A study of the Parasitic Habit of *Paratylenchus projectus* and *P. dianthus*

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*Paratylenchus* spp. are primarily ectoparasites of roots. Although some early workers found individuals of this genus in the cortex (Bally and Reynolds, 1931) and in root lesions (Steiner, 1924), Goodey (1934) determined that they occurred almost entirely on the surface among the root hairs. He found one nematode still attached by its stylet to a processed root.

Linford, *et al.* (1949) published a comprehensive paper on the biology of *P. minutus*, giving particular emphasis to its feeding habits on roots growing in soil.

Observations presented in this paper were made on *Paratylenchus projectus* Jenkins, 1956, and *P. dianthus* Jenkins and Taylor, 1956, parasitizing seedling roots growing in agar.

**Materials and Methods**

The feeding habit of *Paratylenchus projectus* was studied in detail in these investigations. That of *P. dianthus* was studied less thoroughly; therefore, reports given here concern *P. projectus* except where *P. dianthus* is mentioned specifically.

In preparation for observation of the feeding process in agar, seeds of red clover (Kenland variety), Ladino clover, and *Nicotiana alata* var. *grandiflora* Link and Otto (Jasmine Tobacco) were disinfested by wetting briefly with 95% ethyl alcohol and then placing them in an 0.35% sodium hypochlorite solution for 5 minutes. At the end of this time the solution was poured off and the seed rinsed in sterile water. The seeds were then plunged individually, with sterilized forceps, into 1½% water agar that had just solidified. Red and Ladino clover seedlings were grown in Petri plates containing approximately 25 ml of agar. The tobacco was grown both in Petri plates and in small cells made by cementing 2½ cm diameter glass rings with Canada balsam to 46 x 60 mm No. 2 cover glasses. The assembled cells were placed in Petri plates, each of which contained a piece of No. 2 filter paper, and sterilized in an oven. The heat dried the balsam, making a firm bond between the 2 parts. Approximately 2 ml of melted agar was placed in each of these cells before planting. The filter paper in the bottom of the Petri plates was kept moist with sterile water and served to retard drying of the agar.

Red and Ladino clover seed produced a primary root about ½ inch long in 3 days, at which time the nematodes were introduced into the dishes. Tobacco was somewhat slower in germination and growth, therefore, nematodes were not introduced until after 2-3 weeks.

Roots of the seedlings grew along the bottom of the containers, thus making them suitable for inversion onto the stage of the microscope for observation of nematode feeding. All seedlings were grown under fluorescent lights controlled by a time switch set for 12 hours illumination each day.

Infestation with nematodes was accomplished by two methods. Nematodes were obtained by leaching stock culture pots and were further cleaned by passage through an extraction pad. For short term observation, large numbers of nematodes were picked up with a small pipette and placed in a

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droplet of water on the agar a short distance from the growing root. As the water evaporated or was absorbed by the agar, the nematodes entered the agar and apparently were attracted to the growing roots. Plates infested in this manner usually became so contaminated with bacteria that they were of little value after about one week. However, in a few cases microphagous nematodes of the genus *Cephalobus* were accidentally introduced with the paratylenchs and proves quite beneficial by feeding on the bacteria and multiplying. Several of the small cells were then intentionally infested with a few of these cephalobids along with the paratylenchs, and it was in these cells that most of the satisfactory observations on feeding were made. A few such cells remained for study for 6-8 weeks.

For longer periods of observation, 1 to 10 freshly molted females with the old cuticle still intact, were passed through 3 successive changes of sterile water, allowing 2-3 minutes in each change. The nematodes were then transferred to a drop of sterile water on the agar using a nylon needle. This needle was first sterilized by placing it for 5 minutes in an 0.35% sodium hypochlorite solution, and was dipped into it briefly between transfers. Most cultures handled in this way remained free of bacterial growth for a long period. There were a few fungus contaminants but they grew sparsely and had no apparent influence on the results. One particularly good culture, started from a single female nematode on red clover in a Petri plate, was kept for 80 days by occasionally adding sterile water to the surface of the agar.

A 40X water-immersion objective with a free working distance of 1.9 mm was used to observe nematode feeding in the Petri plates. The coverslip used on small cells made possible the use of t 90X oil immersion objective.

**Observations**

*Paratylenchus projectus* and *P. dianthus* were both attracted to the young mature zone of roots growing in agar where they fed ectoparasitically much

![Fig. 1. Female *Paratylenchus projectus* feeding on epidermal cell of tobacco root growing in agar. Note the deep insertion of the stylet with the dome of granular material around the tip. One egg has been laid.](image-url)
as Linford, et al. (1949) reported for *P. minutus*. Females characteristically fed by inserting the stylet into epidermal cells (Fig. 1 and 2) or at the base of root hairs, but they were never observed feeding far out on the latter. Young larvae fed both on epidermal cells and root hairs, and frequently at some distance from the base of the latter (Fig. 3 and 4). The preadult larval stage of both species and the males of *P. dianthus* moved about the roots but made no attempt to feed.

The process of puncturing cell walls of tobacco and red clover by larvae and females was observed several times. It is a slow process requiring from 5 to 40 minutes. The nematode places its head against the cell wall and sets up a characteristic series of 4-16 relatively slow stylet thrusts. At the end of each series of thrusts, a rest period of 3-5 seconds ensues, then another series begins. As penetration of the cell progresses and the stylet becomes more deeply inserted, the series of thrusts becomes much longer with longer intervening rest periods. Counts of 53 to over 200 thrusts in a series, be-
tween rest periods of 16-28 seconds, were recorded near the end of the penetration process for one particular individual.

After the wall had been penetrated and the stylet tip thrust well into the host protoplast, there was always a period of inactivity of some duration (55 minutes to 1 hour and 47 minutes) before pulsation of the median bulb began.

Nematodes feeding on the exceedingly thin and translucent roots of tobacco growing against the cover slip in the small cells could be seen to good advantage and with sharp resolution with a 90X oil-immersion objective lens. The nematode esophagus and the host cell protoplast were observed closely both before and during the process of feeding. In favorably situated individuals, saliva could be distinguished by its finely granular appearance throughout the entire length of the dorsal duct from the basal bulb to the duct opening into the lumen of the esophagus.

After the stylet tip was inserted into a cell and before pulsation of the median bulb began, saliva was seen to flow forward from the dorsal side of the basal bulb, through a slender passage along the dorsal side of the isthmus of the esophagus, through the broader duct that arches dorsally around the valve plates, and into the ampulla adjacent to the opening into the lumen of the esophagus behind the spear base. Commonly, the duct was inconspicuous when the stylet was first inserted, and it characteristically became more readily visible and distended as saliva accumulated, especially within the limits of the median bulb and anterior from that. Only after the duct became filled did pulsation of the median bulb begin. At no time was saliva seen to pass through the stylet or into the host cell, yet just before pulsation of the bulb began, a dome of granular matter was seen to form over the stylet tip. Soon after this pulsation began, the duct became narrower and less opaque, indicating that saliva had been moving out of it.

Fig. 3. *Paratylenchus projectus* larva feeding on a root hair of red clover growing in agar. Granular contents of protoplast are visible on either side of the stylet.

Fig. 4. *P. projectus* larva feeding on opposite side of same root hair as in Fig. 3 one day later.
Activity of the median bulb started gradually and was not limited to the characteristic pulsation that soon followed. The valve plates occasionally were seen to be pulled apart and then closed slowly. Also, sometimes there were muscular contractions that altered the shape of the bulb without opening the valve. Soon after the first activity of the bulb began, a rhythmic pulsation became established with the valve opening wide and closing with each beat. This was readily observable even in the smallest larvae. The rate of pulsation varied between individuals from about 100 to 180 per minute, but seemed relatively constant in one individual until the nematode was nearly ready to stop feeding. It then slowed gradually and stopped, after which the stylet was retracted.

Periods of continuous pulsation were extremely long for females on tobacco, lasting from one hour to 3-4 days. On red clover the period was even longer, often more than a week. One female was observed at 20 minute intervals during 7 hours, and many others were frequently observed after feeding started, without ever seeing an interruption of the pulsation until the nematode was ready to retract its stylet. However, some brief periods of interruption may have been missed. The feeding larva shown in Fig. 3 and 4, for example, was found feeding from the same root hair on two successive days, yet between observations it obviously had withdrawn its stylet and moved to the opposite side of the root hair to resume feeding. These observations are contrary to those of Linford, et al. (1949) for P. minutus, for they reported frequent interruptions and resumptions of pulsation during each prolonged period of feeding.

Rhythmic pulsation of the median bulb characteristically was of such vigor that it moved other parts of the esophagus in rhythm with it, and even caused pulsation of the granular matter over the stylet tip inside the host cell. Saliva throughout the entire length of the duct was seen to oscillate forward and backward. This was true especially in the narrow duct within the isthmus, where the oscillation was of wide amplitude making it impossible to estimate whether there was more movement forward than backward. At times there appeared to be some movement in the basal bulb independent of that imparted to it by the action of the median bulb, but this could not be certainly determined.

The epidermal cells of tobacco and root hairs of red clover were very favorable for observation of the host cell contents during feeding, but epidermal cells of red and Ladino clover were too opaque. As soon as pulsation of the median bulb began, the dome of granular material enclosing the stylet tip beat synchronously. It usually grew somewhat in size and sometimes appeared to occupy nearly half of the cell contents, but seemed always confined to only one cell. In root hairs it commonly occupied a considerable portion of the protoplast. Many of the granules became large oval bodies resembling yeast cells. In addition to the granular material, dark filaments frequently were seen in the cytoplasm and often led into the dome toward the stylet tip.

Cytoplasmic streaming and migration of the nucleus within the cell continued in apparently normal fashion during feeding, with the granular dome and stylet tip serving only as a minor barrier. After the stylet was retracted, the granules remained in somewhat the original position in tobacco epidermal cells. Even after 2 weeks these were still observable with cytoplasm continuing to stream. However, the protoplasts of red clover root hairs contracted and disappeared after being fed upon several days by larvae, leaving them apparently devoid of protoplasm.
Paratylenchus projectus and P. dianthus were relatively sedentary after feeding began. The location of certain females was marked and observed frequently while feeding on red clover roots. They consistently fed for several days at one site. When females were first put into agar containing a young clover seedling, they characteristically moved to the young mature region where they began feeding in 2-3 days. Several days later, after the growing point of the root had advanced some distance, they retracted the stylet and moved up to the young mature region where they began feeding again. A single female placed in a Petri plate fed at four different sites in this fashion during a period of 28 days and laid a cluster of eggs at each feeding site.

Only 2 females were discovered feeding as endoparasites in agar cultures. Both were located within epidermal cells of a secondary root of tobacco at its junction with the primary root and were feeding with their stylets inserted into adjacent epidermal cells. When roots from soil cultures were stained, cleared, and examined, however, both larvae and adults were found within epidermal cells or located either within or between cells of the cortex. These always were few in proportion to those that were present in the rhizosphere and appeared to have entered through wounds caused by other agencies. One of the most frequent portals of entry was through wounds made by emerging lateral roots. Senescent roots with cracked and deteriorating surfaces contained more nematodes than younger roots.

**SUMMARY**

A study was made of the parasitic relationships of Paratylenchus projectus, and P. dianthus. Feeding, observed on roots of seedlings growing in agar, was found to be chiefly ectoparasitic on epidermal cells and root hairs in the young mature region. Stylet insertion was a slow process requiring several minutes, followed by a period of relative inactivity in which saliva flowed forward in and filled the salivary duct and ampulla. No flow of saliva from the stylet was observed, but the salivary reservoir became less opaque and a granular dome built up around the stylet tip inside the host cell during feeding. The nature of this granular mass was not determined. Apparently it caused little disturbance to the host cell protoplast, as streaming continued in seemingly normal fashion during feeding and in epidermal cells of tobacco for many days after feeding had stopped and the stylet had been retracted. The protoplasts of red clover root hairs contracted and disappeared after prolonged feeding. No other evidence of local pathology was observed.

These paratylenchus were relatively sedentary once they began to feed in a suitable cell; certain larvae and females were observed to feed from a few days to over a week from one cell.

**LITERATURE CITED**


