

and control females suggest that the corpus allatum hormone, though not always essential for mating, does enhance the sexual receptivity of females. The difference in the percentage of mating in allatectomized females observed by Roth and Barth (1964) and that by Engelmann (1960a) and this paper remains unexplained. The remote possibility exists that strain differences are found governing the extent to which the corpus allatum hormone promotes sexual receptivity in the females.

Table 1.—Mating in allatectomized and control females of *L. maderae*.

	No. ♀♀ observed	No. ♀♀ mated	
		Within at least 8–10 days after start of observations	Within 10 days after implantation of corpora allata
1. Allatectomized females	a. 36*	11 = 30.5%*	
	b. 29	9 = 32%	
	c. 16	4 = 25%	
	d. 34	17 = 50%	
2. Corpora allata and corpora cardiaca removed	81	35 = 43%	
3. Controls: sham-operated	a. 16*	14 = 87%*	
	b. 23	22 = 95%	
4. Allatectomized females which received 4 active c. allata ^b	a. 11*	—	9 = 82%*
	b. 12	—	11 = 92%
5. Allatectomized females which received 2 active c. allata and c. cardiaca. ^b	35	—	30 = 86%

* Small colonies of 2 pairs (data from Engelmann 1960a).

^b Allatectomized females received active implants 3–4 weeks after the previous operation.

Females sexual receptivity could be controlled also by the pars intercerebralis of the brain. Preliminary experiments on females of *L. maderae* indicate that destruction of this region of the brain by electrocautery eliminates mating behavior completely. However, reimplantation of the pars intercerebralis does not reestablish normal behavior. It is highly likely that the cautery technique used on these specimens caused damage elsewhere in the brain, damage to neural structures necessary for normal sexual behavior that could not be counteracted merely by the implantation of the pars intercerebralis from normal animals. Further analysis of the possible role of the pars intercerebralis in sexual behavior requires more refined techniques.

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The Occurrence of a Nematode, *Diploscapter lycostoma*, in the Pharyngeal Glands of the Argentine Ant, *Iridomyrmex humilis*^{1,2}

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ABSTRACT

Juvenile forms of the nematode *Diploscapter lycostoma* Volk were found inhabiting the pharyngeal glands of laboratory-reared Argentine ants, *Iridomyrmex humilis*

(Mayr). Although the infested glands were destroyed, the nematode did not appear to injure the host or affect its behavior.

Nematodes have been found in many modes of as-

sociation with insects from true parasites (e.g. mermitid parasites of blackflies, midges, and grasshoppers) to simple commensals (e.g., nematode inhabitants of galleries of bark beetles). Their discovery in the pharyngeal glands of ants was first reported by the French myrmecologist Janet (1893) from

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² Accepted for publication June 12, 1967.

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Formica rufa L. and *Lasius flavus* F. With the exception of the work of Wahab (1962), little more has been added to the knowledge of this biological association between ants and nematodes, nor has it until recently been identified from a North American ant (Nickle and Ayre 1966).

While rearing the Argentine ant, *Iridomyrmex humilis* (Mayr), in the laboratory for biology studies, it was found that the detritus in many of the artificial nests contained a saprophagous nematode identified as *Diploscaptor lycostoma* Volk. Further investigation revealed that the juvenile stages of this nematode, in addition to being present in the detritus, could be found in the pharyngeal glands of the worker ants. The purpose of this study was to investigate this ant-nematode association, estimate nematode frequency in the field, and characterize the effect of the nematode on both laboratory and field colonies of the Argentine ant.

METHODS AND MATERIALS

The principal type of nest used for rearing the Argentine ant in the laboratory was a modification of that described by Brian (1951). It consisted of a 10-cm plastic petri dish containing a dry and a moistened surface of plaster of Paris. The moist surface was maintained by a cotton wick suspended in a container of water (Fig. 1). The saturated plaster furnished a supply of water for the ants and maintained a high humidity in the nest; it was also the site on which they deposited their detritus. Within these piles or refuse, the nematodes were generally found in large numbers (Fig. 2).

Nematodes were obtained from the pharyngeal glands located in the head of the ants by dissection. The ants were first anaesthetized with CCl_4 , the head was removed, placed on a slide in a drop of physiological saline, and opened, exposing the glands. The glands were next opened to release the nematodes, which were removed from the slide with a bamboo spear. To prepare the nematodes for microscopic study, they were heat-relaxed by pouring hot 10% formalin into an equal volume of the saline containing the nematodes. After 5–10 minutes, they were either held in the formalin for later processing or mounted directly in 2% glycerine for immediate examination. Permanent slides were prepared by fixing the nematodes in formalin for 24 hr and then processing them through the Baker's rapid glycerine series (Baker 1953). Then the nematodes were mounted on slides in anhydrous glycerine.

TAXONOMIC CONSIDERATIONS

Wahab (1962), in studying the nematode associates of ants, reported the initial insect association for the genus when he found *D. lycostoma* in the pharyngeal glands of all genera of the Camponotinae which he examined. According to Wahab (personal communication) males are rare, as only 8 were found in a culture after 43 days, or nearly 3 generations. In the currently reported studies, males were never recovered from cultures maintained at 80°F in the labora-

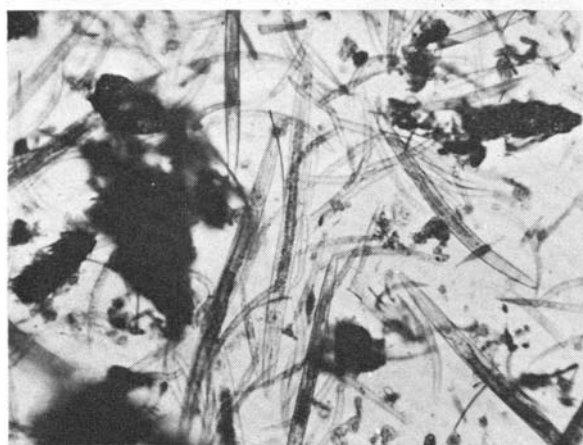
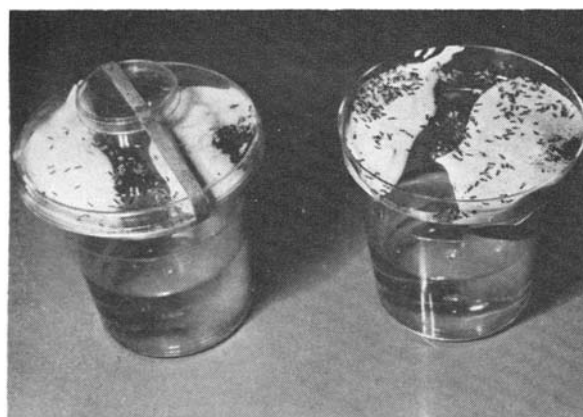


FIG. 1 (above).—Laboratory nests used for rearing the Argentine ant. The nest on the left is open. The dark material on the left side of both nests are the detritus piles.

FIG. 2 (below).—Adult and juvenile forms of the nematode *Diploscaptor lycostoma* present in the detritus in nests of the Argentine ant.

tory. In an effort to stimulate male production, ant cultures containing high nematode populations were exposed to temperatures of 85°, 90°, and 95°F for 14–18 days, a period sufficient for the completion of 2 generations. At all exposures, no males were produced. Maupas (1900) concluded that *D. lycostoma* is an autogamous protandrous hermaphrodite and the males, which are rarely present, are non-functional.

According to Goody (1963), the genus, represented by 7 species, appears to be saprophagous. This would appear to be true if one considers the different conditions it inhabits. For example, *D. coronata* (Cobb 1893), synonymous with *D. lycostoma* Volk 1950, was initially reported from the roots of banana plants. DeMan (1896) reported it in diseased pseudo-bulbs of tropical orchids, while Peters (1930) reported *D. lycostoma* from sewage.

THE ARGENTINE ANT

The Argentine ant is an introduced pest species from South America, now widely distributed in the southern part of the United States. In California, it

is presently the most economically important ant, primarily as a pest of citrus, where it upsets biological control by the coincidental protection of scale insects and mealybugs. Its biology varies somewhat from that of the normal Nearctic ant. The usual concept of an ant colony, a permanent nest with its surrounding territory, does not apply, for not only are individual nests relocated according to the season or soil condition, but there is a continual exchange of individuals between all nests in a given area. The alate queens do not appear to have a nuptial flight but mate in the nest and form new colonies by the budding of an old colony and invasion of new areas. The ant is polygynous and averages a queen for every 500–1000 workers. The queens are quite small and very active and are frequently seen on the surface with the workers when the colony is moving.

The ants used in the laboratory for studying this nematode were collected from nests in citrus groves from 4 widely separated locations in southern California: San Diego, Orange, Ventura, and Riverside Counties. Ants from all localities were found to be infected. After examining several thousand workers from each locality, it was determined that, on an average, less than 1 ant in 200 was infected. A similar low rate of infection was found by Janet (1893) and Wahab (1962).

BIOLOGICAL CONSIDERATIONS

In the laboratory, reproducing female and juvenile nematodes readily were found living in the refuse piles accumulated by the ants. These detritus piles appear to be the primary source of infection for the ants. Uninfested ants placed in contact with infested detritus were found to be infected within 24 hr and continued to pick up juveniles until in a month's time, they averaged 15 or 20 each. After 6 months, individual workers were found which contained more than 100 juveniles.

In general, the nematodes appeared to have no pronounced effect on the worker ants. Infected workers lived and behaved normally for at least 7 months. Colonies containing 95 to 100% infected workers have been maintained at least 10 months, at which time they were behaving normally and had large, healthy broods.

The method by which the juvenile nematode leaves the detritus and enters the ant has not been determined accurately. Perhaps worker ants depositing refuse in the detritus piles might accidentally pick up the juveniles on their mouthparts, thus allowing them to pass up the pharynx to the pharyngeal glands. However, a more likely explanation can be found by considering the behavior of the juvenile in the detritus. The mobile juveniles were constantly observed ascending to high points in the detritus, such as fungus mycelium. At this point they elevate the forward part of their bodies and wave them back and forth as though searching. In this position, they readily attach to any object they touch, such as a needle or presumably the leg or antenna of a living ant. Welch (1965) reported that Dougherty (1960) observed

similar activity in *Pelodera coarctata* (Leuckart), as did Nickle and Ayre (1966) for *Caenorhabditis dolichura* (Schneider). In the latter case, nematode phoresis was commonly observed. Once on the body of the ant, the juvenile could be picked up by the mouthparts during cleaning and then be passed to the pharynx and the pharyngeal glands. The pharyngeal gland was the only location in the body of the ant in which the nematodes were found.

The length of time the juvenile nematodes spend in the ant and the exact method by which the nematode leaves the host and returns to the detritus to complete its development is unknown. Most likely the juvenile nematode returns to the detritus by descending from the pharyngeal gland to the buccal chamber and being passed out with the infrabuccal pellet. Another method would be for the nematode to escape directly from the head of the infected ant after it has died and been deposited in the detritus piles.

Usually the nematodes were found only in workers, but in very heavily infested colonies mature queens, winged queens, and males were found to be infected. Nematodes were not found in any stage of the larvae or in the pupae.

DISCUSSION

In considering the association, in light of the information available, it appears that the nematode, even while widely distributed in the study area, is rather rare and probably of insignificant importance to the wild ant populations. However, the artificial nests used in the laboratory to rear the Argentine ant appear to offer near optimum conditions for its development in the detritus and for infecting the workers of the nest. Also, the nematode appears to be fairly widely distributed among various species of European ants (Wahab 1962), and most laboratory studies are made using nests that would allow the accumulation of detritus and therefore the development of the nematodes.

The question therefore arises as to what effect this nematode has on ants in the laboratory. This is a very important question since it is normally thought that ants in laboratory colonies are reasonably similar, behaviorally and biologically, to those in the field. Janet (1893), the original discoverer of this phenomenon, interpreted the association as a type of phoresy in which the nematode is not a true parasite living at the expense of its host but uses the ant as a means of dispersal. Janet, however, did believe the juvenile nematode grows while in the gland by feeding on the glandular secretions. Wahab (1962) further suggested that the nematodes might feed on and damage the glands themselves.

The authors have found indications of definite damage to the pharyngeal glands in both dissections and histological sections of infected ants. In the healthy glands the individual lobes are short with thick walls containing typical secretory cells (Fig. 3a). In the heavily infected ants the glands have lost all appearance of a secretory organ and are probably no longer functional. The lobes are often reduced to little more

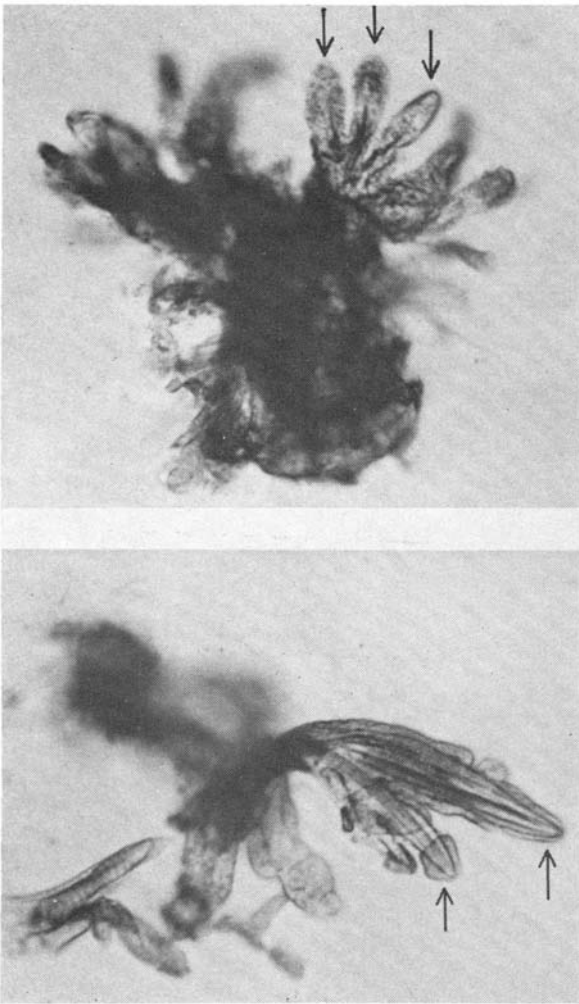


FIG. 3a (above).—Pharynx and pharyngeal glands of a healthy Argentine ant. Arrows indicate several healthy lobes of the gland. The dark, thick walls and short length of the lobes is typical of the uninfested glands.

FIG. 3b (below).—Two lobes from infested glands containing juvenile nematodes.

than a thin and very fragile shell by either feeding or irritation of the 1-4 juvenile nematodes they contain (Fig. 3b). Any effect on the ant would probably be related to the destruction of this gland.

If the gland should have a role in extra-oral digestion as suggested for the ant *Camponotus pennsylvanicus* De Geer, its destruction could effect the nutrition of the individual worker ant. However, Ayre (1963), who examined this as well as other possible digestive glands of another ant, *C. herculeanus* (L.), found no indication that the pharyngeal gland contained any enzyme in concentrations enough to play a role in digestion.

Another possible role of this gland first suggested by Bugnion (1930) is as a source of specially secreted foods involved in trophallaxis. Gösswald and Kloft (1958, 1960) by the use of radioactive tracers have shown that queens of the small red forest ant, *Formica*

polyctena Foerster, were not fed regurgitated food from the crop of the foraging workers but were offered special secretions from a gland in the head of the worker. Naarmann (1963) has since definitely identified this gland as the pharyngeal gland. It is suggested that the secretions of this gland may not be limited to feeding only the queen, but they also may be used in feeding the larvae and have some role in caste determination.

Although the exact role of the pharyngeal gland is far from completely understood, the evidence outlined herein indicates that it may not play an important role in the physiology of the individual worker, but instead may be important in the social biology of the nest. Therefore, the destruction of this gland by the nematode *D. lycostoma*, while not affecting the individual worker, could have a profound effect on the colony as a whole.

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Seasonal Development and Emergence of Two Species of Gall-Forming Aphids, *Pemphigus bursarius* and *P. nortoni*, Associated with Poplar Trees in California¹

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ABSTRACT

Several phases of the biology of *Pemphigus bursarius* (L.), on the primary host, *Populus nigra italica*, and *Pemphigus nortoni* Maxson on *Populus fremonti* were studied at Davis, California. Seasonal development within the galls, formed on leaf petioles, was determined by periodic dissections of the galls, and periods of emergence of the aphids, and numbers emerging were determined by caging galls. Fundatrigeniae of *Pemphigus bursarius* began to emerge in early May, approximately 1-2 weeks before *P. nortoni*. Emergence of *P. bursarius* continued until the end of June. A maximum number of 341 alates emerged from a single gall. August 26 was the latest date

of emergence of *P. nortoni* from a gall. It yielded 1091 alates. The numbers of aphids emerging from the galls of both species were considerably less than the numbers of first instars produced. Physical factors may have contributed to this mortality, but biological agents, such as a fungus and several kinds of predators, were most important. Alienicolae of *P. bursarius* colonized several species of secondary hosts in the family Compositae. The alienicolae were offspring of fundatrigeniae collected from the caged poplar galls. Attempts to establish colonies of *P. nortoni* on potential secondary hosts were unsuccessful.

Two of the commonest species of poplars found at Davis, Calif., are *Populus nigra italica*, the Lombardy poplar, an introduced species, and the native *P. fremonti*, the Fremont poplar. Numerous species of aphids use these trees as primary hosts and form galls on either the leaves or leaf petioles. *Pemphigus bursarius* (L.), the lettuce root aphid, is the only species at Davis found on *Populus nigra italica*, but *P. fremonti* is a host common to *Pemphigus nortoni* Maxson; the poplar petiole gall aphid, *P. populitransversus* Riley; and *Thecabius populimonilis* (Riley).

P. bursarius overwinters on the poplar in the egg stage. The egg hatches in the spring and the fundatrix or stem mother starts the formation of the gall (Fig. 1) in which winged fundatrigeniae are produced. During the spring and summer, fundatrigeniae fly to a secondary host and produce wingless alienicolae. These in turn produce sexuparae which migrate to the primary host and produce wingless sexuales. After mating, the female oviposits on the poplar to start the cycle again. The general life history of *P. nortoni* is believed to be the same, but there is the possibility that its overwintering stage is not on the primary host and that egg deposition by this species may not be initiated until late winter or early spring.

The relationships between *P. bursarius* and its primary and secondary hosts in California were reported by Lange et al. (1957). For *P. nortoni* only the primary host is known. Our study deals principally with the development and emergence of these aphids on the primary hosts and with attempts to initiate colonization on potential secondary hosts.

METHODS

Ten galls of *P. bursarius* and 10 of *P. nortoni* were collected at random from their respective host trees every 6-9 days. Galls that appeared abnormal or had stopped developing were not selected. The collection began the first half of May 1960. The development of early instars was in progress at that time so the sampling was started in mid-April 1961. The galls were placed in plastic bags and frozen shortly after they were collected. Later the galls were measured, weighed, dissected, and the numbers of fundatrices and immature and mature fundatrigeniae recorded. The averages of the counts and the mean temperatures at Davis between collection dates are presented in Tables 2 and 5.

Migrating fundatrigeniae were collected from 19-20 galls of each species of poplar. Each gall was placed in a separate cage. The cages (Fig. 2) were made from 1-pint cylindrical, cardboard dairy cartons that were modified to accommodate the leaf stem. Adequate ventilation was provided with windows of nylon netting. Alates that emerged from the galls were removed with an aspirator every 6-9 days. The average number of alates per gall per period and the mean maximum temperatures for the corresponding periods are plotted in Fig. 3 and 4.

The aphids collected from the caged galls were used in attempts to colonize potential secondary hosts. These fundatrigeniae were placed on the roots of the selected plants according to the methods reported by Grigarick and Lange (1962).

Pemphigus bursarius

Development within Galls and Emergence.—Dissec-

¹ Accepted for publication May 17, 1967.