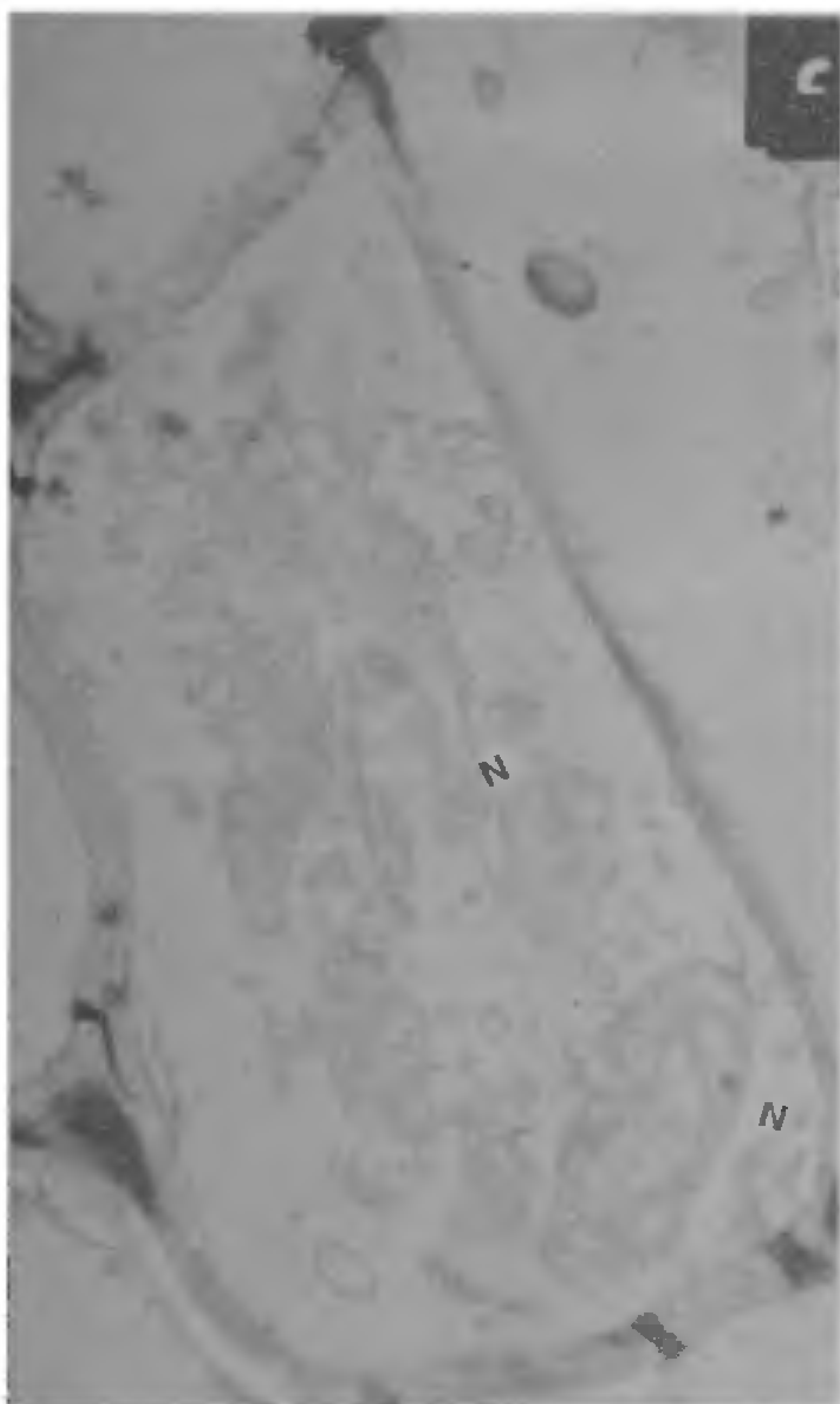

Figure 2. Coiled nematodes in phloem parenchyma × 96000.

treated with Ekalux-25 (Sandoz) active ingredient 0,0-diethyl-0(quinoxalinyl-(2)-thionophosphate) at 0.150% for 60 min before planting.

The treatments were carried out for 12 months in two plots under the same edaphic factors. Hot water treatment gave 10% success; burnt soil treated with fungicides and fumigant gave 2% success. The maximum control was observed with Ekalux-25 soil treatment once in every 3 months, when 80% success was obtained. In the control where no treatments were given all the plants died during the course of 12 months.

Bunchy top of banana is caused by a nematode. Both *Radopholus similis* and *Helicotylenchus multicinctus* were isolated from the diseased soils but the intensity of attack by Radopholus is more. Treatment of the pits with Ekalux before planting and drenching the suckers with the insecticide before planting can control the disease effectively.

21 December 1983; Revised 6 February 1984.

1. Seshadri, A. R., *Agricultural year book*, IARI, New Delhi, 1970, p. 370.
2. Simonds, N. W., *Bananas*, Longmans, England, 1966.

APICAL DISINTEGRATION OF FUNGAL HYPHAE WITH REFERENCE TO THEIR BRANCHING POTENTIAL

T. S. SURYANARAYANAN,
S. MUTHUKUMARASAMY and
E. SIVAMANI
Department of Botany, Vivekananda College, Madras 600 004, India

Bartnicki-Garcia and Lippman[1] showed that hyphal tips of fungi burst when flooded with a variety of dilute solutions of acids, neutral salts or detergents. They suggested that, in the hyphal tip of a fungus, there is a delicate balance between wall-synthetic and wall-lytic enzymes and flooding the hyphae with solutions disturbs this delicate balance leading to violent disintegration of the apices. This finding strengthened the belief that autolytic enzymes play a role in hyphal development.

One of the morphogenetic processes of filamentous fungi that involves localised participation of wall-lytic enzymes is branch initiation[2-4]. It has earlier been reported that some filamentous fungi branch more profusely while growing on a disc of cellophane overlying agar medium[5]. Accordingly, while growing on cellophane, these fungi should posses increased wall lytic activity. To study this, the work of Bartnicki-Garcia and Lippman[1] was repeated for fungi growing on agar medium and on cellophane overlying agar medium.

Single spore isolate of *Aspergillus nidulans* (Eidam) Winter, *Botryodiplodia theobromae* Pat., *Fusarium solani* (Mart) Sacc. and *Syncephalastrum racemosum* Cohn were used. The fungi were grown for 3 days on Czapek's agar medium at 30 ± 1°C. The margin of the colony was cut by a sterilized cork borer and this plug of mycelium was used as inoculum. It was placed mycelium surface down on a semicircular piece of cellophane overlying Czapek's agar medium in a

petridish such that one half of the colony growing from it would be on the agar medium while the other half would be on cellophane overlying agar medium.

The cellophane used was clear, uncoated and was sterilized in an autoclave at 1.05 kg cm^{-2} before being laid on the medium. The number of hyphal apices formed was determined by observing the margin of the growth under the low power field of a compound microscope and by counting the hyphal tips present in one unit width of the margin.

The response of hyphal tips to flooding with various solutions was recorded by flooding the colony with 20 ml of a solution while observing the hyphal apices through a microscope[1].

All the 4 fungi formed a significantly higher number of lateral branches while growing on cellophane (table 1). Cellophane also induced lateral aggregation of hyphae leading to the formation of "strands". It is interesting to note that both aseptate (*S. racemosum*) and septate fungi responded to cellophane in the same way. These results were similar to those obtained by Suryanarayanan and Swamy[5].

The influence of water and a 0.01 M solution of acetic acid, lactic acid, sodium chloride, calcium chloride or EDTA and that of sodium dodecyl sulphate (SDS) 6×10^{-3} M and cetyl trimethyl ammonium bromide (CTAB) 4×10^{-3} M on hyphal bursting was studied (table 2). The concentrations of SDS and CTAB represent their critical micelle concentration. The pH of each solution was adjusted to pH 5.5.

Bartnicki-Garcia and Lippman[1] showed that the bursting of hyphal tips upon flooding with solutions is not an osmotic phenomenon. Our results also support this finding since the solutions varied in their capacity to induce bursting and since water did not induce bursting (table 2). The bursting of hyphae of all the fungi was more rapid and more pronounced in that part of the colony which was growing on cellophane. Thus the lytic propensity of hyphal tips in these fungi can be correlated with the branching potential of their mycelium. The rigid wall of the hyphae has to be made plastic for branch initiation. This is achieved by wall-lytic enzymes[6,7]. It has been shown that cellophane imparts a contact stimulus leading to extensive hyphal

Table 1 *Influence of cellophane on branching of hypae of A. nidulans, B. theobromae, F. solani and S. racemosum.**

Fungus	Number of hyphal tips** (mean) on agar	on cellophane	Critical difference at 5% level
A. nidulans	40.55	113.11	22.85
B. theobromae	87.66	153.66	58.10
F. solani	47.88	94.55	9.81
S. racemosum	42.66	156.66	66.70

*per unit width (1.6 mm) of colony margin after 48 h growth.
**mean of 50 counts from 6 replicates.

Table 2 *Influence of some dilute solutions on hyphal apices of fungi on Czapek's agar and on cellophae overlying Czapek's agar medium**.

Solution	*A. nidulans*		*B. theobromae*		*F. solani*		*S. racemosum*	
	agar	cellophane	agar	cellophane	agar	cellophane	agar	cellophane
Water	e	e	e	c	e	e	e	e
Acetic acid	e	a	c	a	e	a	d	a
Lactic acid	e	e	c	a	e	e	e	e
NaCl	e	e	c	b	e	e	e	e
$CaCl_2$	e	e	b	a	e	e	e	e
EDTA	c	a	c	a	c	a	a	a
SDS	e	a	c	b	e	e	e	e
CTAB	e	e	c	b	e	e	c	c

*incubated for 48 hr at 30 ± 1°C.
a = most of the tips burst; b = over one-half of the tips burst; c = about one-third of the tips burst; d = only a few tips burst; e = none.

branching[5]. Consequently, hyphae growing on cellophane should possess a high degree of lytic activity. Flooding them with solutions disturbs the delicate balance between wall-lytic and wall-synthetic enzymes in the hyphae leading to the violent disintegration of apices. The augmentation of branching and lytic propensity by growing the fungi on cellophane strongly suggests that autolytic enzymes play a role in hyphal branching.

The authors thank Prof. S. Bartnicki-Garcia, Department of Plant Pathology, University of California, Riverside, USA for critically reading the manuscript and for his suggestions.

21 Feburary 1984

1. Bartnicki-Garcia, S. and Lippman, E., *J. Gen. Microbiol.*, 1972, **73**, 487.
2. Mahadevan, P. R. and Mahadkar, U. R., *J. Bacteriol.*, 1970, **101**, 941.
3. Mahadevan, P. R. and Rao, S. R., *Indian J. Exp. Biol.*, 1970, **8**, 293.
4. Thomas, D., Das, J. and Mullins, J. T., *Science*, 1967, **156**, 84.
5. Suryanarayanan, T. S. and Swamy, R. N., *Proc. Indian. Acad. Sci.*, 1981, **B90**, 137.
6. Mehta, N. M. and Mahadevan, P. R., *Indian J. Exp. Biol.*, 1975, **13**, 131.
7. Sukumaran, C. P. and Mahadevan, P. R., *Indian J. Exp. Biol.*, 1975, **13**, 127.

THE USE OF ACETOCARMINE AS A SPECIFIC STAIN FOR NUCLEOLUS

K. GEORGE and C. PRAKASH
Department of Botany, University of Kerala, Kariavattom 695 581, Trivandrum, India.

THE importance of the study of nucleolar structure and function in cytology and cell physiology calls for the development of a proper and easy method of staining this organelle at various stages of cell cycle. The property of the nucleolus reducing silver salts has been originally employed for its specific staining[1-7]. The different fixatives used in these methods modify the affinity of cell structures in a marked way[8,9] and the methods have proved to be unsatisfactory because of artefact produced by metallic inpregnation or poorly differentiated internal cell structures in addition to the fact that these methods are laborious, time and resource consuming[5-7]. Acetocarmine method of staining chromosomes[10] has been modified by Rattenbury[11] for specific staining of nucleoli. But other investigators[12,13] have expressed concern over the inadequacy of acetocarmine to distinguish between nucleoli and chromatin. Therefore, attempts have been made to modify and improve the acetocarmine staining technique to give a satisfactory differentiation between nucleus and nucleolus.

Root tips of *Allium cepa*, *Vicia faba* and *Limnocharis flava* were used in the present study. One set of root tips of these plants were stained with acetocarmine according to the method of Rattenbury[11]. A second set of root tips without any pretreatment in formalin or fixation in Carnoy were directly hydrolysed in 1 N HCl at 60°C for 6 min and washed with distilled water. Hydrolysed root tip is squashed with two drops of 2% acetocarmine on a slide and covered with a coverslip for observation under the microscope. Sufficient staining of the nucleus is ensured by carefully introducing a little more acetocarmine through one edge of the coverslip and drawing it out from the other edge with the help of blotting paper. The slide is then kept at 60°C for 2 min. The edges of the coverslip are sealed with paraffin wax and kept at room temperature (27 ± 5°C) for a minimum period of 48 hr. With complete sealing the prepared slide could be kept for several weeks for observation.

In the first set of squash preparations the nucleoli were not sufficiently differentiating as regards the nucleolar area and the chromatin containing nuclear region. Visibility of the intranuclear and nucleolar structures was obscure probably due to hardening by the formalin used for pretreatment or modification and distortion by the chemicals in the fixative. Destaining of the nuclear chromatin was incomplete and inadequate. On the other hand, in the second set of squash preparations the nucleoli were well stained with optimum intensity at various stages of cell cycle in all the plants alike (figures 1–6). The destaining of the nuclear chromatin was adequate showing distinct differentiation from the nucleolar region. There was even a difference in colour when viewed in transmitted light; nuclear region being light violet and nucleolar region pink. A survey of the interphase cells revealed the existence of mono, di and trinucleolate nuclei (figures 1–3).

In this method the nucleoli as well as nuclear chromatin are simultaneously stained first but on keeping for no less than 48 hr the chromatin gets destained leaving the nucleoli well stained more or less permanently. This indicates that acetocarmine in good