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Induction of red pigment in white petals of *Hippeastrum* x Hybr. Hort. during infection by *Phoma Narcissi* (Aderh.) Boerema, De Gruyter et Noordel., Comb. Nov. and by mechanical injuries

ALICJA SANIEWSKA*, BARBARA DYKI**

*Research Institute of Pomology and Floriculture, Pomologiczna 18, 96-100 Skierniewice, Poland,
**Research Institute of Vegetable Crops, Konstytucji 3 Maja 1/3. 96-100 Skierniewice, Poland

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Abstract

Red colouration of tissues occurs as a result of mechanical injuries to petals of *Hippeastrum* hybr. hort. cv. Winter Carnival or their infection by *Phoma narcissi*.
Microscopic study recorded red pigment formation following mechanical injuries or pathogenic infection of white petals. For comparison, localization of anthocyanins in natural red petals of cv. Red Lion was determined. Red pigment, formed in white petals mechanically injured or infected by *Phoma narcissi*, is different than anthocyanins.
Key words: *Hippeastrum*, petals, *Phoma narcissi*, mechanical injuries, infection, red pigment formation.

INTRODUCTION

Phoma narcissi (Aderh.) Boerema, de Gruyter et Noordel., comb. nov. (syn. *Stagonospora curtisii* /Berk./Sacc.) causes red spot on various organs of *Hippeastrum* and pertains to the most serious pathogens of this species (Tapio 1966, Saniewska and Orlikowski 1984). Reddish colouration of different organ tissues of *Hippeastrum* is also induced by mechanical injuries (Tapio 1966, Saniewska and Saniewski 1992). Tapio (1966) suggested that reddish colouration of *Hippeastrum* tissues is caused by anthocyanins formation. Saniewska and Saniewski (1992) found that reddish colouration in the mechanically injured tissues or in the tissues infected by *Phoma narcissi* is caused by a compound different than anthocyanins.

The aim of the present work was anatomical observation of red pigment formation in white petals of *Hippeastrum* during infection by *Phoma narcissi* and by mechanical injuries.

MATERIAL AND METHODS

Petals of two *Hippeastrum* cultivars: red (Red Lion) and white (Winter Carnival) were used in the study (Fig. 1). The surface of white petals was inoculated with discs (3mm) of *Phoma narcissi* mycelium cultured for 7 days on potato-dextrose-agar (PDA) or with 20 µl inoculum of *Phoma narcissi* spores of a density equal to 600 spores/ml inoculum. The plants with inoculated petals were kept under a polythene tent, placed on glasshouse window-sill. The control consisted of petals provided with discs of potato-dextrose-agar or a drop (20 µl) of distilled water.

For mechanical injuries, white petals were either cut with razor blade on the length about 5 mm or punctured with a needle and incubated 4 days under a polythene tent with about 90% humidity and temperature varying from 20°C--25°C. Red spot of white petals, as result of mechanical injuries, were inoculated by *Phoma narcissi* and incubated as described above.

Fig. 1. Flowers of two *Hippeastrum* cultivars
a – red (Red Lion); b – white (Winter Carnival)

Material for microscopic examination was obtained 1, 2, 3, 4 and 5 days after inoculation. It intended to compare the localization of red pigment (anthocyanin) naturally occurring in red petal cells to that formed in white petals in response to infection by mycelium/spores of *Phoma narcissi* or to mechanical injuries.

The epidermis was isolated with cellotape, from fresh material or stained with toluidyne blue and closed in glycerol (Dyki and Habdas 1996).

RESULTS AND DISCUSSION

Mechanical injuries and infection with *Phoma narcissi* of white petals of *Hippeastrum* cv. Winter Carnival induced red pigment formation (Fig. 2, 3).

Epidermal cells of abaxial (outer) side of a natural red petal (cv. Red Lion) are of a characteristic elongated shape, with pigment (anthocyanin) localized in the vacuole that fills almost an entire cell (Fig. 4). This is particulary conspicuous when some cells lose their turgor and show a tendency to plasmolysis. In such a case the tonoplast keeps a certain distance from the cell wall, limiting the red colour (Fig. 4).

A different localization of the red pigment occurs in a white petal either mechanically damaged (Fig. 2) or penetrated by mycelium of *Phoma narcissi* (Fig. 3). After a needle injury, the pigment appears around the punctured spots, then progresses along the cell walls (Fig. 5). Also, an injury to the epidermis by fungal hyphae induced red pigment formation, beginning at the spot of initial damage to the cell wall and gradually spreading to the cells adjacent to a hyphae (Fig. 6, 7). Cell plasmolysis is observed at the same time.

Epidermal cells of adaxial (inner) side of natural red petals have convex external walls and their basal parts arranged in a compact polygonal system. Anthocyanin(s) is localized in the central part of a cell including the vacuole (Fig. 8). In white petal mechanically damaged the pigment expands from the spot of injury along the cell walls (Fig. 9). Mycelial penetration through the inner epidermis of a white petal also results in pigment formation along the cell walls, accompanied by cell plasmolysis. Stomata cells are also affected by the pathogen and synthesize red pigment (Fig. 6, 10, 11).

In fungus-infected white petals with colouration previously induced by mechanical injuries, mycelium grows towards white (intact) cells, avoiding red areas and necrotic spots (Fig. 12, 13).

There is still unknown mechanism of red pigment biosynthesis in white petals of *Hippeastrum* due to infection by *Phoma narcissi* or to mechanical injury. Results of the initial research imply that such pigment is of a phenolic nature (Saniewska and Budzinowski 1997). Neither is known the role played by this pigment in infection of *Hippeastrum* tissues by *Phoma narcissi*.

Wagner and Hrazdina (1984) documented that endoplasmic reticulum is a site of phenylopropanoid and flavonoid metabolism in petals of *Hippeastrum* cv. Dutch Red hybrid.

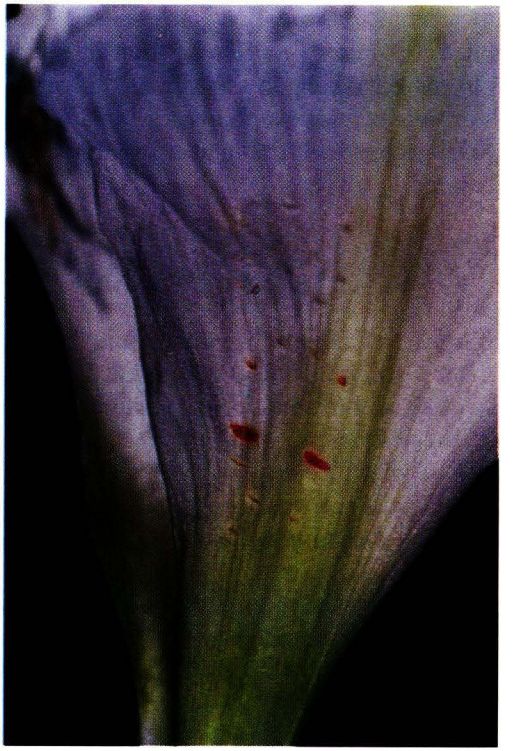


Fig. 2. Reddish colouration on white petals of *Hippeastrum* cv. Winter Carnival after mechanical injuries (cutting).

Fig. 3. Red spot on white petals of *Hippeastrum* cv. Winter Carnival caused by *Phoma narcissi.*

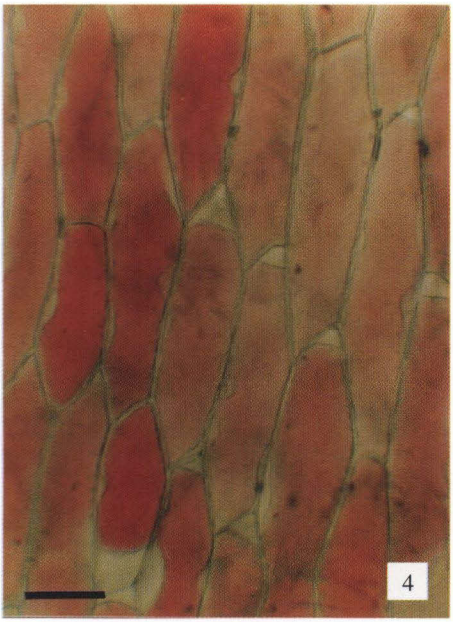


Fig. 4. Abaxial epidermis of natural red petals of *Hippeastrum* cv. Red Lion; anthocyanins can be observed. Scale bar = 100 μm

Fig. 5. Abaxial epidermis of white petals of *Hippeastrum* cv. Winter Carnival after mechanical injuries; red pigment formation can be observed.

Fig. 6 and 7. Abaxial epidermis of white petals of *Hippeastrum* cv. Winter Carnival after infection by *Phoma narcissi*; hyphae and red pigment formation can be observed. Scale bar = 100 μm

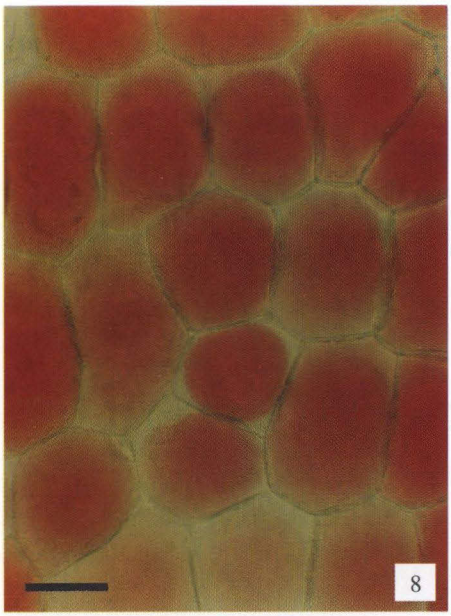


Fig. 8. Adaxial epidermis of natural red petals of *Hippeastrum* cv. Red Lion; anthocyanins can be seen. Scale bar = 100 μm

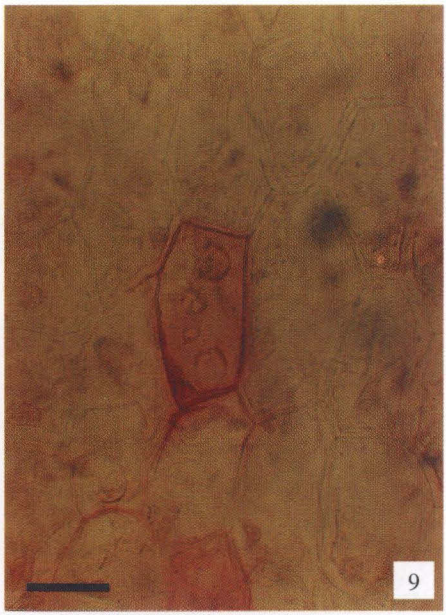


Fig. 9. Adaxial epidermis of white petals of *Hippeastrum* cv. Winter Carnival after mechanical injuries; red pigment formation can be seen. Scale bar = 100 μm

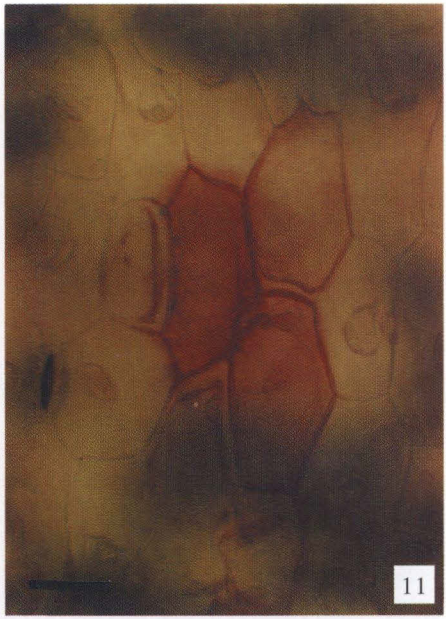
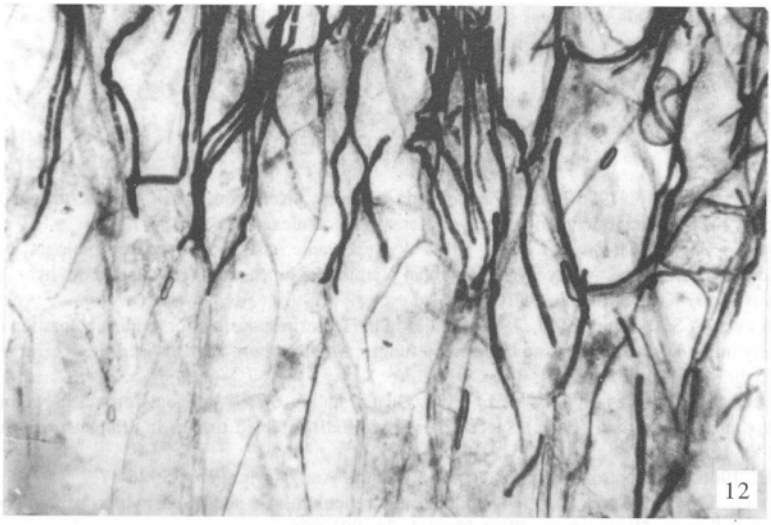


Fig. 10 and 11. Adaxial epidermis of white petals of *Hippeastrum* cv. Winter Carnival after infection by *Phoma narcissi*; red pigment formation can be seen. Scale bar = 100 μm



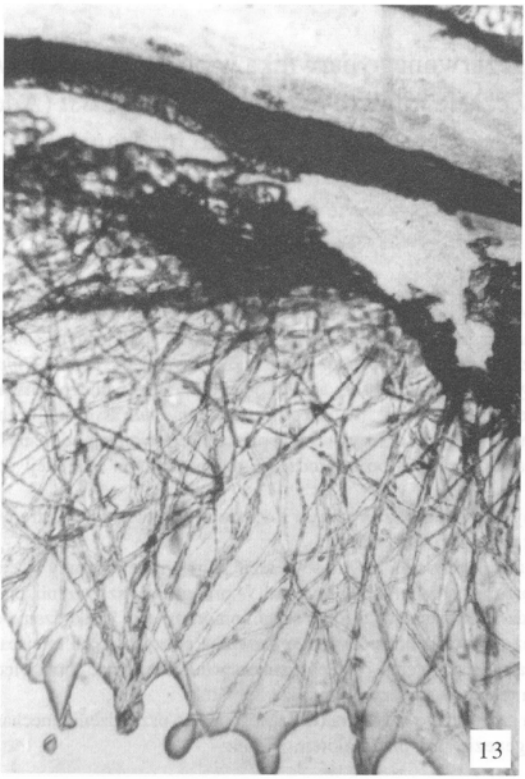


Fig. 12 and 13. Penetration of mycelium of *Phoma narcissi* on inner side of white petals of *Hippeastrum* cv. Winter Carnival following mechanical injury and formation of red pigment; the growth of hyphae occurs in the direction of intact cells. Scale bar = 100 μm

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Tworzenie się czerwonego barwnika w białych płatkach *Hippeastrum* x Hybr. Hort. w czasie infekcji przez *Phoma Narcissi* (Aderh.) Boerema, De Gruyter et Noordel., Comb. Nov. i po mechanicznym uszkodzeniu

Streszczenie

Phoma narcissi (Aderh.) Boerema, de Gruyter et Noordel., comb. nov. (*Stagonospora curtissi* /Berk./ Sacc.) powoduje czerwoną plamistość różnych organów *Hippeastrum* i jest jednym z najgroźniejszych patogenów tego gatunku. Mechaniczne uszkodzenie również powoduje czerwienienie tkanek *Hippeastrum*.

Celem badań były anatomiczne obserwacje tworzenia się czerwonego barwnika w białych płatkach *Hippeastrum* odm. Winter Carnival w czasie infekcji przez *Phoma narcissi* i po mechanicznym uszkodzeniu. Dla porównania określono lokalizację antocyjanów w płatkach czerwonych odmiany Red Lion. Tworzący się czerwony barwnik w płatkach białych po uszkodzeniu mechanicznym i infekcji przez *P. narcissi* nie jest antocyjanem.

Komórki epidermy odosiowej (zewnętrznej) i doosiowej (wewnętrznej) strony naturalnie czerwonego płatka zawierają barwnik antocyjan w wakuoli.

Inną lokalizację barwy czerwonej można stwierdzić w białym płatku przy uszkodzeniu mechanicznym, czy też po uszkodzeniu przez grzybnię *Phoma narcissi*. W przypadku uszkodzenia płatka igłą barwnik tworzy się wokół miejsca uszkodzenia postępując wzdłuż ścian komórkowych. Uszkodzenie epidermy przez strzępki grzyba powoduje również powstawanie czerwonego barwnika poczynając od miejsca, w którym uszkodzona została ściana komórkowa przez patogena i postępuje sukcesywnie obejmując coraz więcej komórek stykających się ze strzępką. Zjawisku temu towarzyszy plazmoliza komórek.

Fizjologiczna rola czerwonego barwnika tworzącego się po uszkodzeniu mechanicznym i infekcji przez *Phoma narcissi* w patogenezie jest przedmiotem badań.