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Relationship between selected morphological, anatomical and cytological characteristics of leaves and the level of tolerance to herbicides in strawberry cultivars

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Abstract

Foliar application of a mixture of herbicides containing phenmedipham, desmedipham and ethofumesate to the plants of nine strawberry cultivars revealed that there were differences in the level of plant tolerance to the applied chemicals. Light, polarized light and scanning electron microscopy were used to explain differences in tolerance to herbicides. The surface of strawberry leaves and cells was examined for stomata, hairs, trichomes, surface structures, cucticle, vacuole and oxalate crystals. The thicker the cuticle on the adaxial leaf surface, the thicker the layer of epicuticular waxes, greater number of large vacuoles and greater number of calcium oxalate crystals in epidermis cells were characteristic for cultivars with very good tolerance to herbicides. The cracking of epicuticular waxes layer was typical to cultivars with respectively low tolerance to herbicides.

Introduction

The variations among strawberry cultivars in their sensitivity to phenmedipham, ethofumesate and their mixtures with other herbicides were reported by Clay and Abernethy (1976), Clay (1982), and Leiteritz and Pilz (1988). The authors, however, did not explain the causes of this phenomenon. In the opinion of other researchers varying sensitivities in strawberry to 2,4-D herbicide were caused both by the rate of its decarboxylation and, during absorption, by its retention and inactivation as water-soluble compounds which were then stored in vacuoles (Luckwill and Lloyd-Jones 1960). The tolerance of strawberry plants to unfavourable external conditions, such as high salinity and drought, is related to the presence of numerous crystals of calcium oxalate in plant cells (Goncharova and Dobrenkova 1981).

An essential role in the absorption of herbicides is played by some of the plant morphological features. The stomata and hairs play only a relatively small role because the active substances are absorbed mainly directly through the cuticle (Bukovac 1976). Plant cultivars differ in their ability to absorb substances. The varied tolerance to acifluorfen in tomato cultivars, and others from the family *Solanaceae,* depends on the number of stomata and also, to a small degree, on the number of hairs per unit of surface area. However, the number and composition of epicuticular waxes and the cuticle thickness do not affect this sensitivity (Ricotta and Masiunas 1992). On the other hand, in some pea and bean cultivars a positive correlation between the amounts of epicuticular waxes and the tolerance to herbicides was reported by King (1980). Certain lipophilic substances, among them some herbicides, are more easily transported within a cuticle covered with a thick coating of wax, *e.g.* in strawberry and rape, than in a cuticle covered by only small amounts of wax, as is the case in sugar-beet and maize (Baker *et al.* 1992). The characteristic features of the stomata, epidermal hairs and epicuticular waxes in strawberry of the Elsanta cultivar were presented by Blanke (1991).

The aim of our investigations, employing light and scanning electron microscopy, was to evaluate selected morphological, anatomical and cytological characteristics of strawberry cultivars in relation to their tolerance to herbicides.

Materials and methods

The morphological features of strawberry plants, particularly leaf appearance, were assessed annually from 1994 to 1996 immediately prior to flowering. The evaluation of the chosen anatomical and cytological characteristics was carried out in 1996. Leaves for the assessments were collected at different times from labelled strawberry plants of nine cultivars. Among the examined cultivars Senga Sengana, Dukat, Kama and Real stood out as those having very high tolerance to the mixture of phenmedipham, desmedipham and ethofumesate. The Syriusz, Redgauntlet and Elsanta cultivars were characterized by an average tolerance while the Dana and Gerida cultivars had low tolerance to the above-mentioned mixture of weed-killing substances. The plants were grown in small fields from which weeds were removed by hand.

The number of the stomata and long straight hairs per unit of surface area of the adaxial side of leaves was determined for nine strawberry cultivars. The leaves were collected on four different dates: 6th and 11th of June, 23rd of July and 4th of September. The number of the stomata per 1 mm^2 of leaf surface was determined by means of an light microscope. Leaf samples were collected from the central leaflet of the main leaf at a place halfway between the main nerve and the right edge of the leaflet, and at a height 2/3 from its base. Specimens for microscopic examination were prepared using the isolated epidermis method (Dyki and Habdas 1996). Strips of transparent tape (of the Scotch brand) were applied onto the collected leaf pieces from both sides. The strips were then pulled apart leaving the epidermis of the adaxial and abaxial side of the leaf secured separately to the tape. Remnants of the mesophyllic tissue were removed with a scalpel. Following a water rinse the tape was stained using toluidine blue (2 % toluidine blue in a 1 % aqueous solution of sodium tetraborate). Counting of the stomata was carried out under the light microscope at a magnification of 500 x. The number of straight hairs in an area of 1 cm^2 on the adaxial side of leaves was determined by means of a binocular microscope at a magnification of 8 x.

Examinations of the anatomical features, with special regard to the thickness of the cuticle, the presence of waxes on the cuticle surface and the cell structure, were carried out using leaf samples obtained from four strawberry cultivars. They included Senga Sengana and Dukat, which in field experiments proved to be the most tolerant to herbicides, and Dana and Gerida, which were the most susceptible to herbicide damage.

The leaves for examinations under the light microscope were collected on the following dates: 11th of June, 23rd of July and 4th of September. The leaf specimens were preserved in a solution of chromoacetoformalin (CrAF) and after dewatering in ethyl alcohol and xylene they were embedded in paraffin. Transverse sections of the leaves, 5 μm thick, were made on a rotary microtome. The specimens for anatomical studies were stained for 4 hours in a 4 % aqueous solution of safranine O, with additional staining for one minute in a 0.4 % solution of fast green in alcohol. The remaining specimens for cuticle studies were stained for 12 hours in a saturated solution of Sudan IV in alcohol. The main subject of examinations under the 'Jenaval' light microscope was the epidermis of the adaxial and abaxial sides of the 12 leaves (for each cultivar), the structure of the stomata and leaf parenchyma of the four strawberry cultivars. Some of the specimens were examined using polarized light.

For the analyses under the 'JEOL JSM-1' scanning electron microscope the 10 leaf samples were collected on 6th of June and 4th of September for each cultivar. They were taken from young, middle-aged and older leaves. The mean temperature of air and precipitation in months prior to sample collecting were noticed, for May: 14 °C and 14.7 mm; for August: 18.2 °C and 50.6 mm. Pieces of the leaves were preserved in CrAF and after dewatering in alcohol, acetone and carbon dioxide they were coated with gold. Both the adaxial and abaxial leaf surfaces were examined in four replications from each leaf. Epicuticular waxes present on the cuticle surface and the hairs and trichomes (small secretory hairs) on the surface of the epidermis were the main subjects of examinations under the scanning electron microscope. The specimens prepared from leaves collected in June were also used to count the straight hairs and the secretory hairs on the lower side of the leaves. Depending on the subject viewed under the scanning electron microscope magnifications from 80 to 4800 x were used.

The results concerning the number of stomata, straight hairs and trichomes were estimated statistically, with analyses of variance – Duncan's t-test at α=0.05.

Table 1. The number of stomata (per mm^2) and straight hairs on the adaxial surface of the leaf (per cm^2) of the nine strawberry cultivars, as the means of four appointed times of counting in 1996.

Cultivar	Number of stomata	Nunber of straight hairs on the adaxial leaf surface
Senga Sengana	328.8 bc	10.4 b
Dukat	352.5 cd	27.5 d
Kama	363.7 d	32.3 d
Real	344.9 cd	17.8 c
Syriusz	333.9 bc	31.9 d
Redgauntlet	295.1 a	13.5 bc
Elsanta	303.7 a	42.6 e
Dana	313.9 ab	1.6 a
Gerida	296.8 a	15.4 c

* Means followed by the same letter do not differ at 5 % level of significance, Duncan's multiple range t-test.

Results

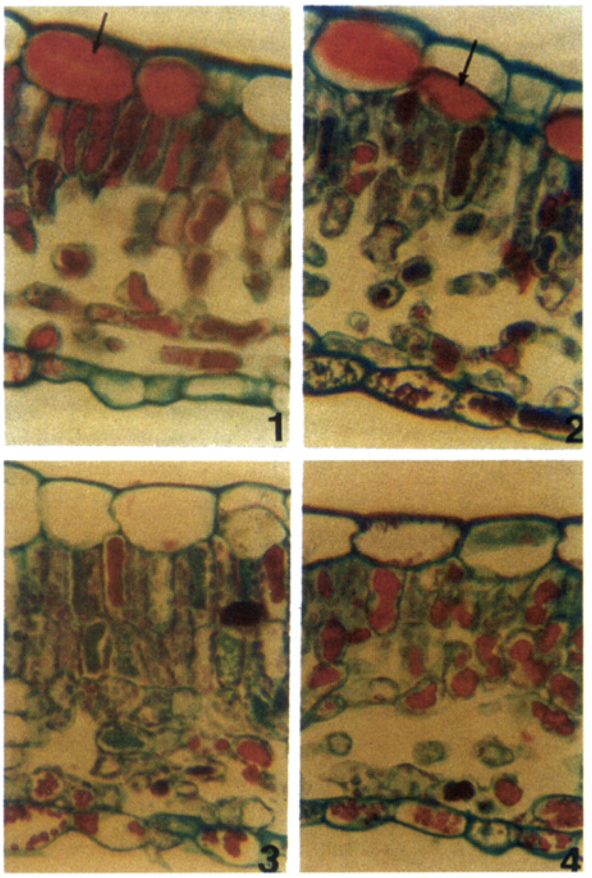
The morphology of the leaves and entire strawberry plants was similar in the nine cultivars examined. The young leaves of all nine cultivars were bent along the main vein in the shape of the letter V and had slightly serrated edges, whereas the older ones were boat-shaped and curved upwards. This feature was most noticeable in the Dana and Dukat cultivars.

Examinations of the epidermis and parenchyma of the leaves showed an absence of distinct anatomical differences between the four cultivars in samples collected on three consecutive dates. The adaxial side of leaves was covered by an epidermis consisting of relatively large cells. Often these cells in the Senga Sengana and Dukat cultivars contained large vacuoles which due to safranine O staining acquired a deep red colour (Figs. 1-4). Palisade parenchyma in all cultivars was composed of two layers of cells under which there were loosely-packed cells of spongy parenchyma and the epidermis of the abaxial side of the leaf. In the cells of the epidermis there were crystals of calcium oxalate which were visible under the polarizing microscope (Figs. 5-8). These crystals were numerous in the Senga Sengana and Dukat cultivars, whereas in Dana and Gerida they appeared only occasionally. In the epidermis of the abaxial leaf side, numerous long straight hairs and short trichomes and numerous stomata could be seen. There were some small differences in the structure of the stomata. In the Senga Sengana and Gerida cultivars they were set deeper below the surface of the epidermis in comparison with the stomata in the Dukat and Dana cultivars.

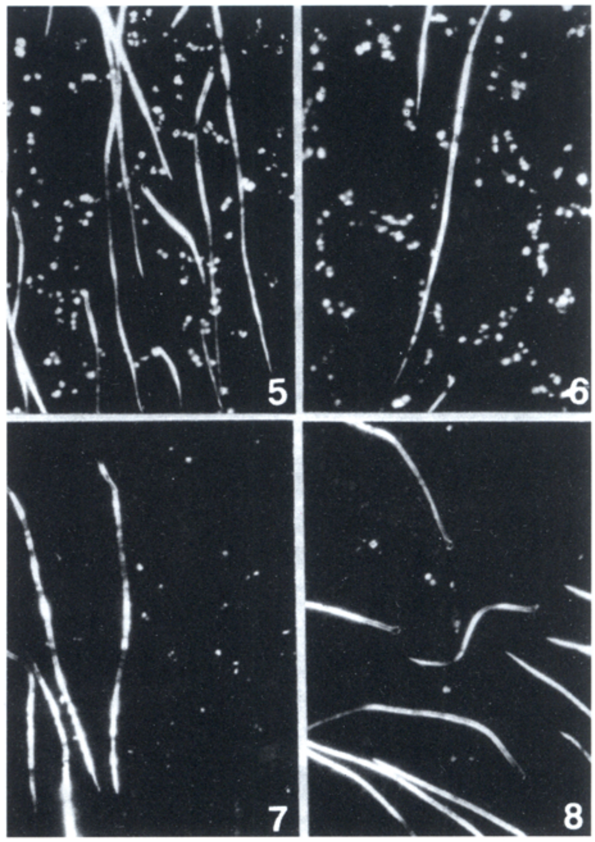
Table 2. Number of straight hairs (per cm^2) and trichomes (per mm^2) on the abaxial side of the four strawberry cultivar leaves.

Cultivar	Number of straight hairs	Number of trichomes
Senga Sengana	862.5 ab	93.8 ab
Dukat	792.5 ab	70.6 a
Dana	1182.5 b	126.0 b
Gerida	552.5 a	106.2 ab

For explanation see Table 1.



Figs. 1-4. Cross-sections of Senga Sengana (1), Dukat (2), Dana (3), and Gerida (4) leaves with visible vacuoles with phenolic compounds (↓). Staining with safranine O and fast green. Light microscopy, 300 x.



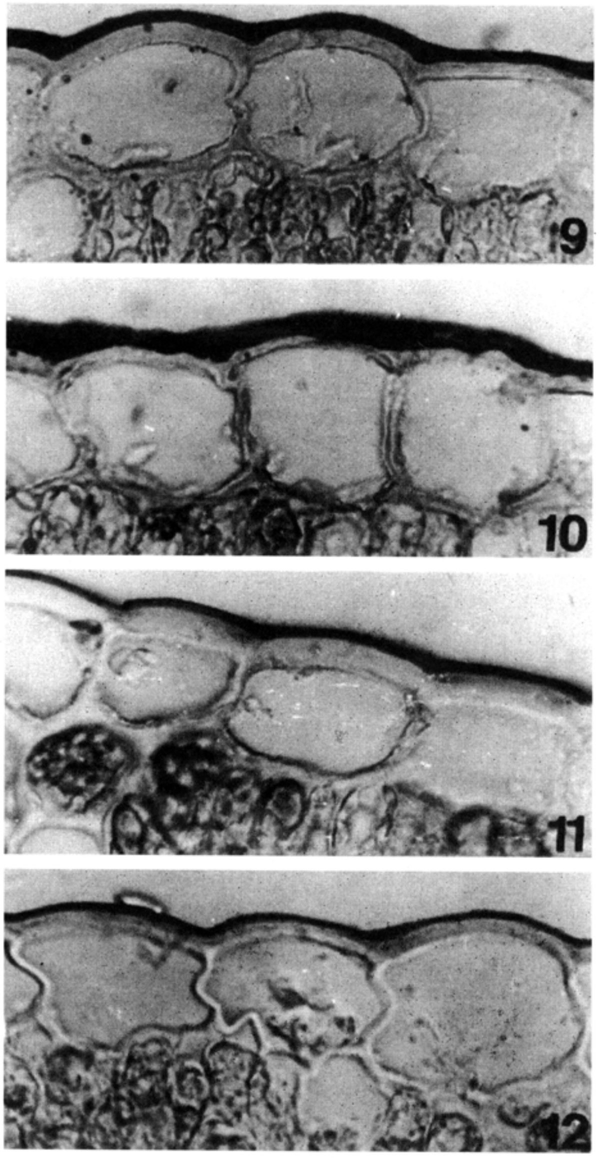
Figs. 5-8. Epidermis of the abaxial surface of the cultivars – Senga Sengana (5), Dukat (6), Dana (7) and Gerida (8) leaves with visible of hairs and crystals of calcium oxalate. Polarized microscopy, 400 x.

The number of the stomata per unit of area was different in each cultivar. The greatest density in their distribution was observed in the Kama cultivar followed by the cultivars Dukat, Real, Syriusz and Senga Sengana (Table 1). Considerably smaller distribution density of the stomata was in the Redgauntlet, Elsanta and Gerida cultivars.

The number of straight hairs per unit of area in the epidermis of the adaxial leaf side was significantly different in the examined cultivars. The greatest density of straight hairs was observed in the Elsanta cultivar and the smallest in Dana (Table 1). The straight hairs appeared in much greater numbers in the epidermis of the abaxial side of the leaf. From the four cultivars examined for that particular feature there were twice as many hairs in Dana than in the Gerida cultivar (Table 2). The number of the trichomes was the greatest in the Dana cultivar and the smallest in Dukat (Table 2).

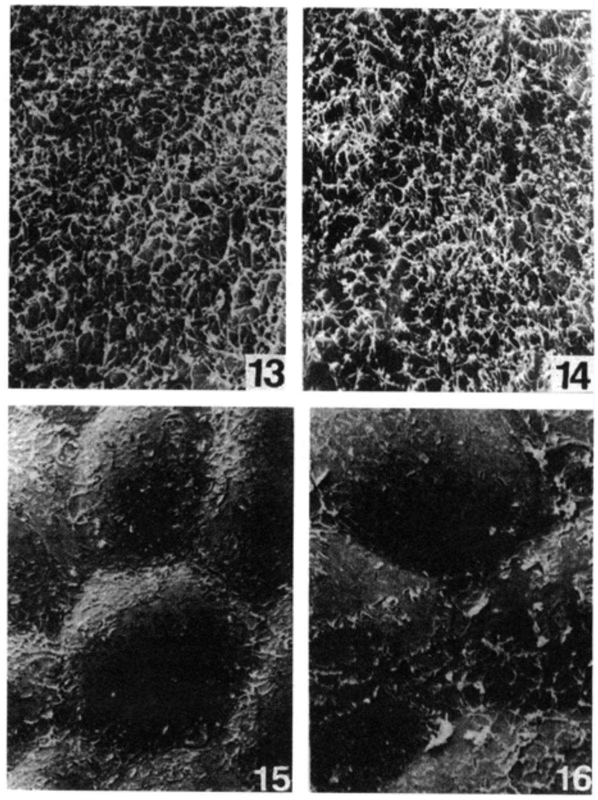
A layer of cuticle covered the leaves of the strawberry cultivars. The cuticle on the epidermis of the adaxial leaf surface in the Senga Sengana and Dukat cultivars was thicker and more intensely stained by Sudan IV than in Dana and Gerida (Figs. 9-12). This difference was particularly noticeable on the leaves collected and preserved in July and September. On the abaxial epidermis a thin layer of cuticle was observed in all the cultivars examined.

The epicuticular waxes were visible under the scanning electron microscope in the shape of fine interwoven threads covering the cuticle of the epidermis to a varying extent. The surface of the epidermis of the adaxial leaf side in the Senga Sengana and Dukat cultivars was covered by large amounts of waxes (Figs. 13-16). In Dana and Gerida the amounts of waxes were relatively small and their



Figs. 9-12. Cuticle on the epidermis of the adaxial leaf surface of Senga Sengana (9), Dukat (10), Dana (11), and Gerida (12) cultivars. Staining with Sudan IV. Light microscopy, 550 x.

surface was frequently seen to be cracked (Figs. 17-18). The epidermis of the abaxial side of leaf was in general covered with a relatively small amount of wax although thickly covered areas were also observed. Also on the leaf's abaxial side in the Senga Sengana and Dukat cultivars the waxes were present in much greater amounts than in Dana and Gerida (Figs. 19-22). The waxes also covered the base of the trichomes. However, they were absent from the base of the straight hairs. Particularly characteristic for the Dana and Gerida cultivars was the frequent occurrence of cracks in the cuticle of the

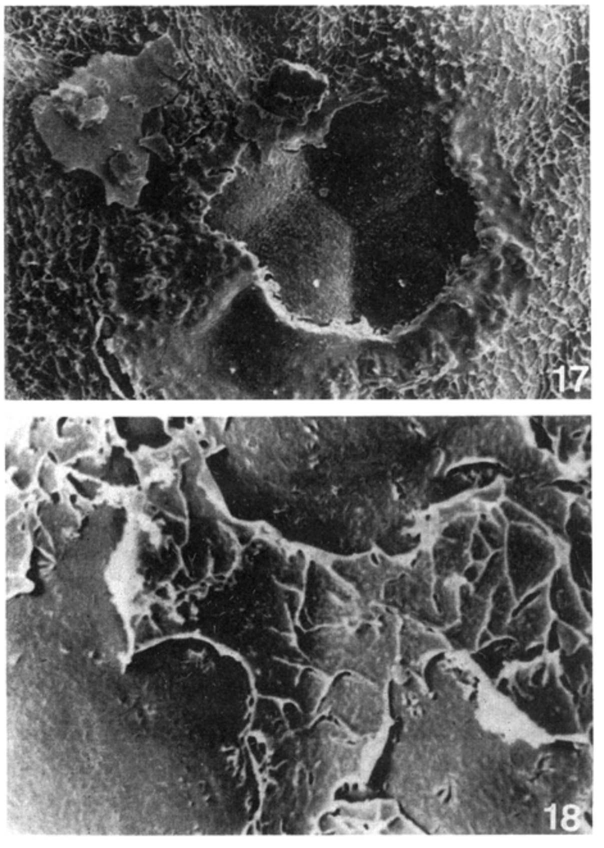


Figs. 13-16. The epicuticular waxes on the adaxial leaf surface of leaves of Senga Sengana (13), Dukat (14), Dana (15), and Gerida (16). Scanning electron microscopy, 600 x.

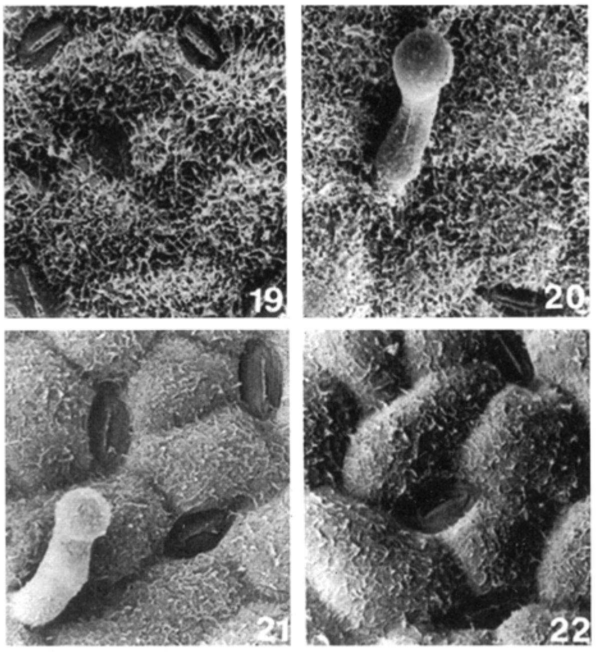
straight hairs (Figs. 23-25). Comparative studies of young, middle-aged and older leaves, carried out on the specimens collected in September, showed the presence of smaller amounts of waxes on younger leaves in all the cultivars examined.

Discussion

During the assessment of the morphological, anatomical and cytological characteristics of the strawberry cultivars the emphasis was placed on the structure of the leaf as the main organ responsible for the absorption of herbicides. The differences between the plants in their appearance and leaf morphology were not significant. The upward curving of the edges of the leaf blade was particularly obvious in the Dukat cultivar having high tolerance to the herbicide mixture as well as in Dana showing low tolerance. Therefore, it is difficult to regard this feature as responsible for the variations in tolerance to the herbicides, *e.g.* because of longer retention of



Figs. 17-18. Damaged layer of epicuticular waxes on the adaxial leaf surface of Dana (17) and Gerida (18). Scanning electron microscopy, 17) – 600 x, 18) – 2400 x.

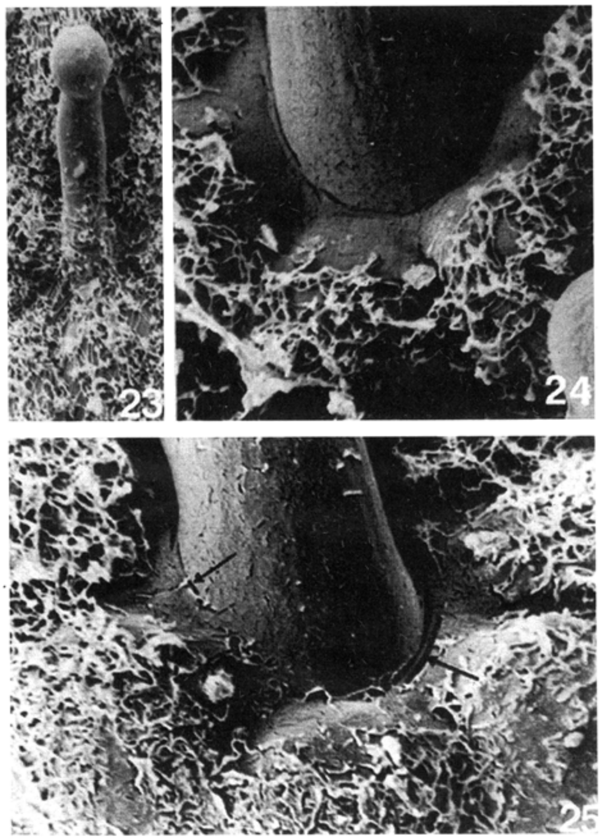


Figs. 19-22. The epicuticular waxes on the abaxial leaf surface of Senga Sengana (19), Dukat (20), Dana (21), and Gerida (22). Scanning electron microscopy, 600 x.

the mixture in the depressions in the leaves. Due to the leaf's curvature the herbicide mixture could have also been absorbed by the lower side of the leaf blades. This was the reason why it was undertaken to examine the anatomy of not only the adaxial but also the abaxial side of the leaves with regard to the number and structure of the stomata (which were present in the epidermis of abaxial side), the number of hairs and the structure of the cuticle and cuticular waxes. The number of the stomata per unit of leaf surface area depended on the cultivar under study. However, the distribution density of the stomata on the leaves of each cultivar, as well as their size and structure, did not play an important role in the absorption of the herbicides by the strawberry plants. The differences in the number of the stomata per unit of area were sometimes greater among the cultivars highly tolerant to the mixture of phenmedipham, desmedipham and ethofumesate than between the cultivars which were tolerant and those which were sensitive. The Senga Sengana cultivar, tolerant to herbicides, and the sensitive Gerida had the stomata situated in distinct narrow depressions in the epidermis while in the highly tolerant Dukat and the sensitive Dana the stomata were located in only slight and shallow depressions. The number of the stomata in an area of 1 mm^2 on the leaves of the Elsanta cultivar obtained in our studies totalled 314 which was in agreement with the number determined for this cultivar by Blanke (1991).

The number of the straight hairs on the adaxial surface of the leaves and the number of the straight hairs and trichomes on the abaxial surface were also difficult to correlate with the tolerance to herbicides. There were too few hairs on the adaxial side of the strawberry leaves to provide a barrier which would make it difficult for the herbicide to cover the leaves properly, as is the case in tomato plants (Ricotta and Masiunas 1992).

The tolerance of the strawberry cultivars to herbicides seems to be related significantly to the thickness of the cuticle and the amounts of epicuticular waxes. The Senga Sengana and Dukat cultivars, which withstand herbicide treatments well, had a much thicker cuticle and a greater amount of epi-



Figs. 23-25. A layer of wax covering the base of a trichoma (23), the absence of wax (24) and cracks in the cuticle (25) at the base of a straight hair of a strawberry leaf (↓). Scanning electron microscopy, 23) – 600 x, 24) and 25) – 2400 x.

cuticular waxes than the sensitive Dana and Gerida. The waxes in our studies were visible on the surface of the cuticle in the shape of small cylinders and threads. A similar description of the waxes in strawberry plants was presented by Blanke (1991). The characteristic feature which enabled absorption of the herbicides in the sensitive Dana and Gerida cultivars was the instability of the cuticle. The cuticle often developed cracks and separated as large sheets from the surface of the epidermis and the straight hairs. The importance of the thickness of the wax layer in the sensitivity to herbicides was confirmed by the fact that the young leaves which showed the worst damage following herbicide treatments had a significantly thinner cuticle than the older leaves in the four cultivars examined for that particular feature. Thus, it must be accepted that the thickness and nature of the waxes covering the strawberry leaves is of importance in determining the level of tolerance to herbicides, just as in the pea and bean cultivars (King 1980).

In the tolerant to herbicides Senga Sengana and Dukat cultivars there were numerous and large vacuoles in the cells of the upper epidermis, which are organelles responsible for, among other things, storing and detoxication of substances harmful to the cell. The vacuoles in the Dana and Gerida cultivars were smaller and not as numerous. In the cells of the epidermis of the abaxial leaf surface, in the Senga Sengana and Dukat cultivars numerous crystals of calcium oxalate were also visible. The presence of such crystals in the epidermal cells of the cultivars sensitive to herbicides, Dana and Gerida, was sporadic. The role of calcium oxalate crystals in the plant tolerance to unfavourable external factors is poorly documented in the literature. However, in the cells of the Festivalnaja cultivar, which is resistant to high salinity and drought, there are significantly more crystals of calcium than in the cells of the Zaria cultivar, which is poorly tolerant to such environmental conditions (Gonczarova and Dobrenkova 1981).

On the basis of the results of our study it should be accepted that the differences among the strawberry cultivars in their tolerance to the applied herbicides are strongly related to some of the features of leaf anatomy and cytochemistry. However, it cannot be ruled out that the tolerance mechanism is of a complex nature, just as in the Talisman cultivar, sprayed with 2,4-D (Luckwill and Lloyd-Jones 1960).

References

Baker E.A., Hayes A.L., Butler R.C. 1992. Physicochemical properties of agrochemicals: their effects on foliar penetration. Pesticide Sci., 34: 167-182.

Blanke M. 1991. Wie sieht ein Erdbeerblatt unter dem Mikroskop aus? Erwerbsobstbau, 33: 79-81.

Bukovac M. 1976. Herbicide entry into plants. In L.J. Audus et al. (ed) Herbicides. Physiology, Biochemistry, Ecology. Academic Press. London, Vol. 1.: 335-364.

Clay D.V. 1982. The tolerance of strawberry cultivars to ethofumesate alone and in mixture with lenacil and phenmedipham. Proc. 1982 Brit. Crop Prot. Conf., Weeds: 291-298.

Clay D.V., Abernethy W. 1976. The response of strawberry cultivars to mixtures of phenmedipham with other herbicides and with mineral oils. Proc. 1976 Brit. Crop Prot. Conf.-Weeds: 265-272.

Dyki B., Habdas H. 1996. Metoda izolowania epidermy liści pomidora i ogórka dla mikroskopowej oceny rozwoju grzybów patogenicznych. Acta Agrobot., 49: 123-129.

Goncharova E.A., Dobrenkova L.G. 1981. Functional role of anatomical changes in strawberry in the course of adaptation to drought and salinity. Trudy po Prikladnoi Botanikie, Genetikie i Selektsii, 71: 100-108.

King J.M. 1980. Methods of testing the reaction to herbicides of cultivars of peas (*Pisum sativum* L.), broad beans (*Vicia faba* L. (partum) and dwarf beans (*Phaseolus vulgaris* L.) and the practical value of the results. Proc. 1980 Brit. Crop Prot. Conf., 453-460.

Leiteritz R., Pilz T. 1988. Einsatz von Herbiziden im Erdbeeranbau und sortenspezifishe Reaktionen. Gartenbau, 35: 151-153.

Luckwill L.C., Lloyd-Jones C.P. 1960. Metabolism and plant growth regulators. II. Decarboxylation of 2,4-dichlorofenoxyacetic acid in leaves of apple and strawberry. Ann. App. Biol., 48: 626-636.

Ricotta J.A., Masiunas J.B. 1992. Relationship of leaf surface characteristics to acifluorfen tolerance in tomato (*Lycopersicon esculentum*) and related species. Weed Sci., 40: 402-407.

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