

## *Paratylenchus minutus*, n. sp., a Nematode Parasitic on Roots<sup>1</sup>

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MEMBERS OF THE GENUS *Paratylenchus* Micoletzky (1921: 605-607) (Criconematinae, Anguilluliniidae) have been reported from soil samples taken about the roots of various plants and, less often, from within the root cortex from a number of localities in Europe, the British Isles, North America, and the Netherlands Indies; but nowhere has the abundance of these nematodes been sufficient to facilitate an investigation of their biology. Certain reports, including descriptions of two species, have been based upon individual nematodes. The most comprehensive and most recent paper on *Paratylenchus* is that of Goodey (1934), who concluded that the genus is monotypic and established *P. macrophallus* (de Man) Goodey as the type species. Although recognizing that some former workers had found this nematode within the cortex, Goodey determined that it occurred chiefly on the surface among the root hairs. He found one individual still attached by its stylet to the surface of a processed root. Goodey regarded this nematode as a semi-parasite, a term that seems to imply incomplete dependence upon the living root for sustenance.

Over a period of years in this laboratory a small representative of this genus that appears to be a distinct species has been taken in moderate numbers from soil samples from pineapple fields and has been found sparingly in or attached to the roots of pineapple plants and of various weeds (Oliveira, 1940: 368). Later

investigations have disclosed that it frequently is the most abundant nematode found about the roots of pineapple plants in certain old fields on the island of Oahu, Hawaii. Methods employed earlier had resulted in grossly underestimating its abundance: its very small size permits it to pass readily through the finest sieves suitable for separation of nematodes from soil suspensions, whether 200-mesh bolting silk or 325-mesh brass screen. Its predominantly ectoparasitic habit results in there being relatively few individuals within roots prepared for microscopic study, even if plants are grown in very heavily infested soil. Utilizing natural infestations, in which other nematodes of known pathogenic nature also occurred, we have investigated certain phases of the biology of this little parasite, including especially its feeding habits. We have not yet established, however, the pure cultures of this species that would be required to determine its significance as a pathogen.

### *Paratylenchus minutus* Linford, n. sp.

*Measurements*.—♀: 0.24-0.31 mm., mean 0.267 mm.;  $\alpha$ =16-24, mean 19.1;  $\beta$ =3.4-4.1, mean 3.68;  $\gamma$ =12-18; V=80-84; stylet 16-21  $\mu$ . ♂: 0.22-0.27 mm., mean 0.258 mm.;  $\alpha$ =22-27, mean 24.4;  $\beta$ =3.5-4.1, mean 3.82;  $\gamma$ =12-19, mean 14.4; spicula 16-19  $\mu$ ; tail 14-19  $\mu$ . Larvae: As small as 0.130 mm. long and 9  $\mu$  in diameter.

*Diagnosis*.—*Paratylenchus* of small size, with fine cuticular annulation interrupted by narrow lateral fields. Buccal stylet only moderately variable in length, degenerate in male. Esophagus

<sup>1</sup> Published with the approval of the Director as Technical Paper No. 182 of the Pineapple Research Institute. Manuscript received April 29, 1948.

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proportionately long; degenerate in male. Excretory pore opposite anterior end of pyriform terminal bulb in living females; slightly posterior when killed. Anus in male protuberant; in female reduced to a faintly distinguishable pore three-fifths to two-thirds distance from vulva to terminus. No postvulvar uterine sac. Spicula slender, nearly straight through proximal four-fifths of length; almost equal in length to the proportionately short tail. Tail of male convex-conoid to the subacute terminus. Female body tapering behind vulva; tail dorsally conoid, ventrally subarcuate, variably subdigitate, with subacute terminus (Fig. 1).

*Type locality*.—Wahiawa, Oahu, Hawaii.

*Type host*.—*Ananas comosus* (L.) Merr. (pineapple).

*Comparisons*.—In size and proportions our specimens closely resemble *P. besoekianus* Bally and Reydon (1931: 92-94). This led to an earlier identification of our material with that species (Oliveira, 1940: 368). *P. minutus* differs, however, in lack of a postvulvar uterine

sac and in a shorter terminal bulb. Major differences occur in size and form of spicula and gubernaculum, and in the male anus.

*P. minutus* is distinctly smaller than *P. bukowinensis* Micoletzky (1921: 606), *P. nanus* Cobb (1923: 367), and *P. macroballus* (de Man) Goodey (1934: 80). From the latter it differs also in its less variable stylet length. Lateral fields bearing four faint incisures are in agreement with *P. nanus* but not with *P. anceps* Cobb (1923: 370). None have been described for the other species.

Males of *P. minutus*, which usually are as abundant as females in extracts from moist soil about roots, are distinctly smaller than those described by Goodey and tend to be more slender and to have proportionately shorter and less acute tails. Spicula and gubernaculum, although smaller, agree well in form with Goodey's 1934 figure 3. The protuberant anus of *P. minutus* distinguishes it from both *P. besoekianus* and *P. macroballus*, the only species for which males have been described. A

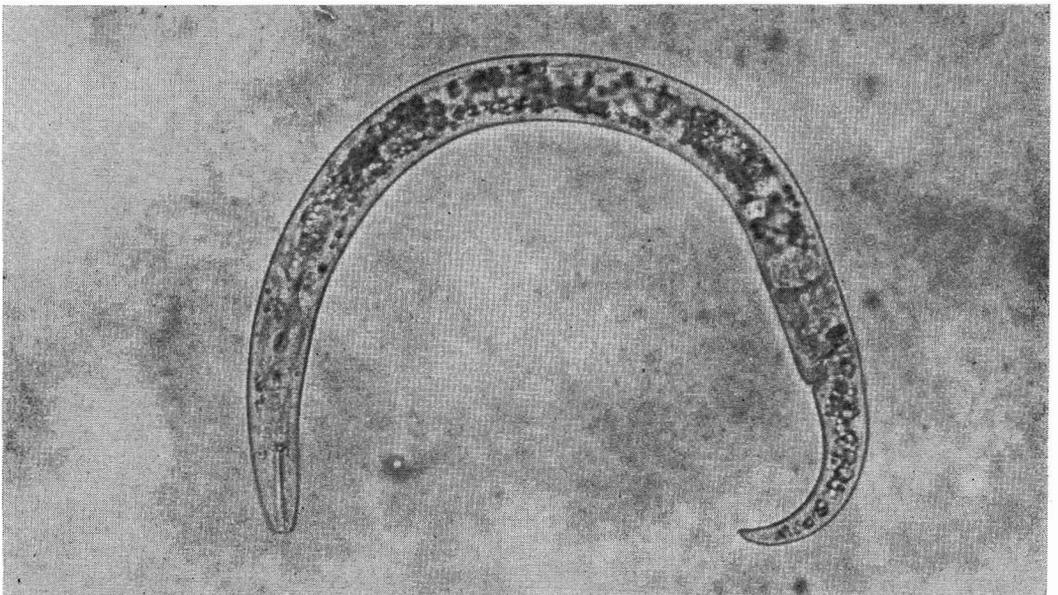


FIG. 1. Female of *Paratylenchus minutus* relaxed in water. Observe the relatively long stylet, the large median bulb, the characteristic distribution of fat globules especially in walls of the intestine, the light-appearing sperm mass in the uterus, and the abrupt narrowing of the body at the vulva.  $\times 570$ .

similar anus has been illustrated for *Criconea squamosum* (Cobb, 1913; Taylor, 1936, pl. 46, fig. 11). In lateral view this appears as two narrow lip-like projections about  $1\mu$  wide that extend somewhat backward about  $1.5\mu$  or one-fifth the width of the body at this position. This character is best seen when the spicula are retracted, although the posterior lip can be distinguished when they are protruded.

#### OCCURRENCE

We have collected this little nematode from three islands of the Hawaiian group: Oahu, Molokai, and Maui. On Oahu it occurs widely and abundantly in pineapple fields in several districts but not in all of the fields where pineapples have long been grown. On Molokai it has been taken from a single pineapple field location among 16 sampled. On Maui it has not been found in a number of pineapple field samples, but it was taken from the roots of

mixed vegetation at elevations of 4,100 and 6,700 feet above sea level. Other habitats generally have not been searched. Limited sampling from mixed vegetation and grasslands adjacent to heavily infested pineapple fields on Oahu, however, has produced few individuals of this species, which suggests that the pineapple field environment is especially favorable to it.

Because of its small size and ectoparasitic habit this paratylench is readily overlooked. We first became aware of its abundance when we found large numbers of these parasites attached to pineapple rootlets that had been fixed, cleared, and stained without washing (Linford, 1942b: 97-100). (Fig. 2.) Because their size permits most of them to be lost through the finest sieves, we adopted the Baermann funnel technique or modifications of it for extraction from roots and soil. In one comparative test, decanting and wet screening recovered 16 paratylenchs per gram of soil, all of them adult females, but the

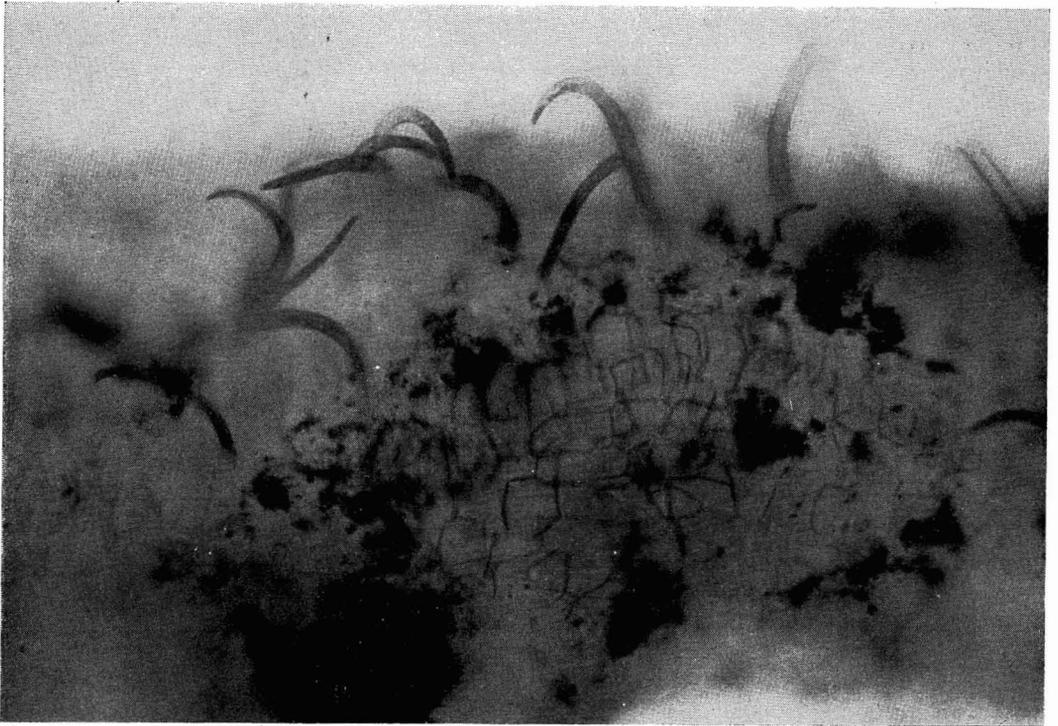


FIG. 2. Numerous *Paratylenchus minutus* projecting from a fixed and stained pineapple rootlet. Most, if not all, of these nematodes were attached only by the buccal stylet. Crescentic posture is typical of this species when relaxed or dead.  $\times 175$ .

Baermann method extracted 209 males, females, and larvae per gram. To retain the soil while allowing small nematodes to crawl through, we have used a fine-textured grade of paper towel that retains its strength when wet.

Soil from under pineapple plants in old fields frequently contains 100 or more paratylenchs per gram. Two samples from separate fields have yielded over 900 per gram. These nematodes are particularly numerous in immediate association with roots. Four composite samples of unwashed roots from each of two fields were held in extraction funnels for 48 hours, then the heaviest nematode population from each field was selected for closer study. The extracted roots were washed free of soil, blotted, weighed, and measured. From one sample, paratylenchs were obtained at rates of 23,800 per gram of root or 570 per centimeter of length. Soil from this source yielded 266 per gram. From the other field, paratylenchs numbered 23,500 per gram of root or 600 per centimeter of root length, and 258 per gram of soil. These and the remaining six samples contained much smaller numbers of other plant parasites in addition to predaceous species of *Aphelenchoides* and *Dorylaimus* (see Linford, 1937a: 42-44, and Christie, 1939).

Within fields, populations sometimes are highly variable, even from plant to plant, but if this nematode is pathogenic to pineapple its effects are overshadowed by other injurious factors, for no apparent correlation exists between populations of paratylenchs and growth vigor of the plants. Very heavy populations are sometimes found around the roots of strong plants.

#### TOLERANCE TO ADVERSE CONDITIONS

Extraction of numerous paratylenchs from dry, loose soil in September in a field that was almost ready for planting after having been plowed several times suggested that this nematode was tolerant to desiccation. Naturally infested soil selected for laboratory tests was of fine texture, with a wilting point in the vicinity

of 27 per cent moisture. When first sampled, at 30.7 per cent moisture, it contained numerous females, males, and larvae. After drying to 20 per cent moisture in 5 days these were only slightly less abundant. At 16.3 per cent moisture, males and larvae were more reduced in numbers than were the females. No living males were recovered at 10.0 per cent, the next drier sample tested. A few larvae were recovered at 9.4 per cent but not at 7.5 per cent. Females revived sparingly after the soil had dried to 7.5 per cent and 7.2 per cent but not to 6.3 per cent moisture. There is no assurance that drying was sufficiently gradual in these tests to measure the maximal degree of tolerance to desiccation. The data are adequate, however, to demonstrate that natural desiccation in the field is not likely to eradicate this nematode, for it occurs in subsoil to a depth of 18 inches or more where drying is much less extreme than near the surface.

Limited information on the sensitivity of *P. minutus* to soil fumigants has been obtained from experiments concerned chiefly with the root-knot and reniform nematodes. Roots of cowpea or tomato grown as indicator plants were washed rapidly from their pots and then held in dishes of shallow water 18 hours or longer before being examined for galls and egg masses. Microscopic examination of the sediment in such dishes frequently detected sufficient paratylenchs for comparative scoring, although the method seriously underestimates populations of this species.

Data collected in this way from laboratory fumigation tests have indicated *P. minutus* to be almost equal to *Heterodera marioni* in sensitivity to various samples of D-D mixture, chloropicrin, and ethylene dibromide. It sometimes has appeared slightly more tolerant. In field tests, following injection of 200 pounds of D-D mixture per acre under mulch paper, this nematode, like other species, has not been recovered from soil samples taken before planting. After 2 years of pineapple growth, however, it has been found even where the soil had been fumigated with 400 pounds per acre or, in one test, with 600

pounds. Survival in subsoil or in non-fumigated spaces between beds, or both, is the probable explanation. Similar reinfestation occurs with other nematodes.

#### PARASITIC HABIT

*Paratylenchus minutus* is chiefly ectoparasitic in the young mature zone of roots. This has been determined by microscopic examination of the undisturbed association of living nematodes with roots, utilizing methods described in detail elsewhere (Linford, 1942*b*). Briefly, roots are grown in infested soil or other fine granular media against a thin coverslip and are then examined under the compound microscope with incident light. A 40 $\times$  water-immersion objective lens permits useful magnifications up to 600 diameters. This method of study has defined the general patterns of the feeding process and of host response. Microscopic examination of stained and cleared roots has yielded information on occurrence within roots.

Paratylenchs are attracted to the piliferous zone where, typically, the larvae and females remain on the surface to feed by inserting the stylet tip into epidermal cells or root hairs (Fig. 3). Males often are observed among them but are unable to feed. The process of puncturing a cell wall has not been observed; but it probably is accomplished rapidly, in contrast with the somewhat prolonged effort required by larvae of *Heterodera marioni* (see Linford, 1942*a*: 582), because few tentative thrusts of the paratylench stylet have been seen. The majority of nematodes found with the stylet tip inserted into a cell have been in process of feeding as shown by rhythmic pulsation of the median esophageal bulb, with its valve opening and closing rapidly.

Periods of sucking alternate with and are preceded by periods of rest during which the median bulb is inactive or only twitches occasionally. Such periods may vary from a few

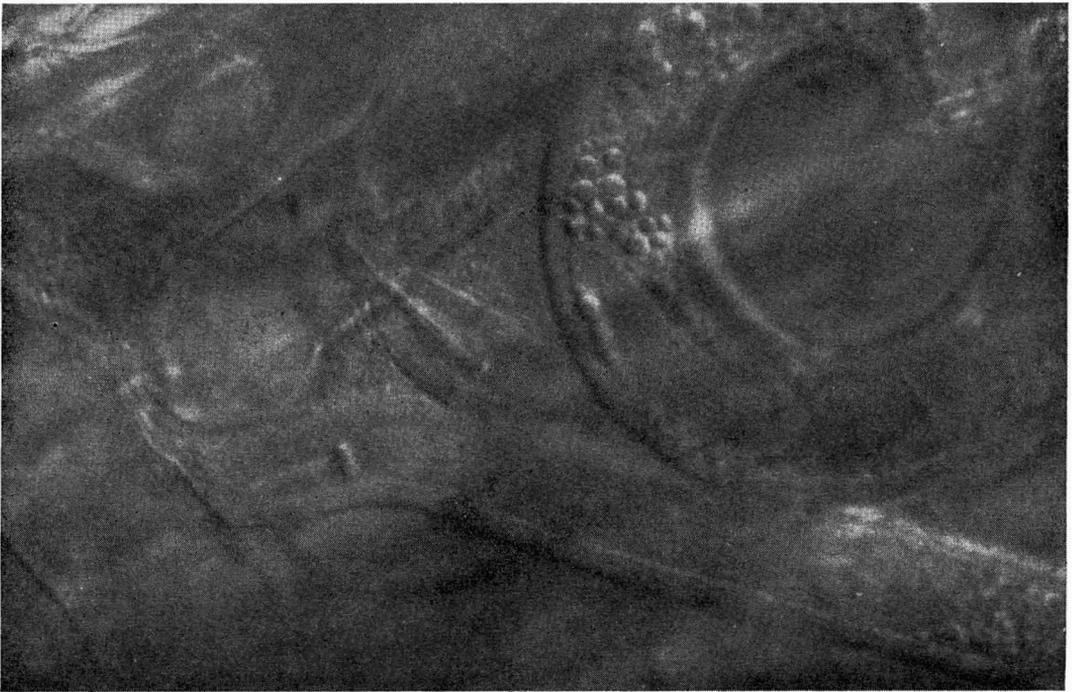


FIG. 3. Two *Paratylenchus minutus* feeding on root hairs of cowpea growing against the coverslip in an observation chamber. Both nematodes have their stylet tips inserted into living root hairs.  $\times 1,500$ .

minutes to over 2 hours. The sucking process also is of long duration, continuing without interruption in some individuals for well over an hour, but it may be interrupted and resumed many times without the nematode's moving to another feeding site. Pulsation of the bulb is vigorous and readily observable even in young larvae, but beyond seeing that the valve plates are pulled well apart and then come back together at each beat it has not been possible to analyze the motion. The rate is variable between individuals, commonly ranging between 80 and 200 beats per minute. Minor irregularities and an occasional beat that may represent a different action of the valve are sometimes seen.

Many observations of host cell protoplasts indicate that no great disturbance results during or soon after feeding. Root hairs are especially favorable for such studies but the active protoplasts of epidermal cells may often be seen when the object is appropriately oriented to the source of light and when the root surface is clean. When the stylet is first inserted, its tip probably extends through the cytoplasmic envelope into the central vacuole. It soon becomes surrounded by a dome of granular material delimited by what appears to be a very thin membrane. During pulsation of the median bulb, this dome enclosing the stylet tip shows synchronous pulsation. Otherwise it is motionless, and no flow from or into the stylet has been detected.

Whether the nematode is sucking or lying at rest, and whether one, two, or even three are feeding simultaneously from a single cell, protoplasmic streaming of apparently normal nature and rate continues without interruption. Sometimes the nucleus moves to a position near a stylet tip; occasionally the dome of cytoplasm over the tip appears to be a focus of streaming strands of cytoplasm; but more commonly the stylet tip and its surrounding mass appear to be only a minor obstruction past which the cytoplasm flows. This has been true of the several host species studied in detail. Streaming continues also after the stylet is retracted and

the nematode moves away. Even after a female had fed from one cell or a small group of cells of a pineapple root long enough to lay 13 eggs, no definite pathology could be distinguished. Very heavily parasitized roots of cabbage seedlings grown in fine sand, fixed, cleared, and stained have shown as many as six larvae and females all with their stylets in a single epidermal cell, without any conspicuous alteration of its protoplast.

This paratylench appears to be relatively sedentary once it has begun to feed in a suitable cell, for certain nematodes in marked locations have been observed during several days to be feeding either in the same cell or in cells lying close together and with the body position changed only moderately. One well-isolated female feeding on pineapple roots was found with a loose cluster of four eggs. Four days later there were 12 eggs. The following day there were 13, and the nematode lay free in the soil with its stylet retracted. Another female apparently laid 11 eggs in two loose clusters very close together, and was still feeding after these had begun to hatch. Between the sixth and seventh days of observation, two larvae began to feed very close to empty egg membranes of one of these clusters.

Observation of the living material gives the impression that this paratylench is strictly ectoparasitic, for the feeding of many individuals during prolonged periods may be observed without detecting any attempt to use the stylet to break down a cell wall or otherwise to force an entry. When roots that have been stained and cleared are examined, however, females, young, eggs, and even a few males may be found within epidermal cells or located either within or between cells of the cortex. These always are few in proportion to the numbers that were present on the root surface, and many if not all of them entered through wounds made by other agencies. Wounds made by emergence of lateral roots allow entry into intercellular spaces deep within the cortex. Sharp sand particles between root and glass in observation

chambers sometimes slit the elongating root, and paratylenchs enter freely. Cracks in the surface of enlarging galls caused by *Heterodera marioni* have been seen to serve in the same way. Entry into epidermal and cortical cells sometimes occurs through penetration wounds made by two species of nematodes consistently present in the soil used in these studies, *Rotylenchulus reniformis* Linford and Oliveira (1940) and a transient rotylech formerly reported from this laboratory as *Rotylenchus multincinctus*. Both species perforate cortical cells as they penetrate somewhat radially to the stele. They sometimes break into an epidermal cell and then move on to another location before penetrating more deeply; and the transient rotylech may penetrate deeply, then withdraw, and move to another site. That some of these wounds are utilized was clear when as many as three paratylenchs were found entering at the side of a single larger form. Many and perhaps most of the paratylenchs found within the epidermis and cortex appear to have entered through one or another of these types of wounds.

#### HOST RANGE

During investigations of feeding habits it became apparent that *Paratylenchus minutus* is able to feed upon roots of a wide range of plants because the feeding process was observed on every plant species that was subjected to adequate observation. No attempt to determine the limits of the host range therefore seemed worthwhile. A few additional species of weeds, crop plants, and ornamentals were grown in miniature root-observation boxes of infested soil and, as before, feeding was observed on all of them that developed sufficient healthy roots in a position suitable for observation. Roots of a few species were stained and cleared and, in some of them, eggs were found associated with females within the cortex. These plants are marked with an asterisk in the list that follows. Plainly this nematode is able to obtain from these plants and also from pineapple plants sufficient nutrient to reproduce. The conditions of

these observations are such, however, that it would be misleading to list plants so examined in which eggs were not found, for the plants were all young, numbers of nematodes within the roots were few, and, judging especially from the pineapple, which was studied in more detail than other hosts, most of the eggs are laid free in the soil or on the root surface.

- Ananas comosus* (L.) Merr.: Pineapple
- Avena sativa* L.: Oat, Vicland
- Bidens pilosa* L.: Spanish needle
- Brassica oleracea* L. var. *capitata* L.: Cabbage, Golden Acre
- Cucumis sativus* L.: Cucumber, Long Green
- Cucurbita Pepo* L.: Squash, White Bush
- \**Emilia sonchifolia* (L.) DC.: Flora's paintbrush
- \**Eschscholzia californica* Cham.: California poppy
- Euphorbia geniculata* Ortega
- Polygonum Fagopyrum* L.: Buckwheat
- Helianthus annuus* L.: Sunflower, Mammoth Russian
- Hibiscus esculentus* L.: Okra, Tall Green
- Medicago sativa* L.: Alfalfa, Chilean
- Oxalis corniculata* L. var. *viscidula* Wieg.: Sticky sorrel
- Portulaca oleracea* L.: Purslane
- \**Phaseolus aureus* Roxb.: Mung bean
- Phaseolus lathyroides* L.: Wild pea-bean
- Raphanus sativus* L.: Radish, Scarlet Globe
- Richardia brasiliensis* (Moq.) Gomez: Richardsonia, false ipecac
- Tagetes erecta* L.: Marigold, Guinea Gold
- Tricholaena repens* (Willd.) Hitchc.: Natal grass
- \**Triticum aestivum* L.: Wheat, Henry
- Vigna sinensis* (L.) Savi: Cowpea, Whippoorwill
- Zea Mays* L.: Corn, maize
- \**Zinnia elegans* Jacq.: Zinnia, Cut-and-Come-Again

#### DISCUSSION

The lack of visible pathology in cells fed upon, and the occurrence of large populations upon roots of apparently normal pineapple plants, tend to indicate that this paratylench is non-pathogenic. Such a conclusion would be premature with present evidence. All observations reported here have been of relatively short duration, and experimental tests of pathogenicity can be made only after pure cultures of this species have been developed, free from other nematodes and root-invading fungi that have been associated with it in these studies.

The pineapple plant is slow to respond to root injuries and, under Hawaiian conditions at least, populations of other nematodes may be markedly reduced by biological agents after they have inflicted root damage but before top growth shows the effect. Lack of correlation between abundance of paratylenchs and plant condition is therefore not proof of harmlessness. It seems improbable that such populations as are encountered around pineapple roots could obtain their entire sustenance from the roots without in some way altering absorptive action or speeding senescence if not even altering the physiology of the entire plant.

Regardless of any minor or delayed pathological effects of its feeding, this paratylench obtains its food without immediately endangering its food supply. It may therefore be considered a well-adjusted parasite. The precise way in which it obtains its food from cells that characteristically are highly vacuolated remains undetermined. By analogy with the predaceous *Aphelenchoides* and the gall-inciting *Heterodera marioni* (Linford, 1937a: 42-44; 1937b; 1942a) the periods of rest of the esophageal bulb, alternating with periods of rapid pulsation, are presumably periods of injection into the host of glandular secretions that selectively prepare nutrients for ingestion. Saliva of predaceous aphelenchs, however, is vigorously digestive, and that of the root-knot nematode stimulates profound changes in the protoplasts of cells into which it is injected in addition to any function it may perform in reducing some of the protoplasm to a state suitable for passage through the narrow lumen of the stylet. In both of those types of nematode the quantity of saliva is relatively copious, and it must distribute freely to perform its functions. In the paratylenchs, however, with small esophageal glands, one would expect the saliva to be limited in quantity or to be secreted only slowly. It is not remarkable, therefore, in view of the minute size of these nematodes and of the limitations placed upon microscopic study by illumination only with incident light, that no flow of saliva

has been detected. Neither could ingestion of food be seen under these conditions even though it obviously occurred during pulsation of the bulb. Unfortunately it was not possible to determine whether the granular accumulation around the tip of the stylet consists only of host cytoplasm or, in part, of secretions from the nematode. That it is not wholly normal cytoplasm was indicated by the temporary persistence of at least part of the mass for a time after the stylet had been withdrawn. If a small quantity of saliva is injected it could probably function to the greatest advantage and with the least disturbance of the host if it remained localized close to the stylet, acting upon cytoplasm clumped about the stylet, rather than distributing throughout the proportionately large host cell. Indicative of extra-oral digestion is the lack of a functional anus and of discernible fecal material in the rectal region. This is in sharp contrast to various other nematodes of somewhat comparable size but different food habits in which discharges of fecal material are readily detected. Even with selective ingestion of almost wholly utilizable nutrients, however, there should be a great excess of water to be excreted. Perhaps this is discharged unnoticed through the excretory pore, carrying with it all soluble waste materials.

There seems no doubt that *Paratylenchus minutus* is an obligate parasite, obtaining all of its nutriment from living cells, yet this fact is not readily determinable by any means other than direct observation of the living nematodes in association with roots. Greater abundance of nematodes near roots than in soil remote from them is not a valid criterion of parasitism, because various microphagous and predaceous nematodes similarly congregate in the rhizosphere where their food is most abundant. Dead rootlets, killed by fungi or other agents, soon are invaded by non-parasitic forms. Similarly, occurrence of moderate numbers of a species within relatively sound roots is not evidence of parasitism since microphagous forms, working over the root surface, may enter

through wounds as does this species. Even infusoria have been found in punctured root cells. There seems, accordingly, to be no substitute for microscopic observation of the living nematodes in association with roots for determination of feeding habits and host ranges of species found superficially associated with roots.

Our studies raise some question concerning the syngonism reported for *Paratylenchus nanus* by Cobb (1923: 369) and Steiner (1924: 1065). Those workers regarded the species as syngonic because, in the absence of males among their specimens, the females consistently contained sperm cells in the uterus. Abundance of males in *P. minutus* makes such an assumption unnecessary. Furthermore, our experience leads us to question whether males may actually have occurred in the populations from which Cobb and Steiner drew their samples. We first recognized the abundance of males after discarding sieve methods of collection and adopting the funnel technique; and the inability of males to feed results in relatively few of them entering a root. Before syngonism is accepted for any paratylench there should be either better evidence that males do not occur or that the female is capable of spermatogenesis.

#### SUMMARY

A very small nematode that occurs in great abundance in certain pineapple fields on the island of Oahu, and less widely on Molokai and Maui, is described as *Paratylenchus minutus* Linford. Over 900 individuals have been extracted from 1 gram of soil and over 23,000 per gram of pineapple root with its adhering soil. This nematode is relatively tolerant of slow desiccation in soil but is essentially equal to *Heterodera marioni* in sensitivity to soil fumigants. It is primarily an ectoparasite of the root hairs and epidermal cells of the young mature zone of roots, but it enters the epidermis and cortex through wounds made by other agencies. To feed, it inserts the tip of its stylet through a wall into a living cell. In this position, prolonged periods of pulsation of the median esophageal bulb for ingestion of food are preceded by and alternate with periods of rest. This feeding, which appears to involve

extra-oral digestion, fails to interrupt normal streaming of the host protoplast. No visible pathology has been associated with prolonged feeding from a single cell. The host range of this paratylench evidently is wide, for limited observation has established 25 known host plants representing 13 families.

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