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Cytogenetic Effects of Vitavax Fungicide on Secale cereale on Allium cepa

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CYTOGENETIC EFFECTS OF VITAVAX FUNGICIDE ON *SECALE CEREALE* AND *ALLIUM CEPA*

by

Jack Ernest Staub

A thesis submitted in partial fulfillment
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of

MASTER OF SCIENCE

in

Botany

Approved:

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Major Professor

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Committee Member

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Committee Member

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Jack E. Staub

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ABSTRACT

Cytogenetic Effects of Vitavax Fungicide on

Secale cereale and *Allium cepa*

by

Jack Ernest Staub, Master of Science

Utah State University, 1973

Major Professor: Dr. W. S. Boyle
Department: Botany

The effects of Vitavax fungicide (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin) on meiotic chromosomes of rye (*Secale cereale*) and mitotic chromosomes of onion (*Allium cepa*) and rye were observed.

The principal aberrations recorded in pollen mother cells of rye were: 1-4 univalents at metaphase I; sticky bridges at telophase I; and micronuclei and misshapen nuclei at the quartet stage.

The principal aberrations recorded in root tip mitoses in onion and rye were: chromosome fragmentation, disintegration, clumping, aggregation and inhibition of mitosis.

All meiotic phases were significantly affected by 1,000, 5,000, 10,000, 33,300, 66,600 ppm Vitavax at the 48, 72, and 96 hour application periods. Heavy application rates (10,000 ppm or more, 96 hours) induced disintegration of pollen mother cells. Spikes grown from seeds treated at recommended field rate (33,300 ppm) and double the field rate (66,600 ppm) showed no significant deviation in chromosomal behavior from controls.

Mitotic cells of onion and rye showed aggregation of chromosomes and inhibition of mitosis at 12 hours, 10,000 ppm, while clumping, fragmentation and disintegration occurred in onion at 48 hours, 66,600 ppm.

Paraffin sections of root tip meristems treated for 48 hours with 10,000 ppm Vitavax disclosed irregularly shaped nuclei and mitotic inhibition in onion and marked splitting of tissues and cell collapse in primary meristems of rye.

(64 pages)

INTRODUCTION

New chemical compounds are continually being commercially produced for control of plant diseases. Although very little is known about the chemical effects of many of these compounds on the growing plant, some have been shown to induce cytogenetic abnormalities (19, 58, 66, 30, 42, 15). The production of abnormal plant tissues and mutant germinal cells which may result from application of some of these highly active compounds is a matter of considerable concern. Additives and pesticides may constitute potential dangers to livestock and human populations (4, 18, 48). Recently it has become increasingly important to better understand the impact of chemicals on the total environment. If a chemical can be demonstrated to be harmless, or relatively so, this also is important information since the control of pests and pathogens is frequently necessary. It is hoped that this study has broadened our understanding with respect to some of these parameters.

The importance of systemic fungicides with relation to the control of various plant diseases prompted the present investigation on a relatively new systemic, Vitavax. Vitavax (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin) is available commercially on a limited scale, and it is desirable to obtain all significant information pertinent to its activity.

Vitavax is considered a specific toxicant (10), yet the conversion of these "biostats" to "biocides" with an increase or decrease in dosage can occur (27). Therefore, one must be extremely careful not only in issuing tolerance limits but also in the formulation of application schedules for these types of chemicals.

The present investigation reports the effects of Vitavax on meiotic chromosome behavior in Secale cereale, mitotic chromosome behavior in Allium cepa and Secale cereale, and anatomical effects on root tip meristems in these two species.

REVIEW OF LITERATURE

Vitavax was first synthesized and tested for its chemotherapeutic action in 1966 (62). The trade name, Vitavax, has been coined by its manufacturer, Uniroyal, Inc. (60), but in the literature one finds it under various designations, i.e., DCMO (22), DMOC (3), D 735 (62). The term carboxin was initiated in 1970 (53, 41, 9, 33) to better describe its mode of action on sensitive fungi. Vitavax has two crystal structures with a molecular weight of 235. Its solubility in distilled water is 0.017 g (grams solute/100 grams solvent at 25°C) (60).

Vitavax may be used in conjunction with other fungicides, insecticides and pesticides without relinquishing its chemotherapeutic effect (60, 43, 6, 14, 13). According to the manufacturers, subacute animal toxicity has been obtained in two-year feeding tests and acute oral and dermal toxic values have been set for rats and rabbits. Harvested seed grown from seed-treated material has shown no detectable residues with methods sensitive to 0.05 ppm (60). Nevertheless, recognized phytotoxic effects have been encountered in plants grown from treated seed (44).

Vitavax has been used effectively as a spray, soil treatment, and seed treatment in controlling various pathogens (62). Foliar spray applications on barley leaves have been reported to produce greener leaves and increased chlorophyll content above controls. It has also been shown that spray application initially inhibits both photosynthesis and respiration. A nonspecific inhibition is suggested (7). Increased protein content in winter wheat grain has been observed following foliar spray applications while seed treatments had no similar effect (45).

Selectivity of fungicides has been studied in some detail (56, 24, 57). The differential fungitoxic action of Vitavax has been studied on barley seed (37, 38). Vitavax has shown selectivity in its fungistatic action, being innocuous to a large majority of Ascomycetes, Phycomycetes, and Deuteromycetes but highly toxic to Basidomycetes. Its isomer, F 427 (2,3-dihydro-5-ortho-phenyl-carbonanilido-6-methyl-1,4-oxathiin), exhibits a much broader spectrum of fungal toxicity, yet "prefers" specific taxonomic groupings (10). Vitavax has been found to be effective in controlling the organisms which cause loose smut of barley and wheat, Ustilago nuda and Ustilago tritici (20, 14, 43, 44, 62), barley leaf stripe, Helminthosporium sorokingianum (25), smut of pearl millet, Tolyposporium penicillariae (63), stem canker of potato, Rhizoctonia solani (11), cotton seedling disease, Rhizoctonia solani (5, 6), red leaf disease of blueberry, Exobasidium vaccinii (17), onion smut, Urocystis cepulae (13), blister blight, Exobasidium vexans (61), leaf and stem rust of wheat, Puccinia recondita and Puccinia graminis (21, 12), bean rust, Uromyces phaseoli (52), flag smut, Urocystis agropyri (22). Vitavax is considerably less effective against rust diseases when compared with DCMOD, F 461 (2,3-dihydro-5-carboxanilido-6-methyl-1, 4-oxathiin-4,4 dioxide) (14, 10, 23), and slightly less effective when used as a foliar spray or dust for some smut diseases (63, 11). This difference between the chemotherapeutic action of Vitavax and DCMOD may not be due to structure alone. The effect of microflora, soil moisture and time of application appears to be of some importance (38). The best explanation, however, for this phenomenon may simply be due to Vitavax's relatively short duration of protective activity (46).

Degradation of fungitoxic compounds in the growing plant and by microbial activity have been studied to some extent (29, 65). Although Vitavax has been shown to completely decompose in the soil within 10-20 days (12), Lane (28) reported that in long term studies Vitavax persisted, at a concentration of 0.2 ppm, approximately 8 weeks after initial application. Hydrolysis in water has never been detected. Vitavax in soil and water has been shown to be oxidized to its sulfoxide (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide-4 oxide) derivative. At high pH values the oxidation to the sulfoxide is inhibited. Once produced, the sulfoxide may further oxidize, yielding at pH's of 2 and 4 a sulfone (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide-4,4 dioxide). After 7 days 20 percent of the initial concentration of Vitavax is changed to the sulfoxide. Subsequent oxidation to the sulfone is detected several weeks after initial application (8).

Uptake and binding of fungicides by intact plant tissue has been reported (39, 55). Vitavax shows acropetal movement with accumulation occurring in the tips and margins of leaves when treated via root and foliage (51). Upward movement has been observed in cotton seedlings with accumulations in the lysigenous glands of the hypocotyl (26). Allam and Sinclair (2) have shown that uptake and translocation occur during the first 12 hours of germination in cotton seeds. Effective uptake of Vitavax appears to be directly dependent on seed treatment, and inhibition of at least one fungus, Rhizotonia solani, is dependent on both concentration and length of germination period (1).

Vitavax, especially within the roots, forms complexes with cellular components. Transpiration appears to govern Vitavax transport, and there is little redistribution after initial accumulation and feeding

of the fungicide has occurred by the roots (52). Kirk and Sinclair (26) found that when applied to cotyledons of cotton, Vitavax was not translocated to the roots, but it did show downward movement into the hypocotyl.

In bean, Vitavax is broken down rapidly in roots, initially yielding its nonfungitoxic sulfoxide (52). This sulfoxide may follow two absorption pathways: either by direct absorption from the soil and subsequent oxidation inside the plant, or by initial Vitavax oxidation in the soil and direct absorption. Only the direct absorption of the sulfoxide has been confirmed (9). Vitavax has been shown in cotton seedlings to form the following breakdown products:

1. F 962, a hydrolysis product
2. F 461 (2,3-dihydro-5-carboxanilido-6-methyl-oxathiin-4,4-dioxide)
3. An aniline derivative
4. Three metabolized, unextractable isomers of Vitavax (3).

Bean root tissue analysis has proven that the aniline residue is formed by a hydrolysis of the carboxamide linkage which subsequently forms conjugation products that are highly soluble in water and bind to plant polymers. Moreover, the aniline formed from hydrolysis is absolutely necessary before binding of Vitavax decomposition products can be achieved (53). Since Vitavax is oxidized to the sulfoxide with such rapidity, the analine moiety probably comes more directly from the sulfoxide than Vitavax itself (9).

Uptake of Vitavax by mycelium and spores of sensitive fungi is also very rapid; maximum uptake occurs in 30-90 minutes. Uptake by Ustilago maydis is not affected by various pH's but is directly proportional to both spore and Vitavax concentration. Active metabolism is not required

for Vitavax uptake. Once inside the fungicide becomes closely associated with the ribosomal-soluble fraction (31).

Shively and Mathre (49) report that ionic materials such as potassium ions, organic acids and phosphate containing materials were being released from mycelia of a sensitive fungus following treatment with Vitavax. Findings indicate that this release is not the result of alterations in the plasma membrane.

Carbon-14 studies by Mathre (32) have shown that low concentrations of Vitavax (2 ppm) stimulated oxygen uptake in various sensitive fungi. Moreover, nucleic acid and protein labeling was observed.

Vitavax inhibits glucose oxidation as much as 50 percent in Rhizoctonia solani and Verticillium alboatrum while acetate oxidation is inhibited 70-90 percent. Vitavax reduces RNA-DNA synthesis and high energy nucleotide phosphate 60-90 percent and 64 percent, respectively. In light, riboflavin and riboflavin phosphate detoxify and reverse these reductions in activity of sensitive fungi (41).

It is interesting to note that inhibition of activity for Ustilago maydis can be attained with Vitavax concentrations as low as 1 µg/g of fungi (31).

Initially in sensitive fungi such as R. solani, U. nuda and U. maydis CO_2 release is inhibited when Vitavax is applied at fungitoxic concentrations. However, after a short lag time (1 hour) CO_2 production is increased. This is suggestive of a recycling of carbon in glucose metabolic pathways (33).

In studies using several fungi, pinto bean, and rat liver mitochondria, Vitavax was shown to have little effect on the oxidation of NADH to NAD^+ or uncoupling of oxidative phosphorylation in the presence

of succinate (35). Vitavax produces immediate inhibition of cytochrome C and succinate oxidation (64). This is initiated by the inhibition of succinate cytochrome C reductase and succinate coenzyme Q reductase. Vitavax has no effect on NADH cytochrome C reductase and NADH coenzyme Q reductase. The site of action of Vitavax appears to reside between succinate and coenzyme Q (59). Genetic evidence has been found that lends support to the above findings. A single gene mutation (oxr) diminishes the inhibitory effect of Vitavax in mitochondria of U. maydis by modifying the succinic dehydrogenase system (15).

Toxicity of Vitavax is greatly decreased with the oxidation of its sulfur atom. As inhibitors of acetate metabolism, the monoxide and dioxide of Vitavax are not nearly as effective as Vitavax itself. Likewise, the oxathiin moiety is not required to induce toxicity in sensitive fungi (34) and the fungicidal spectrum is not increased with substitutions in the molecular architecture. Moreover, electron withdrawing groups such as Cl and NO_2 tend to reduce fungitoxicity when substituted in the aniline ring (54). All fungitoxic activity can be destroyed by the elimination of the benzene ring. The carboxamide moiety along with Vitavax's relatively low partition coefficient plays an integral part in its chemotherapeutic nature (36).

MATERIALS AND METHODS

Preparation of Chemicals

Test solutions were prepared by mixing 0.026, 0.067, 0.133, 0.200, 0.266, 0.532, 1.33, 2.66, 7.525, 15.05 grams of the 75 percent wettable Vitavax with 200 ml. of distilled water to give concentrations of approximately 100, 250, 500, 750, 1,000, 2,000, 5,000, 10,000, 33,300, 66,600 ppm, respectively. All solutions were shaken for two minutes to assure maximum miscibility. Controls were prepared as 200 ml. of distilled water.

The concentration of 33,300 ppm (5 g/15 ml) is considered to be standard rate for seed treatment (60). All above concentrations were freshly prepared not more than 24 hours before application.

Plant Preparation and Treatment

The effects of Vitavax on meiotic chromosomes of rye (Secale cereale) and mitotic chromosomes of onion (Allium cepa) and rye were observed. An anatomical study of root tip meristems of these two species was also conducted.

Meiotic studies

The meiotic study employed pollen mother cells of S. cereale and was subdivided into four treatments: (a) wet pack, (b) flask, (c) petri dish soak, and (d) seed.

In the wet pack treatment, specimens were treated in a field nursery by wrapping absorbent tissue around spikes in the early "boot" stage and

securing it at the base of the spikes with a paper clip. Wrapped spikes were then bagged with plastic sacks and tagged for identification. A pencil-sized opening was made at the side of each plastic bag and concentrations of 2,000, 5,000, 10,000, 33,300, and 66,600 ppm Vitavax were applied with a long nosed spray bottle so as to drench top and sides of the wrapped spikes. Additional spray applications were administered every 24 hours. Plastic bags were used to reduce evaporation of Vitavax solutions, while the opening allowed for gas exchange.

Specimens used for the flask treatment were grown in a field nursery and harvested in the early "boot" stage. The lower end of the culms were then immediately set in flasks containing concentrations of 1,000, 5,000, 10,000, 33,300 and 66,600 ppm Vitavax. Flasks were allowed to have normal day length periods. Solutions in the flasks were changed every 24 hours and concurrently the basal portions of the culms were trimmed with a razor blade to facilitate absorption.

Spikes to be used in soak treatment were dissected from potted plants grown in a field nursery at early and middle "boot" stages. These "naked" spikes were then submerged in sterile petri dishes containing concentrations of 10,000, 33,300 and 66,600 ppm Vitavax. Lids were placed over the open petri dishes but left ajar to assure gas exchange. Solutions in the petri dishes were changed at 24-hour intervals.

Vitavax was applied as a slurry to seeds at the recommended field rate (33,300 ppm) and double the field rate (66,600 ppm) (60). Controls and treated seeds were then potted in a sterile soil, labeled, and planted in a field nursery. Spikes grown from these treated seeds were used to observe effects of Vitavax on meiotic chromosome behavior.

Treated and control spikes used in pollen mother cell analysis were stripped of their leaf sheaths and immediately placed into individual vials of Newcomers fixative (40). Spikes were fixed at 48, 72 and 96 hours in flask and wet pack treatments, while petri dish material was fixed at 8, 12, 24 and 48 hour intervals. Spikes produced from treated seeds were fixed in the middle and late "boot" stages. Observations of meiotic chromosome behavior were made from aceto-carmine squash preparations.

Mitotic studies

Seeds of S. cereale were germinated on moistened filter paper in sterile petri dishes. Bulbs of A. cepa were stimulated to produce roots by immersing basal portions in jars containing tap water. Water was changed every 24 hours.

When A. cepa root tips attained a length of 2 cm. the bulbs were transferred to jars containing 10,000, 33,300 and 66,600 ppm Vitavax. Germinated seedlings of S. cereale were transferred to petri dishes containing the above concentrations of Vitavax. Roots were immersed in the test solutions while seeds rested on a parafilm surface which was perforated (using a No. 3 cork borer) and superimposed on the open petri dishes. Test solutions were changed at 24-hour intervals.

Excised root tips, fixed at 12, 24 and 48 hours in Newcomers, for use in mitotic studies, were soaked in a 1:1 solution of hydrochloric acid and 95 percent ethyl alcohol for 1.5 minutes and washed in a 50 percent ethyl alcohol series. Mitotic cells were then observed using the aceto-carmine squash technique.

Anatomical studies of root meristems

Roots of S. cereale and A. cepa were treated at 100, 250, 500, 750, 1,000, 10,000, 33,300 and 66,600 ppm Vitavax in the same manner as described for the mitotic studies. Vitavax concentrations were changed at 24-hour intervals.

Excised root tips used in anatomical studies were fixed in F.A.E. at 8, 12, 24, 48 and 72 hour intervals and prepared by the paraffin method (47). Paraffin blocks were cut on a rotary microtome providing 13 micron sections. Sections were mounted using Haupts gelatin adhesive (47) and then stained with safranin and fast green. Slides were sealed with canadian balsam resin.

At least four, but not more than seven samples were observed in each experimental unit within a treatment. Meiotic chromosome behavior was analyzed at metaphase I, telophase I and quarted stages. Two hundred pollen mother cells were interpreted at metaphase I and telophase I and 1,000 pollen mother cells at the quartet stage. Telophase II was substituted for the quartet stage in seed treatment analysis. In mitotic studies 50 metaphase and 200 telophase nuclei were observed in each lot of samples. Photomicrographs were made with DuPont 711 Cronar film.

RESULTS AND DISCUSSION

Effects on Meiotic Chromosome Behavior

It is clear that Vitavax induces chromosomal aberrations in pollen mother cells of S. cereale. Aberrations observed included univalents, sticky bridges and micronuclei. Due to inconsistencies in petri dish and wet pack data, conclusions concerning these experiments must be somewhat restricted and therefore further discussion of these matters occurs in the appendices.

In flask treatment (Table 1) 1-4 univalents were observed. A single univalent (Figure 1) was the abnormality found in highest frequency. At 96 hours and 66,600 ppm disintegration of pollen mother cells was recorded. Abnormalities in the controls were observed to rise from 0 and 1 percent at 48 and 72 hours, respectively, to a 4.5 percent level at 96 hours. Univalents may have their origin as asynaptic homologues produced during zygotene. Vitavax may interfere with the formation of the synaptinemal complex but substantiation of this point requires more study.

Telophase I (Table 1) data show that sticky bridges (Figure 2) were the most abundant abnormality observed, while 1-2 lagging univalents were also recorded. Disintegration of pollen mother cells occurred at 96 hours, with 10,000, 33,300 and 66,600 ppm Vitavax. Sticky bridges are most likely due to modification of protein and/or DNA and probably do not represent dicentric chromosomes.

A fairly high frequency of micronuclei (Figure 3) at the quartet stage in treated material was observed (Table 1). With increasing

Table 1. Effects of Vitavax on meiotic chromosome behavior following flask treatment of S. cereale spikes. (200 pollen mother cells observed at metaphase I and at telophase I, and 1,000 at quartet stage).

		Metaphase I					Telophase I			Quartet
	Treatment hours	Univalents				Total no. of cells containing aberrations	Sticky bridges	1 univalent	Total no. of cells containing aberrations	No. of cells containing 1-3 micronuclei
		1	2	3	4					
		Number of cells					Number of cells			
Control	48	0	0	0	0	0	0	0	0	0
1,000	48	23	13	0	0	36	2	0	2	10
5,000	48	11	10	0	0	21	0	0	0	55
10,000	48	10	1	0	0	11	4	3	7	42
33,300	48	12	1	0	0	13	2	0	2	24
66,600	48	17	21	5	3	46	14	2	16	104
Control	72	2	0	0	0	2	1	1	2	2
1,000	72	14	9	1	2	26	0	0	0	164
5,000	72	10	6	0	0	16	0	2	2	166
10,000	72	16	8	0	0	24	0	0	0	145
33,300	72	11	5	0	0	16	2	2	4	173
66,600	72	45	25	2	1	73	37	7	44	308
Control	96	6	3	0	0	9	2	0	2	8
1,000	96	14	0	0	0	14	4	1	5	98
5,000	96	16	8	4	0	28	28	0	28	114
10,000	96	29	8	0	0	37	*P.M.C. disint.			163
33,300	96	9	6	0	0	15	*P.M.C. disint.			---
66,600	96	----------					*P.M.C. disint.			---

*Pollen mother cells disintegrating.

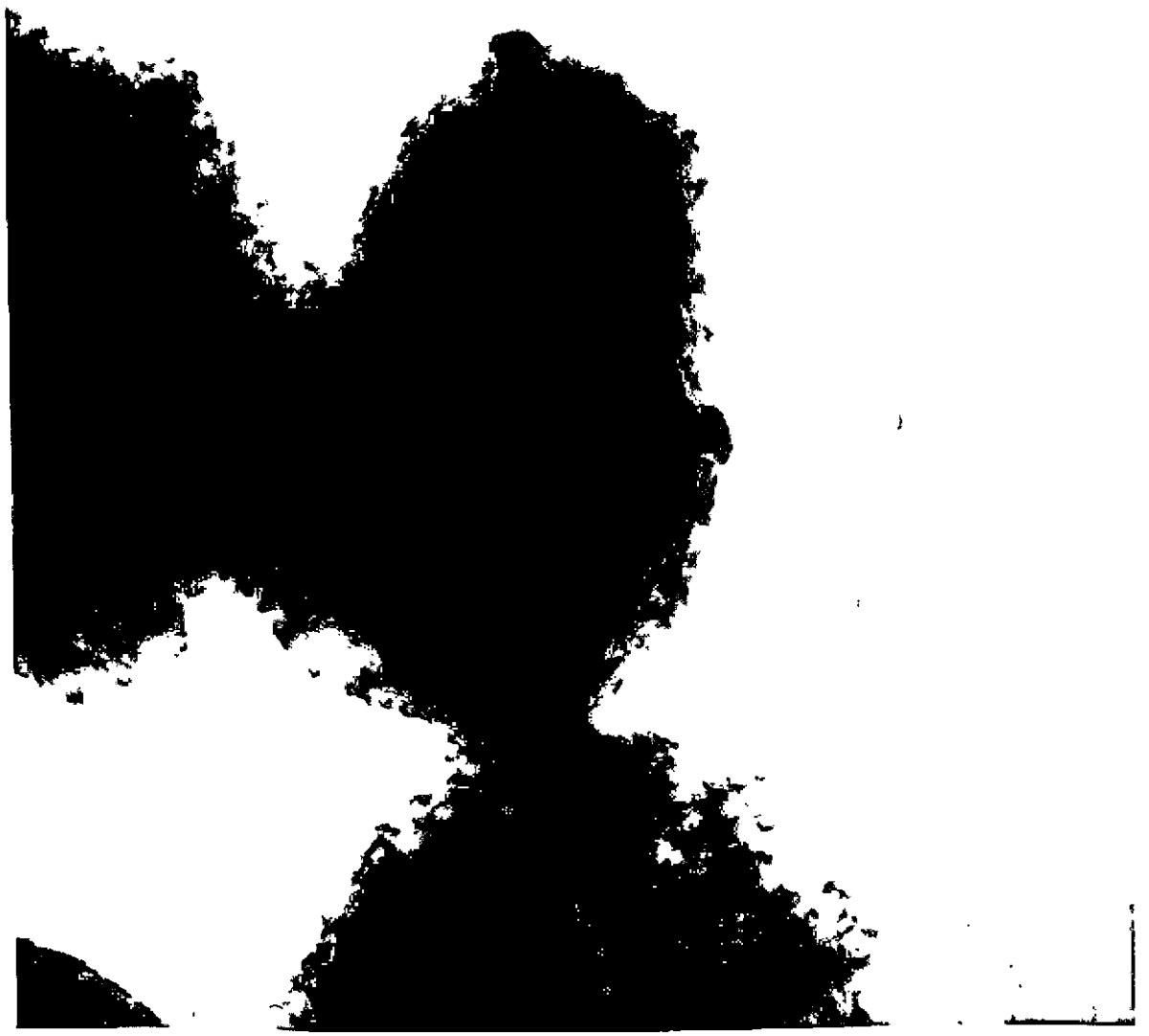

Figure 1. Univalent at metaphase I in pollen mother cell of S. cereale following Vitavax flask treatment at 33,300 ppm, 96 hours. 1070X

Figure 2. Sticky bridge at telophase I in pollen mother cell of S. cereale following Vitavax flask treatment at 66,600 ppm, 48 hours. 1077X

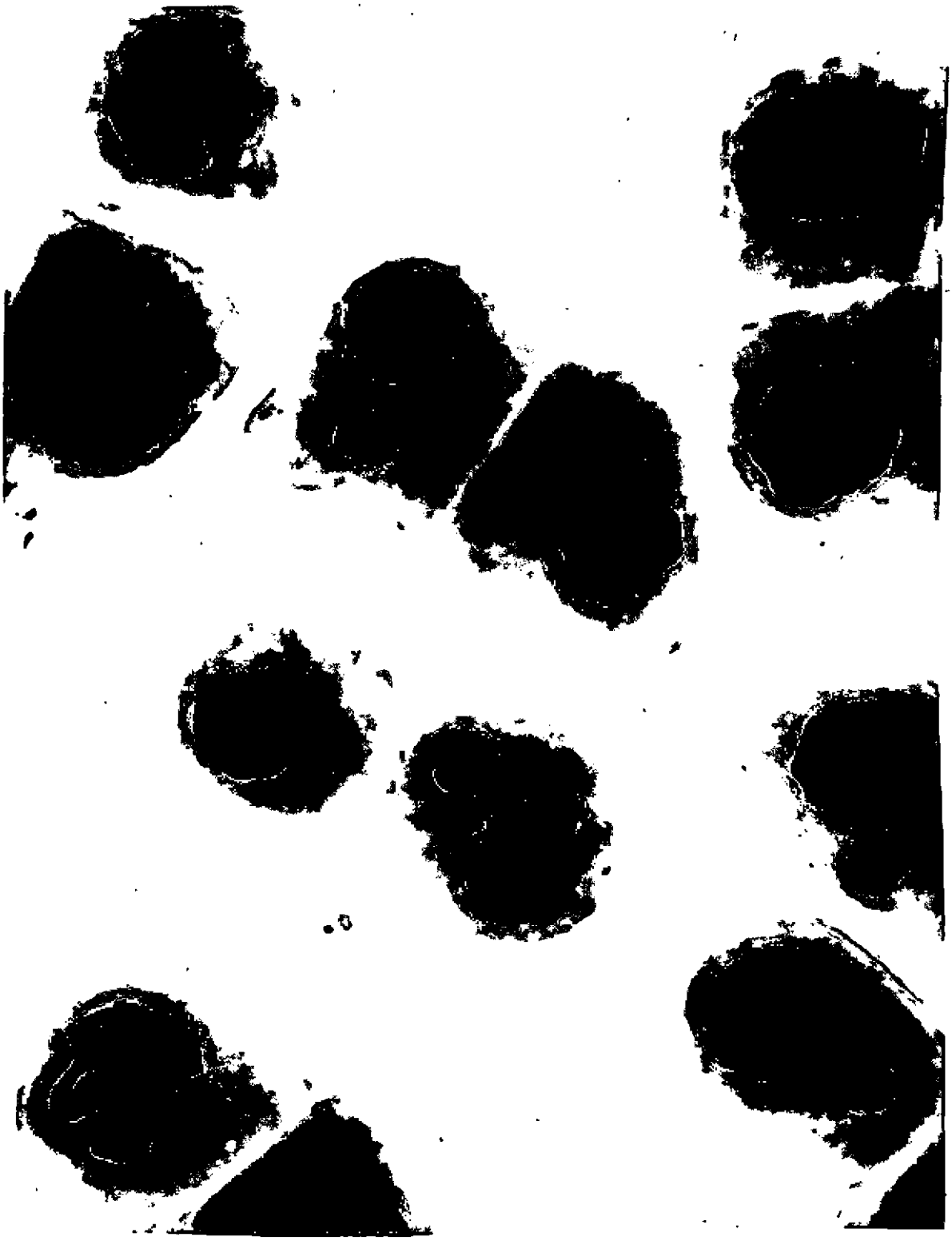

Figure 3. Micronuclei at quartet in pollen mother cells of S. ceréale following Vitavax flask treatment at 5,000 ppm, 72 hours. 1260X

exposure, 96 hours, 33,300 and 66,600 ppm, disintegration of pollen mother cells was observed. It should be noted that the highest application rate, 66,600 ppm, in each treatment time segment produced approximately twice as many aberrations when compared with any other concentration in that particular time segment. Micronuclei have their origin from chromosome fragments or lagging chromosomes. Only sticky bridges were seen at anaphase I and observations of telophase II figures showed the presence of laggards. These observations tend to lend support to the hypothesis that the micronuclei observed have their origin as lagging chromosomes.

Two binomial chi-square analyses (50) were conducted, utilizing flask treatment data, since a single complete test was not possible because of missing data at higher concentrations in the 96 hour application period. The first analysis considered all data in the 48 and 72 hour application periods, while the second analysis considered data only up to and including 5,000 ppm in each of the 48, 72 and 96 hour application periods.

In the chi-square analysis considering the 48 and 72 hour application periods, metaphase I, telophase I and quartet stages were, with respect to total aberrations, significantly different (Figure 4) from controls. Moreover, metaphase I and quarter show, proportionately, twice as many total aberrations when comparing them with telophase I. In these application periods, all concentrations applied differed significantly (Figure 5) from controls in total aberrations. Concentrations of 66,600 ppm Vitavax showed more than twice as many total aberrations when compared with any other concentration. Also, application period seems to be a factor which affects the frequency at which aberrations occur,

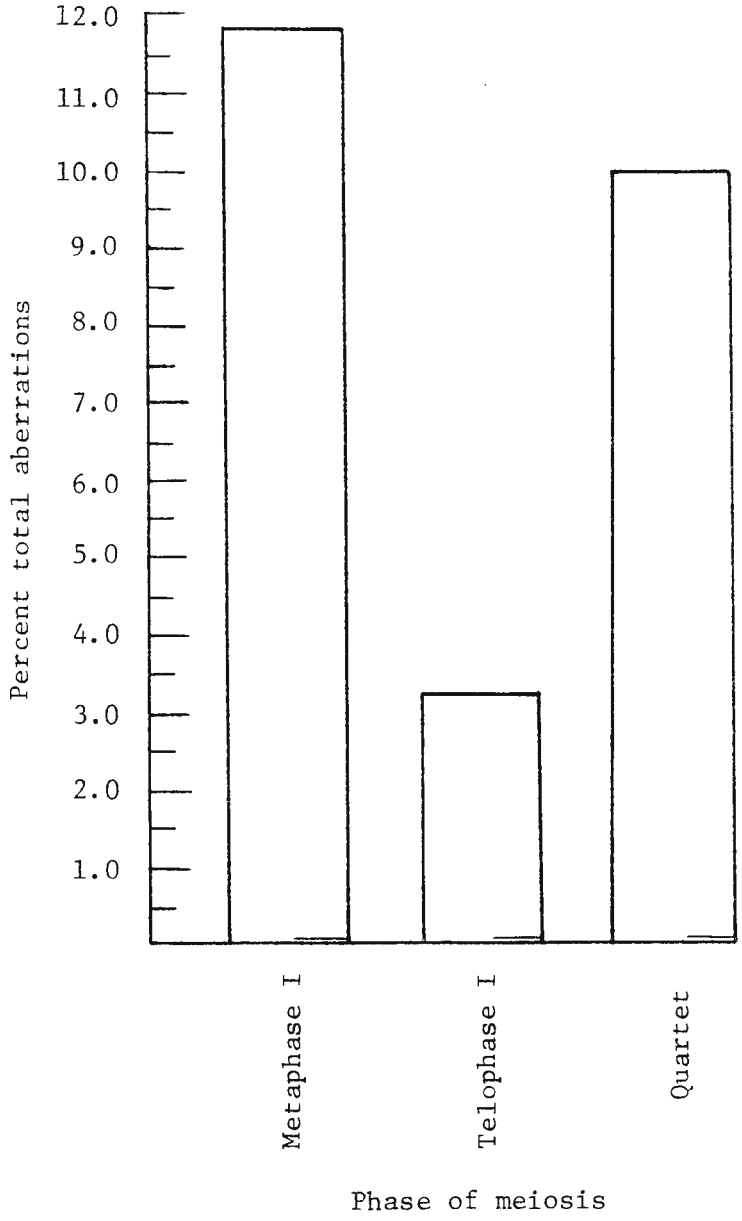

Figure 4. Frequency of total meiotic chromosomal aberrations (combined data) taken collectively at 48 and 72 hours in S. cereale following flask treatment (controls are indicated by indented lines at the base of each column). **Significant at the 0.01 level.

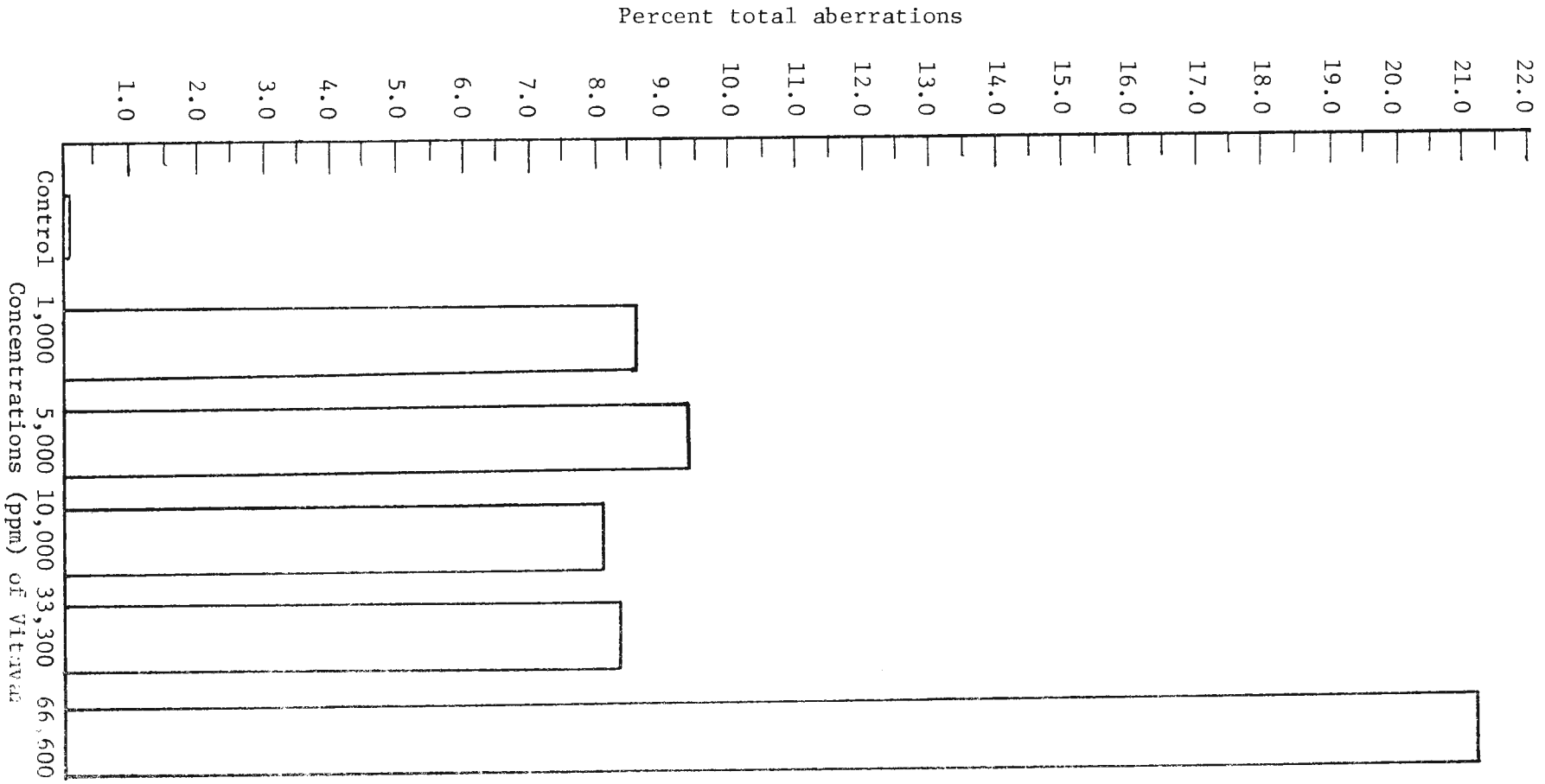

Figure 5. Relative increase in total aberrations with increasing concentrations of Vitavax taken collectively over 48 and 72 hours in pollen mother cells of S. cereale following flask treatment. **Significant at the 0.01 level.

since both application periods, 48 and 72 hours, showed significance (Figure 6). The 72-hour application period had more than twice the number of total aberrations seen at the 48-hour application period.

Close parallels can be seen when comparing the second chi-square analysis with the first. Metaphase I, telophase I and quartet again deviated significantly from controls (Figure 7) with regard to application periods as did the test considering concentrations (Figure 8). Both metaphase I and quarter show, proportionately, twice as many total aberrations when comparing them with telophase I. All application periods, 48, 72 and 96 hours, showed significance (Figure 9) and thus once again indicating that application period may be a factor that affects the frequency at which abnormalities occur. Furthermore, both the 72 and 96 hour applications had, proportionately, more than twice as many aberrations when compared with the 48-hour application.

In the seed treatment (Table 2), materials having been treated at the recommended field rate (33,300 ppm) and double the field rate (66,600 ppm) showed no significant deviation from controls in metaphase I, telophase I or telophase II. Abnormalities observed were: 1-2 univalents at metaphase I, sticky bridges at telophase I, and micronuclei at telophase II.

Effects on Mitotic Chromosome Behavior

Abnormalities observed in treated root tips were mitotic inhibition, aggregation of chromosomes, disintegration, fragmentation and clumping of chromosomes. Control material (Figures 10 and 11) showed no abnormalities.

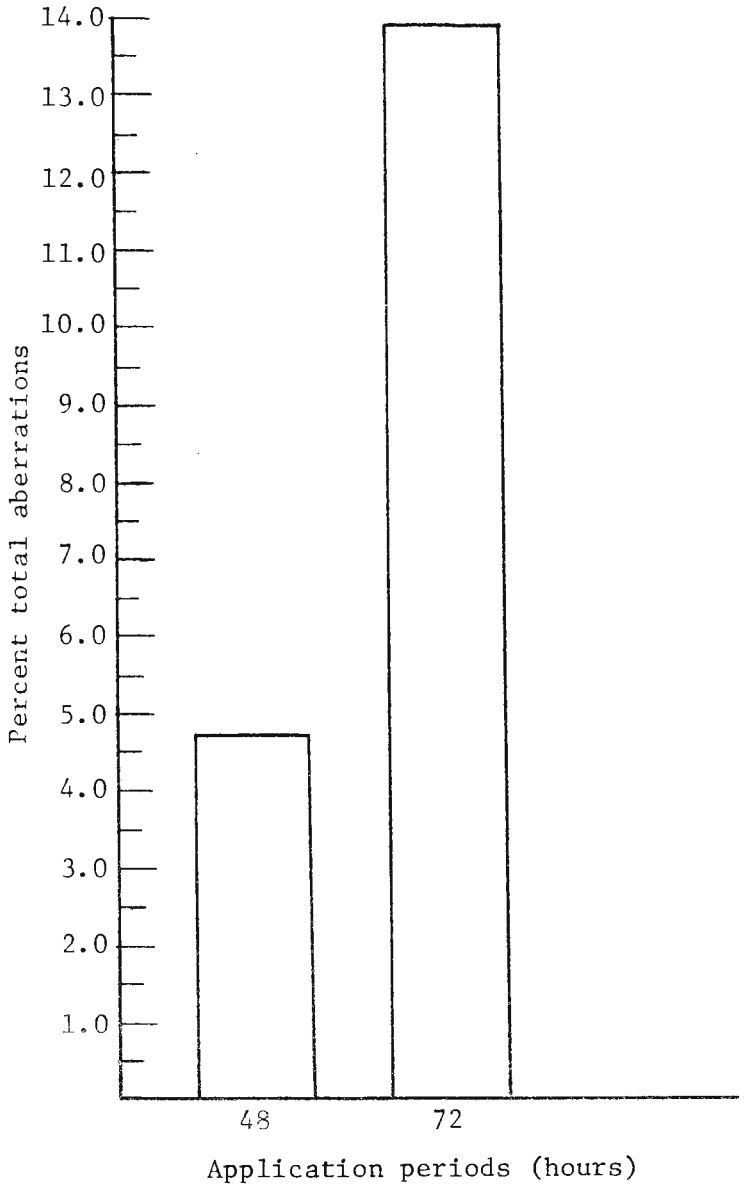

Figure 6. Relative increase in total aberrations (combined data) with lengthening application periods in pollen mother cells of S. cereale following flask treatment. **Significant at the 0.01 level.

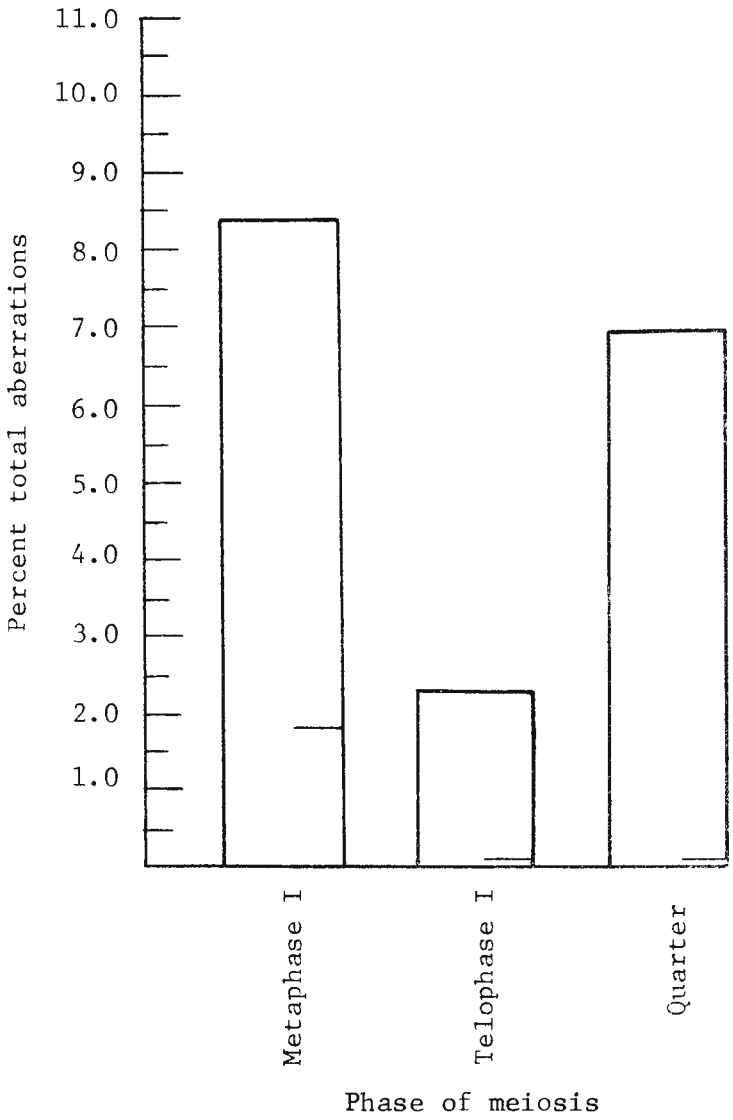

Figure 7. Frequency of total meiotic chromosomal aberrations taken collectively over 1,000 and 5,000 ppm at 48, 72 and 96 hours in S. cereale following flask treatment (controls are indicated by indented lines at the base of each column). **Significant at the 0.01 level.

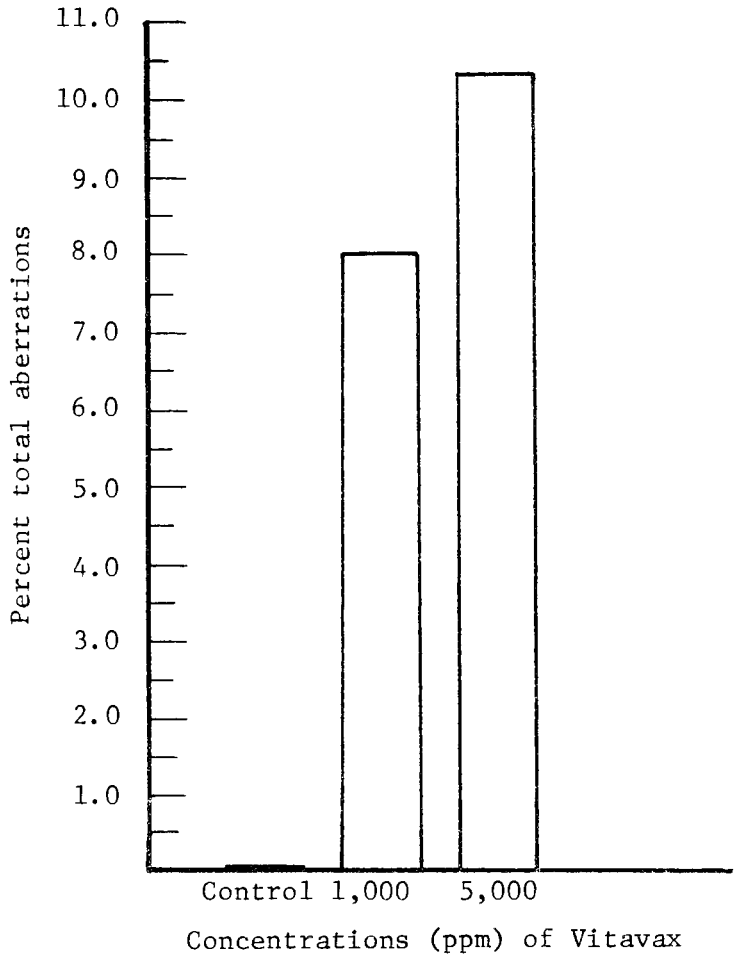

Figure 8. Relative increase in total aberrations with increasing concentrations of Vitavax taken collectively over 48, 72 and 96 hours in pollen mother cells of S. cereale following flask treatment. **Significant at the 0.01 level.

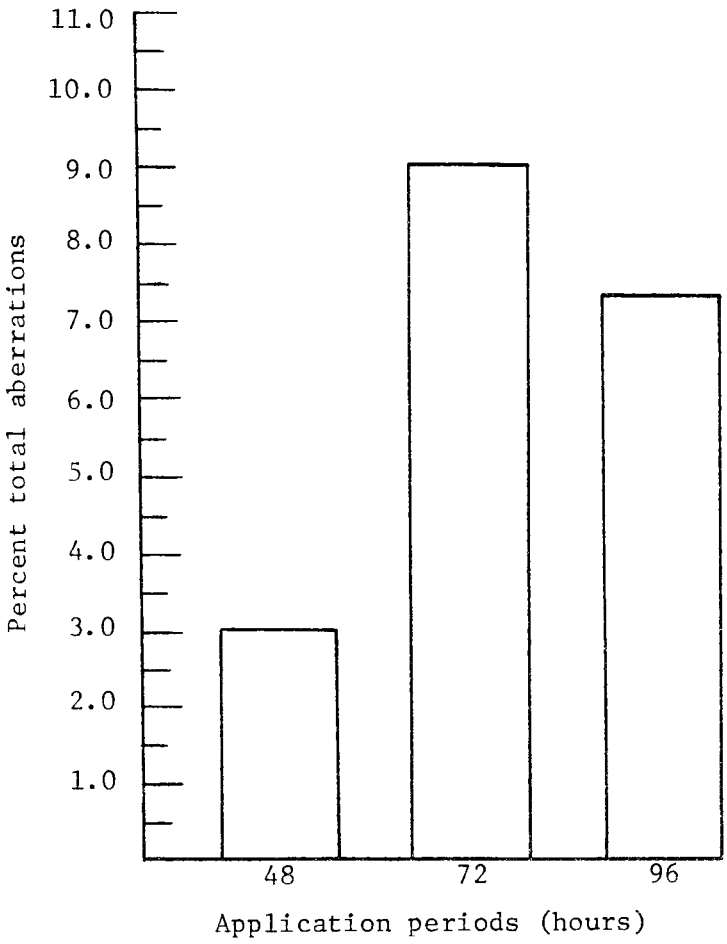

Figure 9. Relative increase in total aberrations (considering 1,000 and 5,000 ppm) with lengthening application periods in pollen mother cells of S. cereale following flask treatment.
**Significant at the 0.01 level.

Table 2. Effects of Vitavax on meiotic chromosome behavior in Secale cereale following seed treatment

	Metaphase I				Telophase I		Telophase II	
PPM	No. cells observed	Univalents 1	Univalents 2	Total aberrations	No cells observed	Sticky bridges	No. cells observed	Micronuclei
Control	200	9	0	9	200	2	1000	8
33,300	200	11	2	13	200	0	1000	10
66,600	200	11	1	12	200	2	1000	7

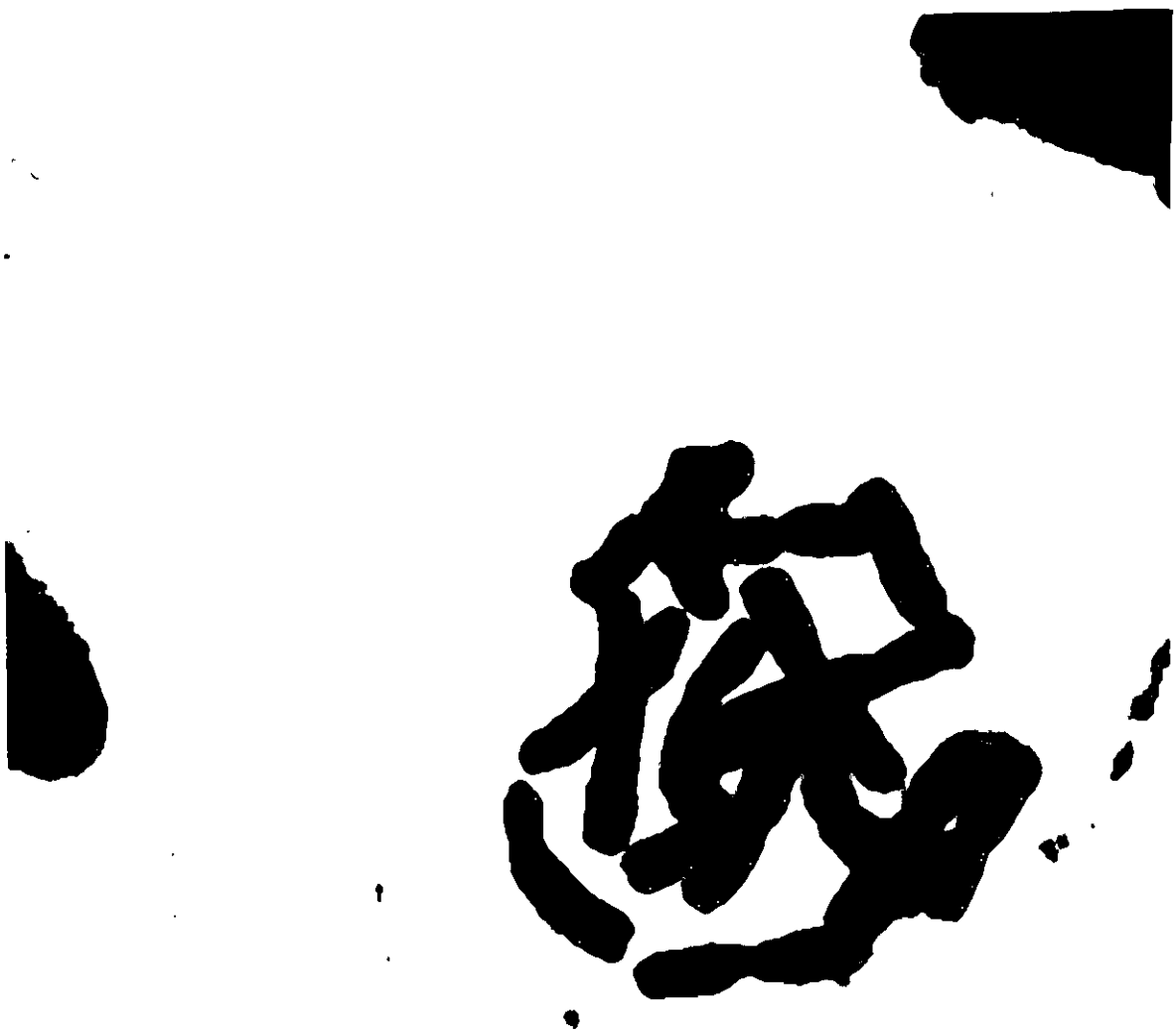

Figure 10. Normal metaphase in root tip of A. cepa control, 12 hours. 1124X

Figure 11. Normal anaphase in root tip of A. cepa; control, 12 hours. 1169X

Vitavax completely inhibited cell division in both S. cereale and A. cepa at 12, 24 and 48 hours with concentrations of 10,000, 33,300 and 66,600 ppm. Chromosomes formed aggregated complexes at metaphase but did not proceed to anaphase. Mitotic inhibition may possibly be attributed to Vitavax's ability to bind with cellular components, i.e. ribosomes, and concurrent inhibition of spindle fiber formation.

Aggregation of chromosomes (Figure 12) was seen in all preparations of the treated material. The slight aggregation seen showed no gradation with respect to increasing concentrations and lengthening of time intervals. Aggregated chromosomes were observed to have a somewhat granular appearance. Aggregation is perhaps due to an inhibition of protein synthesis, histones and associated acidic proteins, thus giving chromosomes a polarity and resulting aggregation.

Treated A. cepa root tissue at 48 hours, 66,600 ppm showed not only aggregation of chromosomes, but also their disintegration and fragmentation (Table 3, Figure 13). The cause of this disintegration and fragmentation is not known. However, if respiration and consequent DNA synthesis is inhibited, as has been shown to be the case for some plants (7), Vitavax may produce these abnormalities as early as the S period in the cell cycle.

Clumping differed from aggregation in the following ways: (a) clumped chromosomes did not have a granular appearance, (b) chromosomes formed amorphous clumps, (c) amorphous clumps were visually smaller when compared to aggregation. The cause of this abnormality is unknown but may again be linked to inhibition of protein synthesis and cellular respiration by Vitavax.

Figure 12. Aggregation of chromosomes at metaphase in root tip of A. cepa following 10,000 ppm, 24 hour treatment of Vitavax. 1070X

Table 3. Chromosome aberrations in Allium cepa root tips treated with Vitavax.

		Metaphase				
	Treatment hours	No. of cells observed	Cells showing slight aggregation	Cells showing aggregation + disintegration and fragmentation	Cells showing clumping	Total number of cells showing aberrations
Control	12	50	0	0	0	0
10,000	12	50	50	0	0	50
33,300	12	50	50	0	0	50
66,600	12	50	50	0	0	50
Control	24	50	50	0	0	50
10,000	24	50	50	0	0	50
33,300	24	50	50	0	0	50
66,600	24	50	50	0	0	50
Control	48	50	50	0	0	50
10,000	48	50	50	0	0	50
33,300	48	50	50	0	0	50
66,600	48	50	9	25	16	50

Figure 13. Fragmentation and disintegration of chromosomes at metaphase in root tip of A. cepa following 66,600 ppm, 48 hour treatment of Vitavax. 1102X

It should be mentioned that due to Vitavax's relatively high rate of disassociation in the soil and decomposition by microorganisms, field rate and double the field rate seed application concentrations would never exist near root tip regions. Moreover, concentrations of Vitavax applied as soil drenches are never used in these proportions. Due to Vitavax's rapid oxidation to its sulfoxide analogue in water the compound absorbed by the roots was undoubtedly not entirely Vitavax.

Anatomical Effects on Root Tip Meristems

Median longitudinal sections of S. cereale and A. cepa root tips were used to determine anatomical effects produced by Vitavax. All control material showed normal development (Figures 14 and 15). Abnormalities observed included inhibition of mitosis, misshapened nuclei, separation of tissues and collapsing of cells.

Mitosis did not occur in either A. cepa and S. cereale at concentrations of 10,000 ppm, and exposures of 48 hours. However, at lower concentrations, 1,000 ppm for 48 hours, mitotic figures were observed. These results supplement the aceto-carmine squash data and give some indication where inhibitory concentrations lie.

A. cepa root tips showed no observable abnormalities after treatment at 48 hours with 1,000 ppm Vitavax. When concentrations were increased to 10,000 ppm at the same time interval misshapened nuclei (Figures 16, 17 and 18) occurred in high frequency. This effect did not appear to assume a logarithmic progression. That is, increasing concentrations of Vitavax did not produce increasing frequencies of this abnormality. Treated roots of S. cereale also showed misshapened nuclei at concentrations of 10,000 ppm, exposed for 48 hours. The small black dots exteri-

Figure 14. Normal development of root tip in S. cereale; control. 269X

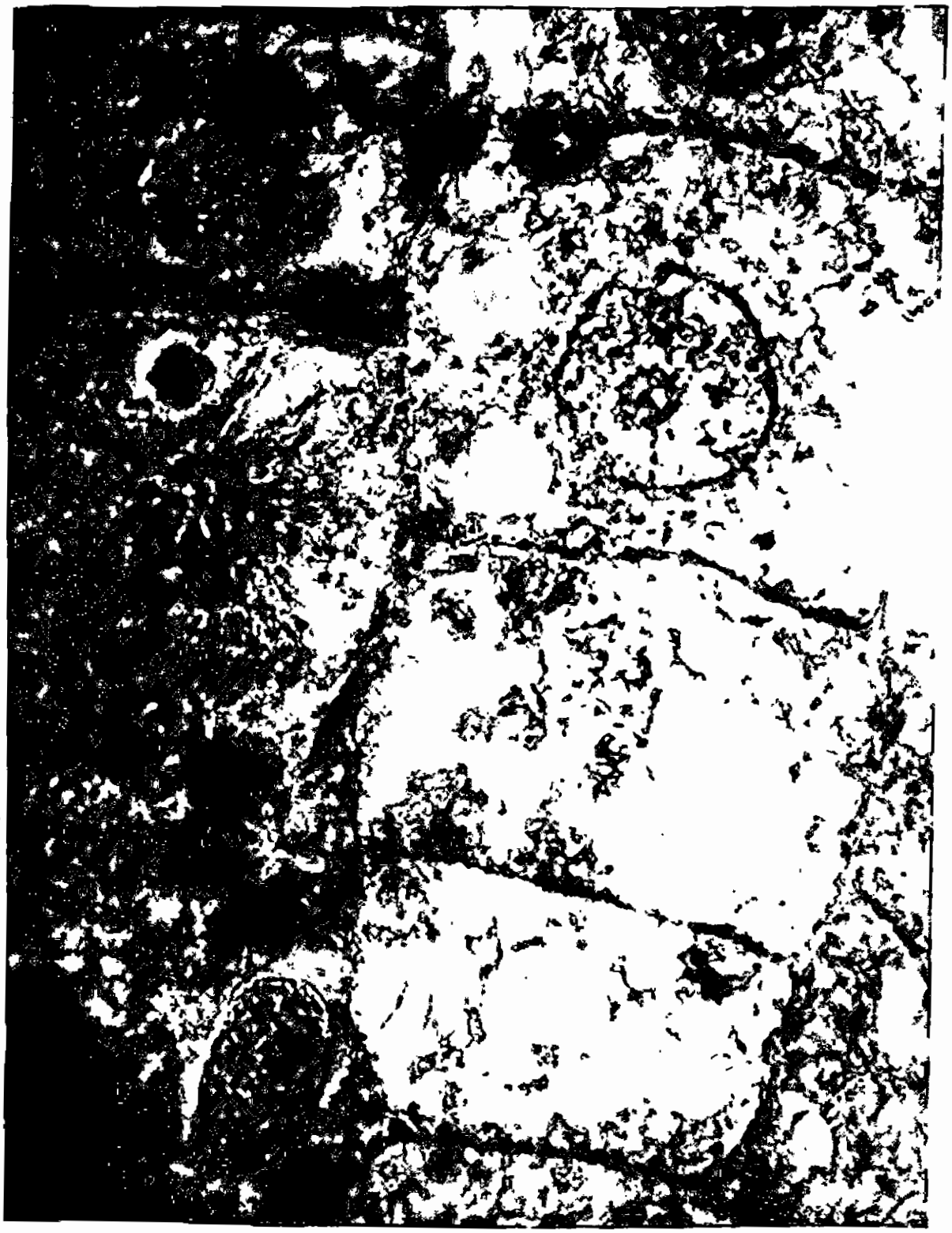

Figure 15. Normal development of A. cepa root tip; control. 596X

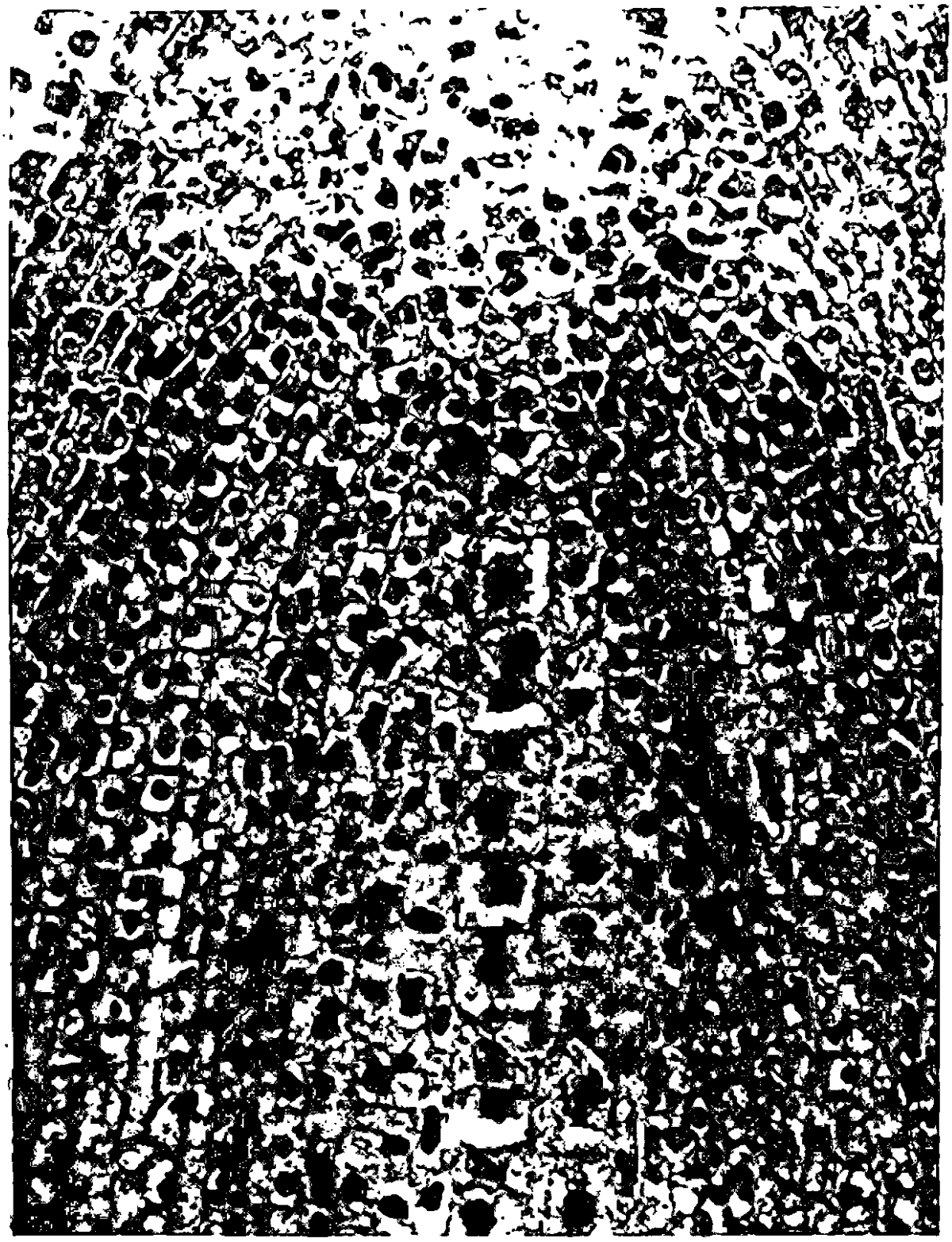

Figure 16. Misshapen nuclei in A. cepa root tip following 66,600 ppm, 72 hour treatment of Vitavax. 256X

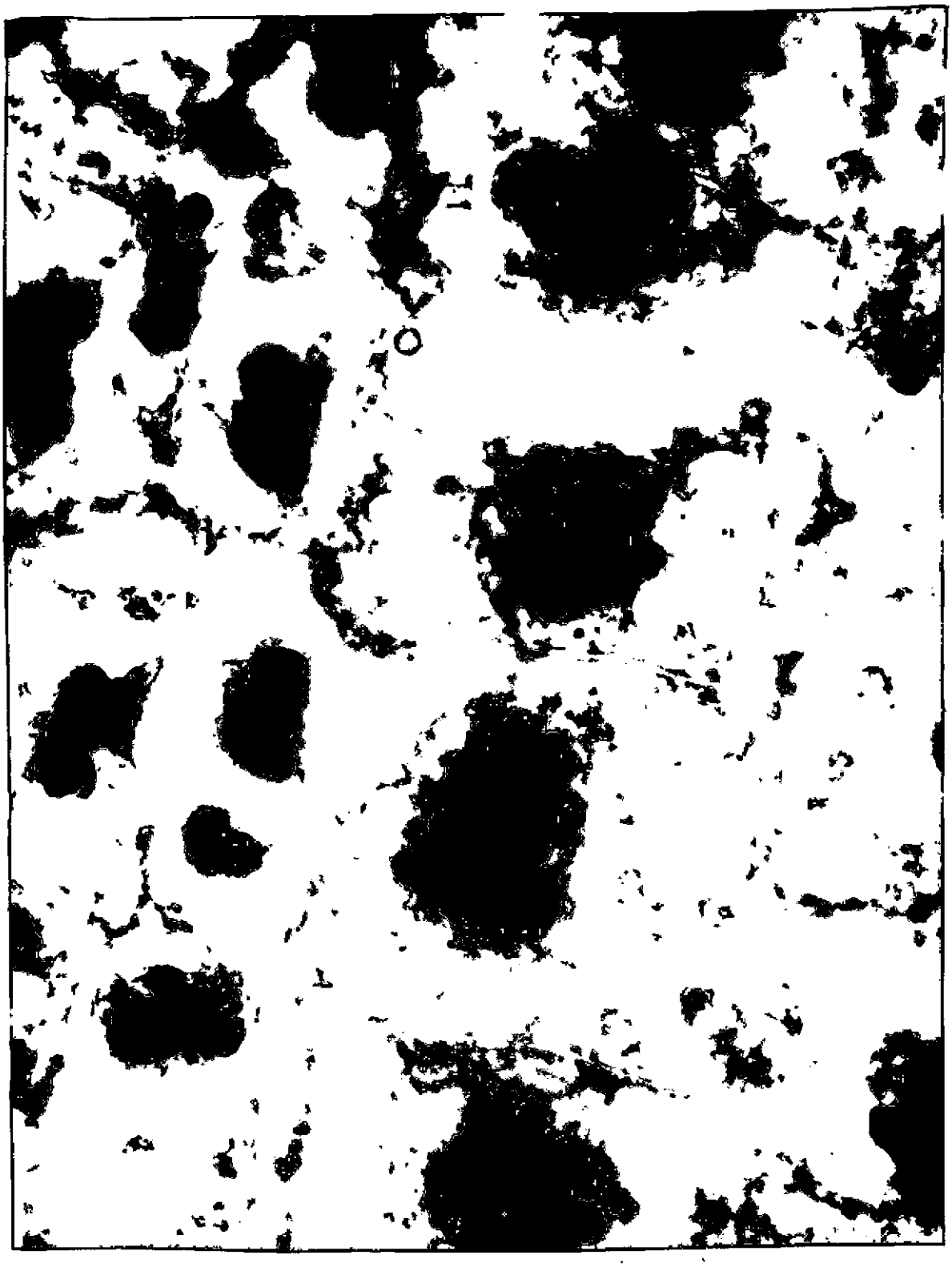

Figure 17. Misshapen nuclei in A. cepa root tip following 66,600 ppm, 48 hour treatment of Vitavax. 596X

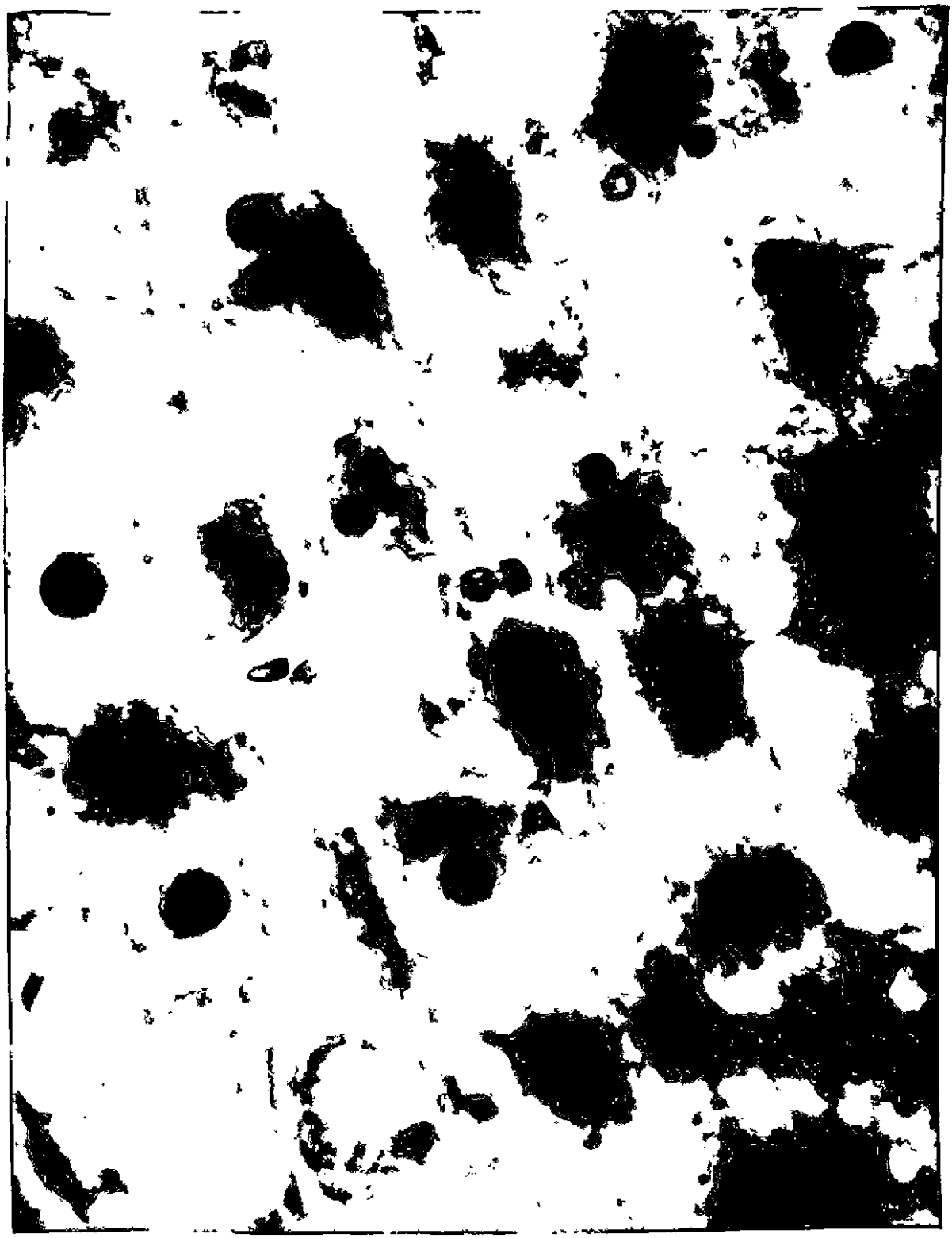

Figure 18. Misshapen nuclei in A. cepa root tip following 66,600 ppm, 72 hour treatment of Vitavax. 604X

to the nuclei may be stained nuclear contents that have diffused out of the nucleus. This may be partially attributed to direct effect by Vitavax on nuclear envelope structure. However, this point is still not clear.

Separation of cells in the procambial (Figure 19) and ground meristem regions (Figure 20) with concurrent collapsing of cells (Figure 21) in procambial, ground and protoderm meristem regions were observed in S. cereale root tips treated at 48 hours with 10,000 ppm Vitavax. Separation of root cap tissue from apical meristem regions (Figure 20) also occurred but with less frequency than the above cited abnormalities. These abnormalities did not occur at 48 hours with 1,000 ppm Vitavax. Separation of cells in these regions may possibly be explained by either inhibition of pectate and/or glycoprotein synthesis or direct Vitavax initiated decomposition of compounds such as calcium pectate found in the middle lamellar region. The former hypothesis seems to hold better with the evidence available, since Vitavax does bind with various cellular components and has been found to be associated with ribosomes (52). Collapsing of cells may also be associated with the inhibition of carbohydrate and protein synthesis, i.e., golgi and polyribosome complexes. Answers to origin of these abnormalities awaits further experimentation.

Figure 19. Separation of cells in the procambial meristem region in root tip of S. cereale following 33,300 ppm, 48 hour treatment of Vitavax. 256X

Figure 20. Separation of cells in the ground meristem region in root tip of S. cereale following 10,000 ppm, 48 hour treatment of Vitavax. 256X

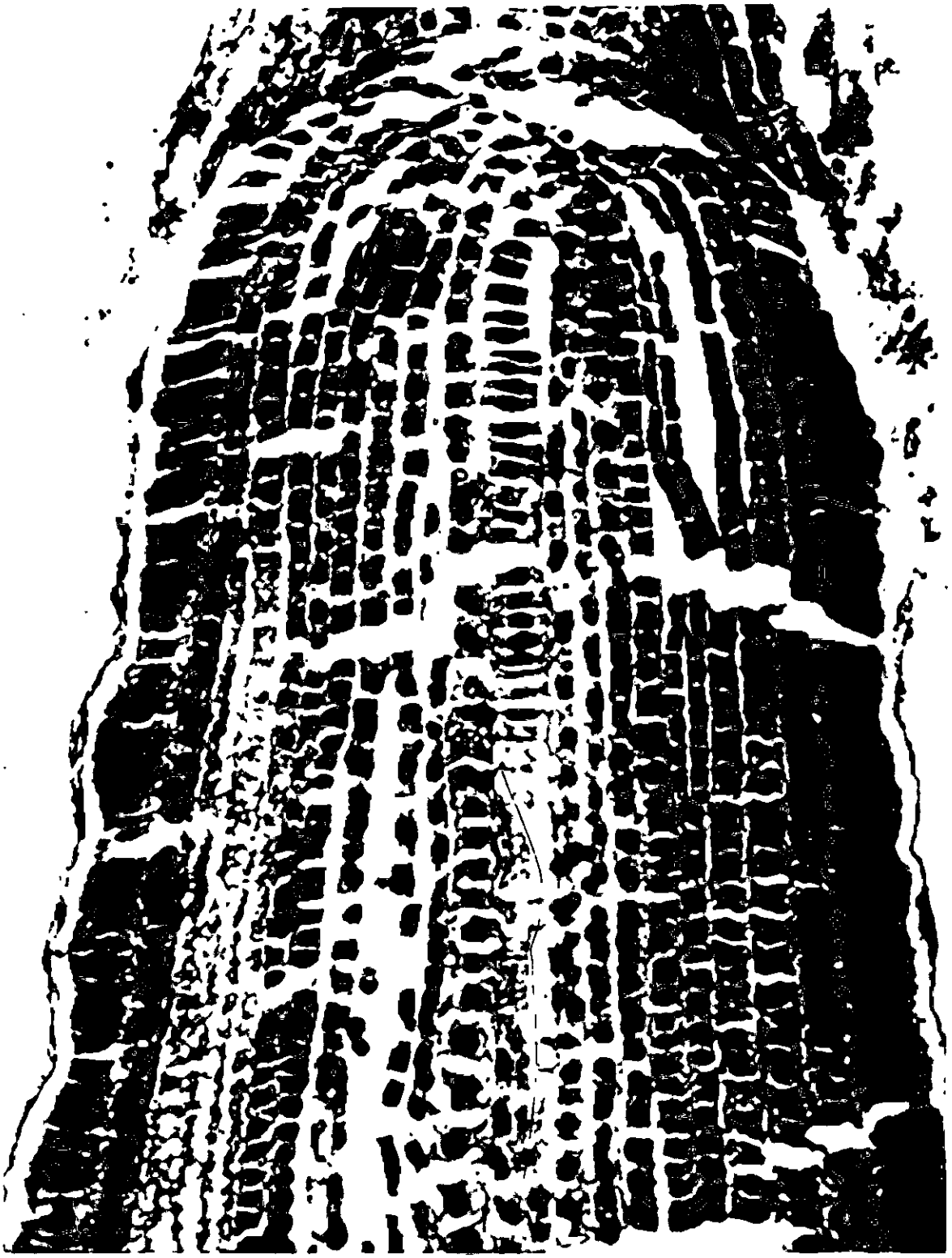

Figure 21. Cell collapse and separation in primary meristem regions in root tip of S. cereale following 66,600 ppm, 72 hour treatment of Vitavax. 256X

CONCLUSIONS

1. Flask treatment indicates that Vitavax or its analogues are translocated acropetally causing pollen mother cells of S. cereale to disintegrate at application rates of 10,000, 33,300 and 66,600 ppm, 96 hours. The principal aberrations recorded at lower concentrations and shorter treatment time were: 1-4 univalents at metaphase I; sticky bridges and single univalents at telophase I; and micronuclei and misshapen nuclei at the quartet stage. Although all meiotic phases were significantly affected by 1,000, 5,000, 10,000, 33,300, 66,600 ppm Vitavax at the 48, 72 and 96 hour application periods, more than twice the number of abnormalities were recorded at metaphase I and quartet stages when compared with telophase I. Also, the 72 and 96 hour application periods produced more than twice as many total abnormalities when compared with the 48 hour application period. The 66,600 ppm application rate produced the greatest increases in the frequency of total aberrations.

2. Treatment of S. cereale seed at recommended field rate (33,300 ppm) and double the field rate (66,600 ppm) did not induce significant abnormal pollen mother cell development.

3. The principal aberrations recorded in root tip mitoses in A. cepa and S. cereale were: chromosome fragmentation, disintegration, clumping, aggregation and inhibition of mitosis. Mitotic inhibition and aggregation of chromosomes was seen in both A. cepa and S. cereale at 10,000 ppm, 12 hours. Disintegration, fragmentation and clumping of chromosomes was observed in A. cepa at 66,600 ppm, 48 hours.

4. Anatomical studies show that Vitavax arrests mitosis in both A. cepa and S. cereale root tips at a concentration of 10,000 ppm for 48 hours. Vitavax did not inhibit mitosis at concentrations of 1,000 ppm, 48 hours.

5. Vitavax has a marked effect on the development of A. cepa and S. cereale root tips causing inhibition of mitosis, misshapen nuclei, separation of tissues and collapsing of cells. A. cepa and S. cereale root tips showed no observable abnormalities at 1,000 ppm, 48 hours. However, at concentrations of 10,000 ppm, 48 hours both A. cepa and S. cereale showed mitotic inhibition and misshapen nuclei. In addition, separation of cells in the procambial and ground meristem regions with concurrent collapsing of cells in the procambial, ground and protoderm meristem regions was observed in S. cereale at 10,000 ppm, 48 hours.

6. Petri dish and wet pack treatment of spikes did not provide consistent enough results to be useful in determining the effects of Vitavax on meiosis.

RECOMMENDATIONS FOR FURTHER STUDY

1. A study to determine the biochemical nature of mitotic inhibition and the concentration at which it occurs. In this study some effort should be made to explain aggregation and the granular appearance of chromosomes at metaphase I.

2. A study to determine the cause(s) for the separation of cells. Initial emphasis should be directed toward cellular metabolism dealing with glycoprotein synthesis.

3. A study to determine the nature of misshapen nuclei. A physiological and biochemical approach may be helpful in such a study.

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APPENDIX

In meiotic studies two types of treatments produced data which is of limited usefulness. These treatments consisted of: (a) young spikes being excised from the "boot" and soaked in petri dishes containing specified concentrations of Vitavax, (b) "boots" containing young spikes being wrapped with absorbent tissue saturated with specified concentrations of Vitavax and bagged with plastic sacks. (see pages 9 and 10 for further details).

Because of the abnormally high frequency of aberrations found in controls following these treatments data is not regarded as useful and dependable. Nevertheless, it has some value and interest and is therefore included here as an appendix.

Aberrations observed following the petri dish treatment (Table 4) included: 1-2 univalents and clumping of chromosomes at metaphase I; sticky bridges and 1-2 univalents at telophase I; micronuclei, misshapen nuclei and fragmented nuclei at quartet.

At metaphase I controls showed a high frequency of abnormalities, especially clumping. During the 48 hour application period total abnormalities in both controls and treated materials ranged between 95-100 percent.

Telophase I data showed on an average 4.0, 33.4, 33.3 and 58.9 percent higher total abnormalities when compared with controls at 8, 12, 24 and 48 hours, respectively.

However, inconsistencies can be observed when comparing experimental units within the treatment, i.e., 33,300 vs. 66,600 ppm at 12 hours.

Quartet data appears to be somewhat more reliable. In the 48 hour application period there tends to be a linear increase in the frequency of total abnormalities. However, inconsistencies did arise at 66,600 ppm during both the 12 and 24 hour application periods.

Table 4. Chromosomal aberrations in pollen mother cells of Secale cereale following petri dish treatment of spikes with Vitavax. (200 pollen mother cells observed at metaphase I and at telophase I, and 1,000 at quartet stage).

	Metaphase I					Telophase I				Quartet			
PPM	Treatment hours	Univalents 1	Univalents 2	Clumping	Total aberrations	Sticky bridges	Univalents 1	Univalents 2	Total aberrations	Micronuclei	Misshapen nuclei	Fragmented nuclei	Total aberrations
	-Number of cells					- - Number of cells - -				- - Number of cells - -			
Control	8	9	0	57	66	2	0	0	2	7	0	0	7
10,000	8	6	2	0	8	5	1	0	6	2	28	12	52
33,300	8	6	5	0	11	14	0	0	14	5	98	43	146
66,600	8	- spikes too young -											
Control	12	12	2	96	110	4	0	0	4	12	0	0	12
10,000	12	6	0	36	42	46	3	0	49	16	274	108	398
33,300	12	7	1	171	179	150	6	3	159	- - - spikes too young -			
66,600	12	10	4	0	14	2	2	0	4	4	8	0	12
Control	24	18	2	123	143	3	0	0	3	21	0	0	21
10,000	24	28	4	0	32	94	7	7	108	- - - spikes too young -			
33,300	24	14	0	141	155	18	0	0	18	11	127	16	154
66,600	24	7	3	190	200	81	2	0	83	25	2	0	27
Control	48	23	3	164	190	3	0	0	3	20	0	0	20
10,000	48	28	5	167	200	93	1	0	94	17	114	0	131
33,300	48	34	5	161	200	111	2	2	115	23	203	0	226
66,600	48	41	11	148	200	147	4	2	53	49	413	0	462

The inconsistencies found with this type of treatment may be attributed to the lack of efficient gas exchange by submerged spikes, resulting in a reduction or inhibition of cellular respiration.

Aberrations observed following wet pack treatment (Table 5) included: 1 and 2 univalents at metaphase I; sticky bridges, 1-2 univalents and clumping of chromosomes at telophase I; micronuclei, misshapen nuclei and fragmented nuclei at quartet.

Total aberrations at metaphase I in treated materials were on an average 11.6, 10.0, 3.0 percent higher than controls at 48, 72 and 96 hours, respectively. Inconsistencies arose between experimental units within the treatment, i.e., 72 hours, 66,600 ppm and 96 hours, 66,600 ppm.

Telophase I treated material showed a high frequency of abnormalities at 33,300 ppm Vitavax during both the 72 and 96 hour applications which is inconsistent with other recorded values.

Data of pollen mother cells in the quartet stage showed inconsistencies at 48 hours, 66,600 ppm, 72 hours, 33,300 ppm and 96 hours, 33,300 ppm. In one case, 72 hours, 33,300 ppm, abnormalities were recorded as occurring at a frequency of 53 percent and then dropping to 6.3 percent at 66,600 ppm.

These inconsistencies may be due to differences in concentrations on the wet pack itself. At high concentrations the solubility constant of Vitavax is surpassed and deposits of Vitavax may have become lodged in the folds of the absorbent tissue, thus providing higher concentrations to localized areas of the spike.

Table 5. Chromosomal aberrations in pollen mother cells of Secale cereale following wet pack treatment of spikes with Vitavax. (200 pollen mother cells observed at metaphase I and at telephase I, and 1,000 at quartet stage).

		Metaphase I			Telophase I					Quartet			
PPM	Treatment hours	Univalents 1	Univalents 2	Tolerance aberrations	Sticky bridges	Univalents 1	Univalents 2	Clumping	Total aberrations	Micronuclei	Misshapen nuclei	Fragmented nuclei	Total aberrations
		- Number of cells -				- Number of cells -				- Number of cells -			
Control	48	6	0	6	2	2	0	0	4	17	0	0	17
2,000	48	17	12	29	2	4	4	0	10	13	0	0	13
5,000	48	22	8	30	4	4	0	0	8	7	0	0	7
10,000	48	15	8	18	8	1	0	0	9	14	0	3	17
33,300	48	25	9	34	3	1	9	0	13	21	0	0	21
66,600	48	28	7	35	1	0	0	0	1	1	0	0	1
Control	72	5	2	7	15	3	2	0	20	15	0	0	15
2,000	72	21	5	26	0	5	1	61	67	32	0	0	32
5,000	72	16	5	21	7	3	1	0	11	73	0	0	73
10,000	72	13	2	15	10	1	1	0	12	65	0	0	65
33,300	72	30	11	41	1	13	9	120	143	270	200	60	530
66,600	72	28	4	32	0	6	0	25	31	21	42	0	63
Control	96	11	4	15	23	2	2	0	27	18	0	0	18
2,000	96	2	1	3	11	0	0	0	11	35	0	0	35
5,000	96	6	4	10	4	2	0	0	6	13	0	0	13
10,000	96	12	6	18	3	3	1	0	7	24	0	0	24
33,300	96	37	16	53	3	0	0	102	105	69	32	0	101
66,600	96	19	2	21	10	4	0	0	14	36	0	0	36

VITA

Jack Ernest Staub

Candidate for the Degree of

Master of Science

Thesis: Cytogenetic Effects of Vitavax Fungicide on Secale cereale and Allium cepa

Major Field: Botany

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