Some structural characteristics of the flowers of apple cultivars with different susceptibility to fire blight

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Summary: Several morphological characters of the hypanthium (size and form of the surface, the shape of the hypanthium) and anatomical traits (number and pattern of stomata) of apple cultivars (*Malus domestica* L.) with different susceptibility to fire blight were studied. The size of hypantium surface was calculated by modelling the hypanthium with a truncated cone. Three types of hypanthium surface form have been revealed: straight, convex and a complex "shouldered" one. The angle between the style and the wall of the hypanthium was narrow or wide. The stomata on the hypanthium surface can be arranged in a zone in the middle third of the hypanthium or dispersed more or less evenly. The number of stomata/flower substantially differed among the cultivars examined. The highest stomata number was detected in the flowers of the tolerant cultivar (Freedom)

No single characteristics of the hypanthium could convincingly be correlated with susceptibility to fire blight. We suggest, however, that combination of morphological properties that sustain moist environment in the hypanthium contribute to susceptibility.

Key words: apple cultivars, hypanthium size and shape, pattern and number of stomata, *Erwinia amylovora*

Introduction

Fire blight as a seriouos disease of plants belonging to the family of *Rosaceae* (*van der Zwet & Keil, 1979; Vanneste, 2000*) has been known for more than a century. Among the economically most important species susceptible to the disease is the apple (*Malus domestica* L.). Research, therefore, focuses mainly on apple cultivars. The flower is the primary site of infection, though leaves, and shoots can also suffer of a serious attack of *Erwinia amylovora* (Burill) Winslow et al. The pathogen is transmitted by the aid of wind, rain or insect pollinators (*Pierstorf & Lamb, 1934; Thomson et al., 1999*). Bacteria colonize primarily the stigma (*Thomson, 1978; Thomson, 1986; Gouk & Thomson, 1999*) and at the optimal level of temperature multiply to a great number. Wetting events in the form of rain or dew facilitate the movement of bacteria from the flower stigmata to the inner part of the floral cup (hypanthium), where infection commonly occur through the nectary stomata (nectarthodes) (*Thomas & Ark, 1934; Pusey, 1999*). Moisture in the floral cup links the stigmatic surface with the hypanthium surface in the form of a watery film. In this thin layer of water develops a concentration gradient of nectary compounds which also helps the further distribution of bacteria on the hypanthium surface (*Raymundo & Ries, 1998*) and the penetration through open nectary stomatas.

It was suggested recently (*Mihalik et al. in press*) that morphological characters of the hypanthium might contribute to susceptibility to fire blight. The aim of this work was to study several of these properties of a number of cultivars to see whether correlation could be found between floral morphology and susceptibility to *Erwinia amylovora*.

Materials and methods

Morphological characteristics

Eight apple cultivars were studied: Freedom, Gala Must, Jonagold Decosta, Idared, Jonica, King Jonagold, Pinova and Sampion. Previous observations revealed that the size of the hypanthium changed with the ageing of the flowers. Therefore the flowers selected were in the same stage of development and from the same position in the inflorescence. The flowers opened on the day of examination were cut in half longitudinally for stereo-binocular microscopy, during which pictures were taken for further analysis. Since the shape of the hypanthium is similar to a

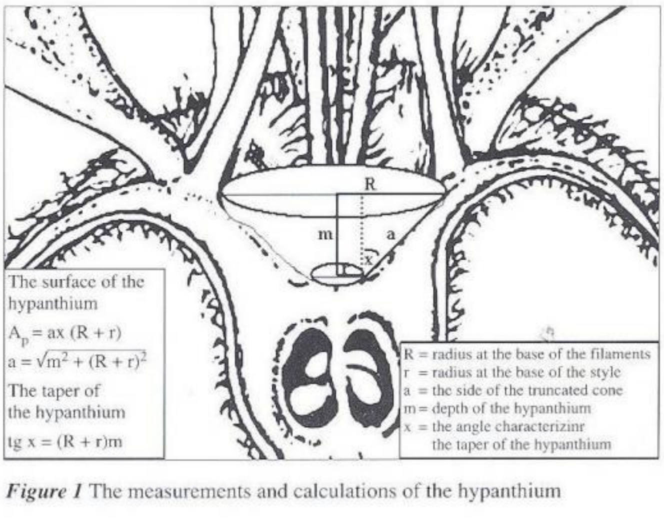

Figure 1 The measurements and calculations of the hypanthium

truncated cone, we characterized their surface accordingly. For this calculation the width (at the level of the base of the filaments and at the base of the style) and the depth of the hypanthium were measured, as well as the surface area of the hypanthium was calculated *(Figure 1)*. The photos were used to detect and measure the shape of the hypanthium surface, the angle between the style and the hypanthium wall and, to estimate the hairiness of the lower part of the style.

Anatomical characters

Hypanthia were separated from the longitudinally cut halved flowers and were fixed. After chemical dehydration specimens were dried in a critical point dryer, coated with gold and viewed in a Hitachi S 2400 scanning electron microscope. We examined the position of the guard cells to the epidermis, and the pattern and number of stomata.

Results and discussion

Hypanthium morphology

For all cultivars tested, the surface of hypanthia varied between 1,6 mm^2 and 8,1 mm^2 *(Figure 2)* and could be divided into two classes. The single member of a group having the smallest area was cv. Idared (1.6 mm^2). For the other cultivars this value varied in the range of 5.8 and 8.1 mm^2 but no significant differences were found in any of the pair-wise comparisons within this class.

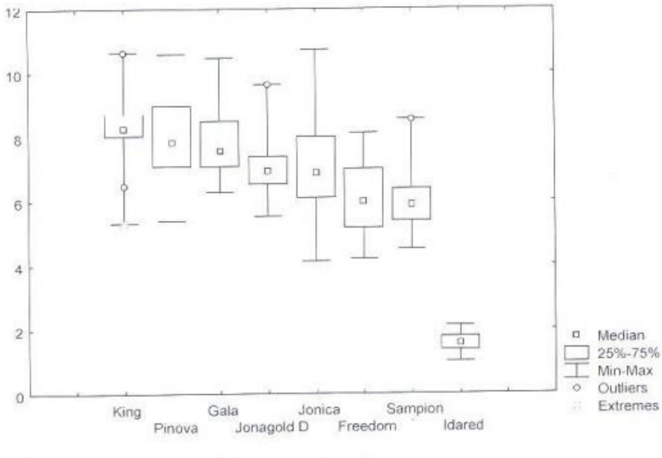

Figure 2 The size of the hypanthium surface

The shape of the hypanthium surface of the cultivars examined fell into three classes. In the case of cv. Gala and Jonica the contour line of the mid-section had a convex shape, and that of the Freedom was straight. The remaining cultivars had a complex shape, that is the contour line run parallel with the style over a short segment and formed a shoulder where the rest of the hypanthium opened wide*(Figure 3)*. We also found that the mean angle between the style and the hypanthium wall varied between 46,5 and 32,8 degrees, the

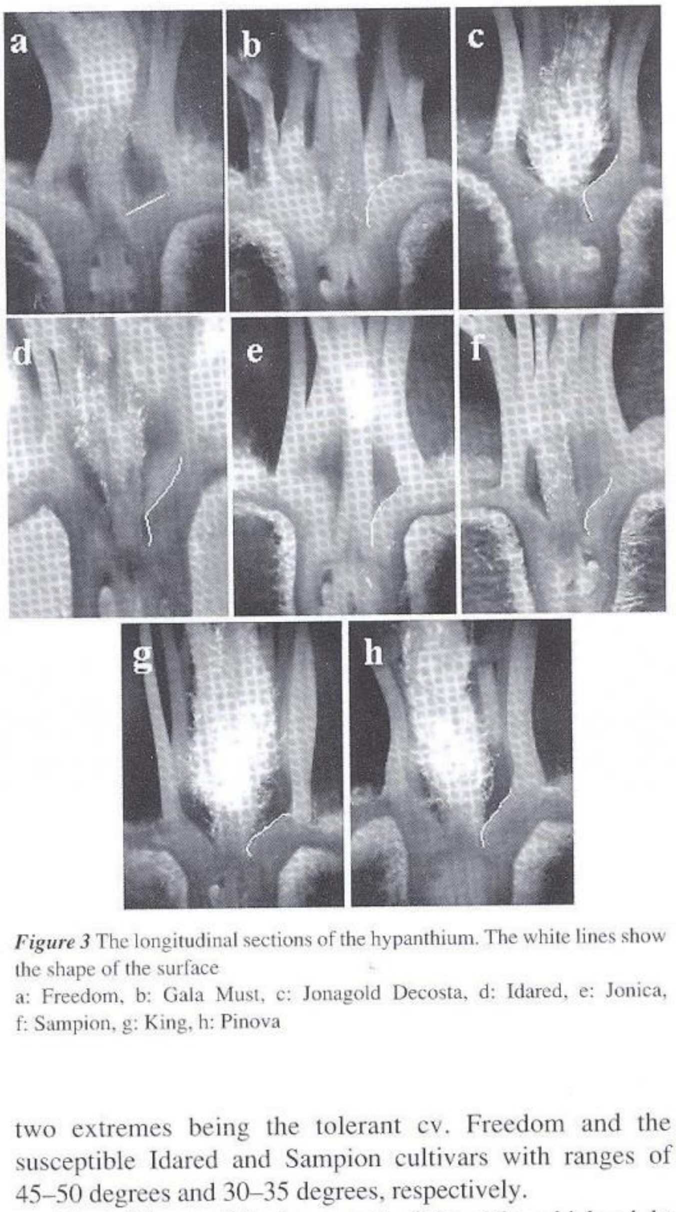

Figure 3 The longitudinal sections of the hypanthium. The white lines show the shape of the surface
a: Freedom, b: Gala Must, c: Jonagold Decosta, d: Idared, e: Jonica, f: Sampion, g: King, h: Pinova

two extremes being the tolerant cv. Freedom and the susceptible Idared and Sampion cultivars with ranges of 45–50 degrees and 30–35 degrees, respectively.

The hairiness of the lower part of the style, which might have a role in keeping humidity around the nectary stomatas, differed among the cultivars investigated. The lower part of the style of Jonagold Decosta, King Jonagold and Pinova were covered by thick hair, and was poorly covered in the case of Freedom, Idared and Sampion, while the rest of the cultivars had no hair at all.

Nectary stomata on the hypanthium surface

The number of stomata varied between 12 and 98 in all the tested flowers with significantly differing mean values between some of the cultivars *(Figure 4)*. Surprisingly the greatest number of stomata were found in the flowers of the tolerant cv. Freedom, indicating that the susceptibility and the anatomical characters are not in simple relation.

The position of stoma guard cells relative to the epidermal cells was also studied *(Figure 5 a)*. We have observed all possible combinations, i.e. the gard cells were at the level, below or above of the epidermis.

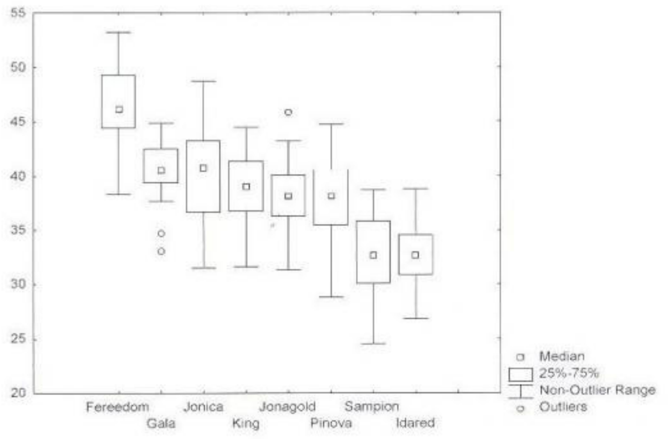

Figure 4 The numbers of stomata in the hypanthium surface

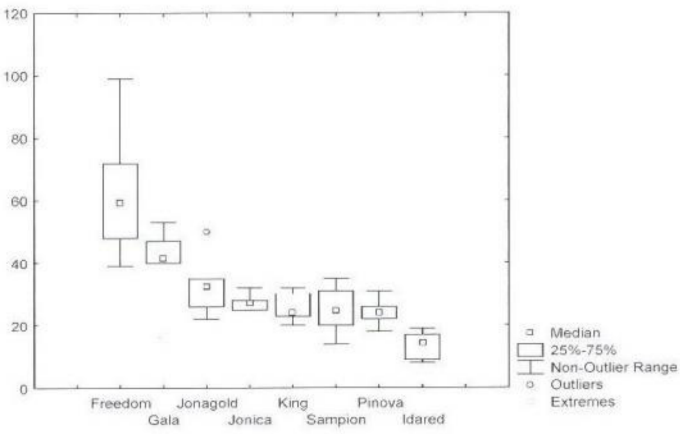

Figure 6 The number of stomata in the hypanthium

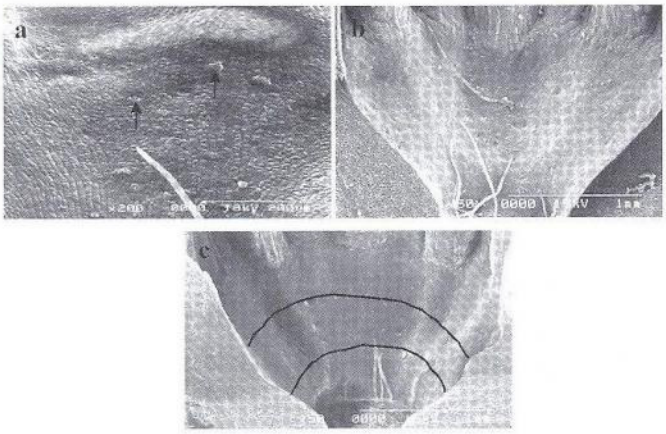

Figure 5 Scanning electron microscope photographs of hypanthium surfaces a: The position of the stoma guard cells (cv. Freedom), b: The uniform distribution of stomata (cv. Jonagold Decosta), c: The stomata in the mid zona of the hypanthium (cv. Idared)

Locating the nectar stomata on the hypanthium surface there were two types we encountered. The distribution was more or less uniform (*Figure 5. b*) in the case of Freedom and Jonagold Decosta. For all the other cultivars the stomata were mainly restricted to the mid third zone of the surface (*Figure 5. c*).

Results of our observation suggest that morphological characters may contribute to the susceptibility to fire blight. It is conceivable that any characteristics that prevent evaporation provide a better environment for multiplication, migration and entry of the bacteria on and into the floral tissues. We investigated these morphological properties. For any of the single characters scored some tendency toward a correlation between susceptibility and moisture maintaining properties, but no significant correlation was found, due to the many exceptions. Therefore we have to conclude that no single characteristics could convincingly be associated with susceptibility to fire blight. However, it is reasonable to think that combination of these characters, like narrow and convex hypanthium, nectar stomatas below the level of epidermis, many stomatas distributed evenly and the rich hairiness of the style could render the cultivar more susceptible.

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