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).

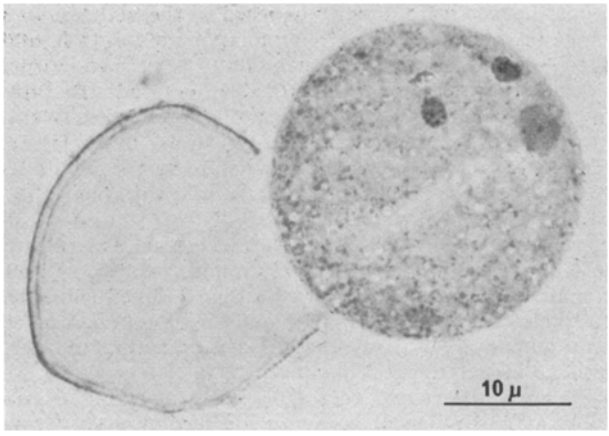


Fig. 1. Pollen grain of *P. nigra* var. *italica* shortly after shedding, fixed in 3% glutaraldehyde in 0.2 *M* cacodylate buffer and stained with lactopropionic-orcein after DYER[6]. The hypotonic fixative solution has caused the rupture of the pollen wall, with the release of the protoplast. The single diffuse vegetative nucleus and the two denser staining, smaller, generative nuclei are apparent.

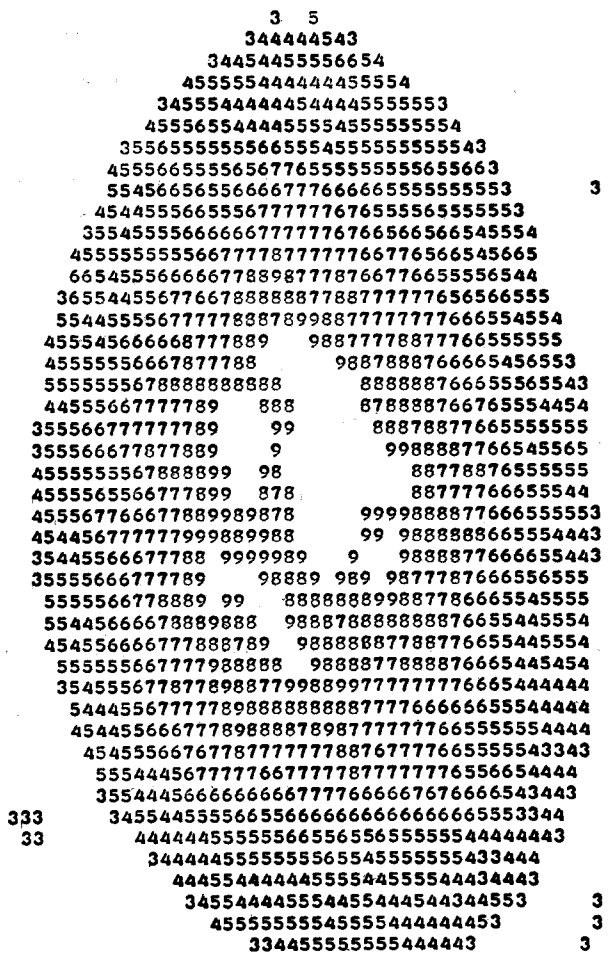


Fig. 2. The distribution of DNA in a single, whole, pollen grain of *P. nigra* var. *italica* as determined by microspectroscopy. The Feulgen staining was after FOX[7] using cold acid hydrolysis. The apparatus used to measure staining intensity was a Zeiss Microscope Photometer, type 05. This was controlled by a Digital PDP-12 Computer under the direction of an APAMOS 2 programme. The figures in the diagram indicate one-tenth of the absorption at 0.580 nm. The clear areas represent staining regions that gave less than 5% transmission. These are areas of DNA concentration; the larger being the vegetative nucleus and the two small areas being the generative nuclei.

*adenopoda* does he report an occasional trinucleate pollen grain. In addition, SMITH[1] quotes CHAMBERLAIN'S[3] observations of *P. monilifera* (in SMITH'S estimation, probably *P. deltoides*); 'the division of the generative cell which presumably takes place, although I was not so fortunate as to observe it, must occur after the pollen tube begins to form'.

NAGARAJ[2] also reported the binucleate nature of pollen of *P. deltoides*. He comments that 'division into separate cells was not observed, but no attempt was made to stain especially for this character. This (the binucleate state) is the condition of pollen at the time of shedding'.

BREWBAKER[4] presents a summary of the distribution of binucleate and trinucleate pollen in almost 2,000 species. He records the genus *Populus* as having binucleate grains. However, BREWBAKER did not examine *Populus* hims elf and he gives no reference for his source of information. Using BREWBAKER'S summary, KIRBY and SMITH[5] again suggest that the pollen of *Populus* is binucleate.

This study reports the prevalence of trinucleate pollen grains in clones of *Populus nigra* L. var. *'italica'*, *P. yunnanensis* Dode, *P. deltoides* Marsh. var. *angulata* Ait. and *P. alba* L. var. *bolleana* Lauche. This conclusion is based upon the light microscopic examination of whole pollen after staining with lactopropionic-orcein and upon microspectrophotometric determinations of regions of DNA concentration within newly shed pollen grains.

In each species, trinucleate grains were commonly observed, although sometimes the division of the generative cell was incomplete. Depending upon the species and the developmental state of the pollen at shedding, 10 to 20% of the grains may be binucleate. Therefore, it would appear that the second mitotic division of the pollen nucleus occurs shortly before, or during, pollen shedding. Both of the accompanying Figures clearly show the presence of one diffuse vegetative nucleus and two, smaller, generative nuclei.

Further support for our findings may derive from BREWBAKER'S[4] observations. He suggested a correlation between pollen cytology (bi- or trinucleate grains), type of incompatibility (gametophytic or sporophytic), and the site of incompatibility inhibitors (surface or stylar) in homomorphic incompatibility.

There are several characteristics of *Populus* pollen behaviour that match those proposed by BREWBAKER for trinucleate, rather than binucleate pollen. For example, the inhibition of incompatible pollen at the stigma surface is a character of the trinucleate class[8,9], as is the difficulty of *Populus* pollen to germinate in artificial media[10], even at short time after shedding. Furthermore, the *Populus* stigma has no copious exudate, a characteristic of plants with a sporophytic incompatibility system. These features, together with the demonstration of trinucleate pollen, indicate a sporophytic type of incompatibility, a conclusion of importance for poplar breeding.

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[6] A. F. DYER, Stain Tech. *38*, 85 (1963).
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[10] D. HAMILTON and M. I. WHITECROSS, in preparation.