametric ANOVA. Numbers of *P. californicum* pollen from insects were ranked and analyzed using ANOVA in Systat. Variation among families was decomposed into independent contrasts (Neter et al. 1996) in ANOVA that compared pollen counts between the taxonomic groups (Triplehorn and Johnson 2005) of Diptera and Hymenoptera, muscoid flies and Aschiza within Diptera, and Calyptratae and Acalyptratae within muscoid flies.
RESULTS

Pollen grains from desert mistletoe viewed in brightfield microscopy were tricolpate. Pollen-grain shape was semiangular with flat corners in polar view and oblate (longitudinally compressed) in equatorial view (Fig. 1b). Dimensions of pollen grains \( (n = 10) \) averaged 23 μm (range 22–24 μm) from corner to opposite side in polar view and 18 (18–21) μm × 23 (22–24) μm in equatorial view. Pollen grains in both views exhibited exines with 2 distinct layers.

I aspirated and identified 145 insects from \( P. \) californicum female flowers that included 13 species of Diptera in 10 genera and 6 families and 2 species of Hymenoptera in 2 families other than Apidae: \( A. \) mellifera (Fig. 2). Fruit flies in Tephritidae were most frequently collected \( (n = 77) \), with 5 species accounting for 53% of the insects aspirated. The most abundant insect aspirated from female flowers was the tephritid Euarestoides acutangulus (Thomson). Other tephritids collected in low numbers were Trupanea actinobola (Loew), \( T. \) jonesi Curran, \( T. \) nigricornis (Coquillett), and \( T. \) pseudovicina Hering. Blow flies (Calliphoridae) were the second-most-frequently aspirated insects \( (n = 24) \) and included 2 species: the
more abundant Phormia regina (Meigen) and the less abundant Calliphora latifrons Hough. Flower flies (Syrphidae) were third most frequently collected (n = 22). Eupeodes volutus Osten Sacken was the most abundant syrphid on flowers followed by Coprostylum marginatum (Say) and Pseudoscaeva diversifasciata (Knab). A species of small (6–7 mm-long) bee in Lasioglossum (subgenus Dialictus) in Halictidae was the fourth most frequently aspirated insect followed by A. mellifera. All bees collected were females. Other species collected in low numbers were the flies Blaesoxipha sp. in Sarcophagidae, Neomyia cornicina (F) in Muscidae, female Delia sp. in Anthomyiidae, and the parasitic wasp Heteroschema sp. in Pteromalidae.

Nearly all (98.5%) of the pollen sampled on insects was from P. californicum. Pollen totaling 106 grains from other plant species was found on 53 insects. Most pollen not resembling desert mistletoe was spherical and smaller, or prolate-rhomboidal and furrowed with the polar axis twice as long as the equatorial axis. Desert mistletoe pollen was found on 119 (82%) of the insects that landed on female flowers on a variety of host-plant species mostly within Asteraceae. Fruit flies aggregated on flowers on a variety of foods. The abundant fruit flies collected on these flowers are phytophagous and develop on a range of genera mostly within Asteraceae (Foote et al. 1993). Fruit flies in Trupanea have varying distributions, with T. pseudovicina being limited to the Southwest and T. actinobola occurring across the United States (Foote et al. 1993). Blow fly

Amounts of pollen in one-quarter of the pollen load differed greatly among insect species other than A. mellifera (Fig. 2). Amounts of P. californicum pollen on insects (n; median, 1st–3rd quartiles) did not differ (F 1,125 = 2.86, P = 0.093) between Diptera (130; 4.5, 1.0–33 grains) and Hymenoptera (10; 36.5, 3.0–70 grains). Within Hymenoptera, halictid bees in Lasioglossum carried more pollen than parasitic wasps in Heteroschema. Pollen loads in Diptera were higher (F 1,125 = 25.2, P < 0.001) on insects in Aschiza (22; 35.5, 20–50 grains), which lack frontal sutures, than on muscid flies (105; 2.0, 1.0–13 grains), which possess frontal sutures. Species of flies in Aschiza, all syrphids, carried varying pollen loads, with C. marginatum carrying the most pollen followed by P. diversifasciata and E. volucris. Within muscid flies, those in Calyptratae with calypters (31; 63.0, 17–114 grains) carried more pollen (F 1,125 = 58.8, P < 0.001) than those in Acalyptratae without calypters, all tephritids (77; 1.0, 0.0–3.0 grains). Pollen loads differed among families and species of Calyptratae, with the calliphorid P. regina carrying the most pollen followed by the muscid N. cornicina, the sarcophagid Blaesoxipha sp., the second calliphorid C. latifrons, and the anthomyid Delia sp. Species of tephritids carried similar, low amounts of P. californicum pollen that totaled 185 grains.

DISCUSSION

The morphology of P. californicum pollen viewed in brightfield microscopy differed from the scanning electron micrographs and description in Kuijt (2003). Pollen grains in both descriptions were tricolporate or tricolpate and 23–24 μm across the equatorial axis. In contrast, the pollen described by Kuijt (2003) was 31 μm along the polar axis, producing a subprolate grain in equatorial view, whereas the pollen I sampled was 18 μm along the polar axis, producing an oblate grain in equatorial view. This difference in polar-axis length cannot be attributed to preservation technique, microscopy, or measurement error. Different morphologies likely resulted from variation in P. californicum pollen. Genetic specialization of P. californicum on different host-plant species has been hypothesized. Desert mistletoes growing on different host-plant species have different allozymes, plant morphologies, and flowering times, and seeds are more likely to establish plants if moved within host-plant species than between host-plant species (Glazner et al. 1988, Overton 1997).

Insects carrying P. californicum pollen to female flowers develop as larvae on a variety of foods. The abundant fruit flies collected on flowers are phytophagous and develop on a range of genera mostly within Asteraceae (Foote et al. 1993). One source of food for E. acutangulus is flowers on H. salicola (Goeden and Ricker 1986), a plant present at the study sites. The species is widespread across western North America (Foote et al. 1993). Fruit flies in Trupanea have varying distributions, with T. pseudovicina being limited to the Southwest and T. actinobola occurring across the United States (Foote et al. 1993). Blow fly
larvae decompose dead animals. Both species of blow flies collected are widespread in North America, with *P. regina* being especially abundant in the West and also occurring in Europe (Cole 1969). Larval diets differ among the Syrphidae aspirated from flowers (Cole 1969). *Eupodetes volucris* is a common species whose larvae eat aphids. Homoptera are likely also eaten by larvae of *P. diversifasciata* (formerly *Ocyptamus diversifasciata*). In contrast, larval *Copestylum* eat decomposing cacti, with *C. marginatum* being a common desert species (Cole 1969). Sarcophagid flies in *Blaesoxipha* include species that parasitize spiders, grasshoppers, or beetles (Cole 1969). Larval diets of Anthomyiidae are mostly unknown, though many species are phytophagous, whereas Muscidae are generally decomposers. Bees in *Lasioglossum* provision solitary or colonial nests of larvae with collected pollen (Michener 2000). The diverse subgenus *Dialictus* contains 340 species in the Western Hemisphere, and many species remain undescribed (Michener 2000). Pteromalidae are minute wasps that parasitize different life stages of insects. The rarity of pollen on insects from plants other than *P. californicum* corresponded with the near absence of flowers on other species during February. Insects obtain pollen, and likely nectar, from desert mistletoe flowers when little else is available. Pollination of the plant is facilitated by the concentration of available insects on flowers. Not all species of *Phoradendron* flower during winter. Two of the species in California deserts flower during winter (December–March), and the other 3 flower during summer (June–September) (Hawksworth and Wiens 2002). Competition between plant species for pollinators is relatively low during summer and virtually absent during winter, allowing *P. californicum* to produce small flowers with minimal visual displays.

Pollen loads on insects visiting flowers generally corresponded with body size. Greatest pollen loads were on 2 of the largest species, *P. regina* and *C. marginatum*. Lowest pollen loads were on the smallest flies, tephritids, and on the minute wasps in *Heteroschema*. Two fly species contradicted this trend; the second calliphorid *C. latifrons* and the sarcophagid *Blaesoxipha* sp, both carried relatively low pollen loads despite larger body sizes. Pollen loads would also be affected by insect morphology and behavior. Pollen on flies would be most likely picked up by the labella during feeding. Pollen, or other material, could be seen attached to the labellum of an *E. volucris* syrphid (Fig. 1d, e) that was dried in HMDS (without being vortexed) and photographed at different focal planes with CombineZP (Hadley 2013). Flies with large setose labella would be expected to transport more pollen between male and female flowers. Flies spending more time on male flowers, increasing their contact with stamens, would also be expected to collect more pollen. Pollen on female bees (e.g., *Lasioglossum* and *Apis*), is mostly transported among hairs on the hind legs (Michener 2000). The *Lasioglossum* females collected from *P. californicum* possessed long hairs on their hind tibiae and femora.

Desert mistletoe in southern Nevada appears to be pollinated by a variety of insects primarily composed of flies. The 2 families of insects most likely to pollinate the plant are Calliphoridae and Tephritidae. Blow flies, especially *P. regina*, were relatively abundant on female flowers and carried large numbers of conspecific pollen. Other dioecious, parasitic plants appear to be pollinated by Calliphoridae. Blow flies visit flowers on *Pilostyles* (Bellot and Renner 2013), a widespread genus of plants in Apodanthaceae with flowers similar to *P. californicum* that occurs in California deserts (Hawksworth and Wiens 2002). Blow flies on female desert mistletoe plants were also observed flying or walking from flower to flower. Tephritidae may significantly contribute to *P. californicum* pollination due to their high relative abundances. Although fruit flies carried less pollen than other families of flies, numbers of pollen grains transported may be adequate for fertilization given the single minute stigma in each desert mistletoe female flower. The 77 tephritids collected would have carried a total pollen load of 740 grains, estimated from the proportion of each pollen load sampled. The abundance of fruit flies on *P. californicum* flowers may be partly due to the fruit fly’s life cycle. One of the species collected, *T. pseudovicina*, overwinters as long-lived adults (Goeden and Teerink 1998), possibly enabling flower visitation during late winter. A third family of insects, Syrphidae, may also be important pollinators of desert mistletoe. One species, *E. volucris*, was relatively abundant.
with moderate pollen loads, whereas a second species, *C. marginatum*, was less abundant but with more pollen. Pollen loads on bees, such as *Lasioglossum* and especially *A. mellifera*, are less predictive of pollination capability. Only pollen on mouthparts, and not the hind legs, would be likely to contact the stigmas within the small flowers of *P. californicum*.

*Phoradendron californicum* is dependent on animals both for pollination and seed dispersal. Seed dispersal by birds would affect the proximity of male plants to female plants, especially if sexes occurred on the same or different host plants. Distances between male and female flowers may affect pollination efficiency. Large, strong-flying insects such as Calliphoridae would likely transport pollen between widely separated male and female plants. Small, weak-flying insects such as Tephritidae may only be able to carry pollen from male flowers to nearby female flowers. Visitation by fruit flies to female, but not male, flowers is suggested by the large number of *E. acutangulus* without pollen. Plant clumping by birds defecating fruits on parasitized host plants (Larson 1996) may facilitate pollination by insects, which in turn would increase fruit set and food production for birds. Successful pollination and reproduction of desert mistletoe would also increase availabilities of food (pollen and nectar) for adult insects during the plant’s atypical winter-flowering period.

*Phoradendron californicum* is an unusual parasite of North American desert trees and shrubs whose male and female plants appear to support a web of mutualism between birds and insects.

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**Literature Cited**


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