

Metaxenic pollen effect of scab resistant apple cultivars on the fruit of apple

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Summary: As a part of an apple breeding program, we have examined the pollen effect on the fruit appearance and inner quality of different apple cultivars in the Corvinus University of Budapest Department of Fruit Science. Five apple cultivars ('Golden Reinders', 'Regal Prince', 'Rewena', 'Renora', 'Idared') have been crossed by the pollen of some new and some traditional cultivars in our experiment. The measurements were carried out in the analytic and fruit examining laboratory of the Department of Fruit Science. The diameter, height and stem length were measured by digital caliper. We have also examined the squash ratio, soluble solid- and acidic content. Sugar content was calculated by a schedule released by the International Sugar Committee (ISC). From the sugar- and acidic content, we could also count the Pomona-value.

We have observed interesting effects according to some attributes in some crossing combinations with the help of statistical analysis. Crossing 'Golden Delicious' with 'Freedom', 'Prima', and 'Baujade' have resulted in smaller fruit size than other combinations. No significant differences could be observed on 'Golden Reinders' fruits according to metaxenic coloration in the year of the examination. On the other hand the color modifying effect of pollen donating cultivars have manifested on the red fruits of 'Regal Prince' (Gala Must). 'Freedom' as a pollen donating cultivar has resulted outstanding value in our consistency measurements. Squash ratio was the highest in combinations pollinated by 'Prima' and 'Reglindis'. We have noticed balanced values when measuring inner qualities, only 'Reka' has caused lower acidic content. Significant differences have been found in combinations according to the number of frivolous seeds. No frivolous seeds were found in the combinations 'Golden Reinders' x 'Freedom' and 'Idared' x 'Prima'. Almost two frivolous seeds were found in the fruits of the combination 'Renora' x 'Sóvári' in average.

Key words: *Malus x domestica*, cultivars, apple, metaxenia

Introduction

The apple cultivars are considered incompatible with themselves; that is why xeno-pollination is essential. When planting an orchard, primary aspects of choosing pollen donating cultivars are compatibility and simultaneous blooming. Among others, agricultural techniques and the date of maturity should also be considered. Metaxenia has been known for a long while. The effect of the pollen on the fruit of the fertilized cultivar, which is so conspicuous in some years that our attention is drawn to the importance of this factor. Previous domestic and foreign experiments pointed out metaxenic interactions among well known cultivars, but the suitability of new scab resistant cultivars as pollen donating cultivars has not been cleared yet. Besides blooming fructification and compatibility parameters, metaxenic effects should also be known before using these cultivars.

Expression xenia was first used by *Focke* (1881) more than 125 years ago. "The opinion is widespread that the effect of a hybrid fertilization is manifested in general in the new individual derived from the sexual union but what is not

well understood is the effect that can be seen already in the fruit, which in higher plants is a part of the mother plant. Hybridization and the changes in appearance of a plant pollinated with foreign pollen are two separate phenomena. Therefore, I suggest that such variations in the normal appearance or in coloration of some parts of a plant through the influence of the foreign pollen should be called xenia – somewhat like a gift from the pollen-donating plant to the pollen-receiving one."

After coining this term, *Focke* goes on to describe two different forms of xenia, "xenoplasms" which are changes in the fruit form and which can be assumed to include effects on size as well as on shape, and "xenochromes" which are changes in fruit color. Over the years investigators have added to the list of characters, notable sweetness or sugar content and time of maturity. Characteristics of internal chemical composition of fruits and seeds other than sugar such as oils, amino acids, proteins and tannins have been added to the list of xenic effects with time and as analytical techniques have improved.

125 years have passed since the observation of *Focke*. Since then numerous scientists have observed this

phenomenon, and expression metaxenia has also appeared and after then people started mixing the two concepts. Some definitions contradict others, while others are partially in synchron with each other.

☐Pollen effects on maternal tissues are more difficult to explain than effects on parts resulting from syngamy. Causals of metaxenia still have not been known. *Swingle* (1928) considered it the effect of hormones produced by seeds.

The term xenia alone is often applied to the embryo and endosperm effects in fruits of species in which pericarp tissue is not economically important, whereas metaxenia is applied to fruit in which fleshy carpel and accessory tissues are more economically important. In albuminous seeds, such as corn and other cereal grains, we find xenia expressed in endosperm. In exalbuminous seeds, such as pistachio and pecan, we find xenia expressed in embryos. In fleshy fruits, such as date, apple and persimmon, we find metaxenia expressed in the maternal tissues of carpels and accessory tissue. It would seem, then, that how xenia and metaxenia are distinguished depends more on what part of seeds and fruits humans find to be of use than it does on the anatomy and physiology of plants (*Denney*, 1992).

We would like to introduce some results from previous metaxenic examinations. Numerous scientists proved the importance of metaxenia, for example *Bach* (1928), *Husz* (1942) and *Nebel* (1930). On the other hand, *Höstermann* (1924), *Krumbholz* (1932), *Muth* and *Voigt* (1928) decline the existence of the phenomena, and *Tufts* and *Hansen* (1933) did not find metaxenic effect. *Nyéki* et al. (2002) and *Kozma* et al. (2003) have also examined the economical importance of the phenomena.

Kovács (1976) carried out crossings in two years. 'Egri Piros' was used as the mother parent. He considered 'Egri Piros' a representative control, while it is a self-incompatible cultivar, and he prepared artificial self-pollination on this cultivar, so he could rule out the effect of foreign pollen. During his examination, he found metaxenic effects not only in size, shape and color but also in skin structure and the date of maturity.

Tóth et al (1985) used *Malus* species and types as pollinators in four years. Flesh firmness increased under the influence of *Malus* pollinators. 'Jonathan' was open calyxed in some combinations, in other combinations, the stem was shorter and thinner. A very long stem was measured in some 'Golden Delicious' and 'Staymared' combinations.

Nyéki (1972) studied the metaxenic effects on pear cultivars. In his opinion, the shape and size of fruits are genetically determined, but can range in a wide scale, influenced not only by the pollen-donating cultivar, but also by much biotic and abiotic factors. It makes difficult to examine this phenomenon. He observed the same pollen effect of some cultivars independent of the year of examination. These differences are expressed through the change of height, largest diameter, neck-length, and shape-and neck index. Metaxenic effects could be observed from just after pollination and early phase of fruit growing.

Let us make a list of observed metaxenic effects according to previous literature references: fruit shape, ribbing, size, coloration, russetting, coloration of flesh, consistency, thickness and structure of skin, date of maturity, taste, chemical composition (pH, acidic- and sugar content, oils, proteins, amino acids), storability, open or closed position of calyx.

We can state that the shape, size, color, date of maturity, and storability of fruits are cultivar characteristics, independent from the pollen-donating cultivar. These characteristics are varying not only due to metaxenic effects but also due to nutrition, climatic conditions, number of seeds etc. In many cases, metaxenic differences are not significant and difficult to prove. We have to measure large samples to get acceptable results. Some examinations give contradictory results. Metaxenic effect can be expressed in only one or more characters. Every possible combination for metaxenia also should be investigated in the future. According to these investigations we can determine the best pollen-donating cultivars. By the result of this we can use metaxenic effects as value increasing device. Mechanisms in the background of the phenomena should be cleared by methodical investigations in the future.

In our study, we introduce the metaxenic pollen effect of scab resistant cultivars as pollen donating partners, and the changes of the fruit of these cultivars.

Material and methods

Our experiment is a part of the breeding program of Corvinus University of Budapest Department of Fruit Science. In the framework of this project, we prepare thousands of crossings year by year. The fruits we have obtained were good material for evaluating metaxenic effects.

We set up our experiment in the research farm of the Faculty of Horticultural Science. The orchard was planted on rootstock M9 in spring of 1999. The slender spindle trained trees are in a 4 x 1,5 m structure. Trees are growing beside a wire supporting system. Water management is solved by the dripping system.

In 2004, more than 3000 flowers were hand pollinated in 25 combinations. In our experiment five apple cultivars ('Golden Reinders', 'Regal Prince' (Gala Must), 'Rewena', 'Renora', 'Idared') were crossed with some new and some old cultivars (*Table 1*). We used fresh and frozen pollen collected in the previous year as well. Crossings were made by paintbrush at balloon stage (just before the petals expand) after preparation and castration. To determine the fertilizing ability of the cultivars, we investigated fruit drop (after fruit setting, in June and before harvesting) and the rate of frolic and fruit drop and determined the number of the viable and frivolous seeds.

The date of harvest was decided with the help of previous proposal (*Szabó* in *Inántszy*, 1992; *Tóth* in *Gonda*, 1995; *Tóth*, 1997; *Soltész* and *Szabó* in *Soltész*, 1998) according to the

Table 1. The crossing codes and the date of harvesting

pollen-donating cultivars I	Golden Reinders	Gala Must	Rewena	Renora	Idared
Florina	11				
Freedom	12				
Prima	13				53
Baujade	14				54
Reka	15				55
Reglindis	16				56
Liberty		27			
Rewena	18	28			
Batul		29			
Sóvári			311		
Sóvári helyi alma			313	412	
Sóvári				414	
Baumann renet				415	
Törökbálint				416	
félvad alma			317		
helyi alma				418	
Ismeretlen magonc			319		
Erdélyi kormos renet				420	
Produkta	121				
date of harvesting (2004)	10. 2.	9. 9.	10. 2.	10. 2.	10. 2.

sequence of ripening time (Table 1). The measurements were carried out during October after harvesting all cultivars. Samples were kept in the storage till the day of measurement. The unregulated air conditioned storage belongs to the research farm. During storage, the temperature ranged between 5–7 °C and the humidity ranged between 90–95%.

The quality and analytic measurements were carried out in the laboratory of the Department of Fruit Science. We measured the size parameters on all fruits obtained from crossings, and we measured 10 piece samples in the case of open pollinated controls. Open pollinated control samples were harvested from the nearby of the crossing combinations (from same branches if it was possible).

Size parameters (diameter, height) and the length of stem were measured with millimeter accuracy with a digital caliper connected to a computer (Mitutoyo CD-15DC). Consistency was measured by a Magness-Taylor penetrometer equipped with a 11.1 mm probe. On the widest diameter the skin was peeled on the two opposite sides of the fruit (the sun exposed and the shaded sides), so the probe of the penetrometer could intrude without difficulty. We measured consistency in kg/cm².

We also needed homogenous squash from the samples to measure inlay contents. Squash was accessed from two apples. The apples were measured by gram accuracy, and the ratio of squash was calculated by the division of fresh- and squash weight (g/100g which equals %). Soluble solid content (refraction) was measured with an ATAGO Palette PR-101 digital refractometer. We received the soluble solid content in Brix % (g/100g).

Acidic content was also measured by filtered homogenous squash. 5 cm³ of squash was pipetted to a test-tube, and was diluted tenfold with distilled water. A few

drops of phenolphthalein were added. We titrated to the end point with 0.1 N NaOH (factor of NaOH was 0,844, malic acid equivalent was 0,0067). Acidic content was calculated by the following formula

$$\text{Acidic content\%} = \text{NaOH (cm}^3\text{)} \times \text{factor} \times \text{malic acid equivalent} \times \text{rate of dilution} / \text{sample (cm}^3\text{)} \times 100$$

We counted further values from the measured data. Sugar content was calculated by a table released by the International Sugar Committee (ISC) according to the value of refraction. Sugar and acidic content was converted into g/l dimension (tenfold of g/100g values). We also calculated the Pomona-value initiated by *Thiault* (1970) from sugar and acidic values.

$$\text{Pomona-value} = \text{sugar content (g/l)} + 10 \times \text{acidic content (g/l)}$$

Data were processed with Microsoft Excel software, average and mean values were also calculated and diagrams were also prepared with this software. During the statistical analysis – while apple cultivars are considered self incompatible and cross pollination is essential – the conception was: samples from crossings on the same mother parent were compared with each other and with the open pollinated control from mother parent. Statistical analysis was carried out with the SPSS software. As the element number of samples and means were different, we used the Kruskal-Wallis test from non-parametric tests for valuating samples in the same mother parentage groups. We got more accurate results with the Mann-Whitney test for comparing only two samples at the same time. We did not use statistical analysis when results could be read from the diagrams.

Results and discussion

During our examinations, in many observed combinations we met with many metaxenic effects. We would like to introduce the most significant results.

We considered diameter the most representative character, as the economic value of fruits is determined by this character. One requirement for first class apple is to

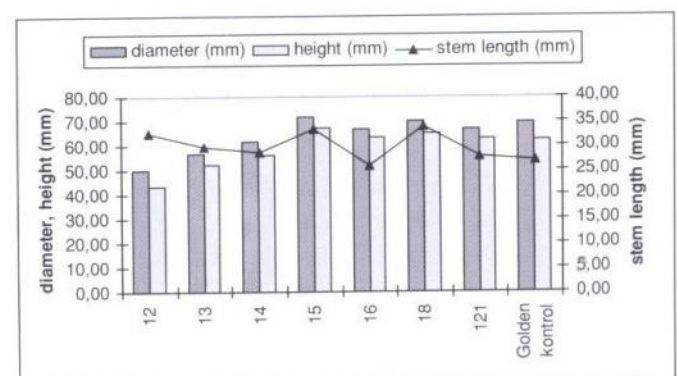


Figure 1. Size parameters of 'Golden Reinders' fruits



Figure 2. Fruit reached by crossing 'Golden Reinders' x 'Baujade'



Figure 6. Fruit reached by crossing 'Regal Prince' (Gala Must) x 'Rewena'

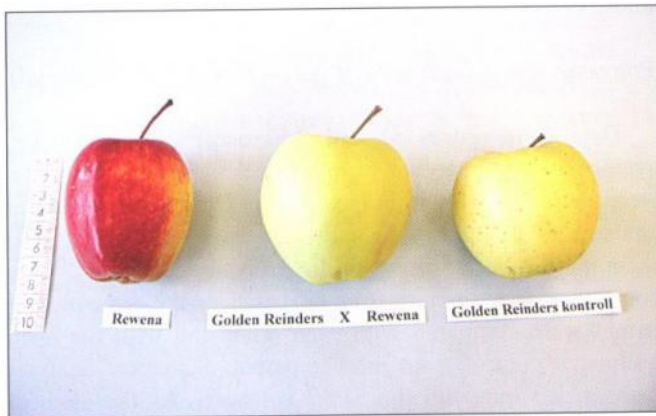


Figure 3. Fruit reached by crossing 'Golden Reinders' x 'Rewena'

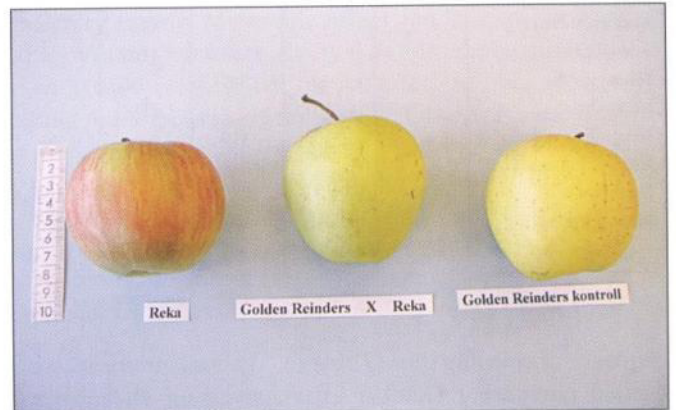


Figure 7. Fruit reached by crossing 'Golden Reinders' x 'Reka'



Figure 4. Fruit reached by crossing 'Idared' x 'Reka'

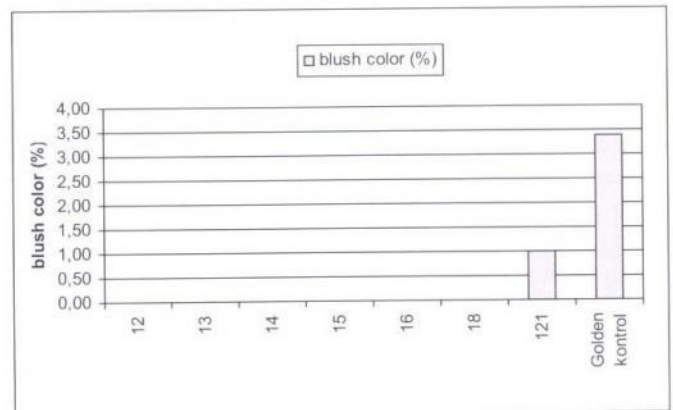


Figure 8. intensity of blush color on 'Golden Reinders' fruits

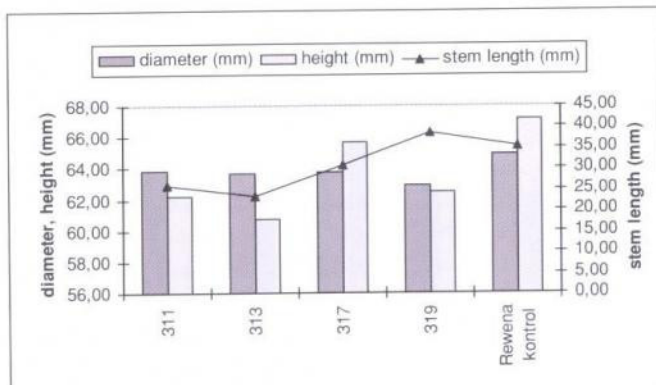


Figure 5. Size parameters of 'Rewena' fruits



Figure 9. Fruit reached by crossing 'Regal Prince' (Gala Must) x 'Batul'

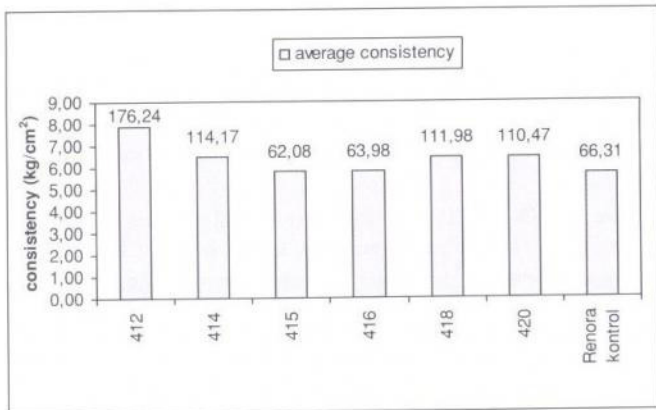


Figure 10. Consistency of 'Renora' fruits

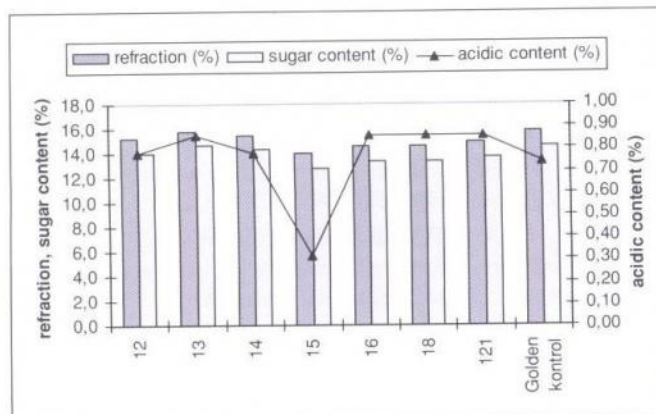


Figure 11. Refraction, sugar- and acidic content of 'Golden Reinders' fruits

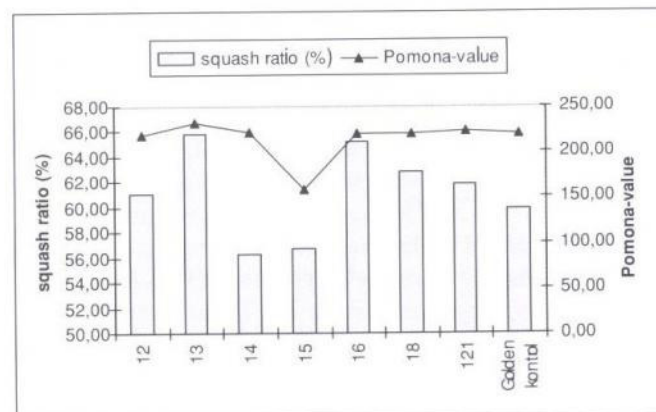


Figure 12. Pomona-value and squash ratio of 'Golden Reinders' fruits

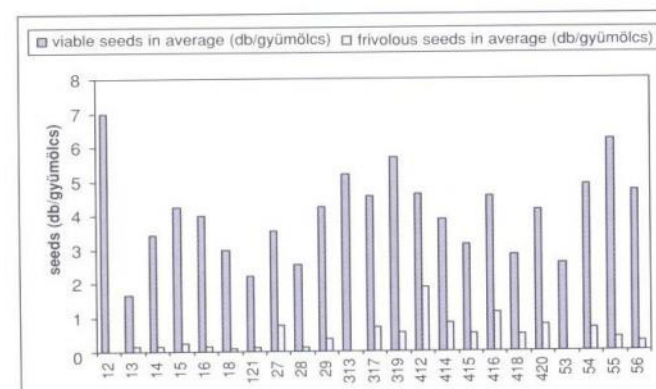


Figure 13. Average number of frivulous and viable seeds

reach 70–75 mm diameter. Fruits of 'Golden Reinders' (Figure 1) were very little in combinations with 'Freedom', 'Prima' and 'Baujade' (Figure 2). Statistical analysis confirmed the results read from the diagram. In combinations with 'Reglindis' and 'Reka' and in open pollinated control we got 65-70 mm fruits in average. The best results were in combinations with 'Reka' and 'Rewena' (Figure 3), we got over 70 mm fruits in both combinations in average. 'Idared' fruits were 70-75 mm in combinations with 'Reka' and 'Reglindis', other combinations (open pollinated control as well) have resulted larger fruits. This can be attributed to the anyway large fruit of 'Idared' cultivar. Metaxenic effect is well shown in Figure 4. The size of the fruit from crossing is among the size of the parent cultivars.

A strange phenomenon was found on 'Rewena' fruits. The diameter of the fruits was approximately the same, but height ranged in a wider scale (Figure 5). This experience also supports the existence of metaxenic effects to the shape. In our experience, we got elongated fruits from open pollination (Figure 6), while pollinating with 'Sóvári' resulted in oblate fruits.

The length of stem plays an important role in the degree of fruit drop. If the stem is too short, fruits can distend off the tree, especially in overcropped trees when fruits also detain each other in growing. Apples like this will be deformed if they do not fall down, and it causes quality loss. A too long stem can worsen the aesthetic value of the fruits. Among 'Golden Reinders' fruits, the stem was the longest when pollinated with 'Reka'. Figure 7 shows that this combination resulted in a longer stem than both parent cultivars had. Figure 3 shows that the fruit from crossings had a obtained stem length among the parent cultivars. Further examinations are needed to decide the proper length of the stem, so we have to emphasize the aesthetic effect of the stem on consumer evaluation tests. We plan to extend our measurements to the depth of the stem cavity, because it could give more representative results and explanation to fruit drop before maturity.

As regard the coloration, 'Golden Reinders' fruits are the most representative ones (Figure 8) while metaxenic effect can be observed most spectacularly on them. In one color cultivars (which do not have blush color), appearance of blush color in the least degree is a disadvantage. Green colored cultivars can have at most 1% of blush color. In our experiment the chance of metaxenic coloration can not be proved in this year. The open pollinated control contained more than 3% blush color, pollinating with yellow 'Produkta' resulted 1% blush color, other combinations resulted in uniformly yellow fruits free from metaxenic coloration. The phenomenon is shown in Figure 3.

On the other hand metaxenic effect was found on red fruits of 'Regal Prince' (Gala Must). 'Batul' as a pollen donating cultivar had the lowest degree of coloration (Figure 9). Further examination are necessary to decide the consumer preference regarding these changes of coloration. Previous results (Nyéki, 1980, Tóth et al, 1985) prove squarely the existence of metaxenic changes in coloration. When planting

an orchard we must consider the effect of pollen-donating cultivars in this view. In our experiment, 'Freedom', 'Florina', 'Prima', 'Baujade', 'Reka', and 'Reglindis' resistant cultivars did not affect the coloration of 'Golden Reinders' fruits, further examinations are needed to draw conclusions.

When measuring consistency we should consider that the higher the value the better consistency, because the firmer apple can be stored better and longer. On the other hand, too firm flesh can reduce consumption value of the fruits. When analyzing previous literature results, we noticed that scientists made categories considering only the cultivars they examined, so different results are difficult to be compared. We would like to introduce only the phenomenon, and we do not want to make new categories. We introduce metaxenic changes on the fruits of 'Renora' (Figure 10). Statistical analysis confirmed the results shown by the diagram. Samples were significantly different from each other according to the Kruskal-Wallis test, and samples can be divided into three groups by right of the Mann-Whitney test. Almost 8 kg/cm² reached sample 412. Over 6 kg/cm² reached samples 414, 418 and 420, and less than 6 kg/cm² reached samples 415, 416 and the open pollinated control. We represent the ranks of Kruskal-Wallis over the columns on the diagram (Figure 11).

During the inner content measurements refraction and acidic content showed outstanding results. As sugar content was calculated – according to the refraction – by a schedule released by the International Sugar Committee, we do not evaluate it apart, because the observations apropos of refraction are also valid for the sugar content. We calculated the Pomona-value from sugar- and acidic content, so it resulted similar values. Inner contents of 'Golden Reinders' fruits are shown in Figure 11. Refraction ranged between 14.0–15.8%. Surprising results were found in combination with 'Reka' – although refraction value was also the lowest in this combination – the fruits have less than half the acidic content than that of other combinations. Earlier studies also investigated inner content of some resistant apple cultivars (Gonda et al., 2000, 2004) and according to lexical data 'Reka' has anyway low acidic content, and this can prove the effect of metaxenic effect.

Squash ratio ranged between 56–66% according to all combinations. These differences are difficult to be analyzed statistically. Juiciness is affected by many other conditions, further examinations are needed to prove metaxenic effects. We introduce only one combination (Figure 12), 'Golden Reinders' x 'Reka' reached lower value, which can be attributed to the lower squash content of 'Reka'.

Significant differences were found according to the ratio of frivolous seeds in some combinations. No frivolous seeds were found in combinations 'Golden Reinders' x 'Freedom' and 'Idared' x 'Prima', while almost two frivolous seeds in average were found in combination 'Renora' x 'Sóvári'. The most viable seeds were found in combinations 'Golden Reinders' x 'Freedom', 'Idared' x 'Reka' and 'Revena' x unidentified seedling (Figure 13).

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