Effect of boron on directionality of pollen tube growth in *Petunia* and *Agapanthus*

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The effect of boron on pollen tube growth was tested using *Petunia* Juss. styles and the semivivo technique, while *Agapanthus* L'Hérit. pollen was used for *in vitro* germination experiments. *Petunia* pollen tubes protruded only from the cut ends of styles incubated in media containing boron. When styles were incised between the cut end and the pollen tube front and either the cut end or the incision was exposed to a boron-containing medium while the other wounded area was exposed to a boron-free medium, the direction of pollen tube growth was changed. Pollen tubes protruded only from those wounds exposed to a boron-containing medium. When a boron gradient was created on agar strips and *Agapanthus* pollen was germinated *in vitro* alternatively on either the end containing a low or a high boron concentration, pollen tubes consistantly grew towards the higher boron concentrations. This is the first demonstration of a possible chemotropic response of pollen tubes to boron.

Die effek van boor op stuifmeelbuisgroei is ondersoek deur gebruik te maak van *Petunia* Juss.-style en die semivivo-tegniek, terwyl *Agapanthus* L'Hérit.-stuifmeel gebruik is vir *in vitro*-ontkiemingseksperimente. *Petunia*-stuifmeelbuise het slegs gegroei uit dié afgesnyde basisse van style wat in boorbevattende media geplaas is. Wanneer 'n klein insnyding in die style gemaak is tussen die afgesnyde basis en die stuifmeelbuisfront en óf die basis óf die insnyding aan 'n boorbevattende medium blootgestel is, terwyl die ander wond aan 'n boorvrye medium blootgestel is, het stuifmeelbuise slegs uit dié wond gegroei wat aan 'n boorbevattende medium blootgestel is. Toe 'n boorgradient op agarstroke geskep is en *Agapanthus*-stuifmeel *in vitro* alternatiewelik aan die kant met óf hoë óf lae boorkonsentrasie ontkiem is, het stuifmeelbuise deurgaans in die rigting van die hoër boorkonsentrasies gegroei. Dit is die eerste demonstrasie van 'n moontlike chemotropiese reaksie van stuifmeelbuise op boor.

Keywords: Chemotropism, semivivo technique, pollination

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Introduction

As far back as 1889 Molish showed that a chemotropic substance diffused from freshly cut styles into gelatin. During the ensuing 70 years, little progress was made towards establishing the chemical composition of chemotropic substances in gynoecial tissues. In 1961 Rosen found chemotropic activity in pistil parts of *Lilium* L. (Rosen 1961) and a year later Mascarenhas & Machlis (1962) demonstrated a pollen tube chemotropic factor from gynoecial tissues of *Antirrhinum* L. In 1964 the same authors reported that calcium had a chemotropic effect on pollen tube growth. Kandasamy & Kristen (1987a) researched possible polarity or chemotropic gradient in the style of *Nicotiana* L. but found none. In the most recent review on pollen tube tip growth, Steer & Steer (1989) referred to the chemotropic effect of calcium on pollen tube growth, but Sanders & Lord (1989) state that, in spite of the '... extensive literature on the theory of chemotropic factors in the style that guide the tubes to the ovary, no substance has yet been isolated'. Rosen (1968) in his valuable review on pollen tube chemotropism criticizes some of the methods that have been used in the past to demonstrate this phenomenon and also proposed criteria for unambiguous assays.

It is therefore clear that except for calcium, no other substance having a chemotropic effect on pollen tube growth has yet been positively identified. In this paper we supply evidence that pollen tubes of *Petunia* Juss. and *Agapanthus* L'Hérit. grow towards an externally supplied boron source and towards the higher boron concentration in an artificially created boron gradient.

Materials and Methods

Boron concentrations (semivivo technique, as applied by Brewbaker & Majumder 1961)

Plants of *Petunia* 'Pink Satin' were grown in a standard Hoagland's nutrient medium in growth chambers under controlled conditions (day phase: 12 h, temp. 25°C; night phase: 12 h, temp. 25°C). Flowers were emasculated prior to anthesis and covered with small paper bags. After anthesis flowers were hand pollinated with fresh pollen and left *in situ*. Twenty hours after pollination the styles were cut above the ovary.

Cut ends of styles were immediately dipped in a simplified version of Brewbaker's medium (Mulcahy & Mulcahy 1985) containing different boric acid concentrations varying with 1-mg dm^{-3} increments from 0 mg dm^{-3} to 10 mg dm^{-3}, with 10-mg dm^{-3} increments to 100 mg dm^{-3} and with 100-mg dm^{-3} increments to 1 000 mg dm^{-3}. The styles were then incubated in the media (Figure 1) for a further 24 h, fixed in alcohol/acetic acid, cleared in 8N KOH, stained with analin blue and investigated using a light microscope fitted with epifluorescence optics (Linskens & Esser 1957; Martin 1959).

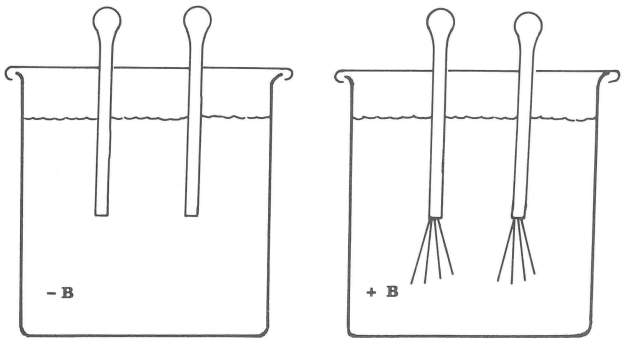


Figure 1 Cut ends of pollinated *Petunia* styles in growth media either containing no boric acid (–B) or boric acid of varying concentrations (+B). Pollen tubes protruded from styles in +B media only.

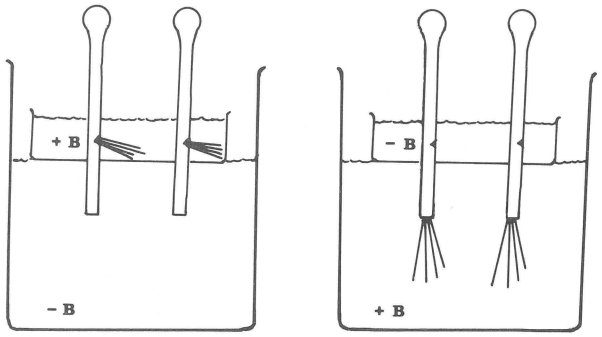


Figure 2 Pollinated *Petunia* styles, cut above the ovary and superficially incised, set through holes in petri dishes with either boric acid-containing media (+B) or boric acid-free media (–B). Petri dishes are floated on growth media with either +B or –B. Pollen tubes protruded only from wounds exposed to +B media.

Directional growth (semivivo technique)

Using the same *Petunia* plants as those described above, pollinated styles cut above the ovary were superficially incised well below the predetermined pollen tube front. The simplified Brewbaker's growth medium was modified either to contain 100 mg dm^{-3} boric acid or no boric acid.

For one part of the investigation, the cut ends of some styles were set through small holes made in plastic petri dishes so that the incision was positioned slightly above the bottom of the dish. Holes were sealed with petroleum jelly.

Half the number of petri dishes with styles were filled with the boron-containing medium and floated on the boron-free medium while the rest were filled with the boron-free medium and floated on the boron-containing medium (Figure 2).

For the other part of the investigation, some incised styles were positioned in the boron-containing medium and some in the boron-free medium, always ensuring that both cut ends and incisions were covered by the medium.

Incubation time and further treatment of styles were performed as above.

Directional growth (*in vitro*)

Hot autoclaved nutrient solutions consisting of 20% sucrose, 3 mM calcium nitrate in de-ionised water, 2% Difco agar and three different concentrations of boric acid (0 mg dm^{-3}, 100 mg dm^{-3} and 300 mg dm^{-3}) were poured into sterile petri dishes to a height of 3 mm and allowed to solidify. Pieces of agar, 3 mm × 5 mm were removed from each plate, transferred to a sterile petri dish and arranged in 5 mm × 9 mm strips in order to create a boron gradient varying from 0 mg dm^{-3} to 300 mg dm^{-3} boric acid. Pollen was placed either on the end with low boric acid concentration or on the end with high boric acid concentration. 'Pollinated' strips were incubated at 25°C for 8 h; pollen tube growth was studied using the light microscope.

Boron analysis

More than 500 *Petunia* flowers were collected. The pistils were separated into stigmas, styles and ovaries which were dried, ground and analyzed according to the ethanol extraction method of Bessinger (1988).

Results

Boron concentrations

In the boron-free medium pollen tubes stopped growing shortly after removal of styles from the plant and no pollen tubes protruded from the cut ends (Figures 1 & 3). In the media containing boron, pollen tubes started to protrude from the cut ends in boric acid concentrations from 9 mg dm^{-3} and there was a relationship between the concentration of boron in the medium and the length of the pollen tubes (Figures 3–6). Good pollen tube growth was obtained at boric acid concentrations between 50 mg dm^{-3} and 500 mg dm^{-3}. Optimal pollen tube growth was found at concentrations between 100 mg dm^{-3} and 200 mg dm^{-3} boric acid while in media with concentrations higher than 500 mg dm^{-3}, pollen tube growth was impaired and the tubes started producing swollen tips or constrictions. At concentrations of 800 mg dm^{-3} and higher no tubes protruded from the cut ends.

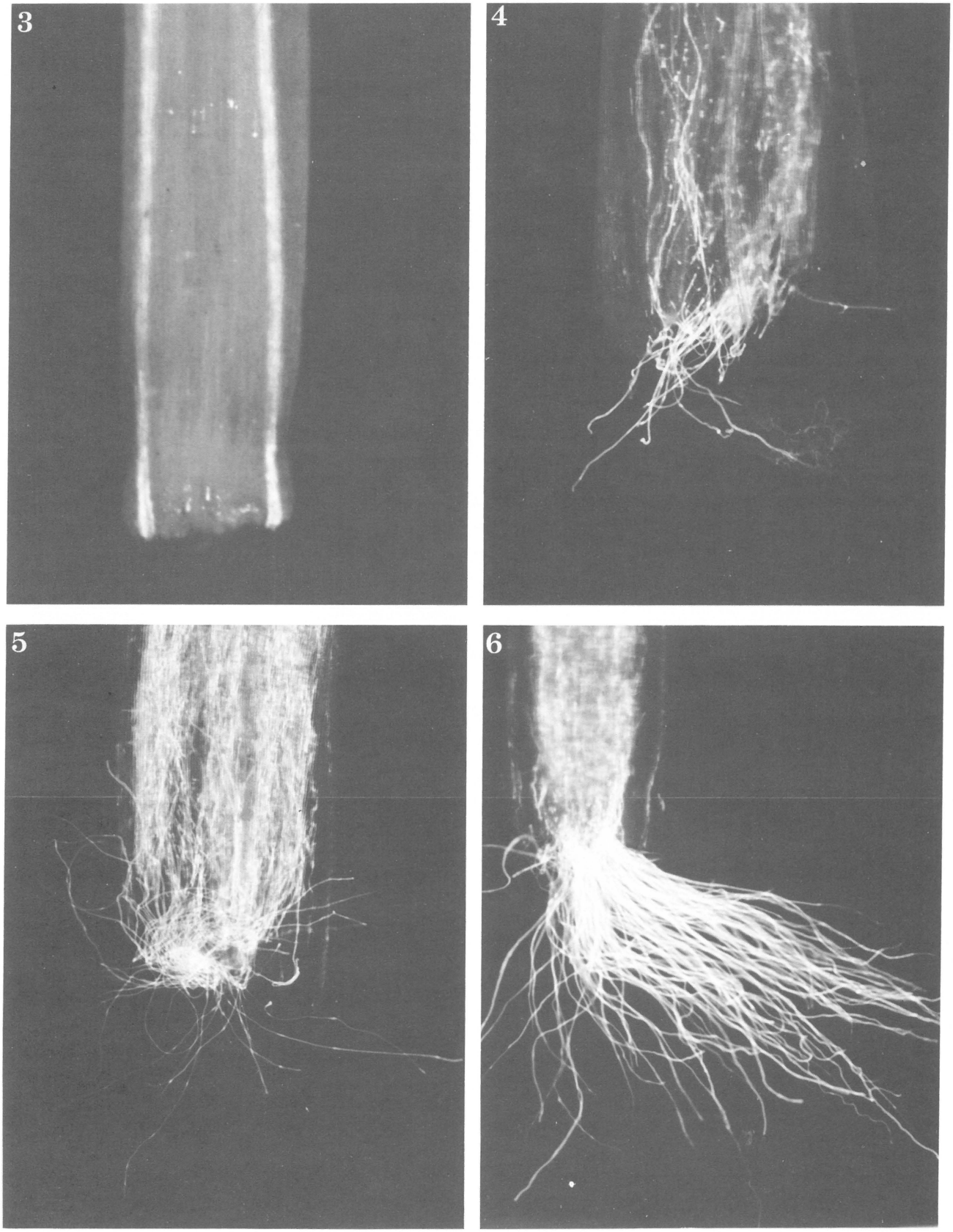
Directional growth (semivivo)

In the experiment where both the cut end of the style and the incision were exposed to boron, and the incision was made well ahead of the pollen tube front, pollen tubes protruded from the lesion only, but when the incision was made at the pollen tube front, tubes protruded from both the cut end and the incision. When either the incision or the cut end alone was exposed to a boron-containing medium, pollen tubes protruded from the lesion exposed to boron only (Figures 2, 7 & 8).

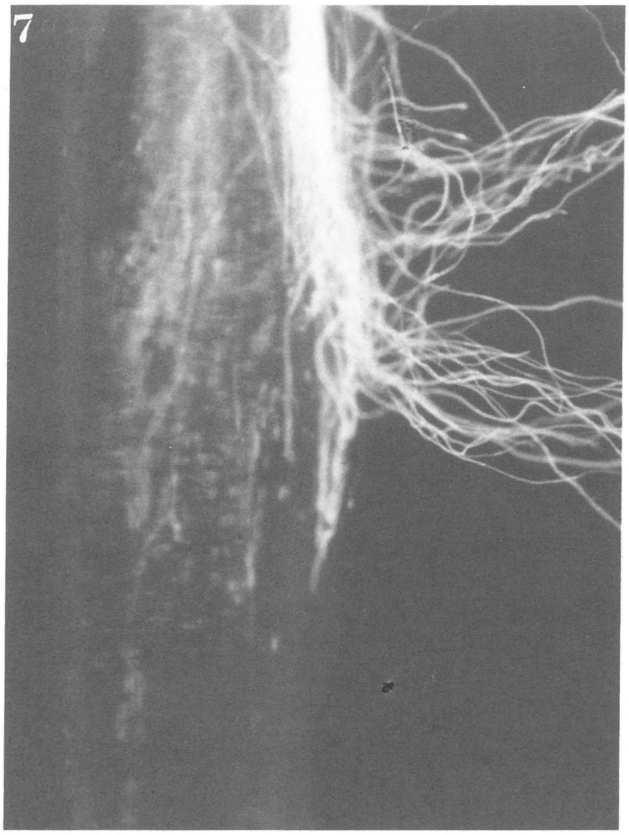
Directional growth (*in vitro*)

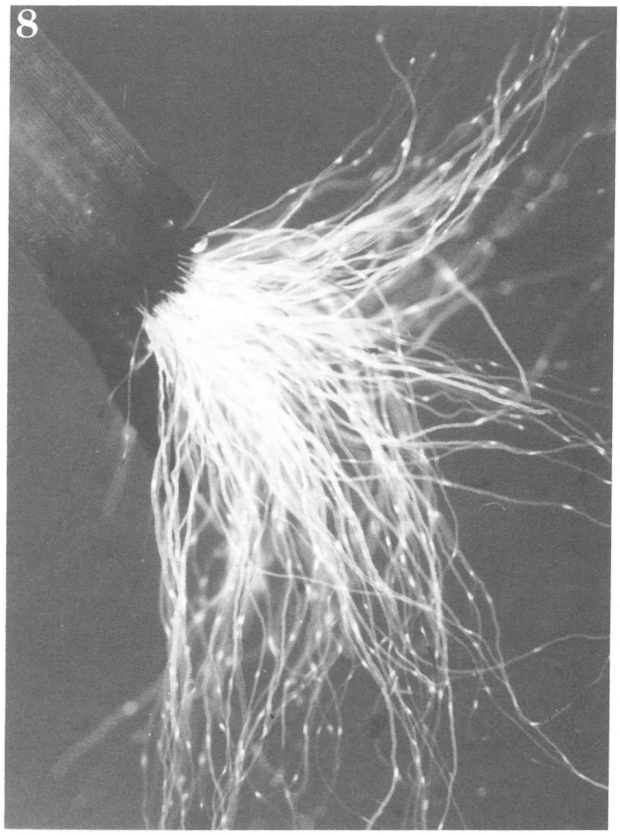
When *Agapanthus* pollen was placed at the agar strip end with the higher boron concentration, most of the

pollen grains germinated, but the tubes grew disorganized in all directions (Figure 9). When pollen was placed on the –B end of the strip fewer pollen grains germinated, but after initial disarranged growth, pollen tubes grew towards the higher boron concentration in the agar strip (Figure 10).



Figures 3–6 Pollinated *Petunia* styles incubated in varying concentrations of boric acid, illustrating the relationship between pollen tube growth and boric acid concentration of the media. 3: 0 mg dm^{-3}; 4: 20 mg dm^{-3}; 5: 50 mg dm^{-3}; 6: 500 mg dm^{-3}.





Figures 7 & 8 Pollinated *Petunia* styles were cut above the ovary and superficially incised well ahead of the pollen tube front. When either the incision (Figure 7) or the cut end (Figure 8) alone was exposed to a boric acid-containing medium (+B), pollen tubes protruded from the lesion exposed to +B only.

Boron analysis

Details are provided in Figure 11.

Discussion

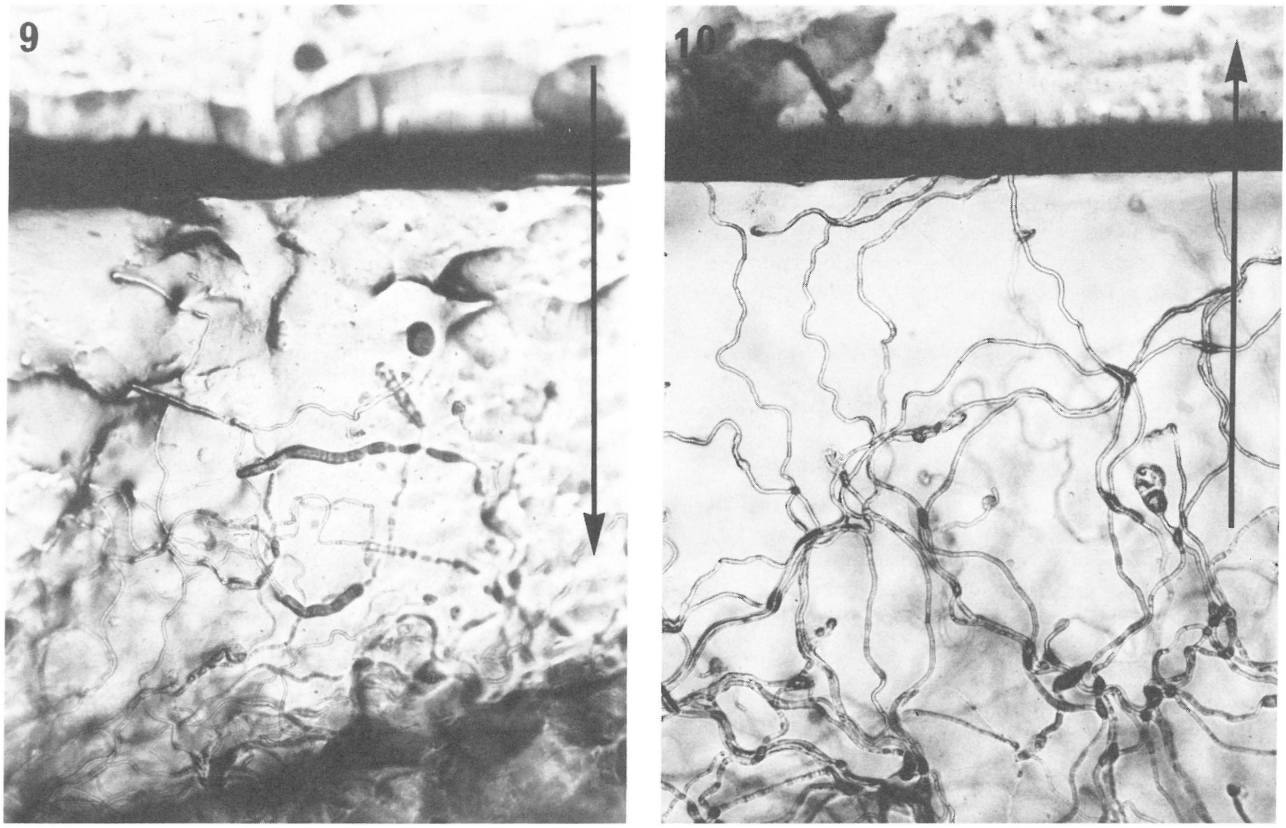
Boron concentration

The *in vivo Petunia* styles contained 37 mg kg^{-1} boron, (Figure 11) which is about 20 mg kg^{-1} more than the 17.5 mg kg^{-1} boron needed for optimal *in vitro* pollen tube growth reported by Dickinson (1978) for *Lilium*, by Kandasamy & Kristen (1987a, b) for *Nicotiana* and by Rosen (1961) for several other species. The arresting of pollen tube growth in the semivivo-cultured *Petunia* styles in a –B medium could be attributed to the fact that most of the boron in plant tissues is bound to cell walls (Dugger 1983) and therefore not available to the growing pollen tube tips. Additional amounts of 17.5 mg kg^{-1} to 35 mg kg^{-1} free boron (100 mg dm^{-3} to 200 mg dm^{-3} boric acid) or a total concentration of between 54 mg kg^{-1} and 72 mg kg^{-1} boron in the styles is therefore needed before optimal pollen tube growth can be attained. This is in line with our results regarding avocado (Robbertse *et al.* in press) where between 50 mg kg^{-1} and 75 mg kg^{-1} boron in the flower parts (with some accumulation in the pistil) is needed for optimal pollen tube growth. In these investigations the boron concentration of the flowers before additional boron was added, was 40 mg kg^{-1}. It could be argued that for *in vitro* pollen tube growth, only the 'free boron' complement would be needed for optimal growth which is 63–37 or 26 mg kg^{-1} for *Petunia* and 72–40 or 32 mg kg^{-1} for avocado, if averages are considered. These figures are in accordance with the results Vasil (1963) obtained with a number of different species. More research is needed to test this 'free boron' hypothesis.

Chemotropism

The epidermis of the *Petunia* style has a fairly thick cuticle. This probably prevents free entrance of the growth medium into the transmitting tissue of the style except through the cut end and the incision. In the experiments with the incision in contact with the +B medium (Figure 2), the part of the style below the incision was supposed to contain 37 mg kg^{-1} boron. In spite of that the pollen tubes grew against the direction of incoming lower concentration (17.5 mg kg^{-1}) free boron from the nutrient solution and protruded through the incision (Figure 7). In the experiments where the cut end of the style was in direct contact with the boron-containing medium, the pollen tubes again grew towards the incoming boron, passed the incision and protruded through the cut end of the style (Figures 2 & 8).

The fact that the pollen tubes consistently followed the shortest route to the boron supply must be regarded as a clear demonstration of directionality, possibly chemotropism. Supporting evidence was obtained with *Agapanthus* where pollen tubes grew towards the higher boron concentrations in the agar strips. We tried both



Figures 9 & 10 Agar strips in which a boron gradient was created were pollinated with *Agapanthus* pollen, either on the end with low or on the end with high boron concentration. On the agar strip end with high boron concentration, pollen tubes grew disorganized (Figure 9); on the end with low boron concentration, pollen tubes, after initial disorganized growth, grew towards the higher boron concentration (Figure 10). Arrows point to high boron concentration.

the 'surface test' (Mascarenhas & Machlis 1962) and the 'depression test' (Mascarenhas & Machlis 1964), but since it was very difficult to create a satisfactory gradient with these methods, better results were obtained with our method. In the experiment where the pollen was placed at the –B end of the agar strip, the pollen tubes grew towards the incoming boron diffusing from the other end of the agar strip, as was the case in the semivivo experiments.

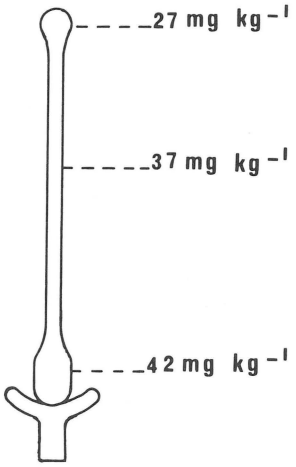


Figure 11 Boron concentration of flower parts of *Petunia* plants grown under controlled conditions.

If it is true that pollen tubes grow towards a higher boron concentration or against the direction of boron flow, the question still remains how this happens in the *in vivo* situation. Mulcahy & Mulcahy (1985, 1987) demonstrated the influence of the ovary on pollen tube growth and pollen tube directionality in *Petunia* hybrids and *Nicotiana alata*, and Mascarenhas (1966) found that the highest concentration of ionic calcium in the pistil of *Antirrhinum majus* occurred in the ovary. We showed that the boron concentration in the ovary of *Petunia* is higher than in the rest of the pistil and suggest that there must be some unknown interaction between boron and calcium in the pistil. The ovary probably acts as a boron sink during flower maturation, creating a boron gradient in the pistil. After pollination the growing pollen tubes form a new drain for boron and boron starts flowing from the ovary in the direction of the stigma causing directional growth of the pollen tubes.

Acknowledgements

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