Original Research

Roadside Larch Trees (*Larix* Mill.) and Its Female Generative Organs as a Biomonitor of Air Pollution

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Abstract

The disturbances in the morphology and anatomical structure of female generative organs were investigated in two species of roadside larch trees *Larix decidua* Mill., *L. decidua* subsp. *polonica*, and *L. kaempferi* (Lamb.) Carr., within the administrative boundaries of the city of Lublin. The irregularities of female cones appear more often at the juvenile stage of their development. Completely dried cones or cones with partially dried scales were observed on the *L. decidua* trees growing in a more polluted environment (in comparison with the place of growing of control trees). Among the more developmentally advanced cones, partially dried and completely deformed or bent female generative organs with a disturbed anatomical structure were visible. Peroxidase activity was significant higher in *L. decidua* subsp. *polonica* located near the street with heavy traffic. The study can be used as a basis for deeper and more detailed investigations of reproduction of larches in different environments with air pollution in the area of Lublin and its vicinity. Maybe roadside larches and its generative organs can be used as possible biomonitors of air pollution in urban conditions.

Keywords: *Larix*, female generative organs, disturbances, guaiacol peroxidase, air pollution

Introduction

Larches are commonly known trees that for a long time have been the subject of interest because of their valuable wood and resin and unquestionable decorative features. The representatives of *Larix* genus occur in the cool and moderate zone of the northern hemisphere – in Eurasia and North America. Some *Larix* species reach far to the north, delineating the northern line of forests, as *Larix occidentalis* [1], and even entering the tundra, an example of which can be *Larix gmelinii* in Asia [2].

Larches are characterized by big or intensive growth, especially in the juvenile period, but in our climatic conditions they terminate growth quickly and do not reach big sizes or heights. Larches have modest soil requirements, although they grow best on soils that are rich enough in nutrients, humid enough, and well-aerated. *Larix* genus trees are light-loving plants. They display high resistance to low temperatures and wind. They also show relatively big

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resistance to air pollution. The resistance of larches to industrial emissions was confirmed in investigations on two sample areas in the Wałbrzych Forest Inspectorate, which is the center of the most industrial region of Low Silesia [3]. It was observed that in the first investigated zone of serious hazards posed to trees by gases and toxic dusts, despite the direct neighbourhood of a coal mine, coke factories, a sulphur acid factory and a steel mill factory, the larch tree stand is characterized by relatively good growth. Spruce that accompany the larch has, however, totally degenerated. In the second sample area, the tree stand of larch growing in the Wałbrzych agglomeration is one of the most efficient in our country. The statement of Karolewski and Białobok [4], Kozlowski [5], and Donovan [6] that the European larch (*L. decidua*) is one of the most industrial pollution-resistant forest-forming species in our country was confirmed in the areas described above. Its great adaptive ability and viability in unfavourable conditions are visible in its quick growth, plentiful health seed production and self-seeded renewal. Thanks to these properties, larches are considered to be pioneer trees and to play a prominent role in the restructuring of the tree stand in regions of industrial emissions; in other places they can facilitate introduction, under their specific protective canopy, of species like fir or beech, which are more sensitive to low temperatures.

There is evidence that urban trees remove large amounts of air pollution and improve urban air quality [7]. Research in recent years has begun to identify how urban tree planting in particular might be tailored to achieve air quality goals while still fulfilling many of the other beneficial functions of urban green space. Not all vegetation positioning yields an equal pollutant removal potential. Local airflows and pollutant concentrations will significantly affect the efficiency with which trees can remove pollution [8]. Where improving air quality outcomes is the primary objective, planting in areas of high pollution, for instance ‘hotspots’ such as traffic junctions and traffic lights, will yield proportionately greater rates of pollutant removal [9]. But care must be taken not to reduce dispersion from local pollutant sources such as traffic, which may lead to local concentration increases, despite the overall reduction in the street canyons [10, 11]. Tree-for-tree, single trees and trees on the edge of woodland collect particles more efficiently than those in the center of woodland [12, 13]. Dance trees in conjunction with understory plants leeward of air pollution sources can maximize pollutant scrubbing by plants. Greatest benefits could be achieved by two or three rows of trees with a relatively high planting density [14]. Species choice has a large influence on the potential for pollutant scrubbing by trees. Evergreen species contribute to pollutant scrubbing year-round, deciduous species are limited to stem deposition only in winter. The contribution of stems to particulate deposition can be substantial, depending on species [15]. When in leaf, broadleaf species may also be more efficient than needle-leaf species due to the higher leaf surface area of broadleaf trees [14]. Tree species selection and positioning are critical initial steps in designing green infrastructure to improve air quality. However, like any infrastructure, vegetation will act more effectively to remove air pollutants if it is properly maintained; the resilience of sustainability solutions like tree planting, in the face of an uncertain future, is often overlooked [16, 17]. Careful maintenance to ensure plant health will increase the leaf area and increase the pollution-scrubbing effect of plants [14]. Careful selection of tree species can also help to ensure that the positive impacts are greatest and any negative impacts minimalized. However, large-scale planting of almost all tree species will have a positive effect on air quality [6]. Careful, but not necessarily onerous, maintenance of tree cover in urban areas will ensure that trees thrive and continue to remove pollutants. Larches, which shed leaves in winter every season, were qualified for the 10 most useful tree species for urban plantings, next to the coniferous *Pinus nigra*, *Chamaecyparis lawsoniana*, and several species of deciduous trees such as *Acer platanoides*, *Betula pendula*, and *Crataegus monogyna* [6].

In recent years the level of air pollution, especially in urban areas in Europe, is increasing. The results of the analysis of trends show that F-NO_2 at the roadside has increased in recent years and that the rate of increase has been greatest since 2000 [18]. Simultaneously, the authors have emphasized the role of roadside greenery in air purification [19, 20]. Trees in urban areas are also exposed to heavy metal and salt stress. It has been shown that enhanced activity of peroxidase is a general response of higher plants to heavy metal stress [21]. Peroxidases are distributed in all parts of plants and involved in regulation of growth, cell formation, fruit development, ethylene biosynthesis, cell wall modification, defense against pathogens, and senescence. They also play an important role in H_2O_2 detoxification, stress response, and salt tolerance. In a number of plant species, peroxidase activity levels have been found to show a positive corelation with levels of Cu, Pb, Zn, and NaCl concentrations [22, 23].

There are known various effects of air pollution on the condition of the trees [5, 24], but there is a little information on disturbances in the development of its generative organs [25], especially of roadside trees as well as about activity of peroxidases in generative organs of coniferous plants exposed to air and soil pollution.

In comparison with angiosperms, the development of female generative organs in gymnosperms is not a favourite subject of investigation. The observations that we undertook had not been conducted in the chosen area. Our preliminary study concerned activity of guaiacol peroxidase and the disturbances in the structure of female generative organs in larches, and a modest attempt at explaining of this phenomenon can be a good basis for deeper and more detailed investigations of larches in the Lublin area.

Material and Methods

The study was carried out in the spring, from the beginning of March to the end of May 2010 within the area of Czechów district and in the Botanical Garden of UMCS in Lublin. After preliminary observation, the selected branches with the female strobili were collected from the larch trees and observed in the laboratory. The observations involved individuals of different larch species growing in urban plant arrangements, whose age ranges between 25 and 30 years. The larches were chosen for investigations from the trees located on both sides of Skrzypcowa Street (Fig. 1). Larches No. 14, No. 15, and No. 17 are located between Skrzypcowa Street and the street with heavy traffic – Kompozytorów Polskich Avenue in Lublin at a distance of several dozen metres from the Lublin transport bus stop. Additionally, the observations of the female generative organ development were carried out in the Botanical Garden of UMCS in Lublin and within the Czechów district (both beyond the direct center of Lublin); the larch from the Botanical Garden of UMCS was considered as a control. Cones were collected from branches located 1.5-2 m above ground.

Cones of *L. decidua* Mill. and *L. decidua* subsp. *polonica* from Skrzypcowa Street and *L. kaempferi* (Lamb.) Carr. from the Botanical Garden of UMCS were collected and used to determine activity of guaiacol peroxidase. Selected cones were cut into small pieces for protein extraction. The tissue was homogenized in an ice-cold 50 mM phosphate buffer (pH 7.0) with the addition of Triton X-100 and polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 15,000xg at 4ºC for 15 min. The activity of guaiacol peroxidases in supernatant was determined spectrophotometrically by measuring the absorbance increase at 470 nm according to the method of Milosevic and Slusarenko [26]. The reaction mixture contained 0.012% (v/v) guaiacol and 0.03% (v/v) hydrogen peroxide in 100 mM phosphate buffer (pH 6.25). The absorbance measurement was conducted at 25ºC for 180 seconds. Protein content was measured following the method described by Bradford [27], using bovine serum albumin as a standard. Peroxidase activity was defined as $\Delta A470$ $mg^{-1}\cdot protein\cdot min^{-1}$.

Results

The larch trees growing on both sides of Skrzypcowa Street (Fig. 2A) in the neighbourhood of the busy Kompozytorów Polskich Avenue in Lublin showed differences in height, general habit, and tree crown shape. The trees growing closer to Kompozytorów Polskich Avenue are more deformed or less regularly built than the trees located on the opposite side of Skrzypcowa Street or the trees growing at a bigger distance from the streets in the Czechów district area. Among them are trees with a more regular crown, like that of the larch tree designated as No. 14 (Fig. 2B). Some of them are characterized by a strong deformation of the habit, e.g. the larch tree designated as No. 15 (Fig. 2C); some, fortunately not numerous, are completely dried (Fig. 2D).

Compared with the formation of the female generative organs of the investigated larches and the larch species investigated during several past years, the development of female cones was slightly delayed this year. Moreover, in late October last year, a short time before the period of dormancy, it was not possible to distinguish morphologically individual vegetative male and female buds. The first and the youngest female cones were observed in March and their structure was not visibly disturbed.

Nearby, on the same twig of larch No. 14, two cones were observed: the upper one – dried and completely deformed, and the lower – normal and healthy cone built out of seed scales overgrowing the bract scales (Fig. 3G). Sometimes the disturbances of the female cone structure were observed at the later stages of development. Fig. 3H shows two cones that are almost adjacent to each other. One of them (on the left) is deformed in the upper part. The place of deformation is brown and consists of tightly pressed scales. The longitudinal section shows or reveals the disturbances in the anatomical structure and in the

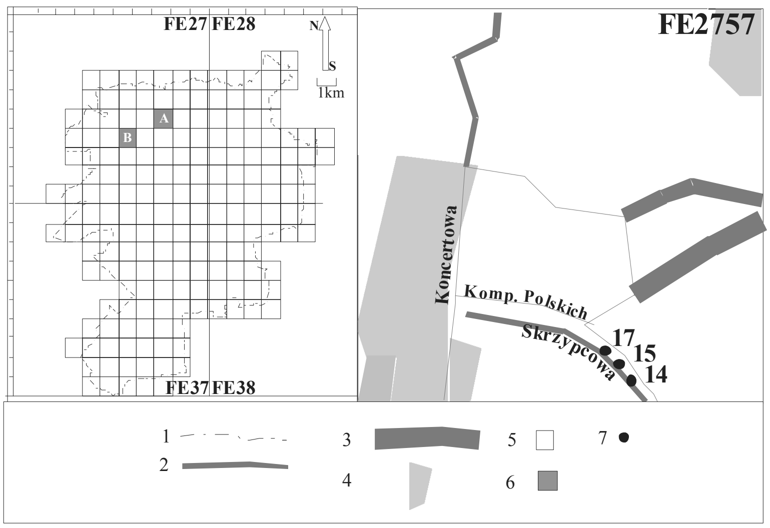

Fig. 1. Location of the investigated larches. *Larix decidua* No. 14, *Larix decidua* subsp. *polonica* No. 15, dried larch tree No. 17 and control larch trees are located in the area of FE2757 square; *Larix kaempferi* in the Botanical Garden of UMCS in Lublin (FE2765 square).
1 – administrative city boundaries, 2 – roads, 3 – main highways, 4 – urban areas, 5 – ATPOL square 1×1 km, 6 – study area (A – FE2757, B – FE2765), 7 – studied specimens

arrangement of both bract and seed scales (Fig. 3I). On larch No. 14, strongly curved cones were found. The longitudinal section of the strongly shortened cone (a natural feature of the investigated larch No. 15) revealed a brown place with degenerated scales in its upper part and a big hole within the long cone axis (Fig. 3J). The strongly curved cone of larch No. 14 completely lacked both bract and seed scales on one side. This disturbance made the cone asymmetric and, undoubtedly, partially sterile (Fig. 3K).

The results of guaiacol peroxidase activity measurements are shown in Fig. 4. The cones of *L. decidua* had the lowest activity. Almost halved activity was measured in cones of *L. kaempferi* in comparison with *L. decidua* subsp. *polonica*. The highest activity was determined in cones of *L. decidua* subsp. *polonica*.

Discussion

Genus *Larix* is composed of ten species occurring in cool environments throughout the northern hemisphere. The morphological and molecular treatment of systematic relationships in this genus is still not established [28-30]. In the present paper, the disturbances in the structure of female generative organs in three species of larches are described. One of the investigated larch trees, *Larix kaempferi,* grows in the Botanical Garden at the edge of the city, while two others, *Larix decidua* and *Larix decidua* var. *polonica,* grow in a more polluted environment. In comparison with the control *Larix* growing in less polluted areas, the investigated larch trees growing in a more polluted environment are more deformed. The deformations are even stronger in

Fig. 2. Habits of the investigated larch trees.
A) larches growing on both sides of Smyczkowa Street, in the neighbourhood of the busy Kompozytorów Polskich Avenue in Lublin, B) *Larix decidua* No. 14, C) *Larix decidua* subsp. *polonica* No. 15, D) dried larch tree No. 17.

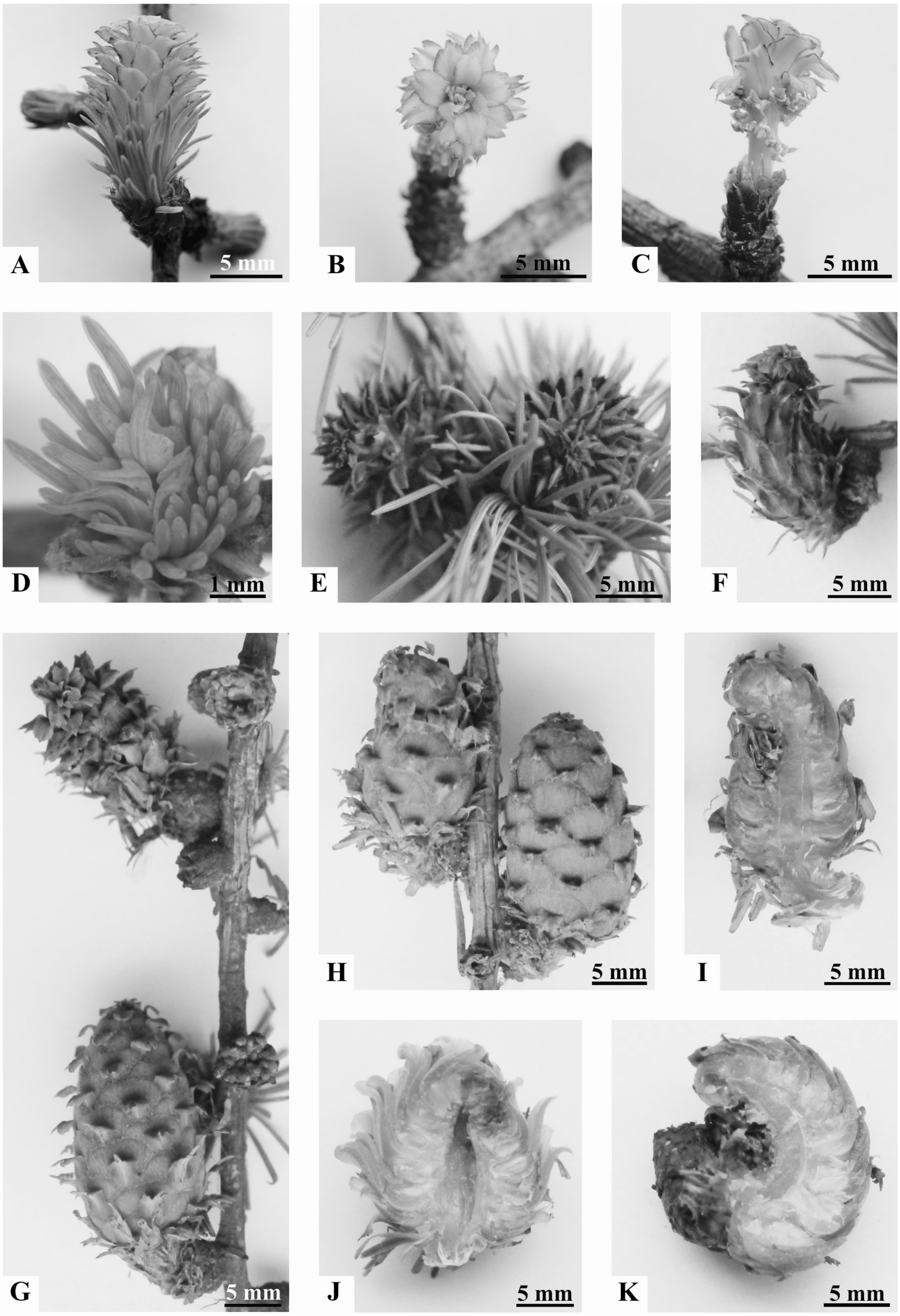

Fig. 3. Female generative organs of larches harvested in May 2010.
A) normal, healthy, young cone of *Larix kaempferi* with visible green-purple bract scales; B) and C) female cones of *Larix kaempferi* with a disturbed structure. Several anthers, enveloped by the bract scales gathered at the top of the cone (B), and the anthers placed in the lower part of the female cone (C); D) deformed short shoot with needle-like leaves at the base and the bract scales in the top part; E) two cones of larch No. 14 with a cluster of dried scales in the top part; F) completely deformed cone of larch No. 14; G) twig of larch No. 14 with two cones: the upper one – dried and completely deformed and the lower – normal and healthy cone build of seed scales overgrowing the bract scales; H) two female cones at the same stage of development as shown in figure (G). One cone, on the left, is deformed or disturbed in its shape; I) the same cone is shown in figure (H) left sectioned longitudinally with the aim to reveal the disturbances of the anatomical structure; J) longitudinal section of a strongly shortened cone of larch No. 15 with disturbed seed scales in the upper part and lack of a big part of the axis; K) Strongly curved cone of larch No.14 in longitudinal section. The inner part completely lacks both bract and seed scales.

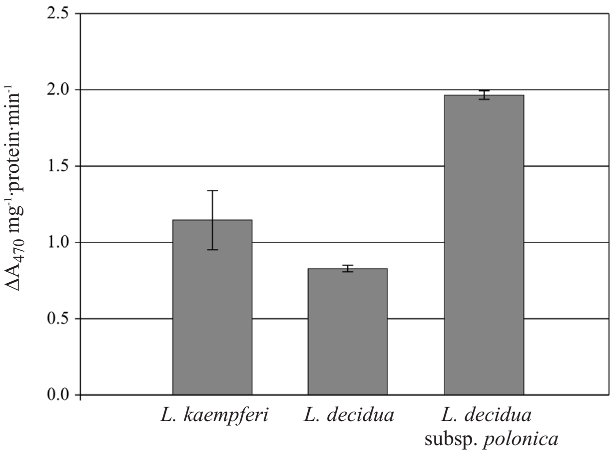

Fig. 4. Guaiacol peroxidase activity in cones of different larch species harvested in May 2010.

comparison with the trees located several meters away on the opposite side of Skrzypcowa Street.

The disturbances found and described in the present paper involve mainly the morphology and, to a lesser extent, the anatomical structure of female generative organs. We can conclude from our observations that the disturbances or irregularities of the female cones appear more often at the juvenile stage of their development and, moreover, at this phase these disturbances are more variably differentiated than in the stages occurring toward maturity of the female cones. In the first and second decades of March, the female cones were observable but the distinct irregularities were not visible. The first disturbances were observed at the end of March and in the middle of April.

The gaseous pollutants and particulate matter have a negative impact on the development of larch. In comparison with evergreen conifers, larches are more resistant to air pollution but are very sensitive to sulfur dioxide, which inhibits photosynthesis, accelerates respiration, and induces adverse changes in the structure of needles. Among different larch species there is the large interindividual variability in pollution resistance, with *L. kaempferi* having higher resistance than *L. decidua* [31].

In plants exposed to stressful environmental conditions, the production of reactive oxygen species (ROS) increases and can cause significant damage to the cells. Among enzymes involved in plant antioxidant defense are peroxidases – enzymes catalyzing the conversion of H_2O_2 into H_2O. Guaiacol peroxidase localized in cytosol, vacuole, cell wall and the intercellular space is involved in lignification, ethylene biosynthesis, defense against pathogens, and antioxidant defense [32-34]. Guaiacol peroxidase activity increases in larches exposed to air and soil pollutants, e.g. the soil salinity increase caused by the use of salt in winter. It should be noted that enzyme activity in *L. decidua* subsp. *polonica* is much higher than in *L. decidua* Mill., which may indicate that the antioxidative defense system works much more efficiently in *L. decidua* subsp. *polonica*. During our investigation of female generative organ development, serious disturbances in the structure of female cones were observed on the larch trees growing in the more polluted environment. Completely dried cones or cones with partially dried scales were observed most often. Among the older or more developmentally advanced cones, partially dried and completely deformed specimens were visible. Such irregularly built cones had a disturbed anatomical structure including lack of scales and seeds. It is not possible to join the obtained results with any specific biotic or abiotic factor. It can rather be assumed that a big range of factors can affect the described disturbances. Among them, the influence of air and soil pollution seems to be especially important. Slobodnik and Guttenberg [35] described meteorological and nutritional conditions as the reason for frequent irregularities in *L. decidua* female cone formation. Watanabe [25] described effects of radionuclide contamination on reproductive organs of forest trees, especially of Japanese cedar (*Pinus densiflora*) and Japanese cypress (*Chamaecyparis obtuse*). Nevertheless, the common effect of all factors on the investigated larches may result in female sterility and a considerable decrease in the seed crop. The biological mechanism affecting the quality and genetic value of produced seeds should be studied in *Larix* in more detail. As suggested by Slobodnik [36] and Carrer and Urbinati [37], such investigations should involve the other genera of coniferous trees.

The female cones with a disturbed structure also were observed in Japanese larch growing in a place evaluated as unpolluted. The structural disturbances found in this species in the present study had a different character than those found in larches from a polluted area. The disturbances appeared sporadically and involved the additional presence of male generative organs (anthers), which were situated at the top or at the base of the female generative organ, and the presence of bract scales placed at the top of vegetative shoots. In Poland, *L. kaempferi* is a species of foreign origin; it is native to Japan. The disturbances of female generative organs described here seem to be explained evolutionarily. All of them can prove or confirm the common origin and the appearance of male and female generative organs through transformation of vegetative organs. Also, we cannot exclude the effect of introduction of this species in the new environment on the appearance of teratological cones. It cannot be excluded that the relatively high level of guaiacol peroxidase activity in *L. kaempferi* indicates that this enzyme is involved not only in stress response, but also in developmental processes. Results of our study suggest that guaiacol peroxidase is involved in the development of the female gametophyte in *L. kaempferi*, enzyme activity increases with the development of ovules and reaches a high level in state of the mature archegonium (data not shown).

The results obtained concerning the appearance and presence of the disturbances in the structure of female generative organs in larches are difficult to explain. Numerous factors have an influence on the cone initiation, and then its growth and development. These factors can be biotic and abiotic such as photoperiodic conditions [38], atypical temperatures, drought, salinity or soil, and air contamination [39-41]. According to numerous data [42, 43], the microclimatic conditions of Lublin are spatially differentiated in a characteristic way. The least favourable microclimate is characteristic for the densely built-up residential and industrial areas as well as railway and road infrastructure. We cannot exclude salinity, either. The soils in the Lublin area are undergoing continuous transformation, being under pressure of gas, liquid and solid contamination. They also undergo a continuous process of drying up due to lowering of the ground water level and drainage of precipitation water. Because of the location of the observed larches, contamination with heavy metals should also be taken into consideration. Due to the big amount of different factors that can affect the disturbances in the female generative organs in larches, we cannot indicate the exact, definite, or key factor of the irregularities at present. Nevertheless, the disturbances appear among cones growing on trees located closer to the place with heavy traffic.

Studies on atmospheric contamination have frequently been limited by high cost of instrumental monitoring methods, and difficulties in carrying out extensive sampling in time and space. Also, the instrumental monitoring technics lack information on impact of atmospheric pollutants on living systems [44]. Hence, there has been an increasing interest in using indirect monitoring methods based on a response of organisms that may act as bioaccumulators. Relatively easy sampling and no need of complicated and expensive equipment contribute to the development of the biomonitoring of air pollution in the foreseeable future. An advantage of plants as biomonitors is that they provide information not only on quality/quantity of air pollutant concentrations, but also about air pollution effects on a living system [45].

In conclusion, we can say that our preliminary studies show that female generative organs of larch can be included in the research as a possible biomonitor of air pollution in urban conditions.

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