17 (9, 12, 13, 14, 15, and 16 short days, respectively), lots of plants were sprayed to run-off with 0.5, 1, and 2% HAN. The mixture of compounds was not soluble in water; an emulsion was prepared by dissolving in the HAN sufficient Triton X-100 (isooctyl phenyl polyethosyethanol) to give 0.1% of the emulsifier in the final spray and then dispersing this concentrate in water.

Treatment with HAN on the 9th and 12th short day caused the death of the apical meristem; the lateral flower buds 7 or 8 nodes down the stem developed flowers. Treatment on the 13th, 14th, and 15th short day caused the abortion of the lateral flower buds. The terminal flower bud was unaffected, and flowered at the same time as terminal flower buds grown with the lateral flower buds removed by hand (Fig. 1).

A 1.0% emulsion of HAN was the most effective dosage. Treatment with 0.5% emulsion caused the abortion of only a part of the lateral flower buds. Treatment with a 2.0% emulsion reduced the number of florets which developed on the flower head. Treatment on the 16th short day, or later, caused the abortion of only a few of the lateral flower buds. HAN was most effective as a 1% foliar spray during the fall; a 2% foliar spray was the optimum one during the summer. The optimum treatment time, as measured by the number of short days, was the 14th or 15th short day. Treatments on sunny days were less effective than those made on cloudy days. Plants placed in chambers held at high humidity, and treated with HAN, developed damaged foilage, but the flower buds were unaffected.

The response of the various chrysanthemum cultivars to treatment with HAN varied greatly. Fred Shoesmith was responsive to 1% emulsion of HAN in the September test (reported here); abortion of all lateral flower buds occurred. Yellow Delaware and Princess Anne responded to 3% and 5% emulsions of HAN, respectively. The treatment caused the abortion of only a portion of the lateral flower buds. Shasta and Improved Indianapolis Yellow developed yellow leaves with black margins in response to treatment with 5% emulsions of HAN. The lateral flower buds were unaffected at all dosages tested.

Dilute auxin sprays are used to reduce the number of flowers which develop on fruit trees (3). Maleic hydrazide has been used to control the growth of lateral buds of tobacco (8) and chrysanthemum (1). Marth and Mitchell (4) reported that Chloro-IPC gave a sucker-retarding effect similar to maleic hydrazide. Tso (6), and Tso, Steffens, and Engelhaupt (7) reported that the methyl esters of fatty acids caused the death of lateral shoots on tobacco. The optimum carbon chain-length was C_{10}, methyl caprate. These chemicals were ineffective in causing the abortion of flower buds on chrysanthemum.

HAN is a selected petroleum fraction of high aromatic content (approximately 87% by weight). About half the aromatics present are alkylnapthalenes, predominantly C_{10} to C_{13} (5). It may act similarly to the methyl esters of the fatty acids by killing all tissues with high rates of cell division (6, 7). The differences in response may be due to the species tested, and to the presence of aromatic compounds which are reported to affect growth.

Various fractions of HAN are now under test to determine which parts of the complex mixture of naphthalenes, indenes, tetralin, indanes, and alkylbenzenes are responsible for the abortion of the flower buds of chrysanthemum, and also to determine their relation to known metabolic inhibitors.

References and Notes

1. R. G. Beach and A. C. Leopold. 1953. *Proc. Amer. Soc. Hort. Sci.* 61:543.
2. G. V. Johnson, A. H. Yeomans, F. F. Smith. 1965. *The Exchange* 143 (8): 34-37, 60-69.
3. L. C. Luckwill. 1953. *J. Hort. Sci.* 24:25.
4. P. C. Marth and J. W. Mitchell. 1964. *J. Agr. and Food Chem.* 12:61.
5 F. C. Nelson and G. W. Fiero. 1954. *J. Agr. and Food Chem.* 2:735.
6. T. C. Tso. 1964. *Nature* 202:511.
7. T. C. Tso, G. L. Steffens, M. E. Engelhaupt. 1965. *J. Agr. and Food Chem.* 13:78.
8. J. W. Zukel, U. S. Rubber Co., Naugatuck Chemical Division, Naugatuck, Conn., MHIS No. 8.
9. HAN was supplied by the Humble Oil and Refining Company, Houston, Texas. Chrysanthemum cuttings were supplied by Yoder Brothers, Barberton, Ohio. Mention of a trade name is for identification and does not imply endorsement by the U. S. Department of Agriculture.

Pollen Nuclear Division Prevented with Toluidine Blue in *Vinca Rosea* L.

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Abstract. A study was made on the effect of toluidine blue on the division of the generative nucleus in pollen tubes of *Vinca rosea* L. grown *in vitro*. Division was prevented in a percentage of pollen tubes, dependent on the concentration of the dye and duration of the treatment.

Haploid sporophytes of higher plants are useful for genetic and cytological research but are of relatively rare natural occurrence (3). A method for producing haploids quickly, with regularity, would be of great value in breeding programs and cytogenetic studies. Production of haploid frog embryos has been reported by Briggs (1) using the basic dye, toluidine blue, as a sperm inactivator. This dye inactivated the frog sperm nucleus without affecting the extra-nuclear parts of the cell. Consequently, at fertilization the sperm nucleus remained condensed and did not fuse with the egg nucleus.

The same type of reaction might occur if this dye were used on pollen. Although the dye might inactivate the generative nucleus, the pollen tube might still discharge its contents into the embryo sac. It is theorized that the egg could then be stimulated to develop into a haploid plant since the male chromosomes would be prevented from fusion with the female chromosomes. Therefore, this study was undertaken to determine the effect of toluidine blue on the division of the generative nucleus in *Vinca rosea* L.

Pollen of *Vinca rosea* L. was treated by immersion in concentrations of from 5-500 ppm of toluidine blue for from 1 to 30 minutes. It was then removed, cultured on an agar-sugar and yeast medium (2), stained and examined for generative or sperm nuclei. Control slides were prepared for each set of slides of treated pollen and examined for nuclei before observation of the treated slides (Fig. 1). When all nuclei had divided on control slides, the slides

Table 1. Percentage of undivided generative nuclei in pollen tubes of *Vinca rosea* after treatment of the pollen grains with toluidine blue. Readings taken after 24 hours incubation.

Treatment time	Concentration in pmm			
	0	5-20 ppm	50-200 ppm	500 ppm
1-9 minutes	0	30	48	57
10-19 minutes	0	39	48	72
20-30 minutes	0	55	55	76

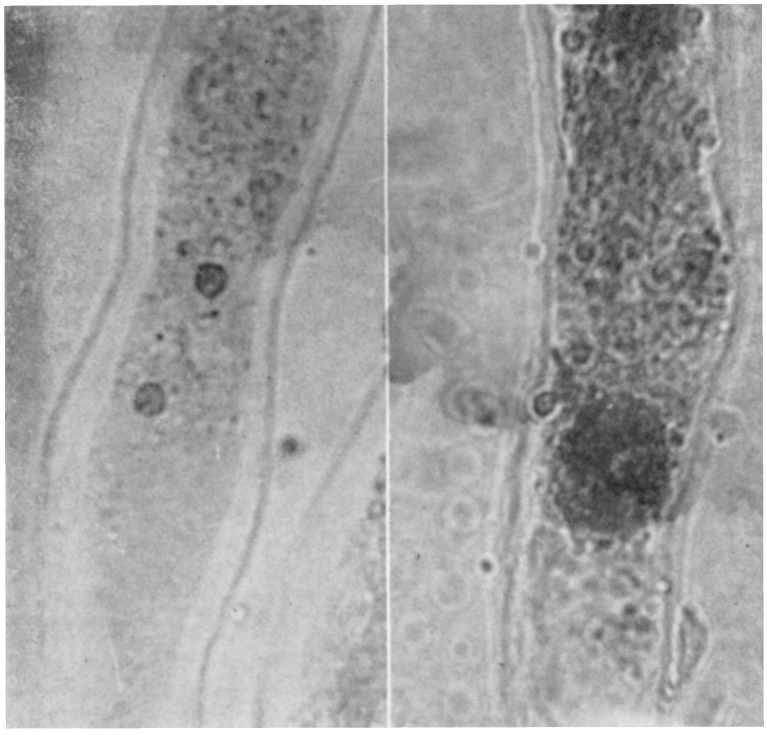

Figure 1. Pollen tubes of *Vinca rosea*: untreated on the left showing two sperm nuclei; and treated (100 ppm for 5 min.) on the right showing undivided generative nucleus. (X970.)

of treated pollen were examined and the results recorded. Germination percentage was based on the first 100 grains examined, and tube length per slide was determined by averaging the lengths of 10 tubes selected at random.

Results show that toluidine blue had little effect on pollen germination of *Vinca rosea* except at the longest treatment time and at the highest treatment concentration. At a treatment time of 20-30 minutes with all concentrations and at 500 ppm concentration with all treatment times, germination was sharply reduced relative to that of control.

When pollen grains germinated, the pollen tubes grew well in all treatments, although some reduction in length was noted as time and concentration increased. The one exception was at 500 ppm for 20-30 minutes, where the average tube length was reduced to 86 μ as compared to an average of 225 μ in the control.

Undivided nuclei were observed (Fig. 1) after all treatments (Table 1). The distribution of undivided among divided nuclei was scattered although a low concentration of the dye greatly increased the time necessary to prevent division of the generative nucleus. There was no evidence of threshold action, and trends were not sharply defined, indicating that the dye had some effect on the nucleus over the entire range of treatments.

The important fact remains that division has been prevented *in vitro* in the generative nucleus of the pollen tube by treatment with toluidine blue over a range of concentration and treatment times. Additional work will be necessary to determine *in vivo* whether these tubes will enter the embryo sac and if the egg will be stimulated to develop into a haploid plant.

References

1. Briggs, R. 1952. An analysis of the inactivation of the frog sperm nucleus by toluidine blue. *Jour. Gen. Physiology* 35:761-780.
2. Brink, R. A. 1924. The physiology of pollen II. Further considerations regarding the requirements for growth. *Am. Jour. Bot.* 283-294.
3. Kimber, G. and R. Riley. 1963. Haploid angiosperms. *Bot. Rev.* 29:480-531.