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# Morphometric Variation in Aphelenchus avenae Bastian, 1865 with Varied Nutrition and Time

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MORPHOMETRIC VARIATION IN APHELENCHUS AVENAE

BASTIAN, 1865 WITH VARIED NUTRITION AND TIME

(TITLE)

BY

John P. Kline

**THESIS**

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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Presented by

John P. Kline

a candidate for the degree of Master of Science and hereby certify that in their opinion it is acceptable.

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# Morphometric Variation in *Aphelenchus avenae* Bastian, 1865 with Varied Nutrition and Time

Abstract: The effect of varied time, nutritional media, and species of fungus on taxonomic characters (L, a, $b_1$, c, and V) of *Aphelenchus avenae* was investigated. The effect of these ecological conditions on morphometrics was variable; however, these characters varied significantly with increased time, decreased nutritional media, and species of fungus. The greatest number of morphometric changes occurred with increased time, and the fewest with species of fungus. With changing environmental conditions, "V" values were the most stable character.

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Variation has been found in the mean de Man values (a, $b_1$, c, and V) and modifications thereof for *Aphelenchus avenae* Bastian, 1865 with altered temperature, kind of fungus utilized for food, and type of nutritional media used for fungal culture (Monoson, 1971; Pillai and Taylor, 1967). Evans and Fisher (1970b) have shown that mean body length of *A. avenae* populations may vary significantly with time as they are maintained on a given fungal culture. These authors suggested that this was a result of a decrease in the quantity of available food, concomitant with increased population size. They also suggested that population size was related to length in that egg production took precedence in nutrient utilization over body tissue building.

This study was an attempt to elucidate the effect of decreased nutrients on body length and de Man values of A. avenae, and to compare these changes with changes due to increased time and utilization of a fungus on which nematodes grew poorly.

## MATERIALS AND METHODS

A population of Aphelenchus avenae growing on Pyrenochaeta terrestris (Hansen) Gorenz, Walker and Larson at 1/4 strength Potato Dextrose Agar (PDA) was obtained from the Plant Pathology Department at the University of Illinois. A single female from this population was surface-sterilized with phenyl mercury acetate (Peacock, 1959) at 0.227 g/liter for 10 seconds, transferred through two successive washes of sterile, distilled water, and transferred to the side of a plate containing 1/4 strength PDA (containing 9.75 g Difco PDA and 15 g Carolina Supply Co. agar per liter of distilled water) on which a small plug of P. terrestris was growing. The cloned population from this female was maintained for 28 days in an incubator at 28° C and in constant darkness, after which samples were taken for innoculation to experimental cultures.

Pyrenochaeta terrestris and Aspergillus nidulans (Eidam) Winters were chosen for experimentation. Aspergillus nidulans (somatic diploid strain) was obtained from the botany department at Eastern Illinois University. Nutritional media used were full and 1/10 strength PDA (Difco) and distilled-water agar (Carolina). All nutritional media were at full agar strength. Fungi were maintained for nine days at identical nutritional and cultural conditions as those to be used in experimentation.

The day before nematode inoculation, fungal inoculation plugs were cut from the side of actively growing cultures with a sterile 8 mm diameter cork borer and transferred face up to the center of 92 mm diameter Petri plates which contained experimental media. Fifteen plates of each nutritional combination were used. Five female nematodes from the cloned population were then transferred to each experimental culture by the sterilizing method previously described.

Five female nematodes were transferred to five replicate plates of every nutritional media, but without fungi, as a check on the effects of starvation. These plates were maintained simultaneously with other experimental cultures. Fifteen plates of every nutritional base were maintained without nematodes, to determine if nematode presence had a measurable effect on fungal colony diameter. All cultures were maintained under conditions described above.

Specimens of Aphelenchus avenae were extracted, counted, and measured from five plates of each nutritional base at the 9th, 18th, and 40th days after inoculation. All fungal colony diameters were measured at the 6th day, as well as at other time periods. Fungal colony diameters were measured to determine their relation to nutrition, nematode length, and as a quantitative measure of nematode presence on fungal cultures. Measurements of fungal colony diameters were obtained by averaging two measurements taken at $90^{o}$ angles to each other. Since fungal growth on water agar nutrition was so sparse, the only practical means of determining fungal colony diameters was by light diffraction. Fungal colony diameters at other nutrient strengths were measured under magnification of a dissecting microscope.

Extraction of nematodes and counting methods were a modification of those described by Monoson (1968). For extraction, only two layers of Kimwipes (disposable wipers manufactured by Kimberly-Clark Corp.) were used to suspend nematodes, fungi, and media in jars of water. This suspension was stirred and flushed at two-hour intervals beginning at 12 hours and ending at 24 hours. Nematodes to be counted were placed in Petri plates marked into 164 equal squares. Dilutions of samples containing nematodes were made at the 40th day after inoculation so that fewer than 2,500 nematodes were counted per dish.

After counting, nematodes were relaxed by heating, killed, fixed, and measured. Heat relaxation was done by placing nematodes in a covered plexiglass dish which contained three drops of distilled water; this dish was then placed in an oven at 70° C for 2 1/2 minutes. Exceptions were nematodes at the 18th and 40th days after inoculation cultured on Pyrenochaeta terrestris at full strength PDA, which did not heat relax until 3 and 4 minutes, respectively. Nematodes were then killed in 5 percent formalin, and 12 hours later, fixed permanently in successively stronger concentrations of FAAGO (Formalin-5ml; 50 percent ETOH-90ml; Acetic acid-2ml; Glycerine-1.5ml; Osmic acid-few drops). Standard taxonomic measurements of nematodes ("L", "a", "b", "c", and "V") were to be used (Hechler, 1962; Goodey and Hooper, 1965; Monoson, 1971); however, poor staining of the upper digestive tract necessitated substitution of the "$b_1$" ratio (Goodey and Hooper, 1965; Pillai and Taylor, 1967) for the "b" ratio.

Data from plates of identical nutrition and times were combined. Differences between means of taxonomic characters and of populations raised with varied time and nutrition were analyzed statistically by

the "t" test. Mean fungal colony diameters were also recorded, but were not analyzed statistically.

## RESULTS

Taxonomic characters, (Tables 1 and 2), total populations (Table 3), and fungal colony diameters (Table 4) varied with altered time and nutrition. Calculations using the "t" test indicated significant differences ($P < 0.05$) between means of taxonomic characters and total populations (Tables 5-12).

Taxonomic characters varied between populations grown under different cultural conditions; however, the "V" value varied least. Of a possible 200 significant differences in length and ratios with altered time, nutritional media, and host fungi, variations occurred in "L" 27 times, in the "a" ratio 20 times, in the "$b_1$" ratio 24 times, in the "c" ratio 15 times, and in the "V" value 6 times (Tables 1, 2, and 5-9).

The effect of altered ecological conditions on length and ratios was not always the same. Taxonomic characters varied most frequently with increased time, less with nutritional media, and least with species of host fungus (Tables 1, 2, 5-9). Of the significant differences which resulted from increased time, decreases occurred in "L" 11 of 12 times, and in the "$b_1$" and "c" ratios 8 of 9 times. The "a" ratio increased in 10 of 11 variations. The "V" value varied significantly twice. With decreased nutritional media, decreases occurred in "L" 11 of 13 times, in the "a" ratio 3 of 5 times, and in the "$b_1$" ratio 11 of 13 times. The "c" and "V" values varied twice.

Table 1. Change in taxonomic characters of *Alphelenchus avenae* populations cultured on *Pyrenochaeta terrestris* using different strengths potato dextros agar and varied time periods.

| Days after Inoculation | (N) | Length (μ) Mean ± SD | a* Mean ± SD | $b_1$* Mean ± SD | c* Mean ± SD | V* Mean ± SD |
|---|---|---|---|---|---|---|
| Full Strength PDA | | | | | | |
| 9 Days | (42) | 755 ± 62 | 24 ± 2.9 | 8.9 ± 0.9 | 30 ± 3.2 | 77.0 ± 6.2 |
| 18 Days | (47) | 655 ± 51 | 27 ± 2.3 | 8.2 ± 0.9 | 28 ± 2.0 | 77.5 ± 6.5 |
| 40 Days | (50) | 665 ± 47 | 28 ± 2.2 | 8.3 ± 0.6 | 29 ± 2.2 | 77.0 ± 2.3 |
| 1/10 Strength PDA | | | | | | |
| 9 Days | (44) | 665 ± 39 | 22 ± 2.6 | 8.1 ± 0.6 | 30 ± 2.7 | 76.5 ± 1.9 |
| 18 Days | (33) | 590 ± 60 | 28 ± 3.2 | 7.3 ± 2.0 | 28 ± 2.6 | 76.0 ± 1.4 |
| 40 Days | (48) | 625 ± 68 | 26 ± 3.2 | 7.5 ± 0.8 | 28 ± 3.1 | 77.0 ± 2.2 |
| Water Agar | | | | | | |
| 9 Days | (33) | 610 ± 45 | 24 ± 1.7 | 7.2 ± 0.5 | 30 ± 1.9 | 76.0 ± 1.2 |
| 18 Days | (26) | 550 ± 31 | 26 ± 3.5 | 7.0 ± 0.7 | 27 ± 2.2 | 77.5 ± 5.0 |
| 40 Days | (40) | 555 ± 26 | 29 ± 3.3 | 7.0 ± 0.3 | 27 ± 1.5 | 76.5 ± 2.9 |

* Characters:
a = length/greatest width
$b_1$ = length/anterior distance to base of median esophageal bulb
c = length/distance from anus to tail
V = anterior distance to vulva/length x 100

Table 2. Change in taxonomic characters of *Aphelenchus avenae* populations cultured on *Aspergillus nidulans* using different strengths potato dextrose agar and varied time periods.

| Days after Inoculation | (N) | Length (μ) Mean ± SD | a* Mean ± SD | $b_1$* Mean ± SD | c* Mean ± SD | V* Mean ± SD |
|---|---|---|---|---|---|---|
| Full Strength PDA | | | | | | |
| 9 Days | (36) | 685 ± 64 | 23 ± 2.5 | 8.9 ± 0.7 | 28 ± 4.0 | 76.0 ± 2.3 |
| 18 Days | (18) | 645 ± 54 | 27 ± 3.1 | 8.2 ± 0.5 | 27 ± 2.3 | 77.0 ± 3.9 |
| 40 Days | ( 1) | 635 | 27 | 7.6 | 27 | 77.5 |
| 1/10 Strength PDA | | | | | | |
| 9 Days | (28) | 665 ± 70 | 23 ± 1.6 | 7.9 ± 0.8 | 28 ± 2.8 | 74.5 ± 2.6 |
| 18 Days | ( 2) | 550 ± 53 | 23 ± 3.0 | 7.0 ± 0 | 26 ± 1.6 | 78.0 ± 1.2 |
| 40 Days | (11) | 520 ± 25 | 24 ± 2.3 | 6.6 ± 0.3 | 24 ± 1.7 | 75.5 ± 7.2 |
| Water Agar | | | | | | |
| 9 Days | (14) | 630 ± 57 | 23 ± 1.8 | 7.2 ± 0.4 | 29 ± 1.9 | 75.5 ± 2.3 |
| 18 Days | (13) | 560 ± 26 | 26 ± 3.0 | 6.6 ± 0.1 | 28 ± 1.4 | 75.5 ± 1.5 |
| 40 Days | (50) | 560 ± 28 | 26 ± 1.8 | 7.0 ± 0.4 | 26 ± 1.8 | 76.5 ± 2.4 |

* Characters:
a = length/greatest width
$b_1$ = length/anterior distance to base of median esophageal bulb
c = length/distance from anus to tail
V = anterior distance to vulva/length x 100

Table 3. Varied nematode population size per plate on *Pyrenochaeta terrestris* and *Aspergillus nidulans* at different strengths Potato Dextrose Agar at varied time periods.

| Days after Inoculation | Full strength PDA Mean† ± SD | 1/10 strength PDA* Mean† ± SD | Water Agar Mean† ± SD |
|---|---|---|---|
| *P. terrestris* | | | |
| 0 | 5 ± 0 | 5 ± 0 | 5 ± 0 |
| 9 | 119 ± 83 | 305 ± 163 | 78 ± 44 |
| 18 | 1,412 ± 868 | 1,079 ± 281 | 386 ± 102 |
| 40 | 14,661 ± 10,139 | 2,930 ± 885 | 241 ± 84 |
| *A. nidulans* | | | |
| 0 | 5 ± 0 | 5 ± 0 | 5 ± 0 |
| 9 | 29 ± 7.5 | 124 ± 67 | 36 ± 15 |
| 18 | 36 ± 23 | 240 ± 191 | 291 ± 160 |
| 40 | 0.6 ± 0.5 | 268 ± 332 | 518 ± 204 |

* Agar was maintained at full strength

† Mean of five replicate plates

Table 4. Variation in colony diameter (mm) of *Pyrenochaeta terrestris* and *Aspergillus nidulans* at different strengths Potato Dextrose Agar at varied time periods.

| Days after Inoculation | Full strength PDA | 1/10 strength PDA* | Water Agar* |
|---|---|---|---|
| | Mean ± SD (N) | Mean ± SD (N) | Mean ± SD (N) |
| *P. terrestris* | | | |
| 0 | 8 ± 0 (30) | 8 ± 0 (30) | 8 ± 0 (30) |
| 6 | 34 ± 6.2 (30) | 34 ± 4.0 (30) | 47 ± 2.1 (30) |
| 9 | 44 ± 8.2 (30) | 43 ± 5.0 (19) | 68 ± 2.9 (30) |
| 18 | 58 ± 13 (24) | 60 ± 7.0 (22) | 92† ± 0 (25) |
| 40 | 68 ± 10 (17) | 67 ± 8.2 (13) | 92† ± 0 (20) |
| *A. nidulans* | | | |
| 0 | 8 ± 0 (30) | 8 ± 0 (30) | 8 ± 0 (30) |
| 6 | 88 ± 5.1 (30) | 84 ± 3.7 (30) | 60 ± 10 (30) |
| 9 | 92† ± 5.2 (30) | 82 ± 6.9 (27) | 85 ± 5.4 (30) |
| 18 | 92† ± 0 (25) | 92† ± 0 (25) | 92† ± 0 (23) |
| 40 | 92† ± 0 (20) | 92† ± 0 (20) | 92† ± 0 (18) |

* Agar was maintained at full strength

† 92 mm was diameter of plate

Table 5. Statistical t values of compared means of characters of *Aphelenchus avenae* at different sampling days on *Pyrenochaeta terrestris*.

| Characters† | 9 + 40 days | 9 + 18 days | 18 + 40 days |
|---|---|---|---|
| Full strength PDA | | | |
| Length (μ) | 7.2** | 8.2** | 1.0 |
| Ratio a | 7.3** | 5.4** | 2.2 |
| Ratio $b_1$ | 3.7** | 3.7** | 0.6 |
| Ratio c | 1.7 | 3.5** | 2.3* |
| Ratio V | 0.0 | 1.1 | 1.5 |
| 1/10 strength PDA | | | |
| Length (μ) | 3.5** | 6.3** | 2.4* |
| Ratio a | 6.6** | 8.8** | 3.3* |
| Ratio $b_1$ | 4.0** | 2.2* | 0.5 |
| Ratio c | 3.3** | 3.3** | 0.0 |
| Ratio V | 1.3 | 1.3 | 2.4* |
| Water agar | | | |
| Length (μ) | 6.2** | 6.1** | 0.7 |
| Ratio a | 8.3** | 2.7** | 3.5* |
| Ratio $b_1$ | 2.0 | 1.2 | 0.0 |
| Ratio c | 7.4** | 5.5** | 0.0 |
| Ratio V | 0.9 | 1.5 | 0.9 |

† Characters: a = length/greatest width
$b_1$ = length/anterior distance to base of median esophageal bulb
c = length/distance from anus to tail
V = anterior distance to vulva/length x 100

* ($P < 0.05$)
** ($P < 0.01$)

Table 6. Statistical t values of compared means of characters of *Aphelenchus avenae* at different sampling days on *Aspergillus nidulans*.

| Characters† | 9 + 40 days | 9 + 18 days | 18 + 40 days |
|---|---|---|---|
| Full strength PDA | | | |
| Length (μ) | | 2.4* | |
| Ratio a | | 4.8** | |
| Ratio $b_1$ | | 1.2 | |
| Ratio c | | 1.2 | |
| Ratio V | | 1.0 | |
| 1/10 strength PDA | | | |
| Length (μ) | 9.5** | 2.9** | 0.8 |
| Ratio a | 1.3 | 0.0 | 0.4 |
| Ratio $b_1$ | 7.4** | 5.9** | 4.4** |
| Ratio c | 5.4** | 1.6 | 1.6 |
| Ratio V | 0.4 | 3.6** | 1.0 |
| Water agar | | | |
| Length (μ) | 4.4** | 4.2** | 0.0 |
| Ratio a | 5.5** | 3.1** | 0.0 |
| Ratio $b_1$ | 1.7 | 5.4** | 6.3** |
| Ratio c | 5.1** | 1.5 | 4.3** |
| Ratio V | 1.4 | 0.0 | 1.8 |

† Characters:
a = length/greatest width
$b_1$ = length/anterior distance to base of median esophageal bulb
c = length/distance from anus to tail
V = anterior distance to vulva/length x 100

* ($P < 0.05$)
** ($P < 0.01$)

Table 7. Statistical t values of compared means of characters of *Aphelenchus avenae* at varied nutritional levels on *Pyrenochaeta terrestris*.

| Characters† | Full PDA + agar | Full + 1/10 PDA | 1/10 PDA + agar |
|---|---|---|---|
| 9 Days | | | |
| Length (μ) | 11.7** | 8.0** | 5.6** |
| Ratio a | 0.0 | 3.4** | 1.8 |
| Ratio $b_1$ | 10.6** | 1.8 | 7.1** |
| Ratio c | 0.0 | 0.0 | 0.0 |
| Ratio V | 1.9 | 0.9 | 1.4 |
| 18 Days | | | |
| Length (μ) | 10.9** | 5.1** | 3.3** |
| Ratio a | 1.3 | 1.6 | 2.3* |
| Ratio $b_1$ | 6.4** | 2.4* | 0.8 |
| Ratio c | 1.9 | 0.0 | 1.6 |
| Ratio V | 1.5 | 0.5 | 1.5 |
| 40 Days | | | |
| Length (μ) | 14.1** | 3.4** | 6.6** |
| Ratio a | 1.7 | 3.6** | 4.3** |
| Ratio $b_1$ | 3.9** | 6.2** | 4.3** |
| Ratio c | 5.1** | 1.9 | 2.0 |
| Ratio V | 0.8 | 0.0 | 0.9 |

† Characters:
- a = length/greatest width
- $b_1$ = length/anterior distance to base of median esophageal bulb
- c = length/distance from anus to tail
- V = anterior distance to vulva/length x 100

* ($P < 0.05$)
** ($P < 0.01$)

Table 8. Statistical t values of compared means of characters of *Aphelenchus avenae* at varied nutritional levels on *Aspergillus nidulans*.

| Characters† | Full PDA + agar | Full + 1/10 PDA | 1/10 PDA + agar |
|---|---|---|---|
| 9 Days | | | |
| Length (μ) | 13.0** | 1.2 | 1.7 |
| Ratio a | 0.0 | 0.0 | 0.0 |
| Ratio $b_1$ | 4.8** | 0.5 | 3.9** |
| Ratio c | 1.1 | 0.0 | 1.4 |
| Ratio V | 0.7 | 3.8** | 1.3 |
| 18 Days | | | |
| Length (μ) | 5.8** | 2.4** | 0.3 |
| Ratio a | 0.9 | 1.8 | 1.3 |
| Ratio $b_1$ | 13.3** | 10.2** | 18.0** |
| Ratio c | 1.6 | 0.8 | 1.7 |
| Ratio V | 1.5 | 0.8 | 2.7** |
| 40 Days | | | |
| Length (μ) | | | 3.9** |
| Ratio a | | | 2.7* |
| Ratio $b_1$ | | | 3.6** |
| Ratio c | | | 3.5** |
| Ratio V | | | 0.5 |

†Characters:
a = length/greatest width
$b_1$ = length/anterior distance to base of median esophageal bulb
c = length/distance from anus to tail
V = anterior distance to vulva/length x 100

* ($P < 0.05$)
** ($P < 0.01$)

Table 9. Statistical t values of compared means of characters of *Aphelenchus avenae* on *Aspergillus nidulans* and *Pyrenochaeta terrestris*.

| Characters† | 9th day | 18th day | 40th day |
|---|---|---|---|
| Full strength PDA | | | |
| Length (ω) | 4.9** | 0.7 | |
| Ratio a | 1.6 | 0.0 | |
| Ratio $b_1$ | 0.0 | 0.0 | |
| Ratio c | 2.2* | 1.6 | |
| Ratio V | 1.0 | 0.4 | |
| 1/10 strength PDA | | | |
| Length (ω) | 0.0 | 1.0 | 8.5** |
| Ratio a | 2.0* | 2.3* | 2.4* |
| Ratio $b_1$ | 1.1 | 0.8 | 6.1** |
| Ratio c | 3.0** | 1.6 | 5.9** |
| Ratio V | 3.5** | 2.3* | 0.7 |
| Water agar | | | |
| Length (ω) | 1.2 | 1.1 | 0.9 |
| Ratio a | 1.8 | 0.0 | 5.2** |
| Ratio $b_1$ | 0.0 | 2.9** | 0.0 |
| Ratio c | 1.6 | 1.7 | 2.9** |
| Ratio V | 0.8 | 1.9 | 0.0 |

†Characters: a = length/greatest width
$b_1$ = length/anterior distance to base of median esophageal bulb
c = length/distance from anus to tail
d = anterior distance to vulva/length x 100

* ($P < 0.05$)
** ($P < 0.01$)

Table 10. Statistical t values of compared means of nematode population size per plate at varied times on *Pyrenochaeta terrestris* and *Aspergillus nidulans*.

| Nutrition | 9 + 40 days | 9 + 18 days | 18 + 40 days |
|---|---|---|---|
| *Pyrenochaeta terrestris* | | | |
| Full strength PDA | 3.2 * | 3.3 * | 2.9 * |
| 1/10 strength PDA† | 6.5 ** | 5.3 ** | 4.5 ** |
| Water agar† | 3.9 ** | 6.2 ** | 2.4 * |
| *Aspergillus nidulans* | | | |
| Full strength PDA | 8.4 ** | 0.6 | 3.4 ** |
| 1/10 strength PDA† | 0.95 | 1.3 | 0.2 |
| Water agar† | 5.3 ** | 3.6 ** | 1.96 |

†At full agar strength

* ($P < 0.05$)

** ($P < 0.01$)

Table 11. Statistical t values of compared means of nematode population size per plate at varied nutrition on *Pyrenochaeta terrestris* and *Aspergillus nidulans*.

| Times after Inoculation | Full PDA and agar† | Full and 1/10 PDA† | 1/10 PDA† and agar† |
|---|---|---|---|
| *Pyrenochaeta terrestris* | | | |
| 9 days | 0.98 | 2.27 | 3.01* |
| 18 days | 2.63* | 0.82 | 5.18** |
| 40 days | 3.18* | 2.58* | 6.76** |
| *Aspergillus nidulans* | | | |
| 9 days | 0.93 | 3.15* | 2.87* |
| 18 days | 3.53** | 2.37* | 0.46 |
| 40 days | 5.67** | 1.80 | 1.43 |

†At full agar strength

* ($P < 0.05$)

** ($P < 0.01$)

Table 12. Statistical t values of compared means of nematode population size per plate on *Pyrenochaeta terrestris* and *Aspergillus nidulans*.

| Nutrition | 9 days | 18 days | 40 days |
|---|---|---|---|
| Full strength PDA | 2.41* | 3.54** | 3.23* |
| 1/10 strength PDA† | 2.30 | 5.52** | 6.30** |
| Water agar† | 3.02* | 1.12 | 2.81* |

† All full agar strength

* ($P < 0.05$)

** ($P < 0.01$)

Taxonomic changes did not occur in direct proportion to decreased nutritional media and increased time. Significant differences in taxonomic characters occurred 19 times between the 9th and 18th days. Between the 18th and 40th days, 8 changes occurred (Tables 5 and 6). More taxonomic changes occurred between 1/10 strength PDA and agar than between full and 1/10 strength PDA (Tables 6 and 8).

The ability of host fungi to sustain nematodes and measurable adults varied with time and nutritional quantity. Pyrenochaeta terrestris cultures produced 106,055 nematodes and 363 measurable adults (Tables 1 and 3), whereas Aspergillus nidulans cultures produced 7,723 nematodes and 173 measurable adults (Tables 2 and 3). P. terrestris cultures produced greatest mean populations at the 40th day after inoculation at full nutritional strength. Smaller numbers were produced on cultures when nutritional strength was decreased (Table 3). A. nidulans produced greatest numbers of nematodes at the 40th day after inoculation on water agar nutrition. With increased nutrient strength, this fungal culture produced decreasing numbers of nematodes (Table 3). The smallest number of measurable adults recovered on P. terrestris cultures was 26 (Table 1), whereas only a single measurable adult was recovered at the 40th day after inoculation on A. nidulans at full nutritional strength, and only two measurable adults were recovered at the 18th day at 1/10 strength (Table 2).

Populations of nematodes on P. terrestris on full and 1/10 strength nutrition were still increasing at the 40th day; but on water agar nutrition, peak populations had occurred by the 18th day (Table 3). On A. nidulans, nematode populations were still

increasing at the 40th day at water agar nutrition; on 1/10 strength nutrition there was no significant difference between populations at the 9th, 18th and 40th days; on full nutritional strength populations decreased after the 18th day (Table 3).

An attempt to determine an adult/larva ratio on the basis of length was unsuccessful because nematodes without gonads and vulva were frequently as large as nematodes with gonads and vulva. Furthermore, even though nematodes had a vulva, they did not necessarily have well-developed gonads.

No living nematodes were observed on nutritional media without fungi at the 9th and 18th days; these plates were discarded at the 18th day.

In this otherwise normally parthenogenic species, a single male was discovered at the 18th day on P. terrestris with water agar nutrition.

Despite large populations of nematodes on certain nutritional bases, their presence did not affect fungal colony diameter except twice. At the 9th day after inoculation, P. terrestris cultures on 1/10 strength PDA, and A. nidulans on water agar nutrition were significantly ($P < 0.05$) smaller when nematodes were present, compared to cultures where nematodes were absent. Since mean fungal colony diameter was affected by nematode presence in only two of thirty measured instances, all replicates at identical times and nutritional bases were combined in Table 4.

Fungal colony diameters increased at every time interval (Table 4) up to 92 mm diameter. At every nutritional strength, most rapid fungal growth occurred between inoculation and the 9th day. Fastest lateral growth was exhibited by A. nidulans on full strength PDA;

this fungus reached the edge of the plate by the 9th day after inoculation. Only slight differences occurred in lateral growth between 1/10 strength and water agar strength nutrition. A. nidulans growth at these nutritional bases was slower than at full nutritional strength (Table 4). P. terrestris grew fastest on water agar nutrition, with colonies reaching the end of the plate at the 18th day. Differences between growth at full and 1/10 strength were slight, and growth was much slower than on water agar nutrition (Table 4).

## DISCUSSION

Previous studies of morphometric differences with varied nutrition using Aphelenchus avenae (Monoson, 1971; Pillai and Taylor, 1967) and other nematodes (Pillai and Taylor, 1967; Evans and Fisher, 1970a; Bird and Mai, 1967; Coomans, 1962; Taylor and Jenkins, 1957; Wu, 1960) have noted both the constancy of the "V" value and the variation of the other de Man values. In the present study, length and the "a", "$b_1$", and "c" ratios were highly variable with altered environmental conditions. The "V" value, however, was the most constant de Man value, and therefore might be a valid taxonomic character. This taxonomic character deviated no more than 1.0 from the 76.5 value of Hechler (1962) at any time period on Pyrenochaeta terrestris at full strength PDA. In addition, mean "V" values varied less from each other than any of the other ratios, and showed no pattern of variation with altered ecological variables.

Criticisms of the "V" value and all de Man values are still extant, however. Monoson (1971) stated that although the "V" value was one of the least variable ratios, it was affected most by

temperature. Others have suggested that two dimensional ratios are poor measures of taxonomic character differences, and that variations in total length and tail length may affect ratios in an allometric fashion (Barraclough and Blackith, 1962; Clark, 1962; Geraert, 1968). Although this study did not evaluate all criticisms of de Man values mentioned above, it did consider the relation between varied length and ratios. Except for variations of the "$b_1$" ratio, no evidence suggested allometric variation.

All of the morphometric changes with increased time could not be accounted for by decreased nematode nutrition or decreased length. The "a" ratios usually increased and the "c" ratios usually decreased with increased time, being similar in this respect to changes observed in Ditylenchus myceliophagous by Evans and Fisher (1970a). Whereas Evans and Fisher (1970a) stated that in Ditylenchus myceliophagous many changes in taxonomic characters with increased time were due to nutrition, the results of the present experiment with A. avenae indicate that the "a" and "c" ratios did not change with decreased nutrients in the same way that they changed with increased time. Of the significant differences which occurred with increased time, the "a" ratio increased 10 of 11 times and the "c" ratio decreased 8 of 9 times, while with decreased nutrients, the "a" ratio decreased 3 of 5 times and the "c" ratio varied significantly only twice. Length and the "$b_1$" ratio, however, appeared to be affected similarly by increased time, decreased nutrients, and altered fungi (Tables 1 and 2). Since decreased length usually resulted in decreased "$b_1$" values, it appears that length and the "$b_1$" ratio are allometrically related.

Evans and Fisher (1970a; 1970b) suggested that decrease in mean nematode length, which occurred with increased culture time and increased

temperature in Aphelenchus avenae and Ditylenchus myceliophagous, was probably caused by decreased nutrition concomitant with increased populations. The results of the present experiment support this suggestion. Mean length of populations, which shortened with increased time, also shortened with decreased nutrients in agar media.

Greatest nematode length on every nutritional base occurred at the 9th day after inoculation. Probably two combined nutritional factors accounted for this: one, a food reserve built up on Pyrenochaeta terrestris at 1/4 strength PDA before actual experimentation and two, improved nematode nutrition up to the 9th day on P. terrestris at full strength PDA. In addition up to the 9th day there existed the best nematode number to fungal food ratios, fewest metabolic by-products, favorable humidity, and fewest density-dependent factors (such as growth-affecting hormones). Evidence which suggested a reserve food supply in nematodes were the facts that growing conditions could not have been ideal on poor host fungi, yet all nematode populations increased up to the 9th day, and mean length of nematodes on all nutritional bases was greatest at the 9th day.

Length and nutritional media were also related to population dynamics at the 18th and 40th days, in that media which produced nematodes of greatest mean length on each fungus at a particular time interval also produced largest numbers of measurable adults. At both the 18th and 40th days, where mean lengths were greatest on each fungus, mean population sizes were also greatest, except on Aspergillus nidulans at the 18th day. The results of Evans and Fisher (1970b) using other fungi, however, indicated that where conditions produced large populations and numbers of measurable adults, mean nematode lengths were

shorter than where smaller populations and numbers of measurable adults were produced. The conclusion suggested by the study of Evans and Fisher (1970b) and the present one was that decreased nutrients resulted in decreased nematode length. It may be that under some conditions total populations are the most important nutritional limiting factor, and under other conditions the amount of nutrients in the media are the most important. Random variation in populations at the 9th day after inoculation, regardless of nutrients in experimental media, probably resulted from reserve nutrition in individual nematodes accumulated before the experiment and differential reproductive abilities of nematodes in the inoculum. It could be expected that differential reproductive abilities would be important initially, considering the small inoculum numbers and the small nematode populations at the 9th day.

The decrease in nematode numbers on Pyrenochaeta terrestris with successive decreases in nutrients in the media was expected, but the increase in nematode numbers on Aspergillus nidulans with decreased nutrients was not. Townshend (1964) found that Aphelenchus avenae reproduced poorly on A. nidulans. However, he implied that A. nidulans was a true host fungus. The results of the present experiment did not indicate that A. nidulans at full strength PDA was a valid host fungus. Since populations at the 40th day on A. nidulans with water agar nutrition were greater than populations on P. terrestris with water agar, however, both fungi must have provided adequate nutrients for reproduction. Evidentally a physical or chemical growth property of A. nidulans which changed with increased nutrients was detrimental to nematode growth. The only growth property measured in this study

was that of lateral growth, and no simple relationships were found between lateral growth and population size or nematode length. With water agar nutrition, aerial mycelia and pigmentation were absent, and except for lateral growth up to the 18th day, there were no observable differences between the two fungi. This lack of observable differences between fungi at this nutritional strength suggests that physiological differences between fungi accounted for differences in nematode populations. It is quite possible that although A. nidulans furnished adequate nutrients for nematode growth, with increased nutrients it also produced a chemical substance which inhibited nematode growth. Two significant chemical products of A. nidulans are Kojic acid and penicillin, (Foster, 1949) and these substances might have had a deleterious effect on nematodes. Production of these chemicals has not been ascribed to P. terrestris (Foster, 1949).

Relationships between fungal colony diameters and nematode length, nematode presence or absence, and nutritional media were unclear. Fungal colony diameters may have been related to nematode length, since all nematode lengths were largest at the 9th day, and most rapid growth of all fungi also occurred up to the 9th day. At the 18th and 40th days, however, measurements indicated that length was most closely related to type of host fungus and its nutrition. It was expected that nematode presence would have a measurable effect on fungal colony diameters; this was not the case. Monoson (1968) indicated that fungal colony diameters generally increased with nematode presence, but not always. Sutherland and Fortin (1968) and Barker (1964) found that reduction of fungal growth was more dependent on the number of nematodes in the inoculum than upon the final number of nematodes in the plate.

Three important growth factors affected interpretation of results in the present experiment. First, the variations with time were more significant than any of the physiological alterations, suggesting that analysis of supposedly physiological alterations might not be appropriate. Second, equal numbers of measurable adults could not be recovered from all nutritional bases, and sample sizes, especially on A. nidulans, were smaller than had been desired. Since cloned populations were used, significant differences between means were naturally fewer than between normal populations. Differences between means revealed by the "t" test might have been greater, especially on A. nidulans, had numbers from these samples been larger. The third significant growth feature affecting interpretation of results was the fact that many of the short nematodes appeared to have poorly-developed gonads. Far fewer short nematodes would have been measured if the limiting criterion for an adult female had been the presence of well developed gonadal material and a vulva, instead of simply the presence of a vulva. Description of an adult female implies the ability to produce eggs shortly after the final molt, but does not limit the property of being an adult to nematodes capable of laying eggs (Hechler, 1962). In the present study it is dubious that a large number of the short, vulva-bearing nematodes could have laid eggs within a few hours of measurement as their gonads were so poorly developed.

The great amount of morphometric variability which occurred simply with increased time, concomitant with the variables inherent with fungal-nutritional media combinations, indicates that it would be wise to conduct additional morphometric studies using only chemically-

defined nutrients. These nutrients do not have growth properties, are currently available, and have been used for experimentation (Hansen, Buecher, and Evans, 1970; Hansen, Buecher, and Yarwood, 1972; Myers, 1967). In addition, the relationship between length and development of gonads found in this study suggests a need to study nematode development and accurately define the reproductive status of nematodes to be measured in morphometric studies.

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# LITERATURE REVIEW

A current problem in nematode systematics is that of determining the validity of using body ratios, specifically the de Man a, b, c, and V values (a is length/greatest width; b is length/anterior distance to base of esophagous; c is length/distance from anus to tip of tail; V is ratio of anterior distance to vulva/length x 100 percent). This problem appears to stem from insufficient knowledge of the causes of variation of linear measurements in nematodes. According to Mayr (1969) phenotypic variation is due to variation of ecology and/or genetics. An additional problem which affects evaluation of de Man values is the difficulty of making accurate observations and measurements (Seinhorst, 1962; Franklin and Goodey, 1949; Hasbrouk, 1959; Geraert, 1960).

Studies of Aphelenchus avenae which utilized other taxonomic tools have revealed no important infraspecific variation with the exception of one population (Evans and Fisher, 1970c). These authors found a variation in a bisexual population which is otherwise normally a parthenogenic species. Studies of protein patterns of whole-body homogenates on starch gels (Evans, 1971) have suggested that this bisexual population was significantly different in the nature of patterns from other populations. Since populations, not individuals, were sampled, it is possible that this variability of protein patterns was due to the inclusion of males in the bisexual population.

Studies of populations of Aphelenchus avenae (Hansen, Buecher, and Yarwood, 1972; Fisher, 1969; Barker, 1964; Barker and Darling,

1965; Hansen, Buecher and Evans, 1970), Ditylenchus myceliophagous (Evans and Fisher, 1969), and species of the genus Meloidogyne (Oteifa, 1953; Marks and Sayre, 1964; Davide and Triantaphyllou, 1967a; Davide and Triantaphyllou, 1967b; Bird, 1960) have shown population dynamics (sex ratios, total populations and fecundity) to be importantly affected by environmental conditions.

Studies done in an attempt to determine host ranges, feeding habits, and pathological significance of Aphelenchus avenae (Barker, 1964; Mankau and Mankau, 1962; Steiner, 1936; Christie and Arndt, 1936; Sutherland and Fortin, 1968; Barker and Darling, 1965; Rhoades and Linford, 1959; Klink and Barker, 1968; Townshend, 1964) did not suggest infraspecific variation. Similar studies involving species of the genus Ditylenchus (Faulkner and Darling, 1961; Goodey and Goodey, 1949; Henderson, 1951; Arrold and Blake, 1966) have been done mainly for agricultural purposes. However, certain host range studies have suggested infraspecific host range variation (Eriksson, 1965; Goodey, 1952a; Smart and Darling, 1963; Webster, 1967). A study by Martin (1954) of Meloidogyne incognita indicated infraspecific host range variation also.

Variation of body dimensions has been noted both in free-living nematodes (Stephenson, 1942) and in parasitic forms. Because of this variation, descriptions have attempted to list de Man values with ranges of variation and include pertinent ecological data (Coomans, 1962; Allen, 1952; Thorne, 1945; Goodey and Hooper, 1965; Hechler, 1962a; Hechler, 1962b; Fisher, 1965; Taylor and Jenkins, 1957; Bird and Mai, 1967).

Within the genus Ditylenchus, variation of body dimensions resulting from varied nutrition has been clearly documented (Wu, 1960; Smart and Darling, 1963; Goodey, 1952b; Pillai and Taylor, 1967). Because of this and other variations, certain authors have strongly suggested that the de Man values are not satisfactory, (Wu, 1960; Barraclough and Blackith, 1962; Geraert, 1968; Clark, 1962). However, most of the studies of dimensional variation of the members of the genus Ditylenchus have indicated that the "V" value is the least variable de Man value, and might be employed legitimately in nematode taxonomy (Pillai and Taylor, 1967; Wu, 1960; Evans and Fisher, 1970a).

Despite the size and dimensional variations which have been noted in Aphelenchus avenae both in descriptions, (Goodey and Hooper, 1965; Hechler, 1962b) and in studies which varied single ecological factors (Pillai and Taylor, 1967; Monoson, 1971; Evans and Fisher, 1970b) most of these authors have noted the relative stability of the "V" value.

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