Autofluorescence of Plant Secreting Cells as a Biosensor and Bioindicator Reaction

Victoria V. Roshchina[1,2]

Autofluorescence of intact secretory cell of plants is considered as a possible biosensor for the cellular biomonitoring. The fluorescence of secretory cells was due to the chemical composition of their secretions. This phenomenon could be recommended: 1. for the diagnostics of secretory cells among non-secretory ones; 2. for the express-analysis of the content of secretory cells at norm and under various factors; 3. for the analysis of cell-cell interactions. Identification of intact secretory cells filled with products of secondary metabolism is based on their fluorescence characteristics. The filling of the cells with a secretion and the removing of the secretions are easily observed under the luminescence microscope—from confocal microscope to various microspectrofluorimeters. The cellular fluorescence can serve as an indicator of the cell state *in vivo.* The following development of the earlier diagnostics based on the autofluorescence may be the area of the remote monitoring of agriculture crops and yields of medicinal plants as well as the remote sensing of the environmental stress.

KEY WORDS: Autofluorescence; confocal microscopy; luminescence microscopy; microspectrofluorimetry; plants; secondary metabolites; secretory cells; spectral analysis.

INTRODUCTION

The term "autofluorescence" is used for a luminescence of naturally occurring molecules of intact cells in visible region of the spectra induced by ultra-violet or violet light [1,2]. Fluorescing world of plant cells, which are well-seen under a luminescent microscope, includes all colours of the visible spectrum. It is not only the emission of chlorophyll (red fluorescence) that located in chloroplasts and the lightening of cell wall (blue or blue-green luminescence [3]. Bright luminescing picture is also observed for specialized secretory cells [4–10]. Moreover, it has been established that many secretory cells, for instance idioblasts and complex glands are not seen on the plant surface without the excitation by UV-light (violet light) or the staining with artificial fluorescent dyes. Secretory plant cells (where secretory function prevail) contain secondary substances, such as phenols, flavins, quinones, alkaloids, polyacetylenes, coumarins, terpenoids and others [11]. The substances in various solutions can fluoresce under ultra-violet radiation [12]. Up to now phenomenon of the bright luminescence emitted from the plant secreting cell was not analysed in literature, and there is necessity to do it. The aim of the review is to show the resources of autofluorescence, peculiar to secretory cells, as a biosensor and a bioindicator reaction for various types of diagnostics—from cellular monitoring of environment to the study of cellular development.

## AUTOFLUORESCENCE OF SECRETORY CELLS AS A PHENOMENON

Secretory Cells

Secretory cells—specialised structures filled with secretory products (S) that are located in secretory vesicles

or reservoirs. Often the secretion moves the cellular organelles to a cell wall (the organelles may be reduced and even disappeared), may be accumulated in free space between plasmatic membrane-plasmalemma and cell wall, and then be excreted out cell, as seen below:

Secreting cell
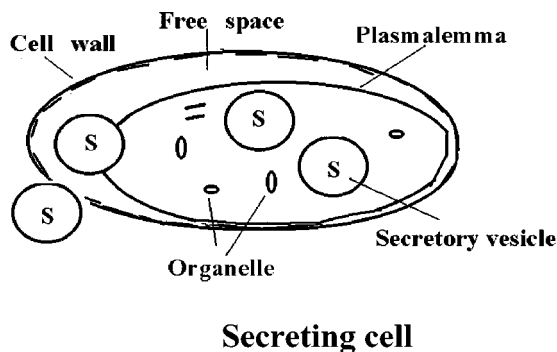
Main plant secretory structures may be distinguished as following groups:

Secretory cells of vegetative organs (leaves, shoots, roots)	Secretory cells of generative organs (petals of flowers, nectaries, pistils, glandular cells)	Microspores for vegetative and generative breeding	Secreting cells of non-differenting tissue

Reader can see terms of some secretory structures in a glossary of Appendix 1. In a detail one should see as sources [11,13–17].

Secretory cells are met as both unicellular cellular structure (unicellular hairs, unicellular spores or microspores that are needed for a plant breeding, single secretory cells among non-secretory ones, etc) or as a part of multicellular secretory structures (multicellular hairs, multicellular glands, resin ducts, nectaries, laticifers, etc). These structures may play roles in plant defence against pest and parasites, in attraction of the insect-pollinators and others [16,11]. Among the secretory organs, which autofluorescence has been studied and well-seen under luminescent or confocal microscope (Fig. 1), were intact single cells such as idioblasts, hairs, generative (pollen or male gametophyte) and vegetative (mainly for species of fam. Equisetaceae) microspores, and cells of multicellular secretory structures of Golosperms and Angiosperms (glands, glandular trichomes, secretory hairs, and others). Various types of secreting cells: (glandular cells, hairs, resin ducts, hydathodes, nectaries, stinging cells, etc.) demonstrate various fluorescence spectra that depends on their chemical composition.

Technique for the Study of the Secretory Cell's Autofluorescence

The phenomenon of autofluorescence may be observed under usual luminescent microscope or confocal microscope, and be registerated by the application of special microspectroflurimeters (see schemes in Appendix 2). Principles of the technique based on modification of a luminescence microscope, in which one can see the fluorescing object in a whole field of view. The luminescence microscopes have a possibility to photo samples. Fluorescence from intact secreting plant cells, that induced by ultra-violet light 360–380 nm, was observed and photographed on the high-sensitive photofilm under fluorescent microscope used for aeroshooting from the aeroplane [10,18,22,23]. Photos, which are represented on the Fig. 1(A–E), demonstrated the examples of the light emission that is seen under luminescent microscope.

Unlike usual luminescent microscope, before catching by photomultiplier, the fluorescence beam from the sample studied passes through confocal aperture named pinhole. Changing the diameter of the aperture, pinhole limits a scattered light from the object points which are out a focal plane and contrasts the volume image [24]. Construction of a confocal microscope permits to observe cellular structures by the mode of a regulation of the depth of an object slide. Cells of algae and some non-secretory cells of higher plants were studied by the method [25,26]. Confocal imaging of secreting plant cells was studied for grass pollen analysis [27]. The example of the fluorescing microspore is shown on Fig. 1(F), where is seen, mainly, internal structure of the *Equisetum arvense* cell (luminescing organelles have been concentrated, perhaps, around non-visible nucleus).

The recording microspectrofluorimetry is also applied for cellular biology. Microspectrofluorimeters were first applied for the studies of fossil pollen [28] and microalgae [29,30]. Microspectrofluorimetric technique is one of the non-invasive methods used for a cellular diagnostics [30,31]. Luminescence of microobjects excited by short wave radiation of an arc lamp and after spectral decomposition is registered by detectors-photomultiplier(s). Microspectrofluorimeters, having a detector with optical probes of various diameter up to 2 μm (the changed areas or probeholes composed with the system of mirrors) have been constructed in the Institute of Cell Biophysics of Russian Academy of Sciences [32–36]. This technique may register the fluorescence spectra or measure the fluorescence intensities at two separate wavelengths. Microspectrofluorimeters can receipt a magnitude fluorescence image of a certain area of the specimen that appears on a spherical mirror. Unlike electron microscopy [16], this method permits the investigation of physiological activity of a secretory process *in vivo*. The emission data may be written in a form of the fluorescence spectra by the help of XY-recorder (could be coupled with a computer). By a using of such microspectrofluorimeters, luminescence
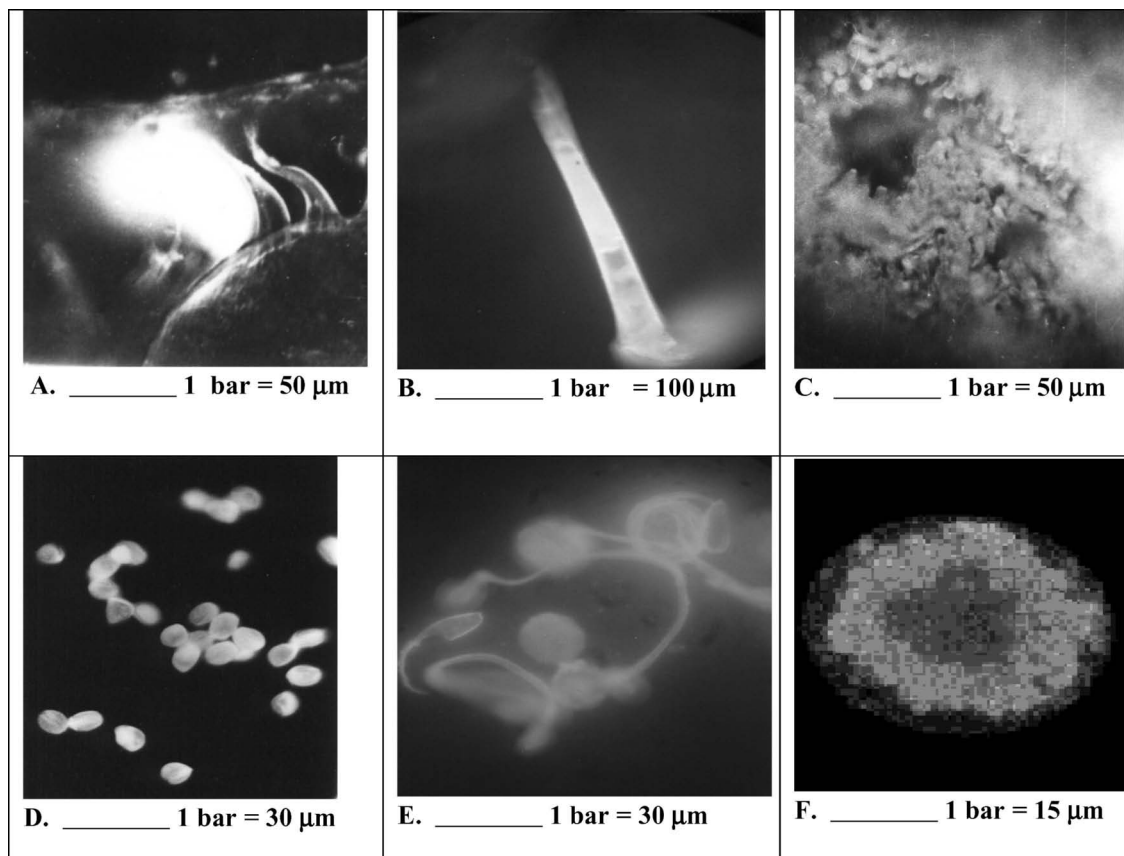
Fig. 1. Autofluorescence of plant secretory cells seen under luminescent microscope at the excitation by ultra-violet 360–380 nm light (A–E) and under laser confocal microscope at the excitation by laser beam 450 nm (F). A. Secretory gland of the bud scale of birch *Betula verrucosa*, B. Secretory stem hair of common nettle *Urtica dioica*, C. Secreting pistil stigma of *Campanula persicifolia*, D. Pollen of birch *Betula verrucosa*, E. Vegetative microspore of horsetail Equisetum arvense with branched elaters, F. Vegetative microspore of horsetail *Equisetum arvense*. Sources: A–after [18], C-after [10,19–21]; B, D, E—unpublished photo of V. V. Roshchina; F-the photo done by V. A. Yashin.

is registered from individual cells and even from a cell wall, large organelles and secretions in periplasmic space (space between plasmalemma and cell wall) as well as from drops of secretions evacuated out of secretory cell on the cellular surface. The fluorescence spectra of secretory cells were registered recently with microspectrofluorimetric technique [7–10,18,22,37–40]. The examples of the fluorescence spectra of some secretory cells are on Fig. 2.

Various modifications of microspectrofluorimeters may not only register the fluorescence spectra, but also measure the fluorescence intensities at two separate wavelengths in a form of histograms related to the fluorescence intensity at λ_1 or at λ_2 as well as to the ratio of their values (See scheme in Appendix 2). A special program "Microfluor" makes it possible to obtain the distribution histograms of the fluorescence intensities and to perform statistical treatment of the data, using Student t-test [36].

By this mode a histogram comparison of the state of different secretory cells were made, especially on the microspores [38,41].

Beside microscopy-related technique, usual standard fluorimeter or MPF fluorescence spectrophotometer with laser 360–380 nm excitation (also in a combination with liquid nitrogen kryostation) was used for the study of the fluorescence spectra on 1–3 mm—film (layer) of pollen at room and temperature of liquid nitrogen [42]. Due to the apparatus, more picks in the fluorescence spectra of the samples have been observed in a comparison with the spectra of pollen from the same species that were registered by microspectrofluorimetry [8,9,23].

Some Characteristics of Fluorescing Secretory Cells

Historical excursion shows that the ultra-violet-excited light emission of secretory cells under
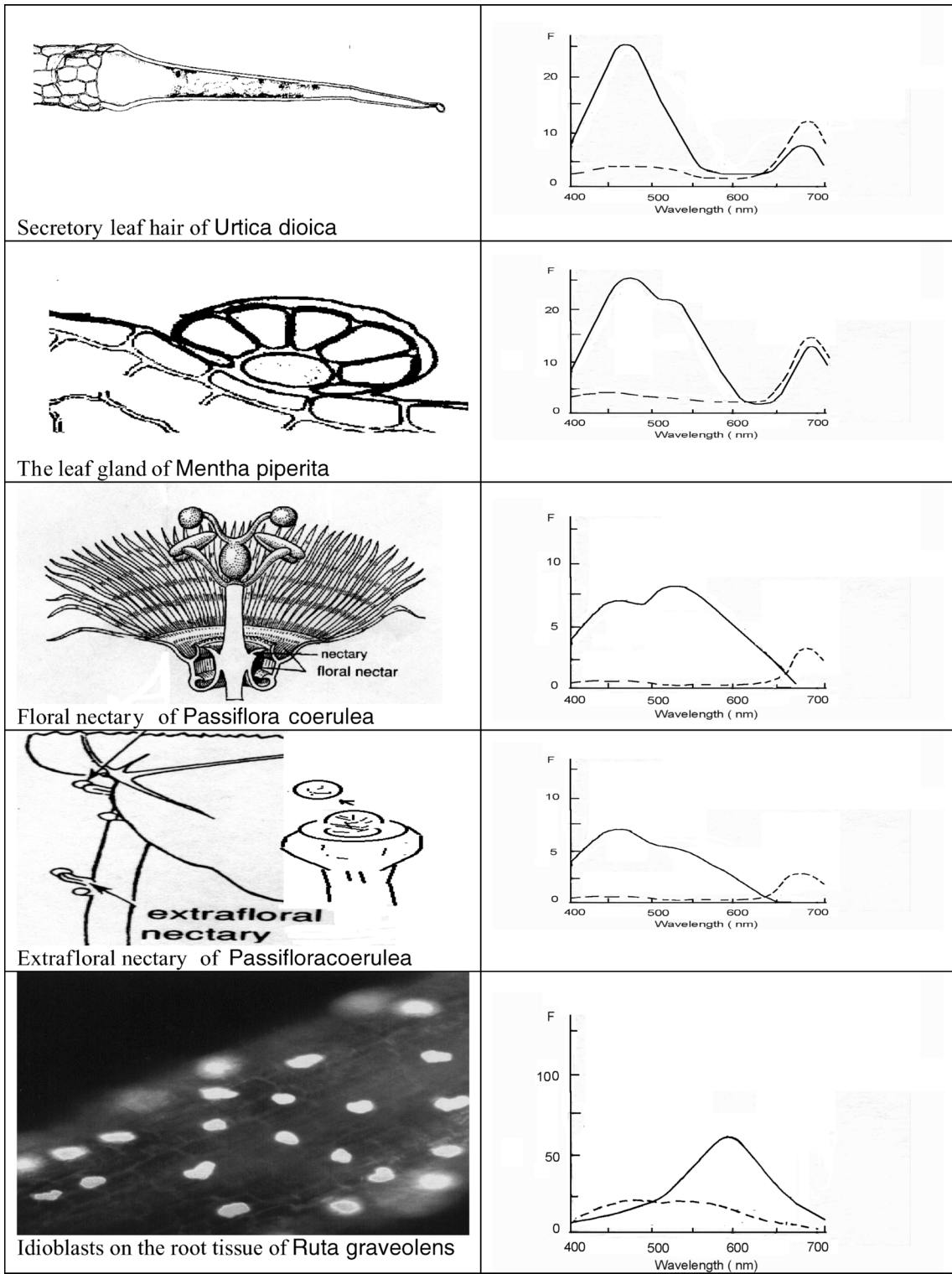
Fig. 2. The examples of fluorescence spectra registered by microspectrofluorimetry from the secretory cells of some plants. F: fluorescence (relative units); unbroken line: secretory cell; broken line: nonsecretory cell.

luminescence microscope has been first demonstrated on pollen [43–45]. Later, using the luminescent microscopy, the fluorescence of secretory cells has been found in leaves [5,46], roots [4,47], in pistil of flowers [48]. Microspores are single secretory cells themselves, and the interest to the fluorescence of pollen grains was maintained by botanists and geologists [49–51], The phenomenon of the autofluorescence of the above-mentioned cells is used for pollen analysis in botany [50,51], in geology [49], meteorology [52], and microphotography of fluorescing secretory structures [5,6]. The beginning of the studies, which are related to a character and a nature of the fluorescence from individual secretory cells and perspectives of the application of the phenomenon to the practice, was in 90s of 20th century [8–10,18–22,39,53,54]. The fluorescence spectra of more than 50 plant species with various secretory cells are characterised in special papers [7–10,18,22,37,39]. The examples of the registered autofluorescence are shown on Fig. 2.

Estimating the fluorescence spectra of secretory cells, where abundant secretion is seen, main fluorescence detected is defined by the composition of secretion and differs from non-secretory cells surrounded (Fig. 2). As shown on Fig. 2, cells of hairs, glands, and nectaries have maxima, related to secretions themselves, in blue, blue-green and yellow-orange regions of the spectra, on the contrary to non-secretory cells of leaf, stem or parts of flower, which either have no maximum at 680 nm, peculiar to chlorophyll or have a small one [9,10]. Due to this characteristic it is easy to distinguish secretory cells from non-secretory ones (Fig. 2). Moreover, floral and extrafloral nectaries differ in their nectar composition that is seen from the different fluorescence spectra. Surrounded non-secretory cells have no maxima in the spectral region, which is shorter, than 650 nm. Chlorophyll-less cells of root tissue (Fig. 2) also demonstrated differences between secretory and—non-secretory cells. Idioblasts, which are secretory cells, fluoresce in orange region of the fluorescence spectra whereas surrounded non-secretory cells—at shorter wavelengths.

What are main evidences that visible fluorescence of secretory cells belongs to, mainly, a secretion? First of all, the evacuation of the secretion from the secretory cells leads to the quenching of the luminescence within the secretory cell, for instance it is well seen for leaf secretory hair of common nettle of *Urtica dioica,* tomato *Lycopersicon esculentum*, and calendula *Calendula officinalis* [18,22]. Empty space within secretory hair is observed as a dark space. Often drop of lipophilic secretion may stay on the end of the secreting hair and can fluoresce, looking as lightening part against the dark background

of empty hair [18]. Only cell walls, when they include phenols, have a weak emission, which is non-measured or measured maximally as less, than 7–10% of total emission of fluorescing secretory cell [10]. Therefore, about 90% of the emission of secretory cell is due to the its secretion. Second evidence of the main contribution of the secretion to the light emission of secretory cell is an analogous fluorescence of the secretion released on the surface such as liquids or crystals of some alkaloids, terpenes and phenols [9,10,22]. For microspores such as pollen or vegetative microspores the most bright fluorescence is observed when the microspores are dry. The contribution to the light emission belongs to both rigid polymer secretion of exine and slightly released secretion of the microchannels [8,37,39]. Water quenched the luminescence of viable pollen, but not non-viable one [8,36]. The liquid secretion is released at the moistening and can fluoresce out of a cell, but more often it is weak or is completely quenched by water.

Characteristic types of fluorescence of secretory cells which more suitable as biosensors may be found among them. This could be observed under luminescent microscope and measured by microspectrofluorimetry.

Main Characteristics of the Fluorescence of Secretory Cells

Fluorescence of intact secretory cells is a sum of emissions derived from several different groups of substances, which either are excreted out or accumulated within cell and linked on the cellular surface (Fig. 3). Beside that, cellular compartments can also contribute in the fluorescence. In non-secretory cells of chlorophyll-containing tissues of leaves, stems and flowers main colour of fluorescence emitted by the cells under luminescent microscope is blue-green, if derived from cellular walls or red, if is collected from chloroplasts, where chlorophyll is located [3]. Except vacuoles, which may be filled with secretory products, the autofluorescence contribution of other organelles is very small, if there is at all. Unlike non-secretory cells, secretions of the specialised secretory cells have visible peculiarities, which differ in their colour and intensity that is well seen from their fluorescence spectra (Fig. 4). About 90% of the emission of secretory cell is due to its secretion.

In multicellular tissues of leafs, stems, roots and seeds fluoresced secretions accumulated within cell and in extracellular space between plasmalemma and cell wall (Fig. 3) and excretions are mainly in liquid state, except the deposition of crystals on the surface. As for microspores, for instance pollen (Fig. 3), they have a complex secretory structure: 1) a cover with outer layer named exine (rigid product of the cellular secretion during the anther

CELL SURFACE

pollen surface
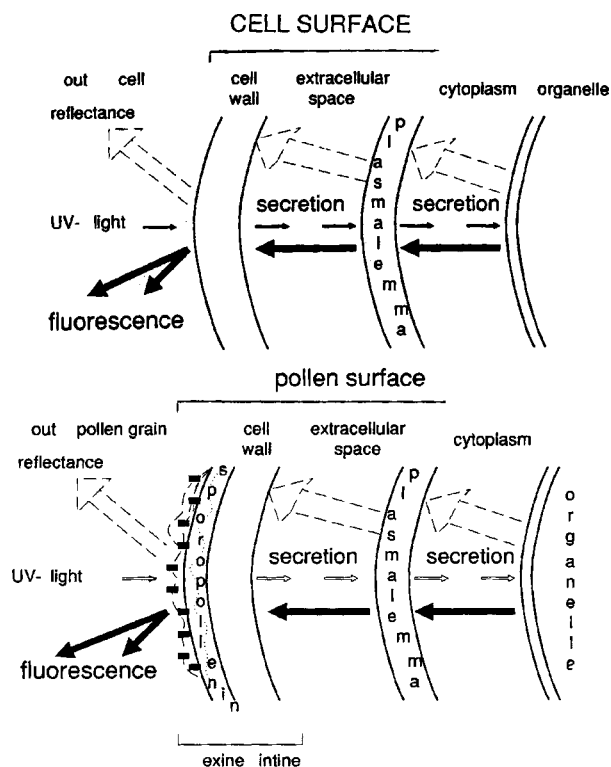
Fig. 3. Scheme of the possible fluxes of UV-radiation (excitation) and fluorescence in plant secretory cell as a whole (upper side) and, in particular in microspores such as pollen (Below).

development) which polymerised material (sporopollenin) brightly fluoresced due to phenolic or carotenoid residues; 2) microchannels in exine filled of liquid secretion [20], consisting of pigments, lipids, proteins, etc, some of which also fluoresce. The contribution to the light emission belongs both to a rigid polymer secretion of exine and, in small degree, to a released secretion of the microchannels [8,10,19,20,37,39]. Rigid polymer material of exine, which is peculiar to microspores, includes pigments such as carotenoids or phenols [13]. The extraction of these pigments of *Petunia* pollen by 80% acetone and then by benzol or chloroform leads to the missing all maxima in green-red region, only the weak blue fluorescence is seen [23].

Water quenches the luminescence of viable pollen, which is capable to germinate in vivo or in vitro, but not non-viable one that is impossible to any development [8,37]. Moreover the emission of non-viable pollen is approximately 3 fold higher. The liquid secretion is released at the microspore moistening and can fluoresce out of a cell, but more often it is weak or is completely quenched by water, if the secretion is lipophilic.
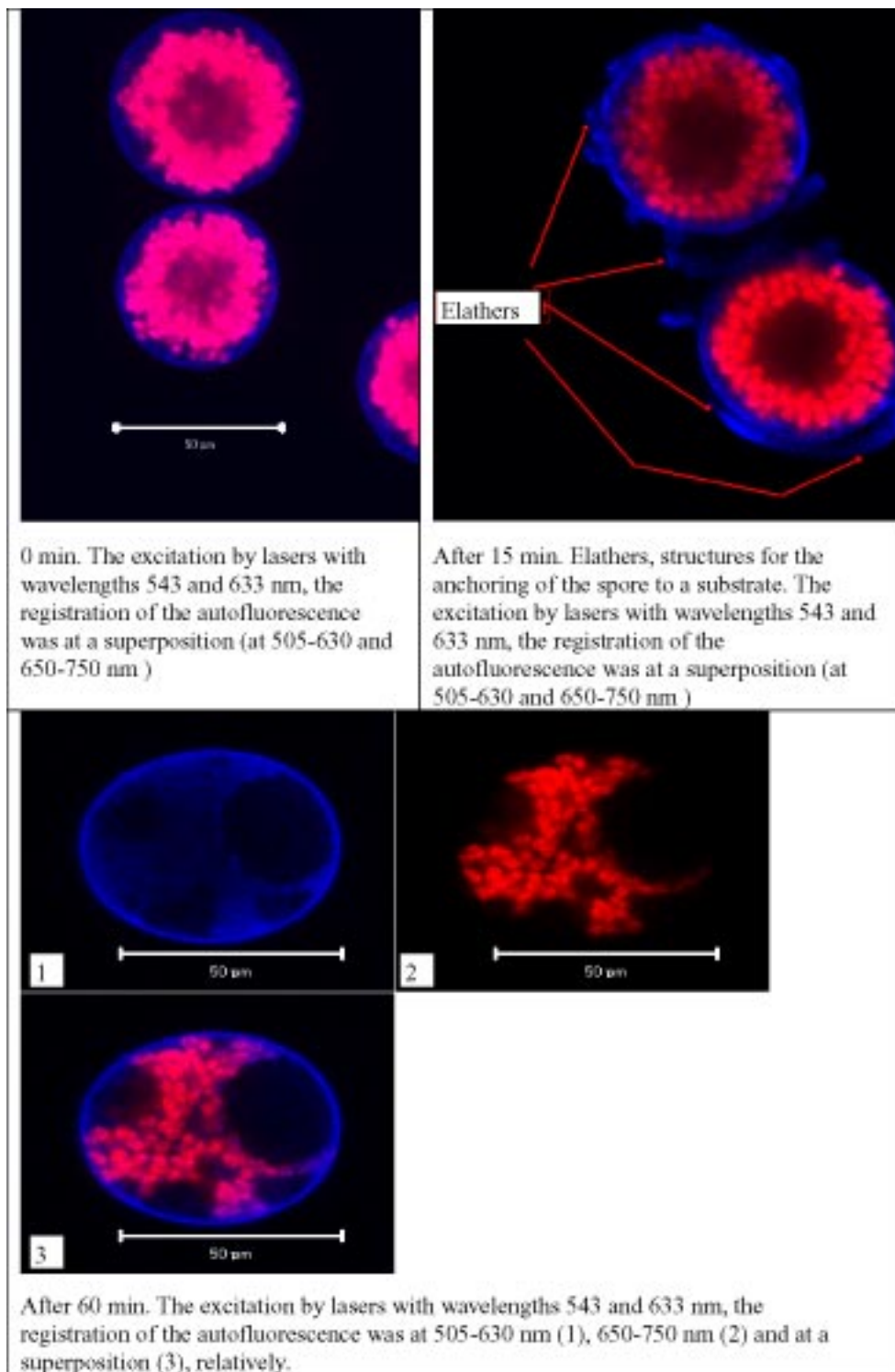
Most studied secretory cells have one or several maxima in the fluorescence spectra: 1—for generative microspores or pollen of some plant species, 2 for generative microspores, vegetative microspores, glandular cells and trichomes on leaves and stems, 3 or 4 for generative microspores, vegetative microspores, glandular cells and trichomes on leaves and stems [8–10,37,38]. It depends on the chemical composition of their secretions and excretions. The multicomponent mixtures in secretions, however, demonstrate luminescent characteristics of prevailing substances. Table I shows the fluorescence maxima of known secondary metabolites which can define the light emission of the secretions in intact secretory cells from various plant species in comparison with their excretions.

In violet-blue region (420–480 nm) of the spectra alkaloids, phenols, terpenes and some aliphatic compounds fluoresce (Table I). The glands and glandular hairs of terpenoid-rich species contain substances, fluorescing at 420–480 nm terpenes such as monoterpenes (monoterpene alcohol menthol), sesquiterpenes (azulenes), and phenols (flavonoids, aromatic acids). The fluorescence scales of the substances which are included in the secretions of secretory hairs and other secretory cells may be also useful for the pharmacological analysis of medicinal plants [55–57]. This permits the fast control the accumulation of active matter without long biochemical procedures. Microspectrofluorimetry permits to analyse both content of intact nectar-containing cells and small drops of the secretions of floral and extrafloral nectaries on *Passiflora coerulea* or *Impatiens balsamina* [10]. Reader can see the fluorescence spectra on Fig. 2. Isolated cellular walls include blue-fluorescing ferulic acid [58], which also may contribute in a whole fluorescence that is seen under luminescent microscope [59]. But, in our practice, the fluorescence of cell wall is not so intensive and is usually observed out the secretory drop [10].

As shown in Table I, in green-yellow (500–570 nm) and yellow (575–585 nm) spectral regions coumarins and some flavonoids, in particular quercetin and rutin, fluoresce (Table I). This is observed in the secretory cells of leaf buds during the development of their secretory structures and accumulation of the secretory products appear to observe by the microfluorescent technique [10,18,22]. Above-mentioned secretory cells may be recommended for cellular control of the development in nature.

In orange (585–620 nm) and red (625–700 nm) region of the spectra the fluorescence of some alkaloids, anthocyanins and azulenes is seen [10,22,60]. Secretory cells of roots contains alkaloids, while anthocyanins and azulenes are met in generative (pollen, male gametophyte) and vegetative microspores (mainly in fam. Equisetaceae). Terpenoid-containing cell often include azulenes which also contribute in the emission when are linked with cellulose [7]. The contribution of chlorophyll at 675–680 nm

0 min. The excitation by lasers with wavelengths 543 and 633 nm, the registration of the autofluorescence was at a superposition (at 505-630 and 650-750 nm)

After 15 min. Elathers, structures for the anchoring of the spore to a substrate. The excitation by lasers with wavelengths 543 and 633 nm, the registration of the autofluorescence was at a superposition (at 505-630 and 650-750 nm)

After 60 min. The excitation by lasers with wavelengths 543 and 633 nm, the registration of the autofluorescence was at 505-630 nm (1), 650-750 nm (2) and at a superposition (3), relatively.

Fig. 4. The changes in the fluorescing microspores of *Equisetum arvense* during their development for first hour after moistening.

Table I. The Fluorescence Maxima of Main Substances Occurred in Plant Secretory Cells (SC) and Excretions (E)

Class of substances	Occurrence			Representative	λ_{ex}/λ_f (fluorophore)
	In plant taxa	In organs	In secretory structures		
Alkaloids					
Acridone type	Rutaceae	Roots	SC	Rutacridone and other acridone alkaloids	381/590–595 (acridone)
Isoquinoline type	*Berberis vulgaris*	Leaves, roots, stems and fruits	SC	Berberine, chelerythrine	380/510, 540 (isoquinolinium)
Tropolonic type	*Chelidonium majus* L. *Crocus autumnalis* L.	Stems and roots Bulbs	Latex and laticifers SC	Berberine, chelerythrine Colchicine	380/510, 540 (isoquinolinium) 360/435
Anthocyanins	*Petunia hybrida, Papaver orientale*	Flower	Pollen	Petunidin	Shoulder 510, 555, 570–585, shoulder 610
Azulenes (Derivatives of sesquiterpene lactones)	*Artemisia, Achillea, Matricaria, Equisetum arvense*	Flowers, leaves, and stems, Microspores	Oil glands, trichomes, pollen Vegetative microspores	Azulenes of pollen bee-collected, Chamazulene 1,3-chlorozazulene Guaiazulene	360–380/420, 620, 725 360–380/410, 430, 725 360/430 60/400, 433 (lactone)
Carotenoids	Many families	Flowers	Pollen cover	Carotenes, xanthophylls	520–560
Coumarins (Dicoumarins)	Many families, *Aesculus hippocastanum*	Woody plant buds	SC and PE	Coumaric acid esculetin 7-hydroxy - and 5-7-hydroxycoumarins	360/405–427, 360/475, (524) 300/479
Cytokinins	Many families	All parts of plants	E	Kinetin	380/410, 430 (adenine)
Flavonoids	Many families	Buds of woody plants	SC or glands	Galangin Kaemferol Quercetin	365/447–461 365/445–450 365/440, (584)
Furanocoumarins	*Heracleum sibiricum., Psoralea corylifolia*	Leaves, stems, flowers, and roots	SC, glands, E	4-Methylpsoralen	360/440–420 (furanocoumarin)
C6–C3 hydrocinnamic acids and their esters	Many families	Buds	E, SC	Caffeic acid, cinnamic acid, Ferulic acid	365/450, 360/405–427, 350/440 (phenolic ring)
Indole derivatives	Many families, *Urtica dioica* L.	Leaves, stems, flowers and fruits	E, stinging trichomes	Serotonin	360/410–420 (indole)
Monoterpenes and their alcohols	Rutaceae, *Mentha piperita,* Asteraceae, Many species, Labiatae *Salvia*	Flowers, leaves, and stems	SC, glands, E	Menthol, camphor, camphor derivatives	360–380/415–420 310–313/404–413
Polyacetylenes	*Cicuta virosa*	Roots	SC, secretory reservoirs	Cicutotoxin	360–380/580 (three triplet bonds)
	Artemisia capillaris	Leaves, roots, and flowers	E	Capilline	360/408, 430 (two triplet bonds)
Sesquiterpene lactones	Genus *Artemisia Gaillardia pulchella*	Flower	E	Artemisine Gaillardine	360/395, 430 (lactone) 360/415 (lactone)
Tannins	Many species	Leaves, stems, fruits, and roots	Idioblasts	Gallic or valonic acid	360–380/500 (polyphenol)

Note. λ_{ex} and λ_f: wavelengths of excitation light and fluorescence. Sources: [12,10,58,62] and unpublished data of Roshchina.

is seen only for chlorophyll-containing cells. Medium also play a certain role in the orange-red emission of intact cells. It can be illustrated in model system—root cells of *Ruta graveolens* which have several secretory structures, filling with lipophilic secretion enriched acridone alkaloids [4], and brightly fluorescing with maximum 595–600 nm among surrounded non-secretory cell with blue (maximum 460–480 nm) fluorescence [61,62]. Lipophilic crystals of rutacridone, representative of acridone alkaloids, and its solutions in non-fluorescing immersion oil also fluoresced orange with maximum 600 nm. Similar orange emission was observed only for the root extract by chloroform or, in less degree, by 100% ethanol, but not for extracts by water or acetone.

Special attention is to microspores. Microspores are usually covered by the multilayer cell wall where outer layer, named exine, consists from polymeric material sporopollenin. The fluorescing sporopollenin components of pollen are phenols (maxima at 440–480 nm), carotenoids (maximum at 500–560 nm), and azulenes (maxima at 440–460 nm, and for azulenes linked with cellulose—at 620–640 nm). The exine of pollen grains also contains anthocyanins (maxima at 450–470 and 600–640 nm), the carotenoid bodies (maxima at 500–560 nm), and other fluorescent products [8–10,63]. Composition of exine of vegetative microspores such as of *Equisetum arvense* is not well studied. It is known that microspores contain fluorescent components, for instance flavonoids quercetin and kaempferol, various alkaloids, carotenoids, chlorophyll [64], and recently azulenes have been found here [38]. The maxima of main components are in Table I.

Although fluorescence of the non-secretory products is not large in a comparison with the secretory ones (only 7–10%) because the cellular fluorescence intensity drops sharply when the secretion is released from the cell [10], it also needs to consider this possibility, besides secretory products themselves. Among excreted metabolites are metabolites, participated in energetic reactions and in the formation of cellular wall cover as well components linked or excreted by cellular membranes which also may contribute in the visible fluorescence.

The light emission of the cell wall is of special interest. Pure cellulose in cell wall demonstrates very weak emission, but the fluorescence is strengthening when phenolic residues (maximum at 440–460 nm) are included [10]. The higher fluorescence of the cell wall in other plants is, probably, due to surface NAD(P)H (maximum at 460–470 nm) or flavin (maximum at 520–540 nm) redox reactions, taking place on the plant surface. Among products secreted through the cell wall is there hydrogen peroxide which induces the accumulation of insoluble fluorescent material, on the surfaces of cell walls. When polymeric material containing phenolic components (cinnamic acid derivatives), accumulates in the cell walls, H_2O_2 takes part in formation of phenolic cross-links, the fluorescence of the surface increases and becomes bright greenish-yellow. The peroxidase-catalyzed polymerization of the pollen sporopollenin also needs H_2O_2 and the pattern of light emission during the process of pollen development [10] may be related with the peroxidation reactions.

The contribution of reactive oxygen species in the autofluorescence of secretory cells, such as superoxide anionradical and peroxides, is also established with the using of the enzymes superoxide dismutase and peroxidase, relatively, which decompose the oxidants [10,41]. The secreting surface of the pistil stigma in a flower shows the decrease in the light emission only in the presence of peroxidase whereas the luminescence of generative and vegetative microspores quenches, in a different degree, by both enzymes studied. Exogenous hydrogen peroxide and some organic peroxides stimulate their autofluorescence [41].

The plasma membrane also produces NAD(P)H and dependent products, therefore the pyridinenucleotides fluoresce. The contribution of internal content of the secretory cell to the total fluorescence also relates to the transparency of epidermal cells. The actinic ultra-violet light easily penetrates through them and excites the luminescence of a secretion located both in periplasmic space and within a cell, as well as luminescence of cellular organelles. The fluorescence of the secondary metabolites of the secretion has been described above. The light emission of microsomes, mitochondria, chloroplasts may be caused by their production of NAD(P)H and flavines. The fluorescence of carotenoids and, mainly, chlorophyll, prevails in a total luminescence of the chloroplasts. Chlorophyll may contribute to the total cellular luminescence of chlorophyll-containing secretory cells, but its maximum at 680 nm differs from the emission of the secretions (<640 nm).

THE BIOSENSOR AND BIOINDICATOR CAPACITY OF AUTOFLUORESCENCE

Autofluorescence could be used: 1. in express-microanalysis of the accumulation of the secondary metabolites in secretory cells during these development without long-term biochemical procedures; 2. in diagnostics of cellular damage; 3. in analysis of cell-cell interactions. These possibilities will be considered below.

Express-Microanalysis of the State and Accumulation of Secondary Metabolites

During the plant development secretory cells may change both the composition of the fluorescing secretions

and their amount that could be measured, estimating their characteristic fluorescence spectra and the fluorescence intensity [8–10]. Most medicinal plants have secretory cells, where pharmacologically-valuable secondary metabolites are accumulated [10,11]. Experiments with leaves and flowers of some plants, which belong to family Asteraceae and contain both sesquiterpene lactones and their derivatives such as azulenes, showed the increase of green-yellow or yellow-orange fluorescence of these intact cells during their development [10,22,65]. For instance, such emission is observed only after the appearance of formed secretory cells on the petals of flower from medicinal plant *Achilea millefolium*. Using double-beam microspecrofluorimeter (see Appendix 2), the ratio of the fluorescence intensities I red (maximum of emission at 640 nm) I green (maximum of emission at 530 nm) was measured. This ratio decreases in petals, which are covered with matured secretory cells, in a comparison with petals of the flower bud, where there are only few or no similar cells. This occurs due to a filling of matured secretory cells with green-fluorescing secretion (Fig. 4). The histogram, which show the distribution of the fluorescing cells shows the appearance of cells with increased green fluorescence whereas red fluorescence of chlorophyll becomes smaller due to the increase of secretory cells on the petal surface (Fig. 4).

Fluorescence of the microspores is also changed during their development. Development of pollen from various plant species on artificial nutrient medium is showed that fluorescence of viable (able to germinate as defined in Appendix 1) pollen is quenched by water medium during first 5–10 min after moistening whereas non-germinating pollen grains have 2–3 times more intensive fluorescence and for a long time [8]. Moreover, fluorescence of dry non-matured and matured pollen also differs. For instance, non-matured pollen of *Tussilago farfara* demonstrated green fluorescence with maxima 465, 518 and 680 nm in the fluorescence spectra whereas matured pollen, which is lack of chlorophyll, showed, mainly, yellow fluorescence with maxima 465, 520 and 535–540 nm [8,10]. Blue fluorescence (maxima at 465–470 nm and small shoulder at 665 nm) of non-matured pollen of *Philadelphus grandiflorus* changed to yellow-orange emission (maxima at 465, 510–520 and 620 nm) in matured pollen grains [8,10] that occurred due to the synthesis of carotenoids (maximum 520 nm) and azulenes (maximum 620–640 nm) during the development of the generative microspores [7,8,10]. Moreover, fluorescence of pollen could be useful at the distinguishing of a self-compatible and a self-incompatible pollen of the same species, like it was seen in *Petunia* clones [7,66]. In the self-incompatible clone there was no maximum at 620–640 nm in the

fluorescence spectra that correlated with the absence of azulenes. The yellow fluorescence at 530–560 nm also was small due to the lower concentration of carotenoids [8,10].

Autofluorescence of secretory cells may be useful for the express—pharmacological analysis of medicinal plants which pollen and secretory cells of vegetative tissues contain physiologically valuable components as an active matter.

Autofluorescence in Diagnostics of Cellular Damage

Damaging factors, such as ageing, ultra-violet light or high doses of ozone and other factors, influence secretory cells [10,11,19,40,67–69]. Changes in their composition are seen from the fluorescence spectra, for instance for intact *Hippeastrum hybridum* pollen and its chloroform-ethanol (2:1) extract (Fig. 5). Ageing, the storage after the collection of *Papaver orientale* pollen from 1994 to 1998 years, shows the formation of new fluorescing products with maxima in more short wavelength region that may be dealt with the ageing [21]. UV-light (300–350 nm) treatment for 1 hr induced the analogous shifts in the pollen fluorescence from orange to green or blue) for *Hemerocallis fulva* and *Dactylis glomerata* [21]. But γ-irradiation (3000 g) only decreased the total green fluorescence by 50% in pollen of *Hippeastrum hybridum* [19].

Secretory cells of glands, glandular trichomes and other structures, localized on the surface of leaf, stem and leaf-derived parts of flower (petal, sepals, anthers, stamens), also interact with ozone (see monograph of [67]). The properties of the individual components are changed [11,70], showing the changes in the fluorescence of secreting cell [40,69]. For instance, in *Raphanus sativus* character of the fluorescence spectrum registered from the secretory hairs themselves, usually blue light fluorescing, changes significantly after the ozone treatment (Fig. 6)—additional green—yellow (510–540 nm) emission arises. Thus, under ozone the changes inside the hair occur that is seen as a formation of fluorescing products [67]. Ozone often induced the green-yellow fluorescence of the dry vegetative microspores of *Equisetum arvense* [41,60,69,71]. Significant green-yellow fluorescence is developed in a response on ozone in vegetative microspores of *Equisetum arvense* (Fig. 7). Perhaps, the phenomenon is related to fluorescing products of a lipid peroxidation that occurred under the oxidative stress [10].

Fluorescing products also arise at ozonolysis in generative cells, for instance in pollen [67]. Pollen, which is lack of the pigments, such as of *Plantago major*, have no significant changes in its fluorescence whereas pigmented pollens, for instance enriched either in carotenoids as *Pas-*
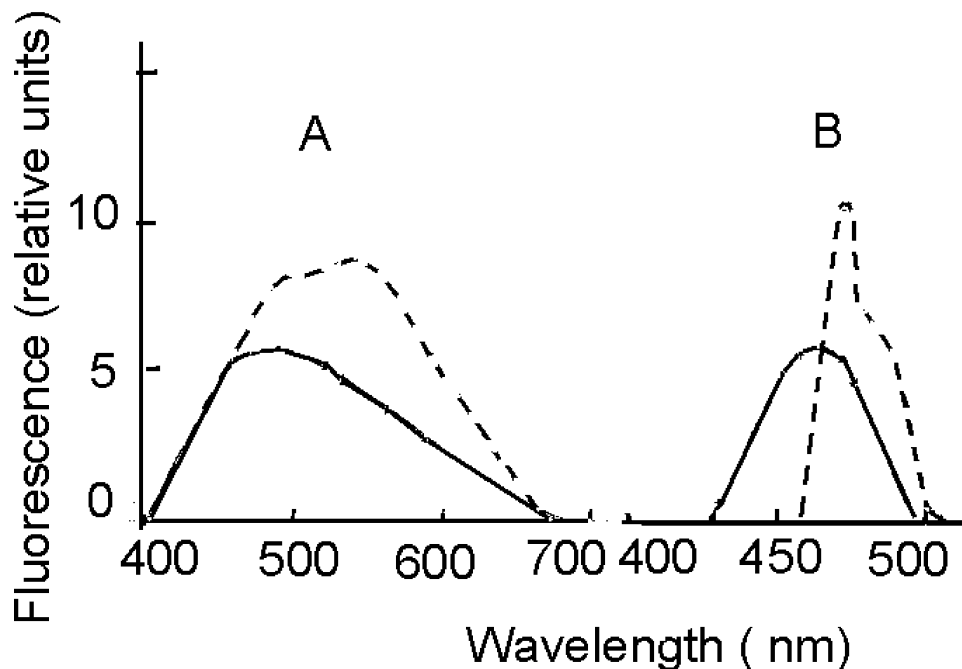
Fig. 5. The fluorescence spectra of the intact pollen of *Hippeastrum hybridum* (A) and its chloroform–ethanol extract (B). Fresh pollen (unbroken line), 1 year aging ((broken line).

siflora coerulea or phenols as *Hippeastrum hybridum*, had the shifts in the fluorescent spectra. The ratio of secreting microspores such as pollen of *Papaver orientale* may be changed under the treatment with ozone, which alters the value of the Igreen/Ired ratio of fluorescence (Fig. 8). Similar changes in the autofluorescence and the orange-blue shift were also observed for the ozonated pollen from many other plant species [40,67].

If the plant cell or tissue is damaged (ageing, UV-light, ozone) formed products of free radical processes,

including a lipid peroxidation, can fluoresce [72]. The nature of fluorescing pots on leaves and microspores of plants is supposed to be associated with the Schiff bases, which are formed at free radical reactions in a lipid fraction of membranes. Deeper changes lead to a formation of fluorescing pigment lipofuscin, which is often found in old tissues of plants as a final product of a lipid peroxidation [73]. The contribution to the autofluorescence at 410–440 nm appear to be attributed to the pigment lipofuscin. The lipofuscin participation in the orange-blue
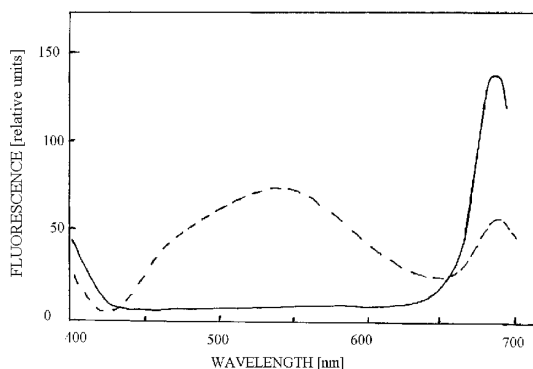

Fig. 6. The fluorescence spectra of the leaf secretory hair of leave from 14-day-old seedlings of *Raphanus sativus* without (unbroken line) and after the treatment with ozone 0.15 ppm for 3 h (broken line).
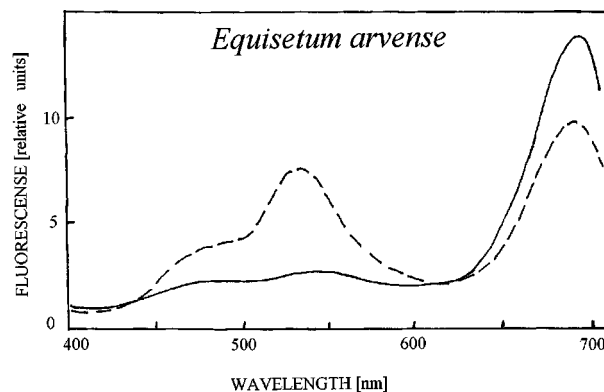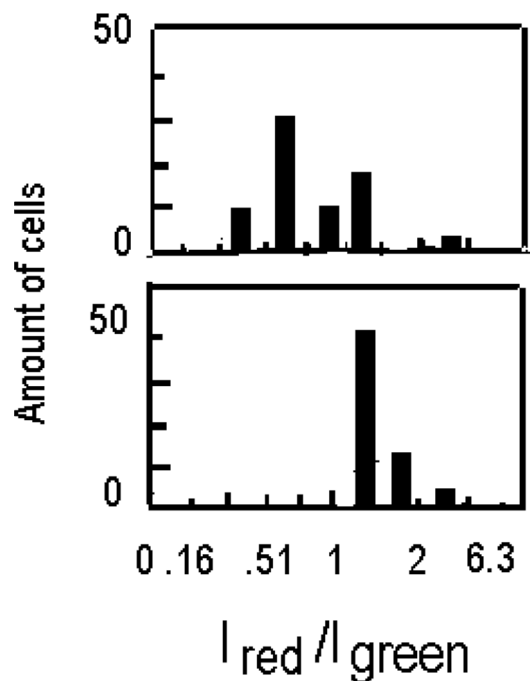

Fig. 7. The fluorescence spectra of vegetative microspore of horsetail *Equisetum arvense* without (unbroken line) and with the treatment by ozone 0.05 ppm (broken line).

Fig. 8. The summed distribution Student t-test histograms of parameter I_{red}/I_{5green} (green-red transition fluorescence intensity) for the dry pollen Papaver orientale without (upper side) and after the treatment with 0.05 ppm of ozone (below). Pollen without any treatment have the ratio equal 0.78 ± 0.1 whereas after the treatment with ozone in total dose 0.2 ppm for 5 days -1.27 ± 0.1. In second variant the histogram shifts to red region of the spectra.

shift (from 560–580 nm to 440–460 nm) in the fluorescent spectra of intact pollen of *Philadelphus grandiflorus* under the ozone treatment has been shown by [68].

Ozone and active oxygen species such as superoxide anionradical and peroxides, which are formed on the secretory cell surface at norm and ozonolysis, also can contribute to the fluorescence changes. It has been demonstrated [10,40,41,54,68] in experiments, in which pollen and pistils from various species treated with ozone, enzyme superoxide dismutase (destroying superoxide radical by dismutation), and peroxidase (cleaving organic peroxides) as well as low-molecular antioxidant ascorbate. The light emission increases when generator of superoxide anion radical noradrenaline or hydrogen peroxide are added on the surface of secreting cells of the *Hippeastrum* pistil stigma [10]. A new maximum 600–620 nm is observed if the antioxidants ascorbate and peroxidase are put on the surface.

Secretory cells of vegetative tissues or microspores are suitable objects for O_3 monitoring due to various fluorescent compounds in secretions [40,67,69]. Low doses of ozone <0.15 $\mu l/l$ induced green-yellow fluorescence

of the leaves of *Plantago major, Zea mays, Raphanus sativus* and *Hippeastrum hybridum* as well as of vegetative microspores of *Equisetum arvense*. The light emission strengthened with the increase of the ozone concentration that showed the damage of the lightening cells, which are in an apoptosis or even in a necrosis. At higher doses of ozone blue fluorescence 420–470 nm was observed in pollen, containing carotenoids such as *Passiflora coerulea, Philadelphus grandiflorus* and *Hemerocallis fulva*, which usually, at norm, fluoresce in yellow-orange region 530–560 nm. These pollen grains have already lost their fertility, and lipofuscin was formed in their cover [68].

Thus, analysis of the autofluorescence could early indicate a damage of the secreting cells induced by a stress factor.

Autofluorescence at Cell-Cell Contacts

Cellular autofluorescence also changes at the interactions between cells, for instance between sexual cells of the one and same plant species or between cells of various plant species (allelopathy). At cell-cell contacts one cell-acceptor by a help of own excretion and sensor systems receipt a chemosignal from a secretion excreted by other cell, which is named a cell-donor. There are a recognition of the signal and the response to the signalling. The fluorescence of both contacting cells may change, for instance at fertilization (interaction between sexual cells—female and male gametophytes such as pollen and pistil in a flower) or chemical relations between cells of different species (pollen of own and foreign species usually have contacts, when are added on the one and same pistil). Therefore, the fluorescence could be considered as a response on the external chemosignal and, simultaneously, as the indicator of the signalling. Cell-donor for chemosignal (fluorescent signalling substance in its excretion) and cell-acceptor of the same chemosignal (recognising systems in its excretion) interact via their excretions that takes place in allelopathic relations between different plant species within biocenosis [57,65]. The changes in the fluorescence of cell-donor and cell-acceptor may be observed simply under the luminescent microscope or with a help of microspectrofluorimetry [18,22,39,53,54]. Similar contacts take place at any chemical relations between species named allelopathic interactions (allelopathy), and the cellular changes as changes in the cellular fluorescence could be also measured [18,22,39]. For instance, such earlier diagnostics of pollen from antagonistic plant species (pollen of which inhibit the germination of pollen from surrounded plant species that prevents a normal fertilization and breeding) helps to design more suitable phytocenosis in the agriculture and the forest
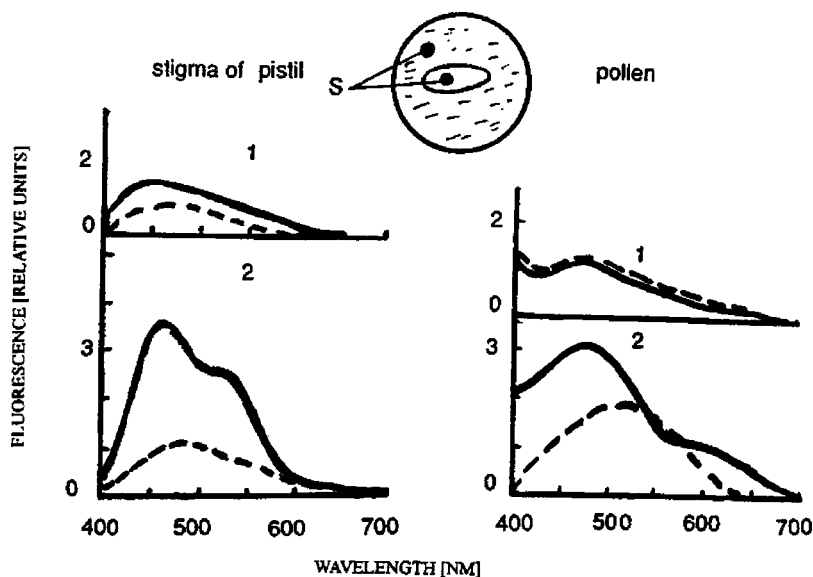
Fig. 9. Changes in the fluorescence spectra at pollen-pistil contacts. Position of optical sound (S) is shown for the pistil stigma and pollen added on the pistil surface. Left. The fluorescence spectra of the pistil stigma of Hippeastrum hybridum without (---) and with (___) pollen grain added. 1. Foreign pollen of *Dactylis glomerata*; 2. Self (own) pollen of *Hippeastrum hybridum*. Right. The fluorescence spectra of pollen with (___) and without (---) interaction with the pistil stigma of *Hippeastrum hybridum*. 1. Foreign pollen of *Dactylis glomerata*; 2. Self pollen of *Hippeastrum hybridum*. All changes is observed only at the contact of pistil with self pollen–pollen of the same plant species.

economy. Below we shall consider some examples of the fluorescence at the cell-cell contacts.

As shown in Table I, the generative structures—pollen and pistil also fluoresce under ultra-violet light. At fertilization of plants generative male microspore, which is named pollen, is added on the surface of pistil by wind, insects or by the human hand. For instance, cell-cell interactions of pollen (Fig. 9) with cell of the pistil stigma at their contacts result in the changes in the fluorescence spectra of cells contacted. The fluorescence of both contacting cells are changed if the cells belong to one and the same plant species. In opposite case, there was no changes [8,10,37]. By this mode of earlier cytodiagnostics, a researcher can know whether cells recognize, who is "own" or "foreign." So the pistil refuse to receipt "foreign" pollen, to permit it to germinate, and then to react with an egg cell. Moreover, pollen can germinate only on the pistil of the same plant species. Possible contacts between "own" and "foreign" pollen grains are also demonstrated out pistil, modelling their interactions on the pistil stigma [22,39]. This is interpreted as models of allelopatic relations between pollen of different plant species that leads to the inhibition or the stimulation of their ability to grow on the pistil stigma [57]. Using the fluorescent characteristics, plant clones with the self-incompatible pollen (although this pollen is given from the flower of the same species that

the pistil is, but a self-pollination is not impossible) may also be distinguished from the clones with self-compatible pollen [8,21,66]. It is necessary to know for the specialists in the plant selection and the genetic practice.

Fundamental studies of the fluorescence in cell-cell contacts under luminescent microscope may be done so that plant is not damaged. For instance, there was similar manipulation with pistil, which was pollinated, but not was separated from the flower of a whole plant *Hippeastrum hybridum* [53,54,74]. In this case, after the pollination and the fluorescence measurement with microspectrofluorimeter plant studied followed its growth and development up to the formation normal fruits and viable seeds.

The difference in the form of the spectra and position of their maxima both for the pistils and pollen grains depends on the plant species and the composition of the surface of the pollen grains. Reviews [10,22] summarized the fluorescence maxima of studied pistil and pollen from many species and shows the variety of peaks, dealing with the different components of their surface and excreta. As seen in Table I, their fluorescence spectra show maxima at 460–490 nm (blue), 510–550 nm (green-yellow) and 620–680 nm (orange-red). Blue and blue-green fluorescence could be related to phenolic compounds, green-yellow—with carotenoids and orange-red—with chlorophyll [12] or azulenes [7]. In a dependence on plant species, the

pistil stigmas also contain phenols in the exudates [10,19–21,22], which can fluoresce both in blue and yellow spectral region.

Beside above-mentioned compounds, the excretions from pollen grains contain acetylcholine and histamine, which are named as neurotransmitters in animals [56,57]. It was shown that both compounds induced the changes in the pistil stigma fluorescence, like is observed at pollen addition [56]. Moreover, this response was absent if the pistil stigma was preliminary treated with antagonists of acetylcholine or histamine such as d-tubocurarine or clemastine (tavegyl) that link receptors on the cellular surface. Thus, autofluorescence of the cells at contacts of pollen and pistil appears to be a biosensor reaction for neurotransmitters and the antitransmitter substances.

CONCLUSION

Autofluorescence of intact secretory cells is changed during the cell development and under various damaging factors. The filling of the cells with a secretion and its removing are easily observed in these conditions that can serve as an indicator of the cell state *in vivo*. Moreover, the cellular autofluorescence could be a sensitive parameter for the analysis of cell-cell interactions. Spectral characteristics of individual substances contained in secretory cells could be measured *in vivo* that is significant for their identification. Microspectrofluorimetry is especially useful for such studies. The fluorescence spectra of intact secretory cells, containing various substances, differ in the maxima position and the intensity. This permits a preliminary discrimination of dominating components in the secretory cell tested. The visible fluorescence of secreting cell depends on chemotaxonomy and metabolism of secreting products, which are stored within secretory structures as well as are evacuated on the surface of the secreting cell. This characteristic may be applied in pharmacology for the analysis of a plant material without expensive biochemical procedures, basing on the scales of the individual secretory products accumulated in similar cells. The fluorescence may be used: 1. for the identification of the secretory cells among non-secretory ones under luminescent microscope; 2. for the identification of the compounds in intact secretory cells if there is an appropriate optical system of registration that enables the fluorimetric analysis of the cell *in vivo*. Autofluorescence of secretory cells may be used for the analysis of changes in a surrounded medium because the cells are sensitive to environmental shifts. The following development of the earlier diagnostics based on the autofluorescence may be the area of the remote monitoring of agriculture crops and yields of medicinal plants as well as the remote sensing of the environmental stress.

REFERENCES

1. D. L. Taylor and E. D. Salmon (1989). in J. L. Wang and D. L. Taylor (Eds.), *Methods in Cell Biology. Living Cell in Culture*, Academic Press, San Diego, New York, pp. 207–237.
2. R. P. Haugland (2000). *Handbook of Fluorescent Probes and Research Chemicals*, 7th ed., Molecular probes, Eugen.
3. F. H. Kasten (1981). in G. Clarck (Ed.), *Staining Procedures*, 4th ed., Williams, Wilkins, Baltimore, pp. 39–103.
4. U. Eilert, B. Wolter, and F. Constabel (1986). Ultrastructure of acridone alkaloid idioblasts in root and cellular culture of *Ruta graveolens. Can. J. Bot.* **64**, 1089–1096.
5. J. D. Curtis and N. R. Lersten (1990). Internal secretory structures in *Hypericum* (Clusiaceae), *H. perforatum* L. and *H. balearicum* L. *New Phytol.* **114**(3), 571–580.
6. A. M. Zobel and R. E. March (1993). Autofluorescence reveals different histological localization of furanocoumarins in fruits of some Umbelliferae and Leguminosae. *Ann. Bot.* **71**(2), 251–255.
7. V. V. Roshchina, E. V. Mel'nikova, N. A Spiridonov, and L. V. Kovaleva (1995). Azulenes are blue pigments of pollen. *Doklady Biol. Sci.* **340**, 715–718.
8. V. V. Roshchina, E. V. Melnikova, and L. V. Kovaleva (1997a). The changes in autofluorescence during the male gametophyte development. *Russ. J. Plant Physiol.* **44**(1), 36–44.
9. V. V. Roshchina, E. V. Melnikova, V. N. Karnaukhov, and B. N. Golovkin (1997b). Application of microspectrofluorimetry in spectral analysis of plant secretory cells. *Biol. Bull.* **2**, 167–171.
10. V. V. Roshchina, E. V. Mel'nikova, L. I. Mit'kovskaya, and V. N. Karnaukhov (1998a). Microspectrofluorimetry for the study of intact plant secretory cells. *J. Gen. Biol.* (*Russia*) **59**(5), 531–554.
11. V. V. Roshchina and V. D. Roshchina (1993). *The Excretory Function of Higher Plants*, Springer-Verlag. Berlin.
12. O. S. Wolfbeis (1985). in S. G. Schulman (Ed.), *Molecular Luminescence Spectroscopy. Methods and Applications*, Willey, New York, pp. 167–370.
13. R. G. Stanley and H. F. Linskens (1974). *Pollen. Biology, Biochemistry, Managements*, Springer, Berlin.
14. K. Essau (1965). *Plant Anatomy*, Wiley, New York.
15. K. Essau (1977). *Anatomy of Seed Plants, Vols.* 1/2, Wiley, New York.
16. A. Fahn (1979). *Secretory Tissue in Plants*, Academic Press, London, 302 pp.
17. E. Werker (2000). Trichome diversity and development. *Adv. Bot. Res.* **31**, 1–35
18. V. V. Roshchina and E. V. Melnikova (1995). Spectral analysis of intact secretory cells and excretions of plants. *Allelopathy J.* **2**(2), 179–188.
19. V. V. Roshchina, E. V. Mel'nikova, R. Ya. Gordon, D. A. Konovalov, and A. M. Kuzin (1998b). A study of the radioprotective activity of proazulenes using a chemosensory model of *Hippeastrum hybridum* pollen. *Doklady Biophys.* **358–360**(1), 20–23.
20. V. V. Roshchina, V. I. Popov, V. I. Novoselov, E. V. Mel'nikova, I. V. Peshenko, S. S. Khutsyan, and E. E. Fesenko (1998c). Transduction of chemosignal in pollen. *Cytology (Russia)* **40**(10), 964–971.
21. V. V. Roshchina, E. V. Mel'nikova, V. I. Popov, A. V. Karnaukhov, L. I Mit'kovskaya, A. A. Gorokhov, and V. N. Karnaukhov (1998d). in V. N. Karnaukhov, and E. N. Gakhova (Eds.), *Cryoconservation of Genetic Resurses. Materials of All-Russian Conference*, 13–15 October 1998, Biological Center of RAS, Pushchino, Russia.
22. V. V. Roshchina and E. V. Melnikova (1999). in Inderjit, KMM Dakshini and C. L. Foy (Eds.), *Principles and Practics in Plant Ecology.*

Allelochemical Interactions, CRC Press, Boca Raton, FL, pp. 99–126.

23. E. V. Melnikova, V. V. Roshchina, and V. N. Karnaukhov (1997). Microspectrofluorimetry of pollen. *Biophys.* (*Russia*) **42**(1), 226–233.

24. J. B. Pawley (Ed.) (1990). *Handbook of Biological Confocal Microscopy*, Plenum, New York.

25. P. C. Cheng and R. G. Summers (1990). in J. B. Pawley (Ed.), *Handbook of Biological Confocal Microscopy*, Plenum, New York, pp. 179–195.

26. N. S. White, R. J. Errington, J. L. Wood, and M. D. Fricker (1996). Quantative measurements in multidimensional botanical fluorescent images. *J. Microsc.* **181**(2), 99–116.

27. Salih, A. Jones, D. Bass, and G. Cox (1997). Confocal imaging of exinefor grass pollen analysis. *Grana* **36**, 215–224.

28. P. van Gijzel (1967). Autofluorescence of fossil pollen and spores with special reference to age determination and coalification. *Leidse Geologische Mededelingen* **40**, 263–317.

29. V. N. Karnaukhov (1972). in G. M. Frank and V. N. Karnaukhov (Eds.), *Biophysics of the Living Cell*, Institute of Biophysics, Pushchino, pp. 100–117.

30. V. N. Karnaukhov (1978). *Luminescent Spectral Analysis of Cell*, Moscow: Nauka, Moscow, 208 pp.

31. V. N. Karnaukhov (2001). *Spectral Analysis in Cell-Level Monitoring of Environmental State*, Nauka, Moscow.

32. V. N. Karnaukhov, V. A. Yashin, V. I. Kulakov, V. M. Vershinin, and V. V. Dudarev (1981). Einrichtung zur Untersuchung von Lumineszenzeigenschaften der Microobjekte. *DDR Patent* **N147002**, pp. 1–32.

33. V. N. Karnaukhov, V. A. Yashin, V. I. Kulakov, V. M. Vershinin, and V. V. Dudarev (1982). Apparatus for investigation of fluorescence characteristics of microscopic objects, *US Patent,* N4, 354, **114**, 1–14.

34. V. N. Karnaukhov, V. A. Yashin, V. I. Kulakov, V. M. Vershinin, and V. V. Dudarev (1983). Apparatus for investigation of fluorescence characteristics of microscopic objects, *Patent of England* 2.039.03 R5R.CHI.

35. V. N. Karnaukhov, V. A. Yashin, V. I. Kulakov, V. M. Vershinin, and V. V. Dudarev (1985). Microspectrofluorimeters. *Proceedings of 1-st Soviet-Germany International Symposium on Microscopic Fluorimetry and Acoustic Microscopy*, Moscow, pp. 160–164.

36. V. N. Karnaukhov, V. A. Yashin, A. P. Kazantsev, N. A. Karnaukhova, and V. I. Kulakov (1987). Double-wave microfluorimeter-photometer based on standard attachment. *Tsitologia* (*Cytology, Russia*) **29**, 113–116.

37. V. V. Roshchina, E. V. Melnikova, and L. V. Kovaleva (1996). Autofluorescence in thge pollen -pistil system of *Hippeastrum hybridum*. *Doklady Biol. Sci.* **349**, 118–120.

38. V. V. Roshchina, E. V. Melnikova, V. A. Yashin, and V. N. Karnaukhov (2002). Autofluorescence of intact spores of horsetail *Equisetum arvense* L. during their development. *Biophysics (Russia)* **47**(2), 318–324.

39. V. V. Roshchina and E. V. Melnikova (1996). Microspectrofluorimetry: A new technique to study pollen allelopathy. *Allelopathy J.* **3**(1), 51–58.

40. V. V. Roshchina and E. V. Melnikova (2001). Chemosensitivity of pollen to ozone and peroxides. *Russ. Plant Physiol. (Fiziologia Rastenii)* **48**(1), 89–99.

41. V. V. Roshchina, A. V. Miller, V. G. Safronova, and V. N. Karnaukhov (2003). Reactive oxygen species and luminescence of intact cells of microspores. *Biophysics (Russia)* **48**(2), 259–264 .

42. T. Butkhuzi, Z. Kuchukashvili, M. Sharvashidze, G. Natsvlishvili, and V. Gurabanidze (2002). Cytodiagnostics on intact pollen using photoluminescence. *J. Biological Physics and Chemistry* **1**(1/2), 53–55.

43. W. Ruhland and K. Wetzel (1924). *Ber. Deutsch. Bot. Ges.* **42**, 3–14 (cited Berger, 1934).

44. F. Berger (1934). Das Verhalten der heufieber-erregenden Pollen in filtrien ultravioleten Licht. *Beitr. Biol. Pflanzen.* **22**, 1–12.

45. F. Asbeck (1955). Fluoreszcezieren der Blutenstaub. *Naturwiss* **4**, 632.

46. L. Ascensao and M. S. Pais (1987). Granular trichomes of *Artemisia campestris* (ssp. *maritima*): Ontogeny and histochemistry of the secretory product. *Bot Gaz.* **148**, 221–227

47. I. N. Kuzovkina, V. V. Chernysheva, and I. E. Alterman (1979). Characteristics of callus tissue strain Ruta graveolens produced rutacridone. *Sov. Union Plant Physiol.* **26**, 492–500.

48. J. Kendrick and R. B. Knox (1981). Structure and histochemistry of the stigma and style of some Australian species of *Acacia. Aust. J. Bot.* **29**, 733–745.

49. P. van Gijzel (1971). in J. Brooks, P. R. Grant, M. Muir, and P. R. Gijzel van (Eds.), *Sporopollenin.* Proc. Symp. at Geology Department, Imperial college, London, 23–25 September 1970, Academic Press, London, pp. 659–685.

50. M. T. M. Willemse (1971). in J. Brooks, P. R. Grant, M. Muir, and P. R. Gijzel van (Eds.), *Sporopollenin*, Proceedings of the Symposium at Geology Department, Imperial College, London, 23–25 September 1970, Academic Press, London, pp. 68–91.

51. M. N. B. M. Driessen., M. T. M. Willemse, and J. A. G. van Luijn (1989). Grass pollen grain determination by light- and ultra-violet microscopy. *Grana* **28**(2), 115–122.

52. M. B. Satterwhite (1990). In R. Mills (Ed.), *Remote Sensing Science for the Nineties, IGARSS' 90: 10th Annual International Geoscience and Remote Sensing Symposium Vol. 3*, Washington, DC, 1990. May 20–24, 1990, Univ. Maryland, The Institute of Electrical and Electronic Engineers, University of Maryland, College Park, pp. 1945–1948.

53. V. V. Roshchina and E. V. Melnikova (1998a). Chemosensory reactions at the interaction pollen-pistil. *Biol. Bull.* **25**(6), 557–563.

54. V. V. Roshchina and E. V. Melnikova (1998b). Allelopathy and plant generative cells. Participation of acetylcholine and histamine in a signalling at the interactions of pollen and pistil. *Allelopathy J.* **5**(2), 171–182.

55. V. V. Roshchina, E. V. Melnikova, and V. N. Karnaukhov (2000). Fluorescing world of plant cells. *Sci. Russ.* **6**, 53–56.

56. V. V. Roshchina (2001a). *Neurotransmitters in Plant Life*, Science Publisher, Enfield, England.

57. V. V. Roshchina (2001b). Molecular-cellular mechanisms in pollen alllelopathy. *Allelopathy J.* **8**(1), 11–28.

58. H. K. Lichtenthaler and J. Schweiger (1998). Cell wall bounded ferulic acid, the major substance of the blue-green fluorescence emission of plants. *J. Plant Physiol.* **152**, 272–282.

59. P. J. Harris and R. D. Hartley (1976). Detection of bounded ferulic acid in cell walls of the Gramineae by ultraviolet fluorescence microscopy. *Nature* **259**, 508–510.

60. V. V. Roshchina (2001c). Autofluorescence of plant cells as a sensor for ozone, in *Abstracts of 7th Conference on Methods and Applications of Fluorescence: Spectroscopy, Imaging and Probes*, Amsterdam, 16–19 September 2001, p. 162.

61. V. V. Roshchina (2001d). Rutacridone as a fluorescent probe, in *Abstracts of 7th Conference on Methods and Applications of Fluorescence: Spectroscopy, Imaging and Probes*, Amsterdam, 16–19 September 2001, p. 161.

62. V. V. Roshchina (2002). Rutacridone as a fluorescent dye for the study of pollen. *J. Fluoresc.* **12**(2), 241–243.

63. V. V. Roshchina (1998). Chemosignaling in pollen. *Uspekhi Sovrem. Biol.* (*Russ. J. Adv. Mod. Biol.*) **119**(6), 557–566.

64. *Plant Resources of Russia and Surrounded Countries.* (1996). in L. E. Budantsev (Ed.), Mir i Sem'ya, Sankt-Peterburg.

65. V. V. Roshchina (1999). in S. S. Narwal (Ed.), *Allelopathy. Basic and Applied Aspects*, *Vol. 2*, Science Publisher, Enfield, England, pp. 3–25.

66. L. V. Kovaleva and V. V. Roshchina (1999). Indication of the S-gene (related to a self-imcompatibilirty) expression based on autofluorescence of the system pollen-pistil, in *Abstracts of 4th All-Russian Congress of Plant Physiology*, 4–9 October 1999, Institute of Plant Physiology, Moscow, p. 598.

67. V. V. Roshchina and V. D. Roshchina (2003). *Ozone and Plant Cell*, Kluwer Academic Publishers, Dordrecht, The Netherlands.

68. V. V. Roshchina and V. N. Karnaukhov (1999). Changes in pollen autofluorescence induced by ozone. *Biol. Plant*. **42**(2), 273–278.

69. V. V. Roshchina and E. V. Melnikova (2000). in V. P. Skulachev and V. P Zinchenko (Eds.), *Mitochondria, Cells and Active Oxygen Species. Materials of International Conference*, 6–9 June 2000, Biological Center of Russian Academy of Sciences, Pushchino, pp. 127–120.

70. V. V. Roshchina (1996). in S. S. Narwal and P. Tauro (Eds.), *Allelopathy. Field Observation and Methodology*, Scientific Publishers, Jodhpur, India, pp. 233–241.

71. V. V. Roshchina, V. A. Yashin, and A. V. Kononov (2003). Autofluorescence of plant microspores studied by Confocal microscopy and microspectrofluorimetry, in *Abstract of 8th Conference on Methods and Applications of Fluorescence: Spectroscopy, Imaging and Probes*, Prague, 24–27 August 2003, p. 142.

72. B. Haliwell and J. N. C. Gutterige (1985). *Free Radicals in Biology and Medicine*, Clarendon Press, Oxford.

73. M. N. Merzlyak (1988). in I. Nagy (Ed.), *Lipofuscin—1987: State of the Art Academ. Kiado*, Elsevier, Amsterdam, pp. 451–452.

74. V. V. Roshchina (1998). Cholinergic mechanisms in plant fertilization. *J. Physiol.-Paris* **92**(5–6), 484–485.

75. I. N. Kuzovkina, V. V. Roshchina. I. E. Alterman, and V. N. Karnaukhov (1999). Study of cultivated cells and pRiT-DNA transformed roots of *Ruta graveolens* by luminescent microscopy, in *Abstracts of 4th All-Russian Congress of Plant Physiology*, *Vol. 2*, 4–9 October 1999, Instiute of Plant Physiology, Moscow, p. 613.

APPENDIX 1. GLOSSARY OF BIOLOGICAL TERMS

Emergencies are termed surface plant projections that arise from sub-epidermal plant cells. They often filled with secretions and functionally similar to trichomes, which have more complex structure.

Glands—specialized multicellular structures on the surface of leaves, stems and flowers (filled, mainly, with terpenoid- or phenol-containing secretory products).

Hairs (trichomes) are unicellular or multicellular (mainly glandular) secretory structures that contain various secretions.

Hydathodes represent modified cells of stomata (cells, regulating aperture for gases) on overgrown parts of plant. They release of water and water-soluble substances out plant.

Idioblasts are single specialized cells which are met among non-secretory cells of vegetative and reproductive organs and differ substantially from other cells of the same tissue in their form, structure, and content . In many cases idioblasts lack organelles, and the secretion is spread within whole interior of the cells that means a prevailing secretory function.

Laticifers are living cells with a latex (includes alkaloids and terpenoids) as a secretion which is located in vacuoles. They are met in many plants, especially in family Euphorbiaceae.

Microspores—secreting unicellular structures for breeding. Vegetative microspores (non-sexual cells) are known for the spore-breeding plants (horsetails, ferns, mosses) whereas generative male microspores, which are called as pollen, pollen grains or male gametophyte, are peculiar to plants, belonging to Golosperms (mainly conifer species) and Angiosperms (species with flowers).

Nectaries—secretory structures filled with a nectar and located on flower (floral nectaries) or on leaf and stem (extrafloral nectaries).

Pollen means male gametophyte (see microspores). When the cell be added (artificially or, as in nature, by the wind or insect-pollinators) to the pistil stigma surface in a flower or on the surface of female Golosperm microstrobils, it can germinate: to form amoebe-like emergence named as a pollen tube [13]. Division of the pollen nucleus leads to the appearance of spermia that move along the growing pollen tube to the egg cell. So the fertilization occurs.

Resin ducts and reservoirs are secretory structures that contain resin (complex terpenoid composition). The structures are especially abundant in conifer plants.

Trichomes (also see hairs) are unicellular or multicellular secretory structures that originate from epidermal cells on plant surfaces.

Vegetative microspores—non-sexual cells served for a vegetative breeding of spore-breeding plants (see microspores). During their development the unicellular organism converts to multicellular tallus, which later forms sexual organs.

Viable or non-viable microspores—cells, which are able to germinate in nature or in the artificial nutrient medium.

APPENDIX 2. TECHNIQUE FOR THE MEASUREMENTS OF AUTOFLUORESCENCE OF PLANTS

LUMINESCENT MICROSCOPY

CONFOCAL MICROSCOPY

MICROSPECTROFLUORIMETRY WITH
REGISTRATION OF THE SPECTRA

DOUBLE-BEAM MICROSPECTROFLUO-RIMETRY
WITH REGISTRATION OF HISTOGRAMS