

## Effect of morphactin (IT 3456) on pollen germination and pollen tube growth in four species of *Angiospermae*

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### Abstract

The action of morphactin in 10, 100 and 1000 ppm on pollen germination and tube growth was studied in four species: *Antirrhinum majus*, *Campanula rapunculoides*, *Eschscholtzia californica* and *Narcissus poeticus*. Pollen grains showed different sensitivity to particular concentrations of morphactin. Although the concentration of 1000 ppm inhibited completely pollen germination in *A. majus* and *C. rapunculoides*, it was not sufficient to suppress this process in *E. californica* and *N. poeticus*. Morphactin has a different effect on the percentage of germinated and ruptured pollen, tube length and its rate of growth. This effect was either inhibitory, neutral or stimulatory, depending on the morphactin concentration, the time of culture and species studied. The action of morphactin on the germination process is discussed.

### INTRODUCTION

It has been reported that morphactins (fluorene-9-carboxylic acid derivatives) are effective in retardation of the onset and progress of the generative phase, i.e. flowering and fruiting (see Schneider 1970, for review).

So far, to our knowledge, only one report on the effect of morphactin on male gametophytes (pollen grains of tobacco) exists (Shivanna 1973). Therefore, it seemed reasonable to investigate whether morphactin influences in a similar way the pollen of different species.

This study was designed to examine the action of morphactin on the *in vitro* germination characteristics of pollen grains of four species of *Angiospermae*. These characteristics include germination time (the period of pollen tube emergence), the per cent of germinated and ruptured grains, pollen tube length and growth rate in subsequent hours of culture.

## MATERIAL AND METHODS

Pollen grains of *Antirrhinum majus* L., *Campanula rapunculoides* L., *Eschscholtzia californica* Cham. and *Narcissus poeticus* L. were used. Fresh pollen grains collected directly from open anthers were cultured on medium prepared according to Brewbaker and Kwack (1963) and containing 1.5% bacto-agar, 0.03% calcium nitrate, 0.01% boric acid, 0.02% magnesium sulphate, 0.01% potassium nitrate and sucrose (for *Antirrhinum* and *Narcissus* — 2%, for *Campanula* and *Eschscholtzia* — 10%), supplemented with morphactin (10, 100 and 1000 ppm). A control containing no morphactin was included. After 1, 2, 3 and 4 h of culture the pollen grains were fixed in vapours of 4% formalin, buffered with phosphates to pH 7.2, during 10 min. and stained with acetocarmin solution for 10 min.

Each pollen grain was classified as either (i) germinated, (ii) ruptured, or (iii) intact. A grain was classified as germinated if a distinct tube had emerged from the single pore. The ruptured category consisted of those grains which ruptured through the pore.

Twenty randomly selected pollen tubes from the control and each concentration of morphactin, after each hour of culture were measured with the aid of a curvometer at  $\times 400$  magnification. Tubes which arose from grains producing more than one pollen tube were not measured.

## RESULTS

The time of pollen grain germination undergoes changes in the presence of morphactin (Table 1). The longest germination time was observed at the highest morphactin concentration (1000 ppm) in the case of pollen grains of *Eschscholtzia* and *Narcissus*. This concentration inhibited practically completely pollen germination of *Antirrhinum* and *Campanula*. With decreasing concentrations of morphactin the time of germination was shorter in all species studied, except *Eschscholtzia* in which emergence of tubes in 10 ppm morphactin and in control material occurs at the same time.

Table 1

Effect of morphactin on the time of pollen tube emergence (in min.). Each value represents the mean of 3 measurements

Species	Control	Morphactin, ppm		
		10	100	1000
<i>Antirrhinum majus</i>	80	85	90	—
<i>Campanula rapunculoides</i>	30	35	40	—
<i>Eschscholtzia californica</i>	40	40	45	50
<i>Narcissus poeticus</i>	25	40	45	50

Large differences in the percentage of germination were visible at three concentrations of morphactin (Fig. 1). In all species studied, each increase in the morphactin level produced a significant decrease in germination. The data in Fig. 1 indicate also that morphactin reduces the number of germinated pollen grains with the exception of *Antirrhinum majus* and *Narcissus poeticus* where it stimulates this process in a concentration of 10 and/or 100 ppm, beginning with 3 or 2 h of culture.

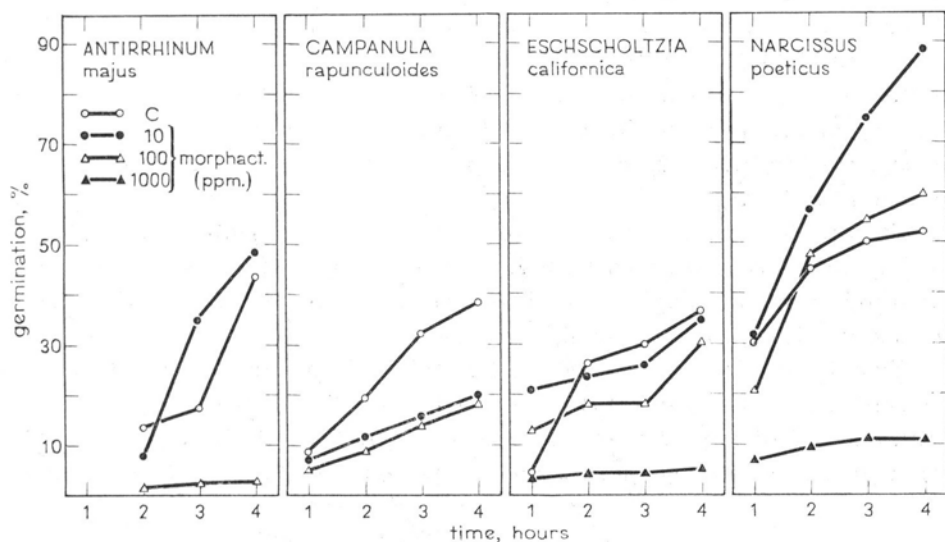


Fig. 1. Effect of various concentrations of morphactin on the percentage of germinating pollen grains

In the presence of morphactin numerous ruptured pollen grains appeared (Table 2). The highest number of ruptured pollen is observed at the lowest morphactin concentration (10 ppm) in all species studied and especially in *Narcissus* in which there is a 13-fold increase as compared with control material. A further increase in the morphactin level caused a diminution in the percentage of rupturing.

Table 2

Effect of morphactin on the percentage of rupturing pollen grains after 4 h culture. Each value represents the mean of 50 measurements

Species	Control	Morphactin, ppm		
		10	100	1000
<i>Antirrhinum majus</i>	5.1	8.5	5.6	—
<i>Campanula rapunculoides</i>	7.2	8.4	7.6	—
<i>Eschscholtzia californica</i>	3.4	6.8	3.5	1.8
<i>Narcissus poeticus</i>	1.1	14.3	9.7	2.4

Morphactin produced a strong effect on the length of pollen tubes (Fig. 2). The length of tubes in all species tested is inverse to the concentration of morphactin. All concentrations of morphactin inhibit more or less markedly pollen tube elongation with the exception of *Antirrhinum* and *Eschscholtzia*, where stimulation of tube length was visible in the presence of morphactin at 10 ppm after 4 h of culture.

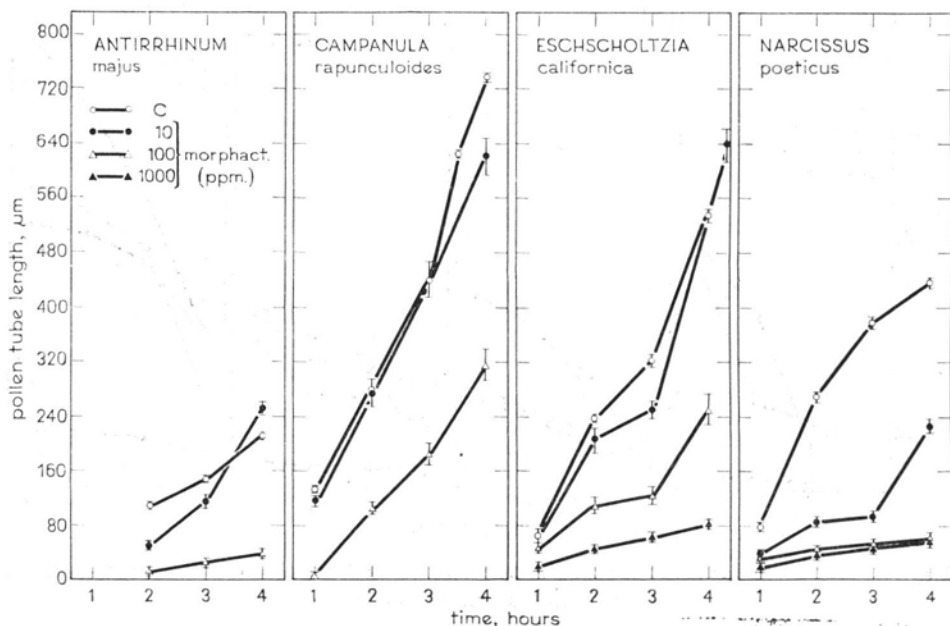


Fig. 2. Effect of various concentrations of morphactin on the elongation of pollen tubes

The rate of growth in the subsequent hours of culture differed significantly (Fig. 3). In general, however, the highest growth rate occurs in the presence of the lowest morphactin concentration i.e. 10 ppm after 4-h culture. The rate of pollen tube growth slowed down together with the increase of morphactin concentration. As indicated by Fig. 3, morphactin delayed the phase of stimulation, i.e. of rapid tube elongation, from 3 to 4 h or later in *Campanula* and from 2 to 4 h or later in *Narcissus*. The phase of stimulation appears in *Antirrhinum* after 4-h culture and it remains unaffected during culture in *Eschscholtzia*.

Cytomorphological observations indicate that the generative and vegetative nuclei, present in the pollen of all species tested at the time of anthesis, immediately after germination move into the tube. No alterations were observed in the nuclei caused by morphactin treatment.

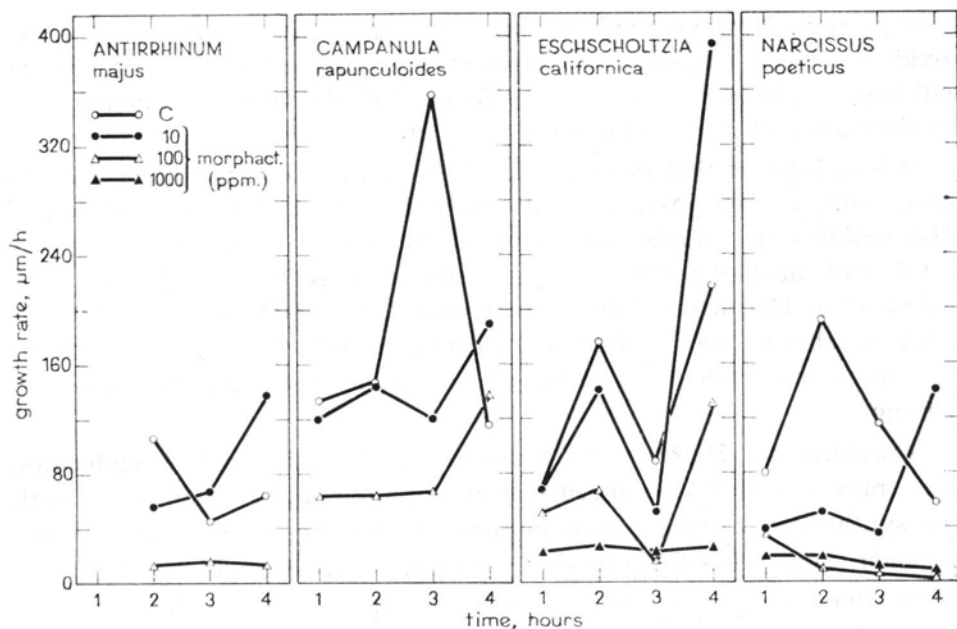


Fig. 3. Effect of various concentrations of morphactin on the rate of pollen tube growth

## DISCUSSION

Pollen grains of the four species tested showed different sensitivity to particular concentrations of morphactin. The concentration of 1000 ppm suppressed pollen germination in *Antirrhinum* and *Campanula* completely, whereas it was not sufficient to inhibit this process in *Eschscholtzia* and *Narcissus*. Morphactin action differed also in its effect on the percentage of germinated and ruptured pollen, tube length and rate of growth. Its effect was either stimulatory, neutral or inhibitory depending on the concentration and species tested.

Somewhat different results were obtained by Shivanna (1973) with tobacco pollen. This author reports that 1 or 10 ppm of morphactin do not affect germination, only higher concentrations (100, 500 and 1000 ppm) inhibit this process. Moreover, at 1, 10 and 100 ppm concentration tube growth is not inhibited up to 3 h of culture.

The nature of morphactin involvement in affecting pollen germination and tube growth as well as other processes in plants is not yet clear. According to Ziegler's (1970) opinion morphactin acts at the post-transcription level. It probably exerts its effect through reduction of the IAA level, by inducing higher IAA-oxidase activity. Thus, it seems possible that such a situation could occur during germination and pollen

tube growth. However, chlorogenic acid which is an inhibitor of IAA-oxidase activity caused only a marginal increase in the growth of crown gall tissue of *Datura innoxia* as compared with the growth on morphactin medium alone (Raste and Johri 1979).

It is of interest that in root cells of *Pisum sativum* growing on morphactin solution (100 ppm) a significant reduction in the incorporation of  $^3\text{H}$ -thymidine into nuclei was observed (Gabara 1982). Moreover, the number of labelled nuclei was diminished in pea root cells treated with morphactin. The above data indicate that morphactin acts rather at the DNA synthesis level. Whether morphactin influences DNA synthesis also in pollen nuclei it is difficult to say because such data are still lacking.

According to Mascarenhas (1975) the stable mRNA molecules, ribosomes and tRNA required for germination and early tube growth are synthesised during pollen maturation. Further pollen tube growth depends on new RNA synthesis in germinated pollen grains. If morphactin would act on the activity of stable RNA or new RNA synthesis one might expect that the early or further period of pollen tube growth should be affected and in consequence the tubes would be shorter. The pronounced increase in tube length and rate of growth in *Antirrhinum* and *Eschscholtzia* does not confirm this suggestion. It cannot be excluded, however, that the concentration of 10 ppm is too low to evoke the above changes.

The specific biological activities associated with morphactin are not clearly understood but the primary site of their action appears to be the membrane (Bridges and Wilkins 1973). It seems probable that the changes in the per cent of ruptured grains reported in this study are related to the morphactin effect on the pollen grain membrane. If this suggestion would be correct, the question arises why is the number of ruptured pollen higher in the lowest morphactin concentration.

#### Acknowledgment

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Wpływ morfaktyny (IT 3456) na kiełkowanie pyłku i wzrost łagiewek  
u czterech gatunków Angiospermae

Streszczenie

Badano wpływ morfaktyny, w stężeniach 10, 100 i 1000 ppm, na kiełkowanie pyłku i wzrost łagiewek u: *Antirrhinum majus*, *Campanula rapunculoides*, *Eschscholtzia californica* i *Narcissus poeticus*. Ziarna pyłku wykazują różną wrażliwość na poszczególne stężenia morfaktyny. Chociaż stężenie 1000 ppm hamowało całkowicie kiełkowanie pyłku u *A. majus* i *C. rapunculoides*, było ono niewystarczające do zatrzymania tego procesu u *E. californica* i *N. poeticus*. Morfaktyna w różny sposób wpływała na procent kiełkujących i pękniętych pyłków, długość łagiewek i tempo ich wzrostu. To działanie było hamujące, neutralne, bądź stymulujące w zależności od stężenia morfaktyny, czasu hodowli i badanego gatunku. Działanie morfaktyny na proces kiełkowania jest przedmiotem dyskusji.