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Cool-weather activity of the forensically important hairy maggot blow fly *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) on carrion in Upstate South Carolina, United States

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ABSTRACT

The hairy maggot blow fly *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) has expanded its range in the United States since its introduction into Texas (ca. 1980) and has been collected in 15 states. We investigated the bionomics of immature and adult *C. rufifacies* collected from carcasses of a raccoon *Procyon lotor* (Linnaeus) and white-tailed deer *Odocoileus virginianus* Zimmerman in Upstate South Carolina during November 2007, and used these insects to estimate the minimum period of insect activity. Puparia of *C. rufifacies* were collected from deer carrion; 28% were parasitized by *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). The mean daily ambient temperature during this study was 11.4 ± 1.02 °C, representing the lowest recorded mean temperature for adult activity of *C. rufifacies*; adults of *C. rufifacies* were observed flying among the carcasses at 9.0 °C. Although *C. rufifacies* is considered a warm-weather blow fly, researchers should be aware of its activity at suboptimal conditions, behavior that might aid its expansion into more northern areas.

1. Introduction

In the continental USA, the hairy maggot blow fly *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) was first detected in 1980 in southern Texas [1]. The hairy maggot blow fly is native to the Australasian and Oriental zoogeographic regions and, through human-assisted transport, has become established in tropical and subtropical regions of the world including Africa, Central and South America, Pacific islands (Hawaii), and the Indian subcontinent [2]. *C. rufifacies* has spread throughout much of the southern and southwestern United States and has been detected in northern states (e.g., Michigan) and southern Canada (e.g., Ontario) [3] (Table 1). The distribution of *C. rufifacies* in North America presumably is limited by an inability to overwinter under the cool conditions of temperate USA and southern Canada. In the southern USA, *C. rufifacies* overwinters as pupae and requires a temperature greater than 15.0 °C to pupate normally [4]. The temperatures that overwintering pupae can tolerate are not known; therefore, the limit of the fly's northern latitudinal distribution in North America is unknown. At northern latitudes, adult activity is thought to cease at 13.0 °C and the development of larvae at 15.0 °C [2].

C. rufifacies has important implications in the fields of forensic science and invasion ecology. The hairy maggot blow fly is considered a secondary carrion fly on decaying animals [5] and consequently not important in determining maximum postmortem intervals (PMIs) when collected alone. The secondary colonization of carrion by *C. rufifacies* makes it useful in predicting the minimum postmortem interval based on the period of insect activity (PIA). Excluding premortem myiasis, the PIA will be shorter than the PMI, as this estimate calculates only time elapsed between initial colonization of the remains and collection of the entomological evidence [6]. We will use PIA to refer to this type of estimate. Second- and third-instar larvae of *C. rufifacies* are facultative predators on other dipteran larvae and can outcompete and displace other blow fly species. In Australia, native *Lucilia* and *Calliphora* spp. are outcompeted and displaced from carrion through predation by *C. rufifacies* [7]. *Lucilia eximia* (Wiedemann) also has been replaced as the dominant carrion fly in some regions of Costa Rica after the introduction of *C. rufifacies* [2]. In Texas, populations of *Cochliomyia macellaria* (Fabricius) were suppressed when *C. rufifacies* was given access to carrion in field experiments [8].

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Table 1
Distribution, seasonal occurrence, and life-history studies of *Chrysomya rufifacies* in the continental USA. NA = unknown; A = adult; L = larva.

State	Year	Month(s)	Stage	Earliest reference
Alabama	1998	September, October	A, L	[23]
Arizona	1982	June	A	[24]
Arkansas	1998	July	L	[25]
California	1987	July, September, October	NA	[26]
Colorado	1994	September	L	[27]
Florida	1991	October	L	[28]
Kentucky	NA	NA	NA	[29]
Louisiana	1995	October	L	[30]
Michigan	NA	NA	NA	[29]
Nebraska	1996	August	A	[31]
North Carolina	2004	June	L	[32]
South Carolina	1993, 2003, 2007	September, November	A, L	This report
Tennessee	1998	June	L	[29]
Texas	1982	April	A	[33]
West Virginia	2006	August, September	A, L	[34]

Understanding the ecology of the invasive hairy maggot blow fly is important to forensic entomologists and ecologists. Here, we document the presence of *C. rufifacies* in South Carolina and describe the bionomics of this species and its association with carrion during cool-weather conditions. Specifically, we investigated *C. rufifacies* under cool-weather conditions with respect to development, associated blow fly species, and parasitism by *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). We also provide an updated distribution for North America.

2. Materials and methods

2.1. Study location and carcasses studied

A freshly killed (ca. <12 h) raccoon *Procyon lotor* (Linnaeus) was discovered as road kill in Clemson, South Carolina, USA (GPS: 34°39′42.00″N 82°49′49.59″W) on 2 November 2007, with no visible adult or immature flies on or near the carcass. On 3 November 2007, the carcass was relocated to the Cherry Farm Insectary, Clemson University, Clemson, South Carolina (GPS: 34°39′11.18″N 82°50′01.91″W), and placed on the forest edge to observe the arthropods associated with its decay. At the time of relocation, no adult or immature fly activity was noted. The study site consisted of a grass field covered with fallen leaves, approximately 2 m from the forest edge. Trees consisted of (in order of highest to lowest relative abundance) oaks (*Quercus* spp.), pines (*Pinus* spp.), maples (*Acer* spp.), and walnut (*Juglans* sp.). Undergrowth in the forest consisted of poison ivy *Toxicodendron radicans* (L.) Kuntze, roundleaf greenbrier *Smilax rotundifolia* L., and Virginia creeper *Parthenocissus quinquefolia* (L.) Planch.

For comparative purposes, a road-killed (dead <12 h) white-tailed deer *Odocoileus virginianus* Zimmerman was observed in tandem with the dead raccoon. The deer was presumed killed (GPS: 34°39′18.96″N 82°50′15.07″W) on 6 November 2007 (CE Beard, personal observation) and placed at the Cherry Farm study site approximately 2 m from the raccoon carrion, on 7 November 2007.

2.2. Weather data collection

Temperature at the Cherry Farm was recorded using a HOBO Micro Station (OnSet Computer Corp., Pocasset, Massachusetts, USA), from 12 to 24 November, 2007. Additional weather information was obtained through a meteorological station operated by the Department of Entomology, Soils and Plant Sciences (ESPS) on the main campus of Clemson University, approximately 3 km to the north of the study site.

2.3. Chrysomya rufifacies collection and rearing

Five days after placement of the raccoon carcass at the study site, third-instar larvae of *C. rufifacies* were noted under the decayed head of the carcass and representative specimens collected. Ten larvae were fixed in sub-boiling (~100 °C) water and preserved in vials containing 95% ethanol. Ten larvae were placed in 20-dram containers on a pupation medium (sand). A moistened paper towel was added to the container to prevent desiccation and the larvae were reared indoors at room temperature (~25.0 °C), 50% relative humidity, and light: dark regime of 12:12 h, so that identification of the larvae could be confirmed with adults. Containers were sealed to prevent parasitism in the laboratory. During the 12 d following placement of the deer carcass at the study site, activity of *C. rufifacies* was recorded with respect to temperature and adults of *C. rufifacies* were collected.

Puparia were collected by searching the dry remains of the raccoon, as well as removing and searching through leaf litter and soil up to 10 cm deep below the carcass in a 30 cm × 30 cm square. Another 30 cm × 30 cm × 10 cm area of soil containing puparia was collected from beneath the carcass and brought into the laboratory for adult emergence. Puparia were collected on and around the deer carcass, brought into the laboratory, isolated in 2-dram vials, and sealed with a cork stop to examine for parasitism. Adult calliphorids were collected by sweep net.

Using keys by [9,10], we identified larvae and adults. Representative specimens of *C. rufifacies*, *N. vitripennis*, and other blow fly species collected were deposited in the Clemson University Arthropod Collection.

2.4. Minimum PIA calculations

To calculate the minimum PIA on the raccoon, we used our collection of *C. rufifacies* and development data previously published for this species [11]. The minimum PIA on the deer carcass was calculated using development data sets for the hymenopteran parasitoid *N. vitripennis* [19] and *C. rufifacies* [11]. Temperature data used for the calculations were acquired from the HOBO Microstation at the Cherry Farm. We also compared these hourly recordings from the HOBO to the hourly temperature recordings from the nearest meteorological station (ESPS). A linear regression was performed on these data, using SAS (Cary, North Carolina, USA) to develop a model for predicting study site temperatures, using meteorological station recordings for 9 d before placement and 4 days after removal of the HOBO Microstation. The temperature readings from ESPS could be used to predict the temperature at the Cherry Farm site ($y = -0.82473 + 1.05957x$, adjusted $R^2 = 0.9366$).

3. Results and discussion

A mass of *C. rufifacies* larvae was present on the raccoon carcass 6 days (8 November 2007) after the presumed time of death (2 November 2007) and was present for an additional 3 days (11 November 2007). The mean ambient air temperature during this time was 12.8 ± 0.84 °C during the day and 8.2 ± 1.70 °C at night. The larvae collected on 8 November 2007 and allowed to continue development in the laboratory pupated on 11 November 2007 and emerged on 17 November 2007. Adult *C. rufifacies* were collected on the dead white-tailed deer 3, 4, 7, and 8 days after presumed death (6 November 2007). The mean ambient air temperature during this time was 14.0 ± 1.37 °C during the day and 8.0 ± 1.66 °C at night. On 12 November 2007, adults of *C. rufifacies* were observed flying around the deer carrion at approximately 9:00 a.m. when the ambient air temperature was 9.0 °C. Blow flies co-occurring with *C. rufifacies* as larvae and adults on the raccoon and deer carcasses were *Calliphora vicina* Robineau-Desvoidy, *Calliphora vomitoria* (L.), *C. macellaria* (Fabricius), *Lucilia coeruleiviridis* Macquart, *Lucilia sericata* (Meigen), and *Phormia regina* (Meigen). Cold-tolerant stages of *C. rufifacies* have not previously been reported. The minimum temperature threshold for flight activity is reported to be 13.0 °C; eggs fail to hatch at 9.0 °C, and at 15.0 °C eggs hatch but larvae do not pupate [2].

In South Carolina, a single third instar of *C. rufifacies* was collected in September 1993 at the Clemson University Horticulture Garden, approximately 2.5 km northeast of our study site. Larvae of *C. rufifacies* also were collected near the Cherry Farm in September 2003 and 2005. Interestingly, *C. rufifacies* was not found during decomposition studies in the same location from January 1995 to April 1996 [12]. Although reported as a primary carrion fly (i.e., a blow fly that initiates the infestation of a carcass; [13]) in southern Queensland and Hawaii [2,14], *C. rufifacies* is generally considered a secondary carrion fly [2,7]. In our study, we also consider *C. rufifacies* a secondary carrion fly. In contrast to the study by Palmer [5], who found that *C. rufifacies* in Australia prefers open pasture areas compared to wooded areas, we found this fly abundant in the latter situation.

Even though this calliphorid has been claimed to be resistant to parasitism [15,16], its puparia were noted to be parasitized by *N. vitripennis* as early as 1914 in Australia [17] and in 1984 in Texas [18]. In our study, a total of 66 adults (12♂, 54♀) of *N.*