In our model, we believe that anchorage is an important step for in vitro growth of trypanosomes of the subgenus *Herpetosoma* for the following reasons: a) Division only starts after 'dedifferentiation' of adult bloodstream trypomastigotes into attached epimastigotes; b) by their solid attachment the haptomonads maintain the infection in the flasks throughout the subsequent changes of medium; c) dividing haptomonads, while invading progressively the bottom of the culture flask, release in the medium intermediate forms which rapidly transform into trypomastigotes or small 'metatrypomastigotes'; d) haptomonads seem to be able to resist better than 'free' trypanosomes to variations of pH (the zwitterionic buffer indispensable for the development of attachment, can be left out afterwards).

These observations suggest that further studies with this model on the mechanism of attachment might enable us to understand the significance of this particular type of differentiation in the life cycle of Trypanosomatidae.

## Nematicidal Activity of Secondary and Tertiary Alkyl Amides and Amines

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*Summary.* Several $C_{11}$ to $C_{15}$ amides and amines that disrupt growth in certain insects showed high nematicidal activity in direct contact tests. Two amides and 9 amines killed *Panagrellus* at 5–10 ppm. Of these, 1 amide and 3 amines killed *Meloidogyne* larvae at 20 ppm.

Certain insect hormonal chemicals affect growth, development, and molting in nematodes[3], and extracts from the nematode *Haemonchus contortus* exhibit juvenile and molting hormone activity in insects[4]. These results, plus the recently reported isolation from a parasitic nematode of 20-hydroxyecdysone[5], a major insect steroid molting hormone, suggest that the hormonal control of development and molting in insects and nematodes may be quite similar. For this reason, a number of secondary and tertiary straight and branched chain amides and amines that disrupt the hormone regulated processes of development, molting, and metamorphosis, and block sterol metabolism in certain insects[6] were tested against nematodes. We now report high nematicidal activity for a group of $C_{11}$ to $C_{15}$ alkyl amides and amines in tests against 2 species of nematodes.

*Panagrellus redivivus*, a saprophytic nematode and a sensitive indicator of nematicidal activity, was exposed for 48 h in water-quartz sand-candidate toxicant mixtures in the standard direct contact test[7] in a range of concentrations for each compound. The compounds were solubilized in a solvent-surfactant-water medium that is non-toxic to nematodes. Approximately 400 nematodes, in all developmental stages, were exposed in each test. Effects were determined during the day immediately after exposure by microscopic examinations[8]. Normal unstressed *Panagrellus* are in continuous rapid motion, and the esophageal areas are hyaline. Exposure to nematicides results in reduced motility, immotility, and death, and esophageal structures in moribund and dead nematodes show disintegration and darkening. Under these test conditions, the $LD_{95}$ for DD (1:1 mixture of 1,2-dichloropropene and 1,3-dichloropropane and related $C_3$ chlorinated hydrocarbons), a standard commercial nematicide, is 36 ppm, and 40 ppm is lethal.

The results of *Panagrellus* exposures to the amides and amines are presented in Tables I and II and are averages of 4 replications. The most active compounds were the straight chain amides and amines, though fewer of the amides were as active as the amines. Of the amides listed in Table I, the dimethyl amides II and III that have a continuous chain length of 11 and 12 carbons, respectively, were the most active and were lethal at concentrations of 5 to 10 ppm. The other amides were active at 20 to 40 ppm, concentrations which approximate the activity of the nematicide standard used in this study.

Most of the amines listed in Table II were active against *Panagrellus* at 5 to 10 ppm. Of the saturated amines, only compounds XII and XVI required higher concentrations to kill 100% of the test nematodes. The 2 unsaturated amines, N,N-dimethyl- and monoethyl-10-undecenamine, XX and XXI, respectively, were active at 10 to 20 ppm. Certain branched chain amides and amines, such as the N,N-dimethyl-3,7,11-trimethyldodecanamine, were also active against *Panagrellus*.

Table I. Range of concentrations of N-substituted amides required to kill 100% of exposed *Panagrellus redivivus* populations in direct contact tests

| Compound | | Concentration (ppm) |
|---|---|---|
| I | $CH_3(CH_2)_9CON(CH_3)_2$[a] | 20–40 |
| II | $CH_3(CH_2)_{10}CON(CH_3)_2$[a] | 5–10 |
| III | $CH_3(CH_2)_{11}CON(CH_3)_2$[a] | 5–10 |
| IV | $CH_3(CH_2)_{13}CON(CH_3)_2$ | 20–40 |
| V | $CH_3(CH_2)_9CON(CH_3)C_2H_5$ | 20–40 |
| VI | $CH_3(CH_2)_{10}CON(CH_3)C_2H_5$ | 20–40 |
| VII | $CH_3(CH_2)_{11}CON(CH_3)C_2H_5$ | 20–40 |

[a] The corresponding mono-N-ethyl amide derivative was not homogeneously dispersed in our test system.

[1] Nematology Laboratory.
[2] Insect Physiology Laboratory.
[3] R. N. JOHNSON and D. R. VIGLIERCHIO, Expl. Parasit. *27*, 301 (1970). – C. S. SHANTA and E. MEEROVITCH, Can. J. Zool. *48*, 617 (1970). – K. G. DAVEY, Int. J. Parasit. *1*, 61 (1971).
[4] W. P. ROGER, Parasitology *67*, 105 (1973).
[5] D. H. S. HORN, J. S. WILKIE and J. A. THOMSON, Experientia *30*, 1109 (1974).
[6] W. E. ROBBINS, M. J. THOMPSON, J. A. SVOBODA, T. J. SHORTINO, C. F. COHEN, S. R. DUTKY and O. J. DUNCAN, III, Lipids, *10*, 353 (1975).
[7] A. L. TAYLOR, J. FELDMESSER and W. A. FEDER, Plant Dis. Reptr. *41*, 527 (1957).
[8] J. FELDMESSER and W. A. FEDER, Proc. Soil Sci. Soc. Fla. *14*, 154 (1954).

Table II. Range of concentrations of substituted amines required to kill 100% of exposed *Panagrellus redivivus* populations in direct contact tests

| Compound | | Concentration (ppm) |
|---|---|---|
| VIII | $CH_3(CH_2)_{10}N(CH_3)_2$ | 5–10 |
| IX | $CH_3(CH_2)_{11}N(CH_3)_2$ | 5–10 |
| X | $CH_3(CH_2)_{12}N(CH_3)_2$ | 5–10 |
| XI | $CH_3(CH_2)_{14}N(CH_3)_2$ | 5–10 |
| XII | $CH_3(CH_2)_{10}N(CH_3)C_2H_5$ [a] | 40–80 |
| XIII | $CH_3(CH_2)_{11}N(CH_3)C_2H_5$ | 5–10 |
| XIV | $CH_3(CH_2)_{12}N(CH_3)C_2H_5$ | 5–10 |
| XVI | $CH_3(CH_2)_{10}NHC_2H_5$ | 20–40 |
| XVII | $CH_3(CH_2)_{11}NHC_2H_5$ | 5–10 |
| XVIII | $CH_3(CH_2)_{12}NHC_2H_5$ | 5–10 |
| XIX | $CH_3(CH_2)_{14}NHC_2H_5$ | < 5 |
| XX | $CH_2{=}CH(CH_2)_9N(CH_3)_2$ | 10–20 |
| XXI | $CH_2{=}CH(CH_2)_9NHC_2H_5$ | 10–20 |

[a] This amine was not homogeneously dispersed in our test system.

Table III. Effects of inoculating tomato seedlings with *Meloidogyne incognita* exposed to several concentrations of N-substituted amides and amines for 48 h

| Compound | Root-knot index | | | |
|---|---|---|---|---|
| | Concentration (ppm) | | | Unexposed check |
| | 20 | 40 | 100 | |
| II | 2.5 | 2.0 | 2.0 | 3.0 |
| IX | 0.0 | 0.0 | 0.0 | 3.0 |
| XVIII | 0.0 | 0.0 | 0.0 | 3.0 |
| XX | 0.0 | 0.0 | 0.0 | 3.0 |
| XXI | [a] | 0.0 | 0.0 | 3.0 |

Results are expressed numerically as root-knot indexes ranging from: 0, no infection; to 4, 100% of roots infected. [a] < 5% infected.

Our results indicate that a number of secondary and tertiary amines of various chain length are highly active against *Panagrellus* and certain of these compounds and/or related aliphatic amines have also been tested against certain animal parasitic helminths including 2 species of nematodes[9]. Additional studies are needed to determine the optimal chain length required for maximum biological activity as well as the activity of the corresponding primary amines.

The active amide II and the amines IX, XVIII, XX, and XXI were further tested against second stage infective larvae of *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 group, a widespread economically significant root parasite that attacks many cultivated crops. Larvae were directly exposed in the vial test to a range of concentrations of test compounds for 48 h, and then washed free of the candidate toxicants. Visual examinations showed darkened, disintegrated structures in the esophageal areas of many of the exposed larvae. Viability determinations, however, were by bioassay. Exposed larvae were used to inoculate small nematode-free tomato seedlings (*Lycopersicon esculentum* Mill., cv Rutgers), growing in nematode-free soil in small containers. 1000 exposed nematode larvae were placed in 3 or 4 small holes in the soil around the stem of each tomato seedling. The holes were then tamped shut and the plants were watered lightly, and thereafter maintained on a regular greenhouse schedule. Unexposed larvae were used to inoculate check plants.

*Meloidogyne* causes root galls or 'root-knots' in the roots at and adjacent to nematode feeding sites. These galls become macroscopically visible, due to host plant reactions involving the proliferation of abnormally large root cell masses. Infections are evaluated on an arbitrary basis, the 'root-knot index', by assigning values of 0 = no infection, 1.0 = 1–25% of the roots galled, 2.0 = 26–50% galled, 3.0 = 51–75% galled, and 4.0 = 100% root infection.

The inoculated tomato seedlings were examined after 3 weeks to determine the viability of the nematode inocula expressed as root infections. Root-knot infections were indexed visually, and the roots were examined microscopically after differential staining to determine the absence or presence of nematodes. The results of inoculation with the exposed root-knot larvae are presented in Table III and are the averages of 2 replications. The amide II was not effective in preventing root-knot infection at concentrations as high as 100 ppm. However, all the amines in Table III except for compound XXI very effectively controlled root-knot larvae at 20 ppm; even at this concentration the amine XXI prevented > 95% of the roots from being infected.

Based on our experience in evaluations of new chemicals for biological activity, an unexpectedly large proportion of these alkyl amines and amides showed activity as nematicides. Further tests are underway to determine the effects of chronic exposure of nematodes to these chemicals and to determine the stability, nematicidal activity, and phytotoxicity of these compounds when they are used as soil drenches and mixes. The general range of nematicidal activity of similar or related compounds as well as the structure – activity relationships of these chemicals are also being investigated. Whether or not these compounds have biological activities or biochemical effects in nematodes analogous to those observed in certain insects[6] remains to be determined.

[9] R. CAVIER and Y. PITON, Chim. therap. *1*, 11 (1965).

## Trinucleate Pollen in the Genus *Populus*

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*Summary.* It is shown by light microscopy and microspectrophotometry that several *Populus* species produce trinucleate pollen. Such pollen seems more widespread than previously acknowledged.

A number of authors have reported that the pollen of *Populus* is binucleate, apparently basing their conclusions on initial studies of SMITH[1] and NAGARAJ[2].

SMITH[1] observed division of the generative nucleus within the pollen tube of *P. laurifolia*. He clearly states that in *P. deltoides*, *P. acuminata* and *P. adenopoda*, the grains are binucleate; only in *P. acuminata* and *P.*

[1] E. C. SMITH, J. Arnold Arbor. *24*, 275 (1943).
[2] M. NAGARAJ, Bot. Gaz. *114*, 222 (1952).