

Research article

Zombie fire ant workers: behavior controlled by decapitating fly parasitoids

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Abstract. Laboratory observations were conducted on four separate red imported fire ant, *Solenopsis invicta*, colonies that contained workers parasitized by the decapitating fly, *Pseudacteon tricuspis*. Parasitized *S. invicta* workers remained inside the nest during parasitoid larval development and left the nest approximately 8–10 hours before decapitation by the parasitoid. When parasitized ants left the nest, they were highly mobile, were responsive to tactile stimuli, and showed minimal defensive behavior. Ants ultimately entered into a grass thatch layer, where they were decapitated and the fly maggots pupariated. This study reveals that parasitized ants exhibit behaviors that are consistent with host manipulation to benefit survival of the parasitoid.

Keywords: *Solenopsis invicta*, *Pseudacteon tricuspis*, host manipulation, host behaviour, parasitoid.

Introduction

Few ecological associations are as intimate as the host-parasite (or parasitoid) interaction (Poulin, 1995). The ability of parasites to influence host behavior is an important feature of host-parasite biology (Price, 1980), because reproductive success of the parasitoid is dependent on the behavior of its host. Parasitoid survival relies on aspects of host growth, development and survival. If the host dies before the parasitoid reaches a critical point of development, then the parasitoid also dies (Fritz, 1982). Consequently, changes in host behavior that minimize premature host mortality during parasitoid development ultimately benefit the parasitoid.

There are many examples reported in the literature of parasitoids that induce behavioral changes in their hosts towards the end of their development. For example, *Chelonus inanitus* (L.) (Hymenoptera: Braconidae) causes

its host caterpillar, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) to dig into the soil at its fourth instar rather than the sixth instar (Rechav and Orion, 1975). Another *Chelonus* sp. causes its host, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) to prematurely initiate metamorphosis by spinning a cocoon but not actually pupating; this way the protective structure of the cocoon is provided to the developing parasitoid (Jones, 1985). Ants that are parasitized by nematodes will drown themselves in water so that the nematodes can emerge (Kaiser, 1986; Maeyama et al., 1994).

In recent years, several species of parasitoids in the genus *Pseudacteon* Coquillet (Diptera: Phoridae), collectively referred to as ‘decapitating flies,’ have been introduced in the United States as biological control agents of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). These parasitoids oviposit in host ants that are engaged in various activities outside of the nest and eventually pupariate inside the decapitated host’s empty head capsule (Porter et al., 1995, 1997).

Morrison and Porter (2005) estimated *P. tricuspis* Borgmeier parasitism rates in *S. invicta* field colonies that were collected and monitored in the laboratory. No *P. tricuspis* puparia were found until approximately eight days after field collection, although they were expected to have appeared within the first few days. Behavioral changes in parasitized ants were hypothesized as being responsible for this effect.

In previous laboratory studies of *S. invicta* and *Pseudacteon* spp., *S. invicta* workers removed the remains of parasitized colony members and deposited them in nearby middens (Porter et al., 1995, 1997) during the course of normal *S. invicta* necrophoric behavior (Howard and Tschinkel, 1976). Porter et al. (1995) posed several questions about the behavior and fate of parasitized *S. invicta* under natural conditions, and the resulting effect of the environment on the phorid

puparium. For example, if this necrophoric behavior also occurs under natural conditions, what would happen to phorid puparia that are exposed to high soil surface temperatures? The upper critical thermal limit of *S. invicta* is reported to be approximately 40°C (Cokendolpher and Phillips, 1990). Our field measurements of exposed soil surface temperatures in the summer showed that thermal limits that are lethal to *S. invicta* are commonly exceeded. In many cases, soil surface temperatures exceeding 55°C were recorded (Henne and Johnson, unpubl. data). Consequently, *P. tricuspis* puparia that are inside *S. invicta* head capsules could be vulnerable to lethal temperatures and desiccation if they are discarded in a middens pile. Moreover, under laboratory conditions *S. invicta* will chew open head capsules containing the parasitoid and kill it (Porter et al., 1997; pers. obs.). These hostile conditions would imply that an alternative strategy exists for these parasitoids to successfully develop into adult flies.

Our observations of *P. tricuspis* parasitized *S. invicta* colonies in a large laboratory arena revealed that these parasitized ants were exhibiting behaviors that appeared consistent with host manipulation to benefit survival of the parasitoid. The objective of this study was to describe the behavior of parasitized *S. invicta* workers in the hours leading up to their decapitation and to determine possible parasitoid pupariation sites.

Materials and methods

Four monogyne *S. invicta* colonies were collected at the Louisiana Agricultural Experiment Station in St. Gabriel, Louisiana (30° 16' N, 91° 05' W) (two in February 2006, two in July 2006). As of 2006, expanding populations of *P. tricuspis* in Louisiana had not yet reached this location. Colonies were separated from soil by the drip flotation method (Banks et al., 1981). Ants from each colony were then sieved to yield 5–6 grams (approximately 600–1,000 ants gram⁻¹) of individuals that were within the preferred size class for *P. tricuspis* females (approximately 1 mm head width (see Morrison et al., 1997)). Ants plus a small amount (approximately 1 gram) of brood were placed inside an open plastic container (Glad® 1.89 liter) lined with Fluon® to prevent ants from escaping. Ants were subjected to continuous oviposition attack by 50–100 *P. tricuspis* females for four days at a temperature of 28°C and 80% relative humidity.

To establish that parasitoid-induced behavior consistently occurred among several unrelated colonies, two initial set-ups were done consecutively during the spring of 2006. These set-ups involved placing a mound of moist potting soil (approximately 50 cm³) in the middle of the arena. After exposure to *P. tricuspis*, ants entered this mound and constructed a nest. Two subsequent set-ups were also done consecutively in the summer of 2006 and involved the placement of inverted plastic containers (Ziploc® 236 ml snap lid containers) in the middle of the arena so that internal observations of the colony could be made (Fig. 1). A moistened plaster block was placed inside each container. Two 15 cm PVC tubes were inserted into opposite ends of each container to imitate foraging tunnels associated with *S. invicta* colonies under natural conditions (Markin et al., 1975). The entire container was covered with a removable cardboard sleeve. The PVC tubes were also covered with cardboard to block light. After exposure to *P. tricuspis*, ants were placed on the floor inside the arena, and they quickly moved into all three containers. Temperature inside the arena was maintained at approximately 25 ± 2°C and 60% relative humidity. Water and sugar water were provided for ants *ad libitum*.

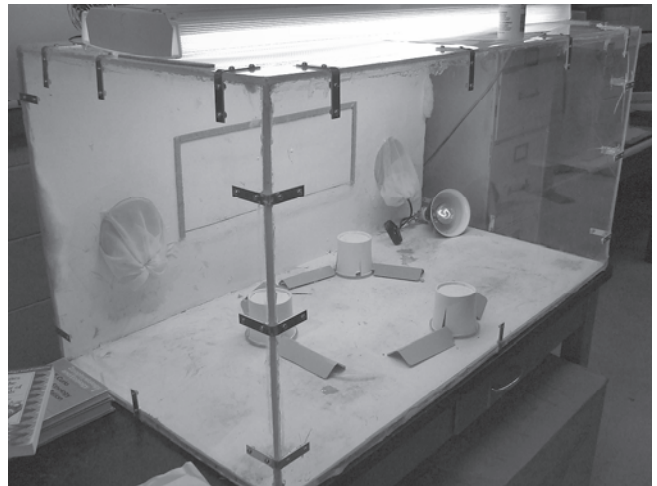


Figure 1. Experimental arena, containing three plastic observation nest units and lateral PVC foraging tubes.

Observations were made through a large enclosed Plexiglas® arena (60 cm × 120 cm × 60 cm) that was illuminated by an overhead fluorescent lamp and heated by a 75 W infrared lamp (Fig. 1). While foraging ants were observed daily inside the arena, parasitized ants did not appear outside of the nest until approximately 15 days after *P. tricuspis* oviposition. Observations continued daily between 0700 h and 1600 h for two subsequent weeks. More than two-thousand ants were randomly collected inside the arena from all four trials combined and examined under a stereo microscope to determine their status as parasitized or unparasitized. The late third-instar maggot was always observed moving around inside the ventral portion of the parasitized ants' head capsule, and the maggot's cephalopharyngeal skeleton could be seen moving as well.

To determine possible *P. tricuspis* pupariation sites, ants (n=100–120) that were confirmed to be parasitized were placed in a Fluon®-lined 31.4 cm × 25.6 cm × 9.7 cm plastic container (Pioneer Plastics, model 395C, Dixon, KY) with a 5 cm thick layer of sod containing grass and thatch. Moist sand and moist potting soil were also placed between two vertical 5 mm thick sheets of clear plastic, with 1 cm between sheets, and the top and sides plugged with cotton. Parasitized ants were placed on the substrate surface to determine if parasitized ants burrowed into these substrates.

To determine the insulating properties of the soil thatch layer, temperature measurements were made on 10 cm × 10 cm × 5 cm pieces of sod and bare soil obtained from a pasture and placed under an infrared lamp. A thermoprobe was placed on the surface of bare soil, and the distance between the soil surface and infrared lamp was adjusted until the temperature stabilized at 40 ± 0.2°C (approximately 5 cm). Then the infrared lamp was placed over sod with the grass and thatch layer intact, with 5 cm between the infrared lamp and the top of the thatch layer. The thatch layer was approximately 2 cm thick, and the thermoprobe was placed at the soil-thatch layer interface. Measurements were repeated 10 times.

Results

Parasitized ants routinely left their nest approximately 8–10 hours prior to decapitation (n>500 observations from four replicate colonies). Initially, their behavior was indistinguishable from unparasitized ants. Unlike unparasitized foragers that were also collected in the arena, parasitized ants never returned to the nest after leaving. After exiting, parasitized ants were observed walking

around the arena floor for 2–4 hours before collapsing. They would then sit motionless for several more hours, sometimes twitching their legs. Parasitized and unparasitized ants were frequently observed inside the PVC tubes and would mass near the exit holes before exiting (Fig. 2). Parasitized ants examined under a stereo microscope were found capable of some degree of defense, since they attempted to sting the forceps being used to hold them. Additionally, droplets of venom were frequently observed exuding from the stinger, and the ants repeatedly rubbed this venom on their legs and the forceps. However, parasitized ants were unable to bite, since damage to the mandibular muscles by the parasitoid was evident. In all cases ($n > 500$), positive identification of the maggot inside the head capsule was made.



Figure 2. Ants inside PVC foraging tubes. These ants left the PVC tubes shortly after this photo was taken. They were later confirmed to contain *P. tricuspis* maggots inside their heads.

More than 100 parasitized ants that were placed in a container with sod were later found in the sod thatch layer, generally within approximately 5 mm of the surface. Results of the plastic vertical sheets observations showed that at least some parasitized ants burrowed into moist sand to a depth of 21 ± 4.2 mm (Mean \pm SE, $n=6$) and in moist potting soil to a depth of 5 ± 0.52 mm (Mean \pm SE, $n=9$), but if no structure was available to hide in most ($n=100-120$) would collapse on the surface, or make feeble attempts to burrow but unable to because their mandibles were no longer functional. Temperatures at the bottom of the thatch layer were approximately 15°C lower ($25 \pm 0.51^\circ\text{C}$, mean \pm SE, $n=10$) than bare soil temperatures 5 cm under an infrared lamp.

Discussion

This study revealed that ants parasitized by *P. tricuspis* probably do not die inside the nest, but instead leave the nest shortly before their decapitation. Other studies

reporting that phorid pupae are deposited in the middens pile involved colonies in small containerized environments, which restricted parasitized ants from wandering away to die.

Parasitized ants seem to be under the control of the parasitoid larva in a way that benefits the survival of the parasitoid and ultimately the adult fly. Once parasitized, ants never leave the nest until the parasitoid has virtually completed larval development. A possible explanation is that the maggot is exploiting the host as a vehicle to locate a suitable microclimate for pupariation. The host's brain is evidently still intact when the ants leave the colony. The brain is reported to be the last structure in the head to be consumed by the parasitoid (Porter et al., 1995, 1997; C onsoli et al., 2001). Presumably the maggot is exploiting the host sensory system to seek out a suitable location for pupariation. Whether other species of *Pseudacteon* affect their hosts in a similar manner is presently unknown.

In our laboratory colonies, parasitized workers remained inside their nests and were among the other ants and brood in a cluster surrounding the moistened plaster blocks. Dead parasitized ants were never observed inside the inverted plastic containers. Unparasitized foragers (25–50 per day) were observed walking around the arena during the first two weeks after exposure to *P. tricuspis*, but the majority of ants remained inside the nest (see Mirenda and Vinson, 1981). Parasitized hosts in social species suffer greater mortality if they behave differently (Curio, 1976; Morse, 1980). Thus, parasitoids should not cause their social insect hosts to elicit unusual behaviors (Fritz, 1982). It has been reported that in laboratory colonies, parasitized workers tend brood, are less aggressive and seldom forage, the last feature that would contribute to the fitness of the parasitoid (C onsoli et al., 2001) since the host would escape environmental hazards outside the nest. Parasitized ants in our study were never observed outside of their nests until they left just prior to decapitation. C onsoli et al. (2001) are correct that, during advanced parasitoid development, these ants are less aggressive. However, our study found that they react to being handled by vigorously attempting to escape and expelling venom.

Precisely where parasitized workers are to be found under natural conditions for the eight days prior to decapitation is still unknown. The setup in the study reported here was not an exact replication of natural conditions and may have constrained some behaviors. Tracking individual parasitized ants with visually detectable markers, such as paint, is not practical, as these marks are scraped off (Mirenda and Vinson, 1979). As Morrison and Porter (2005) hypothesized, it is likely that parasitized ants move into lateral foraging tunnels and, thus, escape collection. Furthermore, it is suggested that behavioral changes in host ants likely begin shortly after injection of the egg into the host's thorax. C onsoli et al. (2001) discuss the role of possible chemicals injected with the egg and/or changes in host hormones or physiology as a consequence of parasitoid development.

In our study, parasitized ants were often observed in the lateral PVC 'foraging' tunnels provided. This behavior, if it also occurs in the field, would seem to ultimately benefit the parasitoid, since it not only reduces the risk of mortality to its host but it also positions the ants near exit holes when it is time to leave the nest. The fact that parasitoids must leave the host at some time to complete their life cycle implies that survival of the parasitoid depends on its location in the environment when it leaves the host (Poulin, 1995). By moving into the thatch layer, a suitable incubation microclimate is achieved for *P. tricuspis* pupariation. Multiple measurements of the temperature at the soil surface thatch layer interface confirmed that the thatch layer is a good insulator against high temperatures that would otherwise be lethal to *P. tricuspis* puparia.

We do not know how far parasitized ants travel once they leave the nest, but it could be up to several meters. *Pseudacteon tricuspis* adults frequently appear at *S. invicta* mounds almost immediately after disturbance (pers. obs.), suggesting that they were already in the vicinity of the disturbed mound. Cõnsoli et al. (2001) reported that the cuticle of parasitized ants darken slightly during the time when the parasitoid is approaching pupariation. This could be interpreted as a precursor to a form of crypsis that enables the parasitoid to avoid detection when the parasitized ant leaves the colony. Fritz (1982) discusses the implications of host behavioral manipulation by parasitoids and suggested that the degree of parasitoid benefit from this is proportional to the intensity of host predation. By remaining in the nest until it is time for parasitoid pupation, the host of *P. tricuspis* escapes superparasitization and predation.

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References

- Banks W.A., Lofgren C.S., Jouvenaz D.P., Stringer C.E., Bishop P.M., Williams D.F., Wojcik D.P. and Glancey B.M. 1981. *Techniques for collecting, rearing, and handling imported fire ants*. U.S. Department of Agriculture, Science and Education Administration, Advances in Agricultural Technology, AATS-S-21, 9 pp
- Cokendolpher J.C. and Phillips S.A., Jr. 1990. Critical thermal limits and locomotor activity of the red imported fire ant (Hymenoptera: Formicidae). *Environ. Entomol.* **19**: 878 – 881
- Cõnsoli F.L., Wuellner C.T., Vinson S.B. and Gilbert L.E. 2001. Immature development of *Pseudacteon tricuspis* (Diptera: Phoridae), an endoparasitoid of the red imported fire ant (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* **94**: 97 – 109
- Curio E. 1976. *The Ethology of Predation*. Springer-Verlag, New York, USA. 250 pp
- Fritz R.S. 1982. Selection for host modification by insect parasitoids. *Evolution* **36**: 283 – 288
- Howard D.F. and Tschinkel W.R. 1976. Aspects of necrophoric behavior in the red imported fire ant, *Solenopsis invicta*. *Behaviour* **56**: 157 – 180
- Jones D. 1985. Endocrine interaction between host (Lepidoptera) and parasite (Cheloninae: Hymenoptera): Is the host or parasite in control? *Ann. Entomol. Soc. Am.* **78**: 141 – 148
- Kaiser H. 1986. Über Wechselbeziehungen zwischen Nematoden (Mermithidae) und Ameisen. *Zool. Anz.* **217**: 156 – 177
- Markin G.P., O'Neal J. and Dillier J. 1975. Foraging tunnels of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). *J. Kansas Entomol. Soc.* **48**: 83 – 89
- Maeyama T., Terayama M. and Matsumoto T. 1994. The abnormal behavior of *Colobopsis* sp. (Hymenoptera: Formicidae) parasitized by *Mermis* (Nematoda) in Papua New Guinea. *Sociobiology* **24**: 115 – 120
- Mirenda J.T. and Vinson S.B. 1979. A marking technique for adults of the red imported fire ant (Hymenoptera: Formicidae). *Fla. Ent.* **62**: 279 – 281
- Mirenda J.T. and Vinson S.B. 1981. Division of labour and specification of castes in the red imported fire ant *Solenopsis invicta* Buren. *Anim. Behav.* **29**: 410 – 420
- Morrison L.W., Dall'Aglio-Holvoicenc C.G. and Gilbert L.E. 1997. Oviposition behavior and development of *Pseudacteon* flies (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae). *Environ. Entomol.* **26**: 716 – 724
- Morrison L.W. and Porter S.D. 2005. Phenology and parasitism rates in introduced populations of *Pseudacteon tricuspis*, a parasitoid of *Solenopsis invicta*. *BioControl* **50**: 127 – 141
- Morse D.H. 1980. *Behavioral Mechanisms in Ecology*. Harvard University Press, Cambridge, USA. 383 pp
- Porter S.D., Pesquero M.A., Campiolo S. and Fowler H.G. 1995. Growth and development of *Pseudacteon* phorid fly maggots (Diptera: Phoridae) in the heads of *Solenopsis* fire ant workers (Hymenoptera: Formicidae). *Environ. Entomol.* **24**: 475 – 479
- Porter S.D., Williams D.F. and Patterson R.S. 1997. Rearing the decapitating fly *Pseudacteon tricuspis* (Diptera: Phoridae) in imported fire ants (Hymenoptera: Formicidae) from the United States. *J. Econ. Entomol.* **90**: 135 – 138
- Poulin R. 1995. Adaptive changes in the behavior of parasitized animals: A critical review. *Int. J. Parasitol.* **25**: 1371 – 1383
- Price P.W. 1980. *Evolutionary Biology of Parasites*. Princeton University Press, Princeton, New Jersey. 256 pp
- Rechav Y. and Orion T. 1975. The development of the immature stages of *Chelonus inanitus*. *Ann. Entomol. Soc. Am.* **68**: 855 – 864

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