Floral nectaries in some apple and pear cultivars with special reference to bacterial fire blight

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The structure of floral nectaries in some apple (Malus domestica Borkh.) and pear (Pyrus communis L.) cultivars, either susceptible or tolerant to fire blight caused by bacterium, Erwinia amylovora (Burrill) Winslow et al. was studied. The surface of nectaries is smooth in tolerant apple cv. ‘Freedom’ as well as in all the pear cultivars investigated. The surface of nectaries was wrinkled and striate in the susceptible apple cv. ‘Sampion’. These features are favourable for the bacterium, because the nectar retained longer in the burrows provides a nutrient-rich environment. The nectary stomata in cv. ‘Freedom’ were meso- or hygromorphic, while those of cv. ‘Sampion’ were usually meso- or xeromorphic and hygromorphic stomata were rare. In pear cultivars, no hygromorphic stomata were observed. The nectary stomata of the tolerant pear cv. ‘Beurré Bosé’ were mostly mesomorphic or were slightly below the level of the epidermis. In susceptible pear cultivars, xeromorphic stomata were observed more frequently.

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Similarly, in the wrinkled nectary surface, cavities around sunken stomata also contribute to nectar-retaining capacity, thus increasing infection.

**Keywords**: Erwinia amylovora, fire blight, Malus domestica, nectary, Pyrus communis.

The present study deals with histological changes in the nectary structure in some tolerant and susceptible cultivars of apple (Malus domestica Borkh.) and pear (Pyrus communis L.) brought about by infection with the bacterium Erwinia amylovora (Burrill.), which causes fire blight.

The present investigation was carried out in northeastern Hungary on two apple cultivars in the experimental orchard of the Research and Extension Centre for Fruit Growing, Újheňértő, and four pear cultivars in a commercial plantation in the vicinity of Sárospatak, during 2001–04. Apple cv. ‘Freedom’ and pear cv. ‘Beurřé Bosc’ are tolerant to fire blight, while apple cv. ‘Sampion’ and pear cvs ‘Hardenpont’, ‘Oliver de Serres’ and ‘Conference’ are susceptible to this pathogen in various degrees.

For histological studies, flowers were fixed in absolute ethanol : glycerine : distilled water (1:1:1), dehydrated in an ascending acetone–xylol series and embedded either in paraplast or synthetic resin. Longitudinal sections (7–10 µm thick) were cut with a sledge microtome. Some samples were also dehydrated in 70 and 96% ethanol, kept in the infiltrating fluid (Technovit 7100) and embedded in glycol methacrylate-based resin. Longitudinal sections (5–8 µm thick) were cut with a rotary microtome. The sections were stained with toludine blue.

The surface of the nectary was observed under SEM. Flowers from healthy and infected plants were fixed in 0.2 M glutaraldehyde, washed in 0.1 M Na-cacodilate buffer and dehydrated in ascending ethanol-series. After critical-point drying, the material was coated with gold in an SCD 020 sputter-coating unit and viewed under ASID-4 SEM.

The floral nectary of apple is receptacular and located between the stamens and the ovary, while in pear it lines the receptacle at the top of the ovary, and is known as receptacular-ovarian nectary (Table 1). Due to morphological differences in the receptacles, the pear nectary is fully exposed, while in apple it is completely hidden. In pear cultivars, a portion of the nectary is located along with the style. Nectar secreted by the nectary along with the secretory product flows down from the exposed surface of the nectary and accumulates in the gap between the style and the nectary, especially if the nectar is abundant and diluted. Accumulated nectar is retained in the flower for a longer time, evaporating less readily than from the exposed gland surface. This nectar reservoir increases the chance of insect pollination, but also bacterial growth. In pear flowers, invasion of fire-blight bacteria occurs more rapidly through nectaries and pistils, whereas in apple flowers, the stigma and anthers are invaded first. Morphological differences of open receptacles in pears with a fully exposed nectarial region versus closed receptacles in apples with an almost completely hidden nectary, appear to account for this variation.

Histologically, the nectary consists of three parts: epidermis, glandular tissue and nectary parenchyma. The epidermis is covered with a cuticular layer, and usually consists of closely arranged square, rectangular or palisade cells. The glandular tissue present below the epidermis consists of tiny, dark-stained cells. The nectary parenchyma is the innermost layer of the nectary. Cells in this part are large with intercellular spaces. In apple cultivars, the thickness of the nectary and that of the glandular tissue is usually smaller in susceptible cvs ‘Sampion’ and ‘Gala Must’ compared to tolerant cv. ‘Freedom’. Such structures are not observed in pear cultivars1. However, in tolerant cv. ‘Beurřé Bosc’, a thicker glandular tissue is present compared to susceptible cultivars.

In apple, the cuticular pattern is highly variable and is quite specific to a particular cultivar. In susceptible cultivars, the cuticle is divided into grooves and wrinkles. This kind of nectary surface is good for nectar-retaining capacity. The surface of the nectary (hypanthium) is much smoother in tolerant apple cv. ‘Freedom’ than in susceptible cv. ‘Sampion’. In pear, on the other hand, the cuticular surface is smooth (Table 1).

**Table 1.** Comparative nectary features of tolerant/susceptible apple and pear cultivars

<table>
<thead>
<tr>
<th>Topography</th>
<th>Apple</th>
<th>Pear</th>
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<tr>
<td>Morphology</td>
<td>Receptacular</td>
<td>Receptacular-ovarian</td>
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<tr>
<td>Hypanthium shape</td>
<td>Plate-like</td>
<td>Plate-like</td>
</tr>
<tr>
<td>Nectary surface</td>
<td>Smooth</td>
<td>Smooth</td>
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<td>Nectary stomata position</td>
<td>Meso/hygromorphic</td>
<td>Meso/xeromorphic</td>
</tr>
<tr>
<td>Nectary size</td>
<td>Large</td>
<td>Large</td>
</tr>
<tr>
<td>Nectar chamber size</td>
<td>Large</td>
<td>Large</td>
</tr>
<tr>
<td>Nectary thickness</td>
<td>Large</td>
<td>Small</td>
</tr>
<tr>
<td>Glandular tissue thickness</td>
<td>Thick</td>
<td>Thin</td>
</tr>
</tbody>
</table>

1. For histological studies, flowers were fixed in absolute ethanol : glycerine : distilled water (1:1:1), dehydrated in an ascending acetone–xylol series and embedded either in paraplast or synthetic resin. Longitudinal sections (7–10 µm thick) were cut with a sledge microtome. Some samples were also dehydrated in 70 and 96% ethanol, kept in the infiltrating fluid (Technovit 7100) and embedded in glycol methacrylate-based resin. Longitudinal sections (5–8 µm thick) were cut with a rotary microtome. The sections were stained with toludine blue.

2. Flowers from healthy and infected plants were fixed in 0.2 M glutaraldehyde, washed in 0.1 M Na-cacodilate buffer and dehydrated in ascending ethanol-series. After critical-point drying, the material was coated with gold in an SCD 020 sputter-coating unit and viewed under ASID-4 SEM.

3. Histologically, the nectary consists of three parts: epidermis, glandular tissue and nectary parenchyma. The epidermis is covered with a cuticular layer, and usually consists of closely arranged square, rectangular or palisade cells. The glandular tissue present below the epidermis consists of tiny, dark-stained cells. The nectary parenchyma is the innermost layer of the nectary. Cells in this part are large with intercellular spaces. In apple cultivars, the thickness of the nectary and that of the glandular tissue is usually smaller in susceptible cvs ‘Sampion’ and ‘Gala Must’ compared to tolerant cv. ‘Freedom’. Such structures are not observed in pear cultivars1. However, in tolerant cv. ‘Beurřé Bosc’, a thicker glandular tissue is present compared to susceptible cultivars.

4. In apple, the cuticular pattern is highly variable and is quite specific to a particular cultivar. In susceptible cultivars, the cuticle is divided into grooves and wrinkles. This kind of nectary surface is good for nectar-retaining capacity. The surface of the nectary (hypanthium) is much smoother in tolerant apple cv. ‘Freedom’ than in susceptible cv. ‘Sampion’. In pear, on the other hand, the cuticular surface is smooth (Table 1).
SEM studies reveal that the surface of the nectary is smooth in apple cv. ‘Freedom’ (Figure 1a) and in all the four pear cultivars (Figure 1b). The smooth surface is attributed to the presence of a waxy layer on the cuticle. Apple cv. ‘Sampion’ is characterized by a wrinkled and striate nectary surface (Figure 1c). The latter type is favourable for bacteria causing fire blight, because nectar can be retained longer in the furrows of the nectary, thus providing a nutrient-rich environment for the pathogen. Similar wrinkled nectary surface has been described in a susceptible apple cv. ‘Jonagold Decosta’1. Pears on the other hand, have a smooth cuticular surface2,3.

The shape of the hypanthium surface varies in apple cultivars, being straight in the tolerant cv. ‘Freedom’ and convex or shouldered in others. The mean angle between the style and the hypanthium wall is the largest in the tolerant cv. ‘Freedom’ and smallest in susceptible cultivars like ‘Sampion’4. The cuticular surface of the nectary in the presently studied pear cultivars is devoid of ornamentation, and an additional waxy layer was observed on top of the cuticle.

In both apple and pear, the nectaries are automorphic (Figure 2a). In pear cultivars however, no hygromorphic stoma position is observed. The glandular tissue protrudes out of the receptacular tissue and the glands are of various shapes, as observed in the median longitudinal section of the flower. The stomata of the tolerant apple cv. ‘Freedom’ are mesomorphic, i.e. guard cells are in the same level as epidermis cells, or hygromorphic, i.e. guard cells are above the level of the epidermis (Figure 2b, Table 1). Hygromorphic stomata accompanied by a smooth nectary surface facilitate evaporation of nectar, and consequently fail to provide nutrients continuously to *E. amylovora*1. In this case bacterial cells fail to get sufficient time to enter the tissues.

The stomata of susceptible cv. ‘Sampion’ are usually mesomorphic (Figure 2c) or xeromorphic (guard cells below the level of the epidermis), and the hygromorphic stoma position is exceptional. The presence of a large number of sunken stomata on a highly ornamented, wrinkled nectary surface further enhances the storage of nectar and increases the possibility of bacteria entering the inner parts of the flower via the nectary stomata. This is in accordance with the findings of earlier workers1,5. The position of the nectary stomata also influences the nectar-retaining capacity of the gland and promotes or hinders access of the pathogen via the stomata. The stoma position refers to the ecological type of the cultivar as well, e.g. sunken stomata indicate drought-tolerance of a cultivar.

The nectary stomata of the tolerant pear cv. ‘Beurré Bosc’ are mostly mesomorphic or are slightly below the level of the epidermis – both on the exposed part of the nectary and in the region adjacent to the style (Figure 2d). Susceptible cultivars tend to have a larger number of xeromorphic nectary stomata, where guard cells may be sunken one, two or even more cell rows below the epidermis (Figure 2e). In less susceptible cultivars, the mesomorphic or slightly xeromorphic stoma position (Figure 2f) with guard cells sunken to half of the epidermal cells occurs more frequently (Table 1). Similarly, on a wrinkled nectary surface, cavities around the sunken stomata also contribute to the nectar-retaining capacity of the gland, thus increasing the possibility of fire-blight infection6.

The present study reveals that morphological and anatomical characters of the floral nectary may contribute to tolerance or susceptibility in apple and pear cultivars to fire blight. A smooth nectary surface with mostly meso- and the hygromorphic stoma is characteristic of tolerant cultivars, whereas a wrinkled surface with sunken stomata is typical of susceptible cultivars. Our results also indicate
that certain quantitative features of various tissue types of the nectary are related to tolerance or susceptibility.


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