

# The effect of oleander glycosides on the germination of pollen grains and the mitosis of the generative nucleus in *Tradescantia bracteata* Small and *Allium cepa* L.

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## Abstract

The effect of water solution of a mixture of glycosides from oleander (*Nerium oleander* L.) on the germination of pollen grains and on the mitosis of the generative nucleus in *Tradescantia bracteata* Small and *Allium cepa* L. has been studied. An inhibition of the germination and of the growth of pollen tubes was observed, proportionally to the concentration of glycosides. The pollen grains of *A. cepa* are more sensitive. The disturbances in mitosis lead to the formation of two or more uneven-sized daughter nuclei, or to the formation of restitution nuclei. These anomalies are more numerous in *T. bracteata*.

From these results it appears that pollen grains of *A. cepa* are characterized by a generally high physiological sensitivity and a small mitotic sensitivity, whereas for *T. bracteata* the opposite is true.

## INTRODUCTION

In the present study were utilized pollen grains and pollen tubes as the experimental material for the testing of the effect of glycosides from oleander (*Nerium oleander* L.) on a cell. Antimitotic properties of these substances have been discovered by T a r k o w s k a (1971a). She has been studying their effect on the cells of apical meristems of *Allium cepa* roots and on the endosperm of *Haemanthus katharinae* (T a r k o w s k a, 1971b) and she reported the following consequences:

1. shortening of the chromosomes
2. disturbances in the formation and development of the mitotic spindle
3. disturbances in the course of cytokinesis

4. formation of polyploid and hypoploid cells capable of further division.

Initial studies under electron microscopy have shown that the glycosides of oleander cause a disorganization of continuous fibres, and after a longer period of action also of the kinetochore fibres of the mitotic spindle (Tarkowska, 1971b).

The purpose of the present study was to establish the effect of this antimitotic agent on the germination of pollen grains and on the division of the generative nuclei in the pollen tubes.

#### MATERIALS AND METHODS

The experiments have been conducted on two species, *Tradescantia bracteata* Small,  $n = 6$  and on *Allium cepa* L.,  $n = 8$ . *Tradescantia* has been used in similar studies by such investigators as Eigsti (1940b), Sax and O'Mara (1941), Swanson (1942, 1944), Venema and Koopmans (1962).

For the study has been taken fresh pollen, collected directly from open anthers. For both species the same culture methods were employed. Pollen grains have been cultured on the agar medium of Kwack and Kim (1967) prepared on distilled water with the following constituents:

- sucrose (for *T. bracteata* 10%, for *A. cepa* 5%)
- agar 1.5%
- microelements:  $\text{H}_3\text{BO}_3$  — 0.01%  
 $\text{Ca}(\text{NO}_3)_2$  — 0.03%  
 $\text{MgSO}_4$  — 0.02%  
 $\text{KNO}_3$  — 0.01%

The pH of the medium has been brought up to 8.3 using 1% KOH.

The provision of optimal growth conditions is essential. This has been treated widely in many studies of culture methods (Uppcott, 1936; Eigsti, 1940a; Sax and O'Mara, 1941; Bishop, 1949; Conger, 1953; Savage, 1957; Darlington and La Cour, 1962; Kwack and Brewbaker, 1961; Venema and Koopmans, 1962; Brewbaker and Kwack, 1963; Maheshwari and Rangaswamy, 1965; Sharma and Sharma, 1965; Kwack and Kim, 1967).

On cover slip was spread a thin film of the above mentioned agar medium, and after it solidified pollen grains were sprinkled on it, and the cover slip was positioned in a growth chamber with a constant humidity and temperature (20—22°C). The pollen tubes of *Tradescantia* were stained and observed at one hour intervals between the 4-th and 22-nd hour of culture. Those of *Allium* were observed between the 2nd and 20-th hour of culture.

For the experiment a mixture of glycosides from *Nerium oleander* L. (Oleander Gesamtglycoside, Laborchemikalien Carl Roth OHG Karlsruhe-West) was used at concentrations of 0.003%, 0.006%, 0.012%, 0.025%, and 0.05%.

The effect of the glycosides was studied in two ways:

1. By the addition of them to the agar medium at the time it was being made — this was the basic method.
2. By culturing the pollen tubes for one hour on agar medium without glycosides, and then by placing them in a fluid medium with the glycosides — complementary method.

The observations were conducted *in vitro* or after staining with acetocarmine.

The numerical data presented in table 1—3 are the means of the observations conducted on three cover slips. Each variant of the culture was replicated 5 times, and the pollen grains originated from different flowers.

## RESULTS

The pollen grains of *T. bracteata* after sowing on the agar medium have germinated in about 10 minutes, while *A. cepa* after 15—20 minutes. The division of the generative nucleus began in *Tradescantia* after 5—7 hours and the largest number of dividing nuclei was observable after 7—9 hours. In *Allium* the division began about 1—2 hours earlier. These data concern both the controls and the material subjected to the action of the glycosides.

In studying the effect of the oleander glycosides on the pollen grains particular attention was given to the following processes:

1. pollen grain germination, growth of the pollen tubes and their survival
2. division of the generative nucleus.

Effect of the oleander glycosides on the germination of pollen grains, on the growth of the pollen tubes and on their survival

Results of these observations are presented in Tables 1 and 2. In order to compare the percentage of germination, the length of the pollen tubes and their survival, the pollen tubes must be in the same developmental stage, and this is the reason why in the tables there are a 2 hours difference in culturing times (in *A. cepa* the division of the generative nucleus starts about 2 hours earlier than in *T. bracteata*).

In both the studied species it was found that under the influence of increasing concentrations of the glycosides the percentage of germinating

pollen grains declined gradually, which was accompanied by a decline in the growth of the pollen tubes and a decrease in their survival (cytoplasmic streaming stops). The critical concentration of the glycosides which completely inhibits germination for *A. cepa* is 0.012‰ and for *T. bracteata* 0.05‰. These results were obtained following culture of the pollen tubes on an agar medium with the glycosides added.

Table 1

The effect of oleander glycosides on the germination and growth of pollen tubes. Culturing time for *T. bracteata* pollen grains — 10 hours and for *A. cepa* — 8 hours. The agar culturing method

|                 | % germination       |                | Max. pollen tube length (mm) |                |
|-----------------|---------------------|----------------|------------------------------|----------------|
|                 | <i>T. bracteata</i> | <i>A. cepa</i> | <i>T. bracteata</i>          | <i>A. cepa</i> |
| Control         | 80                  | 30             | 2.4                          | 0.8            |
| With glycosides |                     |                |                              |                |
| 0.003%          | 70                  | 2              | 1.4                          | 0.2—0.3        |
| 0.006%          | 65                  | 1              | 0.8                          | 0.1            |
| 0.012%          | 45                  | no germin.     | 0.5                          | no germin.     |
| 0.025%          | 5                   | no germin.     | 0.2—0.3                      | no germin.     |
| 0.05%           | no germin.          | no germin.     | no germin.                   | no germin.     |

Table 2

The effect of oleander glycosides on the survival of pollen tubes. Culturing time for *T. bracteata* — 10 hours and for *A. cepa* — 8 hrs. The agar culturing method

|                 | % of living pollen tubes (normal cytoplasmic streaming) |                     |
|-----------------|---|---------------------|
|                 | <i>T. bracteata</i>                                     | <i>A. cepa</i>      |
| Control         | 70—80   | 60                  |
| With glycosides |   |                     |
| 0.003%          | 50  | 5—10                |
| 0.006%          | 30  | single pollen tubes |
| 0.012%          | 15  | no germination      |
| 0.025%          | 5—10  | no germination      |

Employing the method of adding the glycosides to a fluid medium supplied to already germinated pollen tubes it was established that concentrations of 0.012‰ and 0.025‰ which inhibit completely the germination of the pollen tubes in *Allium cepa* are not lethal to pollen tubes from pregerminated grains. They survive for about 7—8 hrs after application. Thus it was possible to study the effect of both these concentrations on the division of the generative nucleus in *Allium cepa* and to compare their

effect on both the studied species. From the observations it appears that the pollen grains of *A. cepa* are much more sensitive to the action of the glycosides than those of *Tradescantia bracteata*. The pregerminated poller tubes are almost equally sensitive to the glycosides.

#### Effect of oleander glycosides on the division of the generative nucleus

*Tradescantia bracteata*. The course of mitosis in the pollen tubes of *T. bracteata* in the control material depends on the diameter of the pollen tube. In wider pollen tubes (sporadically even up to 20  $\mu$ ), mitosis runs a typical course, that is the kinetochores arrange themselves along a line more or less perpendicular to the walls of the pollen tube (Fig. 5). In narrow tubes (6—10  $\mu$ ) the metaphase plate is positioned obliquely relative to the pollen tube walls (Fig. 3) or in the case of very narrow tubes the plate is absent. The metaphase chromosomes are then arranged more or less parallel to walls of the tube (Fig. 1). In such cases as a result of the absence of room for the migrating chromosomes spontaneous disturbances can easily occur. The anaphasae does not always lead to the formation of two male nuclei of equal size. Three or two nuclei of unequal size may form, sometimes joined by chromosomal bridges.

Some of the disturbances observed in the division of the generative nucleus in *Tradescantia* following the action with the glycosides are partially of the same type as the spontaneous disturbances, however the number of disturbances considerably increases under the influence of the glycosides, which is demonstrated by Table 3.

Table 3

The number of disturbances under the influence of the glycosides

|                 | % of divisions with disturbances |
|-----------------|----------------------------------|
| Control         | 12                               |
| With glycosides |                                  |
| 0.003%          | 50                               |
| 0.006%          | 62                               |
| 0.012%          | 73                               |
| 0.025%          | 64                               |

The type of culturing method employed and the concentration of the glycosides did not affect the type of disturbances. The disturbances concern primarily the movemens of the chromosomes, and all the observed mitotic figures are a consequence of this. They can be reduced to the following types:

a) Metaphase chromosomes do not show a tendency to form a metaphase plate. They are spread out along the pollen tube (Fig. 9).

b) The number of chromosomes is reduced. Metaphase chromosomes incapable of normal division and movements form two, three or even four groups with a various number of bi-chromatid chromosomes.

The division of the chromosomes in the kinetochores usually occurs after the formation of one of the above mentioned mitotic figures. This division sometimes forms also before the formation of these groups of chromosomes and may lead to anaphase movements. The chromatids migrating relative to each other may then form chromatid bridges between the forming gametic nuclei (Fig. 7).

c) Diploidal restitution nuclei may form (Fig. 8).

*Allium cepa*. The percentage of spontaneous disturbances in the mitosis of the generative nucleus of *A. cepa* pollen tubes is very low (about 1%), these can therefore be neglect in our considerations of the effect of glycosides on this species.

On an agar medium with the glycosides it was possible to analyse the effect of concentrations 0.003% and 0.006%, below the critical concentration that inhibits germination (0.012%). Making use of the low insensitivity of the germinated pollen tubes to the action of glycosides and using the method of adding a fluid medium with the glycosides it was possible to study also the effect of higher concentrations (0.012% and 0.025%). Analysing the chromosomal patterns after the action of concentrations 0.003% and 0.006% it was established that in *A. cepa* the anaphase chromosome movement to the poles was not completely inhibited yet, the separation of the chromosomes was not normal. The final effect is that two (Fig. 2) or three unevensized male nuclei form which are sometimes linked by chromatid bridges. Restitution nuclei were not observed.

Under the influence of higher glycoside concentrations (0.012% and 0.025%) more numerous and more varied effects were obtained. The frequent disturbances in the mitosis of the generative nuclei lead to the formation of 2-, 3- (Fig. 3) or even 4-groups of chromosomes linked by chromatid bridges. Sporadically restitution nuclei form (Fig. 8). However even using these high concentrations, the anaphase movements of chromosomes were not completely inhibited, as was the case with *Tradescantia bracteata*.

#### DISCUSSION AND CONCLUSIONS

In both the studied species, *Tradescantia bracteata* and *Allium cepa* it was observed that the glycosides of oleander have an inhibitive effect on the germination of pollen grains and the growth of pollen tubes. The critical concentration which causes a complete inhibition of pollen grain germination is four times lower in *A. cepa* than in *T. bracteata*, which would indicate that the pollen grains of *Allium* are more sensitive to the

action of the studied reagent. Similarly the percentage of germinated pollen grains of *Allium*, relative to those of *Tradescantia* was much more reduced from the control value. Even the lowest concentration used (0.003%) causes a 15-fold decrease of pollen germination in *Allium* (from 30% to 2%), while in *Tradescantia* at this concentration the reduction is rather small (from 80% to 70%). A 15-fold decrease in germination percentage is obtained for *Tradescantia* just lately at a concentration of the glycosides amounting to 0.025%. The glycosides must be responsible for the inhibition of some physiological processes related to germination, and the degree of inhibition depends on the plant species. A reduction in the germination percentage and in the length attained by the pollen tubes is also clearly accompanied by a lowering of the viability.

A study of the effect of oleander glycosides on the divisions of the generative nucleus in both the species was not an easy matter. The difficulties concerned primarily *T. bracteata* and resulted from the fact that in the control material numerous spontaneous disturbances were observed in the mitotic figures, frequently identical to those which occur in the pollen tubes under the influence of the glycosides. These were observed by Eigsti (1940b) and by Sax and O'Mara (1941) in *Tradescantia*, one of the two species used in the present study. Thus using *T. bracteata* as the experimental material it was essential for the correct interpretation of the data to compare both the numerical and the qualitative nature of the disturbances occurring in the controls with those observable after the action of the glycosides.

The comparisons made have indicated that in *T. bracteata* not only is there a marked increase in the number of disturbances as a result of the action by the glycosides (from 12% in the control material to 60-70% after treatment with the glycosides), but also that there are differences in the types of disturbances and in the mechanism through which they are formed. It is true that such figures as two or three uneven male nuclei or the presence of bridges has been observed in both instances (in the controls and after glycoside treatment) however in the latter case they could have formed from the unwinding metaphase chromosomes lying along the pollen tube and unable to form a metaphase plate. Since the division of the kinetochores of metaphase chromosomes was not inhibited, the free chromatids (anaphase chromosomes) that did not migrate to the poles of the spindle by adjusting their positions relative to each other could have formed the bridges linking the male nuclei. The movements of these chromatids could have been caused by the slowed down cytoplasmic streaming in the pollen tube during mitosis of the generative nucleus (Venema and Koopmans, 1962), or by disturbances in the functioning of the mitotic spindle (Tarkowska, 1971b). The types of disturbances observed in *T. bracteata* seem to indicate rather the latter possibility. These anomalies and the presence of the restitution nuclei

seem to indicate that the mechanism of the formation of anomalies in the mitosis of the generative nuclei under the influence of the glycosides is different than in the case of the control material. The glycosides disorganize the mitotic spindle, which significantly affects the movements of the chromosomes, while the uneven divisions of the generative nuclei observed in the control material appear to be caused by lack of space in narrow pollen tubes for the migrating chromosomes.

The types of disturbances observed in *T. bracteata* were the same after the use of various concentrations (0.003%, 0.006%, 0.012%, 0.025%), after different durations of exposition to the glycosides and after use of different methods of administering the glycosides. The percentage number of disturbances at the studied concentrations was also similar (50%, 62%, 73%, 64%). The observed difficulties in the movement of the chromosomes in *T. bracteata* towards the metaphase plate and the lack of movement of chromatids to the poles during anaphase, the consequence of which are the figures described above, appear to indicate that the mitotic spindle was very seriously damaged. This is confirmed by the electron microscopic studies on the endosperm cells of *Haemanthus* (Tarkowska 1971b).

The number of spontaneous disturbances in the divisions of the generative nucleus in *T. bracteata* did not exceed 1%, we can therefore ignore them when discussing the abnormal mitotic figures observed after the action with the glycosides.

Conducting the culture on an agar medium with glycosides it was found that the pollen grains of *A. cepa* are very sensitive. The critical value completely inhibiting germination was 0.012% (for *T. bracteata* it was 0.05%). Using this method the effect glycoside concentrations of 0.003% and 0.006% was studied. These however, as was expected, were too low to completely disorganize the mitotic spindle. The movements of the chromosomes were not completely inhibited. Making use of the fact that glycosides are less toxic to the already germinated pollen tubes, and using the method of sprinkling glycosides contained in a fluid medium, it was also possible to study the effect of concentrations 0.012% and 0.025%. The effects of the glycosides were more distinct. More frequently it was possible to observe three male nuclei, occasionally diploid restitution nuclei. This seems to indicate that the mitotic spindle was more seriously damaged than following the treatment with the lower concentrations (0.003% and 0.006%). However the injury was not as high as was observed even when using the lowest glycoside concentrations on *T. bracteata*. On the other hand if one considers the effect of the glycosides on the germination and growth of the pollen tubes *A. cepa* is the more sensitive species. Thus the general physiological sensitivity does not go parallel to the mitotic sensitivity. Pollen grains and tubes of *T. bracteata* have a considerable mitotic sensitivity (the mitotic spindle is not very



resistant to the glycosides), and low physiological sensitivity. For the pollen grains and tubes of *A. cepa* the relations are opposite.

From the conducted studies it can be concluded that:

1. Glycosides of oleander inhibit the germination of pollen grains and *Allium cepa* is more sensitive in this respect than *Tradescantia bracteata*.

Pollen grains and tubes of *A. cepa* have a considerable physiological sensitivity and low mitotic sensitivity. In *T. bracteata* the relations are opposite.

2. Oleander glycosides significantly alter the course of mitosis of a generative nucleus in both the studied species.

a) The effect of the glycosides on the division of the generative nucleus depends on the concentrations used — that is the degree of damage of the mitotic spindle.

b) The following effects of the action of the glycosides were observed:  
— two or more uneven size male nuclei are formed, sometimes likened by chromatid bridges  
— restitution nuclei form.

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