Agricultural, Forestry, and Veterinary Sciences

Division V – Agricultural, Forestry, and Veterinary Sciences covers a variety of research fields, including agrophysics, plant physiology, plant genetics, animal genetics and breeding, animal physiology and nutrition, animal reproduction and veterinary sciences, human nutrition, ecological management, and agricultural and forestry environment.

The Division's Chairman is Prof. Andrzej Grzywacz; the two Deputy Chairmen are Prof. Tomasz Brandyk and Prof. Zygmunt Reklewski. There are 33 national members (18 full members and 15 corresponding members) as well as 25 foreign members of Division V. New foreign members of Division V are Prof. Josse de Baerdemaeker (Belgium), Prof. Winfried E.H. Blum (Austria) and Prof. Gerhard Oesten (Germany). It is with great sorrow that we note that the following full members of Division V passed away in 2005: Prof. Tadeusz Kobak-Nowacki, Prof. Witold Niewiadomski and Prof. Tadeusz Wolski.

Within the Division, there are 9 research institutes employing 304 researchers. The scientific research staff published 1,269 papers in total.

Under the auspices of the Division, there are 16 Scientific Committees associating 452 members. Throughout the reporting period, the main task of the Committees was to assess the achievements for the years 1994-2004 and to draw up the future development of the directions for agricultural, forestry, veterinary and food sciences. In 2005 the Scientific Committees of Division V organized several plenary meetings as well as scientific sessions and seminars.

Division V plenary meeting – assessment of the B. Dobrzyński Institute of Agrophysics in Lublin (16 March 2005)

The scientific activities of the Division, its research units and Committees resulted in the publication of 23 scientific titles of journal status, including: *Acta Agrophysica, Acta Physiologiae Plantarum, Animal Science Papers and Reports, Annual Review of Agricultural Engineering, Folia Forestalia Polonica (Series A and B), International Agrophysics, Inżynieria Rolnicza* (Agricultural Engineering), *Journal of Animal and Feed Sciences, Journal of Applied Genetics, Journal of Plant Protection Research, Journal of Water and Land Development, Polish Journal of Food and Nutrition Sciences, Polish Journal of Soil Science, Polish Journal of Veterinary Sciences, Prace i Materiały Zootechniczne* (Livestock Research Papers and Communications), *Problemy Inżynierii Rolnicze* (Problems of Agricultural Engineering), *Problemy Zagospodarowania Ziem Górskich* (Problems of Management of Mountain Areas, Reproductive Biology), *Roczniki Nauk Rolniczych (Seria G)* (Annuals of Agricultural Sciences – Series G), *Zagadnienia Ekonomiki Rolnej* (Problems of Agricultural Economics). The Division also issued the bimonthly *Postępy Nauk Rolniczych* (Advances of Agricultural Science) as well as *Zeszyty Problemowe Postępów Nauk Rolniczych* (Advances of Agricultural Sciences – Problem Issues), which is published non-periodically.

The scope of the Division's statutory tasks covered the coordination of numerous activities of the Division's Scientific Committees and institutes, publishing activities, international cooperation and knowledge implementation. On the behalf of the Academy's Presidium, the Division acted also as a scientific advisor on issues concerning legislation in various fields related to comprehensive problems of science and education, as well as agriculture, forestry, animal health protection and nature conservation.

Two plenary sessions of Division V were held in 2005. During the first session, held in Lublin from 16 to 17 March 2005, the Division members evaluated the scientific activities of the PAN Institute of

Agrophysics in Lublin. Moreover, they accepted the annual report of the Division's activities in 2004 and delivered information on the financial situation of the institutes, Committees and the Division as a whole in 2005. During the same session, new foreign members of the Polish Academy of Sciences were elected and candidates for the Oczapowski Medal were approved. Information was also provided on the present status of the restructuring of the Popielno Experimental Station and the organization of conferences and the preparation of joint assessment by the Committees. Moreover, Prof. A. Byczkowski was nominated an honorary member of the PAN Committee of Drainage and Environmental Engineering, and Prof. Marek Łukaszewicz was elected the Division's representative to the Disciplinary Committee of the Polish Academy of Sciences.

During the second session held in Warsaw on 16 November 2005, an evaluation was made of the scientific activities of the PAN Institute of Plant Genetics in Poznań and its outstanding employees were honored with state medals. Moreover, the Division members agreed upon the rules for conferring the Division's scientific awards. They debated the regulations for awarding the "Division V Laurel Leaf" statue and assessing the scientific categories of institutes and scientific journals. Moreover, they were presented with information concerning the current state of the Popielno Experimental Station's restructuring.

In addition to the plenary sessions, on 26th October 2005 a scientific conference of Division V entitled "Biotechnology in Food Production" was organized by the PAN Committee on Food Sciences. On 5 December 2005, a ceremony of conferring the Division's scientific awards, diplomas and nominations took place. Furthermore, the "Pro Scientia et Vita" foundation created by the Division members has now continued to financially support young scientists for 4 years.

On 20 June 2005, the 50th anniversary of the Jan Kielanowski Institute of Animal Physiology and Nutrition (1955-2005) was celebrated in Serock. During the celebrations a ceremonial Scientific Council session was held, the Institute was honored with the Oczapowski Medal and the PAN 50th anniversary medal, and outstanding employees of the Institute were honored with state medals.

On 8 September 2005 the PAN Institute of Genetics and Animal Breeding in Jastrzębiec celebrated its 50th anniversary (1955-2005). These celebrations included the ceremonial scientific session: "The Significance of Basic Sciences in Domestic Animal Improvement." On this occasion, the Institute was honored with the Oczapowski Medal and the PAN 50th anniversary medal, while outstanding employees of the Institute were honored with state medals.

Ceremonial Session held on the occasion of the 50th anniversary of the PAN Institute of Genetics and Animal Breeding in Jastrzębiec. From the left: PAN Vice-President Emil Nalborczyk, Minister of Agriculture and Rural Development Jerzy Pilarczyk, Department Director at the Ministry of Agriculture and Rural Development Wojciech Wojtyra, PAN Presidium member Marian Truszczyński, Director of the Institute (1961-1969) Henryk Jasiorowski, Chairman of Division V Andrzej Grzywacz

The Division granted scientific awards and diplomas for outstanding books and research papers: The Oczapowski Scientific Award was bestowed upon Prof. Brożek and Dr. Maciej Zwydak from the H. Kołłątaj Agricultural University of Cracow for the book *Atlas gleb leśnych Polski* (Atlas of Forest Soils in Poland); a Diploma of Recognition was conferred upon Prof. Jerzy Wiśniewski and Prof. Bohdan Kiełczewski from the A. Cieszkowski Agricultural University of Poznań for the book *Kulturotwórcza rola lasu* (Culture-Forming Role of the Forest). The Division's scientific award went to Prof. Jacek Osek, Marcin Weiner and Kinga Wieczorek, M.Sc., from the National Veterinary Research Institute in Puławy, for their research on shigatoxic *Escherichia coli* O157 strains.

Diplomas of Recognition were conferred upon: Dr. Tomasz Misztal, Dr. Katarzyna Romanowicz, and Prof. Bernard Barcikowski (posthumously) from

The ceremony of conferring the Division V scientific awards (Warsaw, 5 November 2005)

the Jan Kielanowski Institute of Animal Physiology and Nutrition in Jabłonna for their research "Hormonal and nutritional factors modulating LH-secretion at the level of the central nervous system in sheep"; Dr. Teresa Doroszewska from the Institute of Soil Science and Plant Cultivation in Puławy for "Distant cross and genetic transformation in obtaining tobacco immunity to Potato Virus Y (PYV)"; Prof. Jolanta Kurył, Joanna Wyszyńska-Koko, M.Sc., Dr. Mariusz Pierzchała, Dr. Agnieszka Kossakowska, Paweł Urbański, M.Sc., and Dr. Tadeusz Blicharski from the Institute of Genetics and Animal Breeding in Jastrzębiec for their study "Identification of marker genes influencing the utility of pigs."

Oczapowski Medals were granted to Prof. Jan Krzymański (Plant Breeding and Acclimatization Institute in Poznań), Prof. Lech Ryszkowski (Research Center for Agricultural and Forest Environment in Poznań), Prof. Maciej Żurkowski (Research Station for Ecological Agriculture and Preservation of Native Breeds in Popielno) as well as to the following institutions: the Central Agricultural Library in Warsaw, the Forest Research Institute in Warsaw, the Jan Kielanowski Institute of Animal Physiology and Nutrition in Jabłonna and the PAN Institute of Genetics and Animal Breeding in Jastrzębiec.

Influence of cell size on failure properties of plant tissue

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Fruits and vegetables are built mainly of thin walled parenchyma cells highly susceptible to mechanical damage. Loading caused by agricultural machines or careless handling can cause irreversible changes in structure and subsequently in the color and taste of the tissue. Mechanical properties of a tissue depend on properties of the cellular skeleton, including its geometrical properties. This subject is now being intensively researched due to the development of new microscopic and image analysis techniques. The goal of our research is to find quantitative relationships between the geometrical and mechanical properties of cellular structure, focusing in particular on failure conditions.

Plant organs are very complex structures that consist of different types of tissues. Due to their heterogeneity, observation methods should be effective, and thus as simple as possible, with minimal alteration in natural structure. Relatively simple sample preparation protocols without any changes in natural structure can be used for observation by confocal microscopes. Another advantage of confocality is high contrast between the object within the focal layer and the area out of focus, which simplifies the image analysis process. The contrast can also be increased by sample staining and observation of fluorescent images.

We developed a special sample preparation protocol for use with a confocal laser microscope, consisting of: (1) cutting into slices of a thickness depending on the tissue (0.3 mm for carrot and 0.5 mm for potato), (2) staining in Coryphosphyne

a) b)

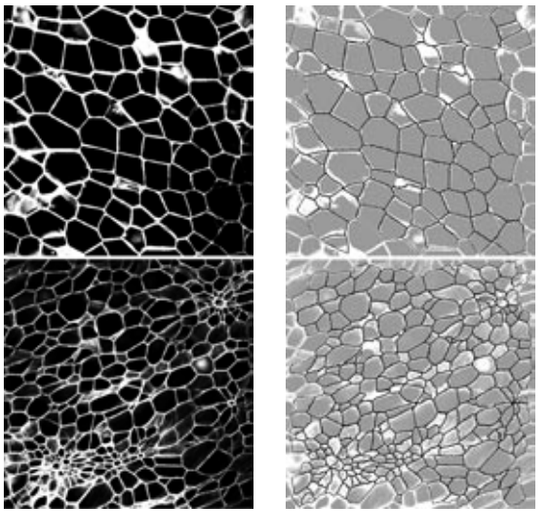


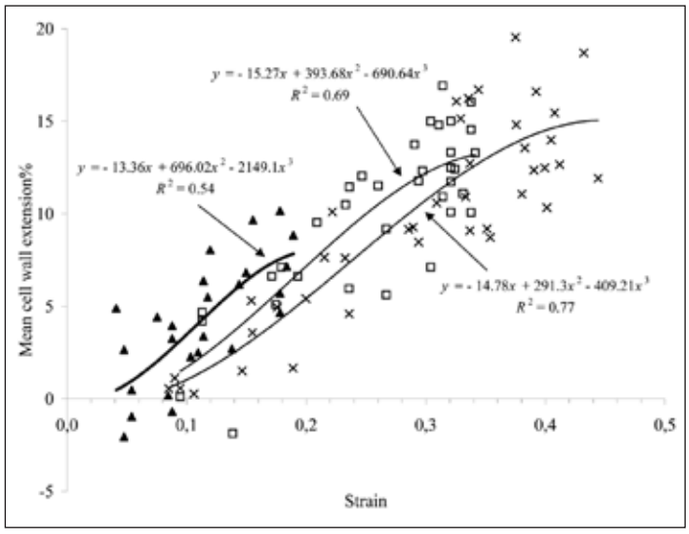
Fig. 1. a) Images of potato (above) and carrot (below) tissue obtained by confocal laser microscope. b) Computer reconstruction of cellular structure (solid lines).

O for 10s, (3) washing in water for 10s, (4) slide mounting and drying off using tissue paper. The protocol allows observation the slice surface using low magnification lenses (about 10x) without cover glass for about 3 min. About 100 cell faces of parenchyma tissue are visible within a single image (Fig. 1a). For such images, a special image analysis procedure was developed using Aphelion® software. The procedure reconstructs cellular structure (Fig. 1b) and measures geometrical properties for each cell face: area, perimeter, elongation, etc.

This method was applied to potato and carrot tissue in order to observe changes in cellular structure after deformation and to find a relationship between cell size (in a linear negative relationship with cell wall fraction) and failure properties of the tissue. The cellular structure was characterized in the plane perpendicular to loading. Fig. 2 shows the mean cell wall extension as a function of sample strain. The wall extension increases after deformation until a certain strain that depends on a rate of deformation. Following Henry's model (2000), a third-order polynomial model was fit to the data in Fig. 2. The strain at a maximum secant modulus equal to $-b/2c$, where b and c are coefficient of the model, is a theoretical strain at the beginning of failure and decreases when the strain rate increases. It was confirmed by microscopic observation, therefore, that the values can be used as the maximal permissible strain that can be applied to these tissues. After this, the energy of loading is used for cracking propagation and wall extension is stopped.

The influence of cell size on failure properties of potato and carrot tissue is presented schematically in Fig. 3. Generally, a tissue built of bigger cells shows lower failure parameters. However, details

a)



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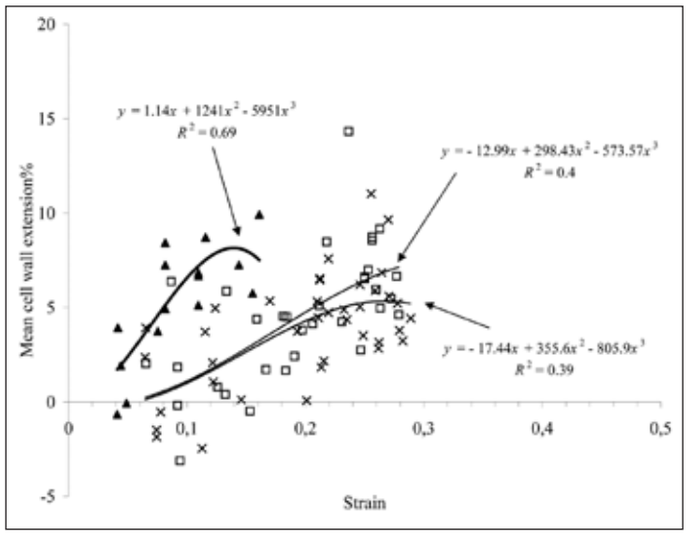

Fig. 2 Mean cell wall extension as a function of strain at different strain rates for (a) potato and (b) carrot. ×, quasi-strain rate 0.012 s^{-1}; □, quasi-static strain rate 0.71 s^{-1}; ▲, dynamic strain rate 60 s^{-1}; R^2, coefficient of determination; -----, third-order polynomial curves at 0.012 s^{-1}; ——, third-order polynomial curves at 0.71 s^{-1}; ——— third-order polynomial curves at 60 s^{-1} . The third order polynomial $y=ax+bx^2+cx^3$ is fit to the data.

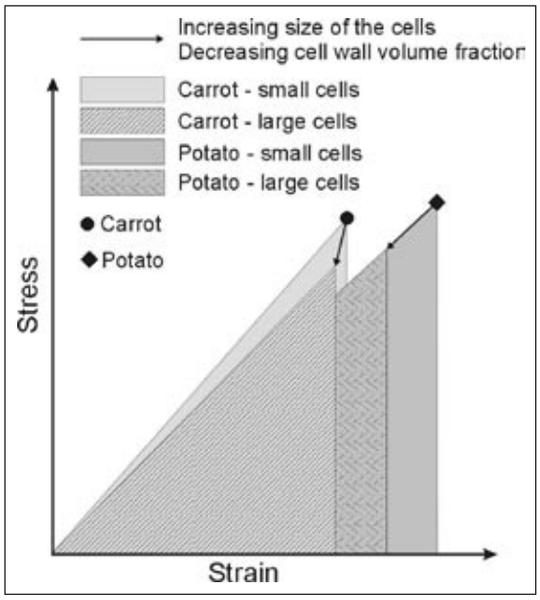

Fig. 3 The influence of cell size on failure properties of potato and carrot tissue. Arrows show the direction of changes for the failure point if the tissue is built of larger cells.

depend on type of tissue. For potato, both failure stress and strain decreases simultaneously with increasing cell size. As a consequence, firmness (the secant modulus) does not change. For carrot, the influence of cell size on the failure strain is less strong than on the failure stress, thus the firmness also decreases if the tissue is built of larger cells. For both materials, toughness (the work necessary to cause tissue failure) decreases if the tissue is built of larger cells.

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Visualization of microtubules and actin filaments in fixed BY-2 tobacco suspension cells

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Excellent visualization of microtubules and actin filaments was obtained in fixed tobacco suspension cells after developing a protocol for whole mount immunolabeling.

Microtubules (MTs) and actin filaments (AFs) are the elements of plant cell cytoskeleton that play integral roles in cells architecture and in the processes of cell division, expansion, intracellular transport and morphogenesis. The MT cytoskeleton is present in four distinct arrays: (1) The pre-prophase band, marking the future division site, (2) spindle, the mitotic apparatus, segregating the chromosomes, (3) the phragmoplast, involved in producing the cell plate, and (4) the interphase cortical MT array, involved in cellulose-microfibril deposition in the cell wall. AFs are not as well characterized as MTs.

MTs in living cells can be visualized such as by injection of fluorescently labeled proteins or by expressing a chimeric protein consisting of green fluorescent protein fused to the microtubules, although such methods require special equipment. Immunolabeling methods offer a good alternative.

Fig. 1 Distribution of cortical microtubules during the formation of the preprophase band from early (**a**, **b**) to late stage (**c**, **d**), and during spindle formation (**e**, **f**) in tobacco cells, visualized with the developed immunolabeling protocol. Images are projections of sequential optical sections at 1µm intervals. Part **a** of this figure gives an overview of a cell with a preprophase band at an early stage of formation, with a detail in **b**. Full projection of preprophase band in **c** at a later stage of formation. Microtubules outside the preprophase band radiate from the nuclear surface and are well visible in **d** which is a part of the projection of **c**. **e** and **f** show a mitotic spindle at metaphase and anaphase, respectively. Bars **a**–**d**: 25 µm; **e**, **f**: 10 µm

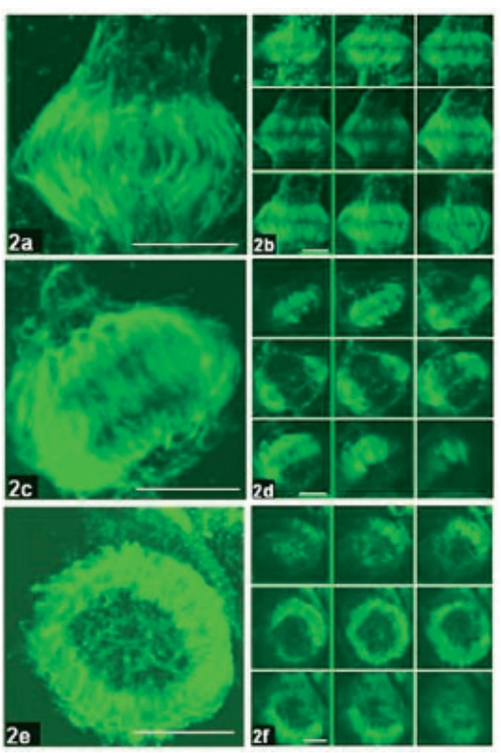


Fig. 3 and 4 Cortical microtubule (MT) configurations in young (**a**) to old, elongated tobacco cells (**d**) visualized with the developed immunolabeling protocol. Images are projections of sequential optical sections at 1 µm intervals. Note that cytoplasmic MTs only occur in **a** and **b** and that cortical MTs attain an orientation more and more transversal to the long axis of the cell. **c** is a projection of a Z-series of a whole cell and **d** is a projection of a Z-series of the cortical cytoplasm alone. Note in d that single MTs can be seen as single fluorescent lines. They sometimes come close to neighboring MTs and sometimes separate from them. Free ends of single MTS are in the focus plane (arrow-heads). Bars: 25 µm.
Cortical actin filaments in tobacco BY-2 cells visualized with developed immunolabeling protocol (Fig. 4) Note the parallel orientation of the actin filaments. Images are projections of sequential optical sections at 1µm intervals. Bars: 25 µm

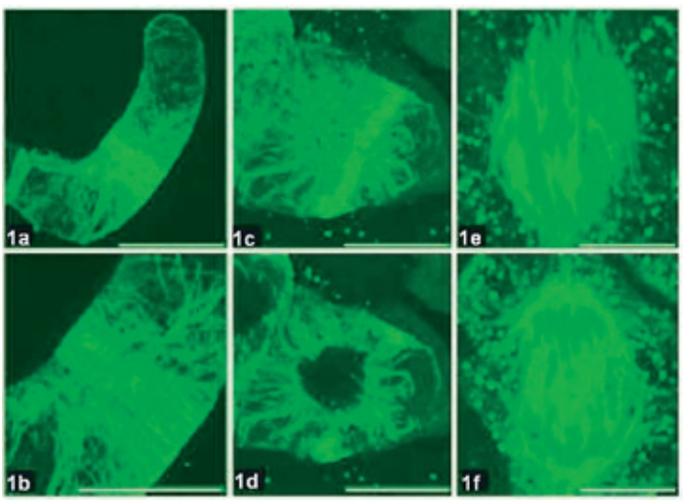
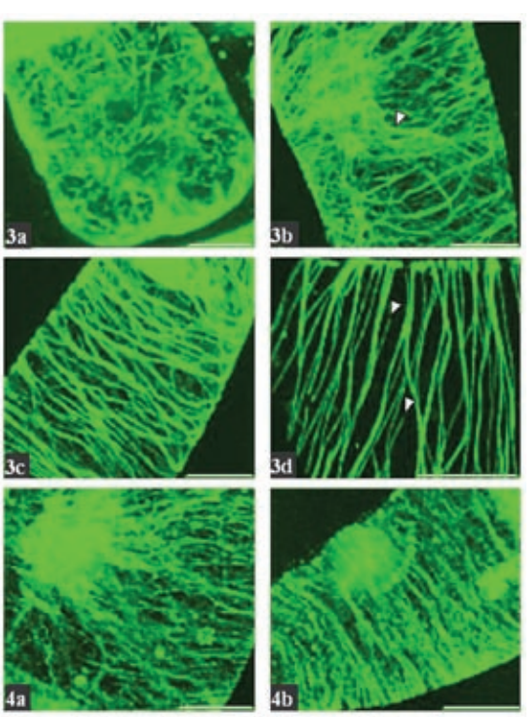


Fig. 2 Distribution of microtubules during phragmoplast formation in tobacco BY-2 cells visualized with the developed immunolabeling protocol from early (**a**, **b**) to late stage (**e**, **f**). **a**, **c** and **e** are projections, and **b**, **d** and **f** sequential optical sections at 2 µm intervals. Note that in the early phase microtubules run to the cell plate region throughout the phragmoplast, although with lower numbers in the central zone than in the periphery (series in **b**). From **d** it is clear that the number of microtubules in the central zone decreased but did not disappear completely. **e** and **f** show a phragmoplast at 90° tilting. Note that not all MTs are in the phragmoplast cylinder but some are at the caps of the cylinder at the two sides of the phragmoplast structure shown in the sequential images of **f**. Bars: 10 µm



Fluorescent dyes are attached to antibodies raised against specific proteins (antigens) present in the biological material. Immunolabeling permits specific light microscopic detection of MTs in animal cells but in plant cells the cell walls are barriers for antibodies.

A standard whole mount immunolabeling procedure consists of five steps: fixation, cell wall permeation, blocking with a blocking agent, incubation with antibodies and signal detection. An original protocol was developed for the detection of mRNAs and proteins in seedlings. Its application to tobacco cells was hindered by several problems resulting from poor permeability of reagents through the cell wall and the cell membrane, a high level of fluorescence background and trembling of cytoskeletal elements. However, modifications to the basic protocol lead to immunolabeling results with excellent resolution and sensitivity. Here the fixation consisted of a three-step fixation, as well as successful digestion of the cell walls with enzymes decomposing cellulose and pectins. Following cell wall digestion, the plasma membrane permeabilization was optimal to enable antibodies to enter the cells.

Microtubules visualized in detail during cell division clearly demonstrate the change of MTs from a pre-prophase band (Fig. 1a and b) and perinuclear organization at prophase (Fig. 1c and d), a spindle organization at metaphase and anaphase (Fig. 1e and f), to a phragmoplast organization at telophase (Fig. 2). In interphase cells the cortical MT cytoskeleton is shown in detail at high resolution in which individual MTs were clearly identified (Fig. 3b–d).

It had been reported that AFs are very difficult to preserve especially at the ultrastructural level. However, in our experiments preservation of the AFs and their visualization proved possible (Fig 4a, b).

Although we present the application of our protocols for cytoskeleton labeling, the excellent results show the potential of using this method for the analysis of various proteins and molecules in plant cells.

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Back to tradition: germination and fermentation – production of food with increased antioxidative power

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A plant-based diet – composed mainly of vegetables, fruits and whole grains – has become one of the most important guidelines for lowering the risk of human diseases for which an increased level of free radicals is implicated. The free radical levels *in vivo* are modulated by a wide range of antioxidative compounds, assisted by repair systems. Many natural antioxidants exhibit a wide range of biological effects and it has been suggested that such biological functions as antimutagenicity, anticarcinogenicity, and antiaging may originate from antioxidative properties of bioactive compounds present in the diet.

Germination and fermentation are economical and simple methods for improving the nutritive

value of food. Several studies have reported higher levels of nutrients and lower levels of antinutrients in germinated seeds. Sprouts, in addition to being a good source of basic nutrients, may also contain important phytochemicals with disease preventive and health promoting properties. Seed germination is common in Asian countries, and their fresh, crunchy and sweet characteristics together with healthy aspects have led sprouts to gain popularity among Western consumers. Fermentation is an ancient technology that remains one of the most practical methods for preserving foods and enhancing their nutritional value, simultaneously providing acceptable and diversified flavors for human consumption. Lactic acid fermentation of legumes also causes desirable changes in texture and decreases some antinutritional compounds, thus providing improved food with enhanced nutritional properties.

Due to the presence of many bioactive compounds in germinated and fermented foods with possible antioxidative activity and increasing concern for the relation between antioxidative actions and disease risk reduction , we have studied how antioxidant content and overall antioxidative activity are modified during seed processing such as germination and fermentation in legumes and cruciferous seeds.

As a beneficial example of germination, despite the presence of a wide array of bioactive compounds, the changes in vitamin C content during *Cruciferae* germination are shown on Figure 1. During sprouting, the vitamin content gradually increased in an almost linear manner. The changes in vitamin C content were positively correlated with SOD-like activity, which represents the ability of sprouts to scavenge superoxide anion radicals, the key radicals when oxidative stress is considered (Figure 2). Sprouts have a higher ability to scavenge different types of free radicals, highlighting their overall antioxidant capacity expressed in terms of Trolox – a water soluble analogue of vitamin E widely used as a standard in free radical research. For example, germination of lupine seeds was found to clearly

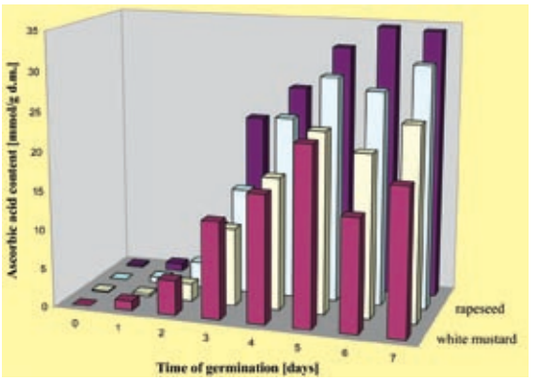

Fig. 1 Time course of ascorbic acid changes during germination of cruciferous seeds under light conditions.

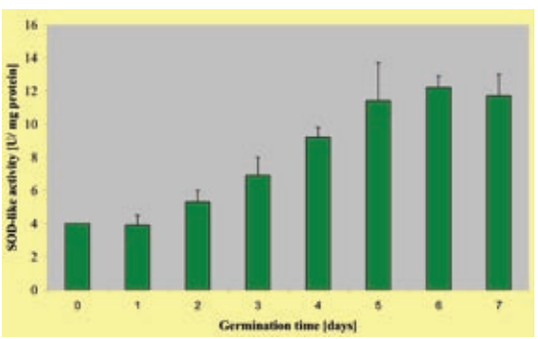

Fig. 2 The changes of SOD-like activity during rapeseed germination.

Table 1. Effect of germination on peroxyl radical-trapping capacity (PRTC) of *Lupinus angustifolius* var. Zapaton.

Lupinus angustifolius var. Zapaton	PRTC [µmol Trolox/ g d.m.]
Raw	2.12 ± 0.12[a]
Germination time (days)	
2	2.69 ± 0.11[b]
3	2.77 ± 0.06[b]
4	2.81 ± 0.11[b]
5	3.10 ± 0.15[bc]
6	3.44 ± 0.59[c]

The same superscript in the column means not significant differences (P≤0.05).

Table 2. Effect of processing of cowpea *Vigna sinensis* var. Carilla on the inhibition of peroxidation in phospholipids bilayers (PC) and Trolox Equivalent Antioxidant Capacity (TEAC).

Sample	Inhibition of PC peroxidation* [%]	TEAC [µmol Trolox/g d.m.]
Raw seeds	48 ± 2[a]	27.95 ± 0.05[b]
Not fermented flour	46 ± 2[a]	26.99 ± 1.33[a]
Flour fermented with *L. Plantarum*	53 ± 11[ab]	60.28 ± 0.41[e]
Flour fermented with *L. Plantarum* and autoclaved	61 ± 1[bc]	57.38 ± 0.83[d]
Flour naturally fermented	61± 9[c]	55.85 ± 0.73[c]
Flour naturally fermented and autoclaved	78± 8[d]	59.71 ± 0.00[e]

*) Peroxidation inhibition after 2 h incubation of liposomal suspension against control sample. The same superscript in the columns means not significant differences (P≤0.05).

increase the peroxyl radical-trapping capacity when compared to non-germinated seeds (Table 1).

Another recently studied process aimed at improving antioxidative properties of seeds is fermentation. Cowpeas (*Vigna sinensis* L. var. Carilla) flours were fermented with inoculum *Lactobacillus plantarum* (PF) or with the natural microorganisms present in flour (NF). Additionally, the fermented flours were autoclaved in order to reduce the antinutritional factors. The effect of fermentation on different nutritional aspects was studied, including antioxidative properties. Antioxidative activities were monitored in phospholipids bilayers by measuring the inhibition of phosphatidylcholine (PC) peroxidation in large unilamellar vesicles (liposomes) and by measuring the Trolox Equivalent Antioxidative Capacity (TEAC) of the products. Table 2 shows the percentages of peroxidation inhibition after 2 h of incubation of liposomes suspension and TEAC values, and clearly demonstrates that fermentation increased both. The oxidation of PC was inhibited by all cowpea fermented flours. The extracts of fermented flours (PF and NF) provided stronger inhibition than the extract from raw seeds and extract from NF flours was better than extract from PF. Autoclaving of fermented flours increased the ability to inhibit PC oxidation but it was significant only for NF. These improvements in antioxidative properties caused by fermentation were supported by TEAC values; a significant increase in the TEAC of fermented flours was noted.

Summing up, fermentation and germination are easy and effective processes to obtain products with higher antioxidative capacity and could be recommended for application in functional food preparation/industry to provide consumers with products with added value.

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The role of cytokines in sickness behavior and depression

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Cytokines are proteins secreted by the cells of the stimulated immune system, for example, during an infectious or malignant disease; they are hormones of the immune system. Apart from their recognized role in immunological and inflammatory processes, it is now known that cytokines have effects outside the immune system and may mediate communication between the immune and nervous systems. In recent years it has been postulated that cytokine secretion, associated with activation of the immune system, may be among the causes of human depression. Precise mechanisms have not been well delineated but there is substantial experimental evidence that cytokines do indeed affect behavior and neurochemical activity of the central nervous system. Pro-inflammatory cytokines, mainly interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) are implicated. In our research we test the hypothesis that cytokines affect brain functions and mediate sickness and depression-like behavior. We also study the underlying neurophysiological mechanisms.

Infections and the administration to mice of bacterial endotoxin (lipopolysaccharide, LPS) induce sickness behavior which includes depressed locomotor and exploratory activities, decreased feeding, and disturbed social interactions. The mechanisms underlying sickness behavior have not been fully elucidated, but it has been suggested that IL-1, IL-6 and TNFα are involved. All three cytokines may be secreted in response to infections and LPS. The cytokine hypothesis of depression would be supported if cytokines and LPS administration induced depression-like behavior in classical tests for depression. The two most commonly used tests for depressants and antidepressants are the forced swim test (FST) and the tail suspension test (TST). In several experiments we administered mice with LPS or IL-1. The results indicate that both IL-1b and LPS can induce depression-like effects in the TST and the FST. However, the doses necessary to induce these changes reduced feeding and locomotion, so that the effects observed in the FST and TST could be attributed to a general reduction in activity. Thus the results obtained in these tests do not provide strong support for an IL-1 hypothesis of depression.

However, IL-1 administration to animals induces secretion of IL-6. Thus IL-6 could be responsible for the behavioral effects of IL-1. Also, LPS administration initiates a cascade resulting in the secretion and consequent increase in plasma concentrations of IL-1, IL-6 and TNFα. Because IL-1 and TNFα can both induce IL-6 production, complex interactions among these three cytokines may occur *in vivo* following LPS administration or infection. Increases in brain concentrations of IL-6 have also been associated with certain stressful treatments and depression. Thus it has been suggested that IL-6 mediates some aspects of sickness behavior and plays a role in some disorders of mental health, including depression and anorexia nervosa. However, support for these hypotheses is circumstantial. We studied, therefore, a range of different behaviors in transgenic mice lacking a functional gene for IL-6 (IL-6-

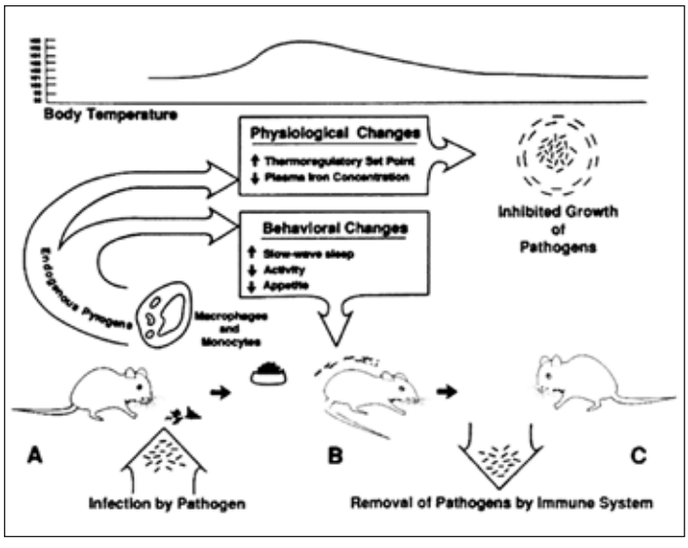

Fig. 1 Possible mechanisms of communication between the brain, immune and endocrine systems. Stimulated immune cells secrete cytokines (IL-1, IL-6, TNFa and others) which either indirectly, via peripheral innervation, for example the vagus nerve, or directly affect cerebral functions. Brain noradrenergic system (NE) is activated and behavioral and endocrine responses can be modified. Also, the hypothalamus (PVN) in the brain can be stimulated and corticotropin-relasing factor (CRF) released to further stimulate secretions of "stress hormones" (ACTH and glucorticoids). Stress hormones, in turn, can directly affect cerebral activity.

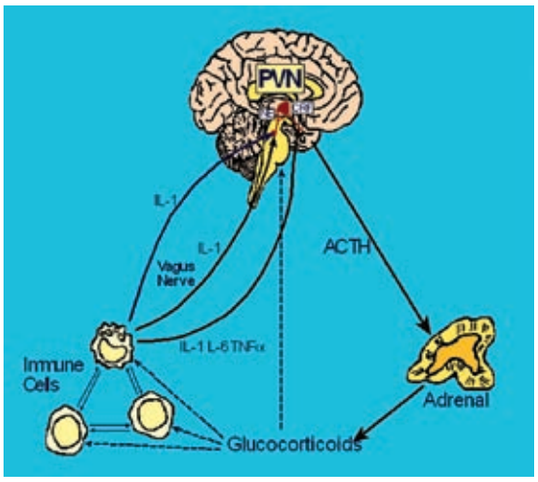

Fig. 2 Adaptive sickness behavior as depicted by Hart. An animal infected by a pathogen decreases activity, becomes lethargic and sleeps more (conserves energy), decreases feeding (exposure to a potentially dangerous environment), and develops fever (increases its body temperature). These physiological and behavioral changes, mediated by cytokines, facilitate combating the infection and are considered beneficial to the animal. At the same time, these responses resemble symptoms of depression and allow sickness behavior to be considered as an animal model of depression.

knockout, IL-6ko) and in "normal" mice (wild-type, WT). The behavioral, neurochemical and endocrine results indicated no major differences between WT and IL-6ko mice. Also, IL-6 did not appear to be critically involved in mediating behavioral responses to IL-1 or LPS or to stressful stimuli. Furthermore, IL-6 deficiency did not affect behavior in the tail suspension and forced swim tests, i.e. paradigms reflecting behavioral despair or behaviors relevant to problems of depression.

As far as the neurophysiological mechanisms of depression-like behavior are concerned, a prevailing hypothesis is that depression is caused by the disorganization of brain serotonergic systems. IL-1, IL-6 and TNFα have been shown to affect the serotonergic system. Currently used antidepressants, unfortunately not always effective, are presumed to work by normalizing a defect in brain serotonergic transmission. There are several hypothetical mechanisms by which cytokines could act on brain serotonergic systems and thus contribute to the pathophysiology of depression. However, relationships between these potential mechanisms and depression remain to be demonstrated in patients with depression. In this context, an interesting facet of our studies is that we perform them in mice with a genetically modified opiate (morphine-like) system. We thus go beyond the serotonergic system, attempting to find new mediators of sickness and depression-like behaviors.

The results of our research suggest that cytokines mediate certain aspects of sickness behavior, mainly changes in locomotion and feeding. The results are significant within the context of improving animal welfare. Furthermore, it appears that the induction of IL-1 within the brain has the potential to induce depression-like behavior and this mechanism is worthy of further investigation. The existing evidence, however, does not provide a firm empirical basis for a cytokine network within the brain that causes depression. The EU funded FP6 NEWMOOD integrated project aimed at identifying molecular targets for novel antidepressants is currently in progress, with our research team being responsible for several animal and genetic models of mental health disorders.

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Transfer of stress resistance genes within the *Lolium-Festuca* grasses by conventional backcross breeding programs

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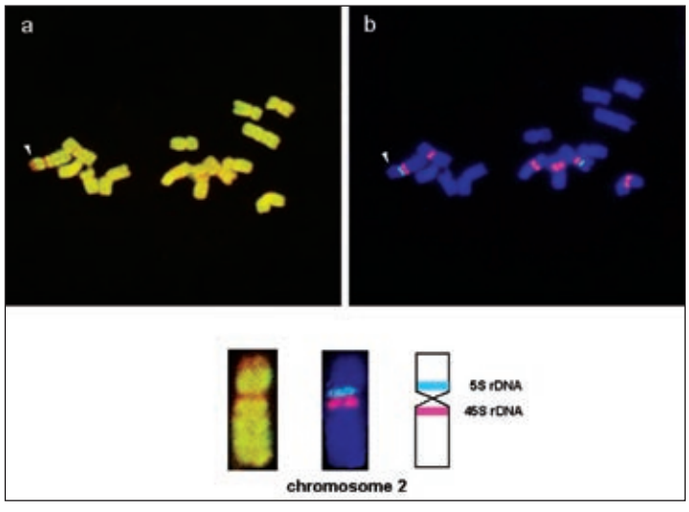
The *Lolium-Festuca* complex contains a number of species of forage grasses, which are of key importance for temperate grasslands. The four major agricultural species – Italian ryegrass (*Lolium multiflorum* Lam.), perennial ryegrass (*L. perenne* L.), meadow fescue (*Festuca pratensis* Huds.) and tall fescue (*F. arundinacea* Schreb.) – offer a considerable range of desirable and complementary traits. *Lolium* species have high productivity and forage quality but rather poor persistency under stress conditions. Conversely, closely related *Festuca* species, although with comparatively poor seedling vigor and nutritive value, can provide greater persistency and tolerance to climatic stresses than *Lolium*. These *Lolium* and *Festuca* species hybridize readily and their homoeologous chromosomes pair and recombine at high frequency, enabling the assembly of their complementary characters within a single genotype. One approach of combining desirable traits from *Lolium* and *Festuca* species is amphiploidy, in which complete parental genomes are combined in intergeneric hybrids. However, the production of grass cultivars based on synthetic amphiploids has been limited because of homoeologous chromosome pairing, leading to genetic instability in later generations. The alternative to the amphiploidy approach at combining traits from *Lolium* and *Festuca* is introgression, which allows a limited number of genes to be transferred from a donor species into the reconstituted genome of the recurrent species by recombination and selection. Since recombination between homoeologous *Lolium* and *Festuca* chromosomes occurs frequently in hybrids, opportunities for gene introgression arise whereby desirable *Festuca* genes may be transferred to *Lolium* through a conventional backcross breeding program. The targeted inclusion of desirable *Festuca* gene combinations can be accompanied by the targeted exclusion of other *Festuca* genes considered detrimental to the desirable forage quality traits found in *Lolium.*

Molecular cytogenetics has not just revolutionized the genetic analysis of plant genomes, but also provided plant breeders with new tools to identify genes involved in resistance to abiotic and biotic stresses. The development of the genomic in situ hybridization (GISH) technique for cytogenetic analyses in Lolium-Festuca hybrids has represented a major advance in the genome analysis of these genera. GISH, where whole genomic DNA of one parental species of the intergeneric hybrid is used as a probe to detect complementary sequences on chromosomes, enables the parental chromosomes of Lolium and Festuca to be distinguished, and the sites of any genome recombination identified. GISH also enables the monitoring of the introgression of alien chromatin from one species to another in successive backcross generations.



GISH (a) and FISH (b) analysis of the same mitotic chromosome spread of freezing-tolerant introgression form. GISH image created using total genomic DNA of *Lolium* as a probe labeled with digoxigenin and detected by anti-digoxigenin conjugated with fluorescein (yellow), with blocking genomic DNA of *Festuca* (red). Chromosomes were counterstained with propidium iodide. FISH image created using as a probe (i) 45S rDNA labeled with biotin and detected by streptavidin conjugated with Cy-3 (red), (ii) 5S rDNA labeled with digoxigenin and detected by anti-digoxigenin conjugated with fluorescein (green). Chromosomes for FISH analysis were counterstained with DAPI (blue). GISH and FISH analysis indicate that the *Festuca* introgression was located on the non-satellite arm of chromosome 2 of *Lolium* (arrows).

One of the limiting factors for the successful widespread use of high yielding Lolium cultivars is their susceptibility for winter stresses. Thus combining the nutritive quality of L. multiflorum and the winter-hardiness of F. pratensis or F. arundinacea in one genotype is considered a primary grass breeding objective.

In our latest papers we demonstrate, for the first time, how a backcross breeding program enables transfer of Festuca genes for freezing tolerance – the main component of winter-hardiness, into diploid freezing-sensitive L. multiflorum cultivars by using partially fertile hybrids, triploid – F. pratensis (2n = 2x = 14) × L. multiflorum (2n = 4x = 28) and pentaploid – F. arundinacea (2n = 6x = 42) × L. multiflorum (2n = 4x = 28), as an initial plant material in the backcrosses with L. multiflorum. The approach described includes the cytogenetic mapping of stress resistance genes using GISH and additionally fluorescence in situ hybridization (FISH) with rDNA probes to assist with chromosome identification (Fig. 1). The targeting of diploid (2n = 2x = 14) freezing-tolerant introgression forms of L. multiflorum that contained only one Festuca chromosome segment allowed the location of putative Festuca-derived genes for freezing-tolerance.

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The importance of young shelterbelts for biodiversity in an agricultural landscape

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Research on the plants and animals of midfield afforestations and adjacent crop fields has been carried out in the area of the village Turew (in the western Wielkopolska region) over the last 50 years. Several new shelterbelts and tree-lines were planted in this region in 1993 to 1996 (with total length of 50 km) to complete the afforestation network created in the 1920s by General Dezydery Chłapowski, which became impoverished after WWII. The introduction of new afforestations into the agricultural landscape has created a unique opportunity for researching the dynamics of processes occurring in afforestations as well as in the adjacent crop fields in relation to afforestation growth and changes resulting from the natural succession. These opportunities have been successfully utilized: two projects were carried out and have been completed, resulting in the publication of several papers summing up their results. Here we present the results of investigations dealing with the role of these afforestations for biodiversity conservation in an agricultural landscape in respect to wintering insects, butterflies and breeding birds.

One study investigated the role of recently established shelterbelts (Phot. 1) as refuges available for

wintering insects (Karg 2004) over the years 1994-2002. Soil and litter samples were taken (the material being sorted manually) from five young (up to 7 years old) and two older midfield shelterbelts, from the ecotone zones and from the adjacent croplands. Shelterbelts newly introduced to the agricultural landscape are very soon (already in their first winter after planting) used as refuges available for wintering insects (Fig. 1). High numbers (250-400 ind. m^{-2}) of insects, with biomass varying between 950 and 2300 mg dry weight·m^{-2}, were found to overwinter in young (7 year old) shelterbelts. In such shelterbelts the insects formed communities (dominated by Coleoptera) representing over 50 families. The total biomass of insects there was comparable with the biomass found in old (over 100-150 year old) shelterbelts. The species composition of the shelterbelt affects the numbers of wintering insects; this should be taken into account when designing shelterbelts. Patches (several m^2 in area) inside the shelterbelts planted with a mixed composition of birch, rowan, elm and linden with admixtures of coniferous larch and pine are preferred by wintering insects. The ratio of insects wintering in the shelterbelt to those wintering in the ecotone and open field is 10:2:1 on average (Tab. 1).

Another study looked at butterfly communities (Sobczyk 2004) in 1999-2000 in midfield shelterbelts using the transect method (with a total length of 4520 m). Five shelterbelts (transect length of 3070 m) were planted in 1993, one (1450 m) in 1998. All the shelterbelts were planted on arable land. Twenty seven species of butterflies (imagines)

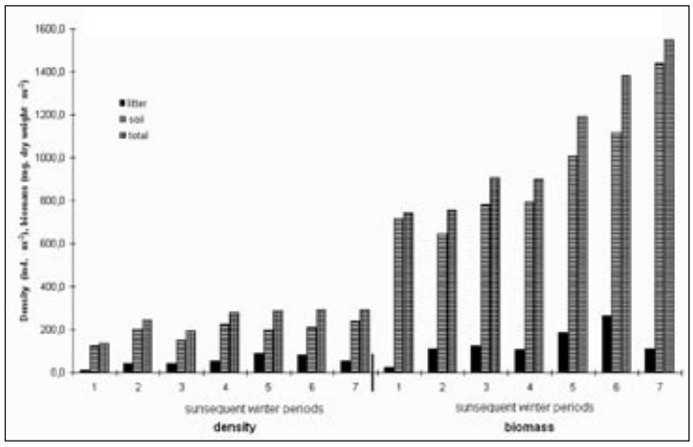

Fig. 1 Mean density and biomass of insects wintering in litter and soil of new young shelterbelts in subsequent years (after Karg 2004).

were recorded during the study period. *Pieris napi* and *P. rapae* dominated in all the shelterbelts. Their dominance stood at 30 and 24%, respectively, in 6-7 year old shelterbelts, and at 34 and 32% in the younger shelterbelt (1-2 years old). The total mean density of butterflies in older shelterbelts was equal to 91.9 ind. km^{-1} of transect (width 5 m) – and was about 40% higher than in the younger one. The lower dominance of *Aphantopus hyperantus* (8%) observed in 6-7 year shelterbelts (when compared to ones several decades old) was most likely caused by a small share of grasses and perennial dicotyledons, which are important for this species. The butterflies recorded in shelterbelts may be assigned to four ecological groups: ubiquistic species (7 spp.),

Table 1. Density (ind. m^{-2}) and biomass (mg dry wt · m^{-2}) of insects wintering in litter and soil of shelterbelts of different age and on adjacent fields (after Karg 2004).

Shelterbelt age (years)	Inside shelterbelt	Ecotone zone (0.5-10m)	Open field (50-100m)	Number of subsequent winters and studied shelterbelts
Density (ind · m^{-2})				
1-3	190.9	63.5	32.4	5
4-7	285.6	162.8	27.9	4
>100	313.4	113.3	21.5	2
mean	263.3	133.2	27.3	-
Biomass (mg. dry weight · m^{-2})				
1-3	802.0	274.8	59.9	5
4-7	1148.6	618.2	110.2	4
>100	878.0	397.0	61.6	2
mean	942.9	430.0	77.2	-

Young shelterbelt near the village of Turew (in the Gen. Dezydery Chłapowski Landscape Park) – one of the afforestations studied

species typical for open areas (10 spp.), species linked to afforestations (3 spp.) and woodland species (7 spp.). In all studied shelterbelts, ubiquistic species and open areas species (*Pieris rapae* and *P. napi*) were most abundant, with no respect to shelterbelt age. Some rare species with higher environmental demands were also recorded, i.e., *Polyommatus amandus* and *Carterocephalus palaeon*, which had not been previously observed in the study area.

A third study on birds (Kujawa 2004) was aimed at recognizing breeding bird community dynamics in young (1-4 years old at the beginning of the study) shelterbelts (N=9) and at estimating their importance for farmland birds. Bird density was estimated by a mapping method in successive years 1996-2001 and the data were combined and analyzed in respect to shelterbelt age. Eighteen breeding species were found (5-8 pairs km^{-1}), the most abundant among them being the Corn Bunting (*Miliaria calandra*) (with dominance of 33%), Yellow Wagtail (*Motacilla flava*) (19%) and Whitethroat (*Sylvia communis*) (12%). No trend was recorded in variations of species richness and total density. The density of species preferring to build nests and/or feed in the herb layer (like the Yellow Wagtail, Skylark *Alauda arvensis* and Whinchat *Saxicola rubetra*) decreased during the study period while the density of species associated with higher layers of vegetation like the Yellowhammer (*Emberiza citrinella*) and Red-backed Shrike (*Lanius collurio*) increased. According to an earlier study, bird species richness and abundance in young shelterbelts were lower than in ones several decades old. However, in relation to species colonization of both classes of shelterbelts (species building their nests on the ground or in low shrubs), young shelterbelts were as important as old ones.

The results presented show that young, several-year-old afforestations play a very important role in the preservation of biodiversity in an agricultural landscape. It should be stressed that this habitat is colonized very quickly – 1-2 years after planting – by relatively species-rich communities of different organisms.

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