APPENDIX NO. 2b

SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

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CONTENTS

1. INFORMATION ON EDUCATION AND EMPLOYMENT

1.1. PERSONAL DATA

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1.2 DIPLOMAS AND DEGREES

1998: **M.Sc. in environmental protection**; Faculty of Zootechnology, Agricultural Academy in Lublin (at present Faculty of Biology, Animal Sciences and Bioeconmy, University of Life Sciences in Lublin);

Master's thesis: „Estimation of Cd, Zn and Pb loads in soils used for agriculture in central and southern Poland in the years 1955-1995", Master thesis were completed at the Institute of Soil Sciences and Environment Shaping

Supervisor: prof. dr hab. Halina Smal
Reviewer: prof. dr hab. Tadeusz Filipek

2003: **Ph.D. in Agricultural Sciences in the field of Horticulture, specialization – plant protection, entomology**; Faculty of Horticulture Agricultural Academy in Lublin (at present Faculty of Horticulture and Landscape Architecture, University of Life Sciences in Lublin);

Doctoral dissertation: "Occurrence of leafroller moths (Lepidoptera, Tortricidae) in apple orchards at varying intensity of plant protection treatments", Doctoral dissertation was completed in Department of Entomology

Supervisor: dr hab. Władysław Huszcza
Reviewers: prof. dr hab. Bożenna Jaśkiewicz,
prof. dr hab. Kazimierz Wiech

COMPLETED VOCATIONAL COURSES AND OTHERS:

1997-1998: graduated from the Interfaculty Pedagogy College of Agricultural Academy in Lublin

2008: Certificate in Advanced English: **TELC ENGLISH B2**

1.3. INFORMATION ON EMPLOYMENT

October 1998 – June 2003	Doctoral program participant at the Department of Entomology, Agricultural Academy in Lublin (at present University of Life Sciences in Lublin)
August – December 2003	professional internship at the Department of Entomology, Agricultural Academy in Lublin (at present University of Life Sciences in Lublin)
November 2004 – February 2005	contract for the completion of classes at the Department of Entomology, Agricultural Academy in Lublin (at present University of Life Sciences in Lublin)
1 April 2005 – 31 March 2006	assistant lecturer – full-time - at the Department of Entomology, Agricultural Academy in Lublin (at present University of Life Sciences in Lublin)
1 April 2006 – until now	assistant professor at the Department of Entomology, Agricultural Academy in Lublin (at present Department of Plant Protection, Subdepartment of Entomology, University of Life Sciences in Lublin)

2. Indication of the achievement according to 16 paragraph section 2 of the act of law from 14 March 2003 on academic degrees and titles in the arts (official Journal of Laws of 2016, item 882 – uniform text with amendments announced in official Journal of Law of 2016 item. 1311)

2.1. TITLE OF THE SCIENTIFIC ACHIEVEMENT

The achievement submitted in the application for the academic degree of habilitated doctor is a series of five research publications under the title:

"Effect of insect feeding on physiological and biochemical changes in host plants on the example of gall-inducing Cynipidae - oaks model"

2.2. PUBLICATIONS THAT MAKE UP THE SCIENTIFIC ACHIEVEMENT

H1. Kot I., Kmieć K., 2013. Galls induced by insects on oaks and elms in the Lublin region, Poland. Electronic Journal of Polish Agricultural Universities, Topic:

Forestry, Vol. 15(3). http://www.ejpau.media.pl/volume16/issue3/art-04.html [7 points]

H2. Kot I., Jakubczyk A., Karaś M., Złotek U., 2018. Biochemical responses induced in galls of three Cynipidae species in oak trees. Bulletin of Entomological Research, 108, 494–500. doi:10.1017/S0007485317001055 [IF = 1.721; 35 points]

H3. Kot. I., Rubinowska K., 2018. Physiological response of pedunculate oak trees to gall-inducing Cynipidae. Environmental Entomology, 47(3), 669–675. doi: 10.1093/ee/nvy047 [IF = 1.661; 30 points]

H4. Kot I., Rubinowska K., Michałek W., 2018. Changes in chlorophyll a fluorescence and pigments composition in oak leaves with galls of two cynipid species (Hymenoptera, Cynipidae). Acta Scientiarum, Polonorum Hortorum Cultus, 17(6), 147–157. doi: 10.24326/asphc.2018.6.15 [IF = 0.448; 20 points]

H5. Kot I., Sempruch C., Chrzanowski G., Czerniewicz P., 2019. Changes in amine levels and amino acid decarboxylase activities induced in galls of three Cynipidae species in oaks. Biochemical Systematics and Ecology, 83, 26-32. https://doi.org/10.1016/j.bse.2018.12.016 [IF = 0.847; 15 points]

Statements of the co-authors of the publications with information on their personal contribution to the papers are included in Appendix 6. None of the publications submitted here have been included in a single-topic series in another habilitation proceeding application.

3. DESCRIPTION OF THE SCIENTIFIC AIM OF ABOVE-MENTIONED PUBLICATIONS AND THE OBTAINED RESULTS WITH DISCUSSION OF THEIR POSSIBLE USE

3.1. INTRODUCTION

Many herbivorous arthropods can affect the development of plant organs which they infest. Their attacks trigger translocation of metabolites and phytohormones in plant tissues, which causes abnormal cell elongation and proliferation thus inducing gall formation (Oliveira et al. 2016). Galls are specific structures regarded as a new plant organ and a site of larval feeding and development. The gall structure varies depending on the phytophagous species inducing them, even on the same host plant (Stone et al. 2002). The high-specialised gall-inducing insects include Hymenoptera from the family Cynipidae. In the world, this family comprises over 1 300 species, while there are at least 163 of them in the Palearctic region (Rokas et al. 2003). In terms of trophic, they are associated primarily

with various species of oaks (*Quercus* spp.), although some species induce galls on herbaceous plants, mainly from the Asteraceae family (Melika 2006).

The life cycle of Cynipidae, referred to as heterogony, is an obligatory alternation of sexual (♀♂) and agamic (♀♀) generations within the year. These two generations may differ in the morphological features of adults, choice of the plant organ attacked, and structure of formed galls (Harper et al. 2004). Galls that bisexual generation is developed are formed in spring and/or early summer. In turn, galls in which the agamic generation develops are formed in late summer and autumn (Stone et al. 2002). Cynipidae induce galls with highly diverse morphotypes on different parts of plants: roots, shoots, leaves, buds, flowers, and fruits (Stone and Schönrogge 2003). Nevertheless, they are all an archetype of nutritional galls composed of three layers: nutritive, parenchymatous and epidermal tissues (Oliveira et al. 2016).

The earliest plant response to gall induction involves increased production of reactive oxygen species (ROS), which may cause harmful oxidation of macromolecules, including nucleic acids, proteins, and lipids (Isaias and Oliveira 2012). On the other hand, reactive oxygen species are produced in plant metabolic processes and have a key role in cellular signalling, which can determine the extent of gall cell differentiation (Carneiro et al. 2014). One of the reactive oxygen species, i.e. hydrogen peroxide (H_2O_2), plays an important role in plant defence response due to its relatively stable form and the ease of free translocation. However, excessive production of H_2O_2 in plant cells leads to oxidative stress (Maffei et al. 2007). Accumulation of hydrogen peroxide and other reactive oxygen species enhances lipid peroxidation in cell membranes, exerting an effect on their structure and membrane permeability as well as change of enzymatic activity (Gill and Tuteja 2010). Excessive production of ROS is buffered by several non-enzymatic (e.g. ascorbic acid, glutathione, tocopherol, and carotenoids) and enzymatic (e.g. peroxidases, dismutases, catalase, polyphenol oxidase) mechanisms. They protect plant cells against oxidative damage by conversion of ROS into less toxic products (Pandey et al. 2017).

Interactions between plants and insects, including gall inducers, consist in complex metabolic signalling in host plants. On the one hand, plants produce secondary metabolites and proteins that are toxic and/or repellent to herbivores (War et al. 2012). On the other hand, the biochemical reactions induced in the plant during gall formation comprise processes that protect the needs of gall-inducing insects. For instance, gall

development induces local accumulation of nutrients, also amino acids (Giron et al. 2016). Therefore, changes in the metabolism of these compounds may be an important element of the biochemical response of plants to the presence of gall-inducing insects. In turn, the decomposition of amino acids as nutrients for phytophages may be associated with to the activity of polyamine biosynthesis enzymes, mainly ornithine decarboxylase (ODC; EC 4.1.1.17) and lysine decarboxylase (LDC; EC 4.1.1.18) (Sempruch et al. 2014). In turn, tyrosine decarboxylase (TyDC; EC 4.1.1.25) is involved in the biosynthesis of aromatic monoamines and classes of compounds involved in plant defence response (Miller-Fleming et al. 2015).

The mechanisms determining the impact of insects on the host plant include changes in the content of assimilation pigments and photosynthetic activity (Guidi and Degl'Innocenti 2012). On the other hand, galls exhibit a high demand for assimilates. Some gall-inducing species stimulate the intensity of photosynthesis, while others diminish this process, which is probably dependent on plant tolerance to insect infestation (Nabity et al. 2009, Haiden et al. 2012). The most common method for investigating the changes in the photosynthetic apparatus and the photosynthetic efficiency in higher plants relies on an analysis of chlorophyll *a* fluorescence (Kalaji et al. 2012).

The views regarding the formation of galls have evolved. Previously, it was reported to be a plant defence response to insect attack. Current theories propose that the development of galls is a result of manipulation of the gall inducer in the host plant. In aspect of these data, it was reasonable to undertake comprehensive studies on the impact of gall inducer feeding on the physiological and biochemical changes occurring in host plants. Elucidation of the basics of the specific plant response to insect attack provides important contribution to the knowledge of the ecology at the individual level. Moreover, the insect species selected for the investigations have not been analysed in Poland or elsewhere in such great detail.

3.2. RESEARCH AIM

Gall induction seems to be the most highly specialised form of interaction at the insect-plant level, and the Cynipidae-oak system can be a model for investigation of the interaction between the gall inducer and the host plant. This is related to the fact that these insects exhibit high food specialisation, i.e. they are outstanding monophages, and their galls are an archetype highly specialised nutritive tissues that form a separate microhabitat.

The presented research series is focused on the analysis of the species composition of Hymenoptera gall inducers and examination whether the physiological and biochemical plant response to insect feeding on the same host plant varies depending on the species of the Hymenoptera gall inducer. The specific aims of the research included:

1. Analysis of the qualitative and quantitative structure of Hymenoptera gall inducers from the family Cynipidae inhabiting oaks growing in habitats with various degrees of anthropopressure in the Lubelszczyzna region;
2. Analysis of changes occurring in galls and in leaves with galls in comparison with control leaves to elucidate the interactions between gall inducers and host plants. To this purpose, the following were examined and analyzed:
   - ✓ cytoplasmic membrane condition;
   - ✓ changes in hydrogen peroxide (H_2O_2) concentration;
   - ✓ changes in antioxidative enzyme activities, including guaiacol peroxidase (GPX) and ascorbate peroxidase (APX);
   - ✓ changes in protein and phenolic compounds content;
   - ✓ changes in peroxidase (POD) and polyphenol oxidase (PPO) activity;
   - ✓ activity of 'pathogenesia-related' (PR) proteins, such as chitinase and β-1,3-glucanase;
   - ✓ polyamines content and activity of decarboxylases involved in biosynthesis thereof;
   - ✓ changes in photosynthetic pigments content;
   - ✓ intensity of chlorophyll *a* fluorescence.

3.3. DESCRIPTION OF THE OBTAINED RESULTS WITH DISCUSSION OF THEIR POSSIBLE USE

Qualitative and quantitative structure of Hymenoptera gall inducers on oaks

Publication:

H1. Kot I., Kmieć K., 2013. Galls induced by insects on oaks and elms in the Lublin region, Poland. Electronic Journal of Polish Agricultural Universities, Topic: Forestry, Vol. 15(3). http://www.ejpau.media.pl/volume16/issue3/art-04.html

The research was carried out in 2009-2011 in habitats characterised by different anthropopressure degrees. The observation objects were oaks (*Quercus* spp.) growing in natural localities (forests, mid-field tree stands) and in urbanised areas in Lublin and its surroundings. The forest localities were situated in Świdnik, Puławy, and Lubartów Forest Districts. Additionally, the investigations were conducted in mid-field tree stands near Lublin and urban greeneries of Lublin and Świdnik. Ten oaks in each habitat type were inspected every 7 days throughout the vegetation season. Qualitative and quantitative analyses were performed on 200 leaves and 100 shoots randomly collected from a tree (one sample). Three classes of abundance were established: high intensity (the presence of galls induced by a given species on more than 60% of leaves or shoots in one sample), medium intensity (the presence of galls induced by a given species on 30-60% of leaves or shoots in one sample), and small intensity (the presence of galls induced by a given species on less than 30% of leaves or shoots in one sample).

In Lubelszczyzna region, observations regarding gall inducers were carried out fragmentary and dated several dozen years ago. The investigations revealed the presence of galls induced by 11 Hymenoptera species from the family Cynipidae. Species inducing galls on leaves were represented by *Neuroterus quercusbaccarum* (L.) (♀♂ and ♀♀), *N. numismalis* (Fourc.) (♀♀), *N. tricolor* (Hartig) (♀♀), *N. albipes* Schenck (♀♀), *Cynips quercusfolii* (L.) (♀♀), *C. longiventris* (Hartig) (♀♀), *C. divisa* (Hartig) (♀♀) and *Andricus anthracina* (Curtis) (♀♀). Other species, i.e. *A. fecundatrix* (Hartig) (♀♀), *A. inflator* (Hartig) (♀♂) and *Biorrhiza pallida* (Ol.) (♀♂), induced galls on the lateral and apical buds of the shoots. Galls induced by both generations of *N. quercusbaccarum* and galls induced by the agamic generation of *N. numismalis* and *C. quercusfolii* were the most numerous.

N. quercusbaccarum galls in which larvae of the sexual generation develop are spherical and juicy and have a smooth surface. They have a diameter of approx. 4 mm and change colour from green to red. They were found on oak leaves and flower pedicels in May and June. In turn, galls induced by the agamic generation have a diameter of approx. 5 mm, a single chamber, and a lenticular shape with an elevated centre. They are covered by red hairs and are located on the lower surface of leaf blades. The galls were collected between August and October. Galls induced by the agamic generation occurred with comparably high intensity on the oak trees from the forests, urban habitats, and

mid-field tree stands. In turn, the highest number of galls induced by the sexual generation was noted only on urban trees.

N. numismalis galls induced by the agamic generation are button-like with a characteristic concavity of the upper surface and covered by silky hairs. They were found on the lower lamina surface in trees from each analysed locality in August and September. In the same period, large spherical galls induced by the agamic generation of *C. quercusfolii* were found. However, these galls were present only on the forest trees. Very high intensity of this type of galls was noted on *Quercus robur* 'Fastigiata' trees in a decorative tree nursery located in the immediate vicinity of the forest near Puławy.

In the analysed forest habitats, there were no *A. inflator* and *A. antharacina* representatives, which were present only in the urban localities. A relatively high number of galls in the form of dilated ends of young shoots induced by the sexual generation of *A. inflator* were observed on *Q. robur* 'Fastigiata' in spring. So far, this species had not been recorded in Lubelszczyzna region.

After finding which species were the most abundant, an idea was formulated to investigate physiological and biochemical changes occurring in galls and in leaves with galls, in comparison with control leaves. This was aimed at acquisition of knowledge of the interactions between gall inducers and their host plants. A research hypothesis was proposed that the diverse structure of galls induced by the different species may trigger different response of plant cells. The analysis involved galls induced by agamic generations of *C. quercusfolii*, *N. numismalis* and *N. quercusbaccarum*.

***Analysis of changes in leaves with galls and in galls induced by Cynips quercusfolii* L., *Neuroterus numismalis* (Fourc.), *and N. quercusbaccarum* L.**

Changes in physiological processes

Publication:

H3. Kot. I., Rubinowska K., 2018. Physiological response of pedunculated oak trees to gall-inducing Cynipidae. Environmental Entomology, 47(3), 669–675. doi: 10.1093/ee/nvy047

The investigations of changes in physiological processes occurring in leaves with galls and in galls induced by *Cynips quercusfolii* L., *Neuroterus numismalis* (Fourc.) iand *N.*

quercusbaccarum L., consisted in analyses of the content of hydrogen peroxide (H_2O_2), assessment of cytoplasmic membrane condition, and changes in the activity of antioxidant enzymes: guaiacol peroxidase (GPX) and ascorbate peroxidase (APX). The results demonstrated that the presence of galls induced by the agamic generations of *C. quercusfolii*, *N. numismalis*, and *N. quercusbaccarum* on oak leaves caused an increase in the level of H_2O_2, in comparison with the control leaves, but these changes were significant only in the case of *C. quercusfolii*. This indicates that gall induction causes oxidative stress in oak leaves. In turn, the H_2O_2 level in the gall tissues varied and was dependent on the gall inducer species. A significant decline in the content of this free radical was noted in the case of *C. quercusfolii* galls, compared with that in leaves with galls. In contrast, the level of H_2O_2 increased significantly in the galls induced by the other two species. Its content in the *N. quercusbaccarum* galls was even 2.4-fold higher than in the control. The results of these studies suggest an ambiguous role of free radicals in gall cells. On the one hand, their high content in these structures may be related to oxidative stress in response to the attack of gall inducing species. On the other hand, gall cells may be involved in the generation of reactive oxygen species, which trigger a morphogenesis process and, consequently, formation of galls. In turn, the decline in the ROS content may be caused by specific scavenging mechanisms.

Changes in the integrity and stability of cytoplasmic membranes are the first visible symptoms of the effect of biotic and abiotic stress on the plant cell. The degree of cell membrane disintegration during the gall formation process was assessed by measuring the value of the indicator of electrolyte leakage (E_L) from the cells. The obtained results showed that the presence of galls induced by all the three Cynipidae species contributed to a significant 22-27% increase in the value of the measured parameter, depending on the species, compared with the control. The increase in the E_L value is observed almost immediately after the impact of the stressor on the plant organism and may increase or persist at an elevated level from a few minutes to several hours. The present results indicate that increased electrolyte leakage from cells exposed to biotic stress may persist for even several weeks, as indicated by the results of measurements in mature galls.

An increase in the degree of membrane lipid peroxidation is observed in plant cells exposed to oxidative stress, which often occurs as secondary stress accompanying biotic stresses. The level of membrane lipid peroxidation in this study was determined by analysis the content of thiobarbituric acid reactive substances (TBARS). An increase in

the TBARS content was observed in the leaves with galls induced by all the three investigated species; however, these changes were statistically significant only in the case of *N. numismalis* and *N. quercusbaccarum*. In these two species, there was an almost 80% difference in the content of the analysed substances, in comparison with the control leaves. The increase in lipid metabolism evidences that the presence of galls induces oxidative stress.

In plant tissues, peroxidases are a group of enzymes with an ability to inactivate reactive oxygen species. As shown by the research, there was an increase in the activity of guaiacol peroxidase (GPX) in the leaves with galls of all species, compared with the control. The leaves with galls induced by *N. numismalis* exhibited a nearly six-fold increase in the activity of this enzyme. The study results indicate that the induction of leaf galls by all the analysed species triggers plant stress. In turn, the GPX activity in the galls induced by all the examined Cynipidae was significantly lower than in the control. The activity of this enzyme was reduced by 66-88%, depending on the species. The low guaiacol peroxidase activity in the galls confirms the thesis that Cynipidae-induced galls are an archetype of nutritional galls with high rates of gall cell metabolism.

The analysis of the ascorbate peroxidase (APX) activity in the leaves with *N. numismalis* galls revealed a 34% decline in this activity, compared with the control, whereas no significant differences were noted in the case of *C. quercusfolii* and *N. quercusbaccarum*. The enzyme was also characterised by low activity in the tissues of galls of three species, in comparison with the control leaves. Depending on the species, the decline in the activity of this enzyme reached 63-89%. Higher APX activity in plant tissues exerts an adverse effect on insect growth and development. In turn, the lack of ascorbate in the insect midgut increases oxidative stress, which leads to generation of highly unstable reactive oxygen species. Additionally, APX reduces excessive amounts of H_2O_2 and oxidises phenolic compounds to quinones, which inhibit insect feeding. The present study demonstrated reduced APX activity in both the leaves with galls and the galls.

Changes in biochemical processes

Publication:

H2. Kot I., Jakubczyk A., Karaś M., Złotek U., 2018. Biochemical responses induced in galls of three Cynipidae species in oak trees. Bulletin of Entomological Research, 108, 494–500. doi:10.1017/S0007485317001055

H5. Kot I., Sempruch C., Chrzanowski G., Czerniewicz P., 2019. Changes in amine levels and amino acid decarboxylase activities induced in galls of three Cynipidae species in oaks. Biochemical Systematics and Ecology, 83, 26-32. https://doi.org/10.1016/j.bse.2018.12.016

The investigations of changes in biochemical processes consisted in analysis of the content of proteins and phenolic compounds, changes in peroxidase (POD) and polyphenol oxidase (PPO) activity, assessment of the activity of plant PR proteins such as chitinase and β-1,3-glucanase, changes in the polyamine content, and activity of decarboxylases involved in biosynthesis thereof.

Since the whole development of gall-inducer larvae takes place inside the gall, the larvae must always be provided with a sufficiently high level of nutrients (e.g. proteins) and energy. In addition, insect feeding itself can induce local accumulation of proteins. However, the results indicate that the content of these compounds was higher only in *C. quercusfolii* and *N. numismalis* galls, compared with the control, but the differences were not statistically significant. In turn, the content of these biomolecules in the tissues of galls induced by *N. quercusbaccarum* was significantly lower than in the control leaves. These results indicate that larvae modify the level of proteins in the surrounding tissues, and the general statement that galls are “sinks” for these compounds cannot be applied to whole Cynipidae family. It was also found that the protein content in the tissues of leaves with galls was highly variable and depended on the gall-inducing species. Leaves with *N. numismalis* galls contained higher protein levels than the control. In turn, the content of these biomolecules in the leaves with *C. quercusfolii* and *N. quercusbaccarum* galls was by 23-38% lower than in the control tissues. This was probably associated with the fact that plant stress reduces the protein synthesis rate.

In deciduous plants, shikimic acid derivatives (phenols, phenolic acids, flavonoids) play a major role in plant resistance to phytophage attacks. Plant phenols limit insect feeding by increasing leaf hardness and reducing their nutritional value. Polyphenol oxidase (PPO) and peroxidase (POD) are involved in reactions of oxidation of phenols to quinones, which bind to the leaf proteins thereby making them less readily digestible to insects. Chitinase and β-1,3-glucanase are one of the less intensively investigated PR proteins, which play a role in the mechanism of plant resistance to insect feeding. These enzymes are usually contained at low levels in most plant tissues (stems, flowers, seeds,

tubers). Their increased activity is observed in plant response to many abiotic and biotic factors.

In this study the content of phenolic compounds was similar in the case of all the Cynipidae species. The leaves with galls and galls contained a significantly higher level of soluble phenols than the control tissues. An extremely high level of phenolic compounds of 349.02 mg GAE g^{-1} DW was detected in the *C. quercusfolii* galls. This value was 5.5-fold higher than that in the *N. quercusbaccarum* galls. The increase in the content of phenolic compounds in the leaves with galls was probably an effect of plant defence response to insect feeding. In turn, the high phenol content in the galls can be regarded as insect manipulation with the host plant targeted at limitation of the risk of attack by natural enemies or pathogen infection.

In the present study, the PPO activity increased significantly in the leaves with galls of all the analysed species, which may indicate plant defence response caused by the gall induction. Very high PPO activity was noted in *C. quercusfolii* gall tissues, i.e. it was 3.2-fold higher than in the control leaves. In turn, these enzymes did not exhibit any activity in the tissues of *N. numismalis* and *N. quercusbaccarum* galls. This may have been related to the action of PPO on phenolic compounds, which alter various cellular components, including proteins, leading to reduction of their nutritional value for insects.

The chitinase activity varied depending on the Cynipidae species. A significant increase in the activity of this enzyme was observed both in the leaves with galls and in the *C. quercusfolii* and *N. quercusbaccarum* galls, compared with the control. The presence of the *N. numismalis* galls on the leaves significantly increased the chitinase activity, whereas this activity in the galls induced by this species was by 70% lower than in the control. A similar trend was noted for β-1,3-glucanase. Only *N. numismalis* galls exhibited reduced β-1,3-glucanase activity, in comparison with the control leaves. Significantly higher activity of this enzyme was detected in the leaves with galls of *C. quercusfolii* (by 73.12%) and *N. numismalis* (by 106.92%), as well as in the *N. quercusbaccarum* galls (by 81%) in comparison with the control leaves. PR proteins such as chitinase and β-1,3-glucanase can protect galls and feeding insects against pathogen infections. On the other hand, chitinases can negatively influence insect growth and development through their ability to destroy the insect chitin cuticle.

Polyamines are compounds that play an important role in plant tolerance to stress. One of the key enzymes in the biosynthesis of these compounds is ornithine decarboxylase (ODC). Lysine decarboxylase (LDC) catalyses the conversion of lysine to cadaverine, and tyrosine decarboxylase (TyDC) is involved in the biosynthesis of aromatic monoamines. The presented results indicate that induction of galls by Cynipidae on oak leaves exerts an impact on accumulation of polyamines, but the direction and intensity of changes in the content of these compounds depends on the insect species. The analyses did not reveal the presence of putrescine in any of the samples. This polyamine exerts a direct effect on insect growth and development and plays an important role in plant response to oxidative stress. In turn, cadaverine was detected only in the control leaves. The level of spermidine was significantly lower in all galls induced by the examined species and in the leaves with galls induced by representatives of the genus *Neuroterus*, in comparison with the control. The highest i.e. nearly 10-fold decline in the level of this amine was found in the *C. quercusfolii* galls, whereas the leaves with galls induced by this species were characterised by a substantially higher spermidine concentration than the control leaves. Additionally, an increase in the spermine content was detected in the leaves with *C. quercusfolii* galls and in the *N. quercusbaccarum* gall tissues. In the other experimental variants, the content of this polyamine was reduced. In stress conditions, the accumulation of spermidine and spermine in plants is rarely observed, whereas the accumulation of cadaverine can compensate for the decline in the putrescine content. However, only very small amounts of cadaverine were detected in some control samples in the present study. Therefore, it should be assumed that changes in the content of putrescine and cadaverine are less important in the regulation of the mechanisms of formation of galls induced on oaks by species from the family Cynipidae. Probably, this process is more strongly associated with changes in the content of other plant amines.

The changes in the content of tryptamine was similar for all the three Cynipidae species. Both, the leaves with galls and the gall tissues exhibited a significantly lower level of this amine, in comparison with the control. The largest 16.5-fold decrease was noted in *C. quercusfolii* galls. In turn, the level of histamine increased significantly in response to the induction of *N. numismalis* galls; however, higher amounts of this amine were found in the leaves with galls than in the galls. In comparison with the control, higher histamine content was detected in the leaves with *C. quercusfolii* galls, but these

differences were statistically insignificant. The tissues of the galls induced by this species were characterised by a significant reduction in the amount of this compound, whereas the *N. quercusbaccarum* galls did not contain this amine at all. The lower amine content, especially in the galls, may suggest a reduction in their biosynthesis rate and/or an increased degradation rate. It was demonstrated that the trends in the polyamine content changes were partly consistent with the changes in the activity of key enzymes involved in their biosynthesis.

Among the leaf tissues from oaks infested by the analysed gall-inducing species, the activity of lysine decarboxylase (LDC) was found only in the experimental variant including *C. quercusfolii*. The leaves with galls were characterised by a nearly 5-fold lower activity of this enzyme than the control leaves. The lack of cadaverine in the analysed samples may have been associated with the low LDC activity.

A decrease in the ornithine decarboxylase (ODC) activity was detected in the galls and leaves with galls induced by both *C. quercusfolii* and *N. quercusbaccarum*, whereas the presence of the *N. numismalis* galls contributed to an increase in the activity of this enzyme. In turn, the activity of tyrosine decarboxylase (TyDC) in the leaves with galls and in the galls of *C. quercusfolii* was insignificantly higher than in the control samples, whereas the induction of galls by the other two Cynipidae species resulted in a decline in the activity of this enzyme. It was by over 50% lower in the gall tissues of both species relative to the control. ODC is directly involved in the putrescine biosynthesis; however, this diamine was not detected in any of the experimental variants. Therefore it should be assumed that it is quickly converted into other products and/or degraded. A similar situation may have occurred in the case of tyramine, which was not detected despite the TyDC activity. This confirms previous reports that this amine serves as an intermediate metabolite the biosynthesis of secondary metabolites, e.g. amide derivatives emerging through binding with hydroxycinnamic acids (HCAAs) or some groups of alkaloids.

Effect of galls on the photosynthetic activity in oak leaves

Publication:

H4. Kot I., Rubinowska K., Michałek W., 2018. Changes in chlorophyll *a* fluorescence and pigments composition in oak leaves with galls of two cynipid species (Hymenoptera,

Cynipidae). Acta Scientiarum Polonorum, Hortorum Cultus, 17(6), 147–157. doi: 10.24326/asphc.2018.6.15

The investigations of the effect of the presence of galls on plant photosynthetic activity consisted in evaluation of the content of pigments involved in the photosynthesis process and analysis of chlorophyll *a* fluorescence parameters in the oak leaves. The experiment was conducted in leaves with galls induced by agamic generations of *N. numismalis* and *N. quercusbaccarum*. The research demonstrated similar changes in the content of chlorophyll *a* and *b* and the total chlorophyll content in the case of all the Cynipidae species. The average content of these pigments was significantly lower in the leaves with galls in comparison with the control leaves, which may have been caused by their accelerated degradation or disturbance of their biosynthesis. The leaves with *N. numismalis* galls exhibited an exceptionally high decrease in the content of both chlorophyll *a* and *b*, as their amount decreased by more than 60% compared with the control leaves. Furthermore, the analysis of the chlorophyll *a* to chlorophyll *b* ratio indicated that the degradation of chlorophyll *b* caused by the presence of *N. numismalis* galls was significantly higher than in the case of chlorophyll *a*. In higher plants, chlorophyll *b* is a precursor of chlorophyll *a* in the synthesis thereof; hence, it is possible that the presence of galls exerts an effect on the transformation of these pigments

Carotenoids play an important role as auxiliary pigments in the photosynthesis process supporting chlorophyll *a* during absorption of light energy and as substances with antioxidant activity. In the present research, the content of carotenoids was significantly lower in the leaves with galls. The presence of *N. numismalis* galls caused a greater reduction (over 2-fold) in the content of these pigments compared with the mean control value.

The accumulation of anthocyanins, which also act as potent antioxidants, is induced in plants by a number of adverse environmental stresses, including biotic stresses. The presence of the galls of both Cynipidae species on the leaves contributed to an increase in the content of these compounds compared with the amount determined in the control leaves. The leaves with the *N. numismalis* galls exhibited an almost 3-fold increase in the content of these pigments.

Analysis of chlorophyll *a* fluorescence provides rapid and precise information about disturbances in the function of the plant photosynthetic apparatus, thus creating an opportunity to assess its condition. The initial fluorescence (F_0) is an indicator of loss of excitation energy during the transfer thereof from energy antennas to the PSII reaction centre. High F_0 values indicate lower efficiency of excitation energy transfer between pigment molecules in PSII, and this phenomenon is characteristic of thermal and salinity stresses. The maximum fluorescence (F_m) value determined after dark adaptation depends on e.g. the chlorophyll content in the tissue. A decline in the F_m value indicates that the analysed object is under the influence of stress, through which not all electron acceptors in PSII can be completely reduced. The measurements carried out in the leaves with galls induced by both Cynipidae species demonstrated a significant decrease in the value of both measured parameters in comparison with the control. These results confirm the thesis that low F_0 and F_m values reveal the presence of stress caused by arthropod feeding. The decline in the F_0, F_m, and F_v value (variable fluorescence), which is expressed in equation $F_v = F_m - F_0$, leads to reduction of the maximum quantum yield of photosystem II (parameter F_v/F_m). This was confirmed in the present study. The leaves with *N. numismalis* galls exhibited a 34% decline in the value of this parameter, whereas a 20% reduction was detected in leaves with *N. quercubaccarum* galls. Such results suggest that the gall formation process influences the photochemical activity of the photosynthetic apparatus. In optimal plant growth conditions, the F_v/F_m value should be approx. to 0.83. The reduction of the value of this parameter accompanying the presence of oak leaf galls proves the occurrence of stress. The presence of galls induced by both species also caused a significant reduction of the effective quantum yield of the energy conversion process in PSII (parameter Y). The decline in the value of this parameter is associated with increased of excitation energy quenching from the chlorophyll area of the energy antenna to the PSII reaction centre.

Fluorescence quenching parameters, such as photochemical quenching (qP) and non-photochemical quenching (qN), are commonly used for assessment of the photosynthetic efficiency of plants exposed to insect feeding. qN is a substantially more sensitive indicator of the photosynthetic apparatus response to stress than qP. The present study demonstrated that the presence of galls induced by both Cynipidae species on the oak leaves contributed to the reduction of the qP value and to the increase in the qN parameter value in comparison with the control. The decline in the qP

value was approximately 30% in both species, whereas the increase in the qN value in the leaves with *N. numismalis* galls was higher than in the presence of *N. quercusbaccarum* galls.

Summing up the obtained results, it can be concluded that the presence of galls induced by the agamic generations of *N. numismalis* and *N. quercusbaccarum* on *Q. robur* trees exert an effect on the content of photosynthetic pigments in the leaves and on the value of chlorophyll *a* fluorescence parameters. The leaves with galls induced by both Cynipidae species were characterised by significantly lower chlorophyll *a* and *b* content and carotenoid levels. Furthermore, a significant decrease in the values of chlorophyll *a* fluorescence parameters was noted (F_0, F_m, F_v/F_m, Y, qP, qN). These results indicate a significant impact of the development of *N. numismalis* and *N. quercusbaccarum* galls on the physiological, biochemical, morphological, and anatomical parameters of the oak leaves. The decrease in the values of parameters F_v/F_m, Y, qP, and qN shows that the photosynthetic reaction centres, in particular PSII, may have been seriously damaged, which directly affects the intensity of the photosynthesis process. The obtained results suggest considerable suitability of chlorophyll fluorescence measurements in investigation of plants growing in natural habitats. The photosynthetic apparatus, and especially the PSII structure, are regarded to be remarkably sensitive to various types of stress, including stress caused by gall inducing species. Therefore, measurements of chlorophyll fluorescence parameters can reveal whether the plant is exposed to stress conditions before other symptoms emerge.

The major findings of the presented research include:

1. Verification and updating of fragmentary data on the species composition and intensity of the occurrence of gall-inducing species from the family Cynipidae in the Lubelszczyzna region. The galls observed in this study were induced by 11 Hymenoptera species, among 52 noted in Poland. They induced galls on leaves (8 species) as well as on lateral and apical buds (3 species).
2. Demonstration of the presence of *Andricus inflator* (Hartig) (♀♂), a species that had not been previously recorded in Lubelszczyzna.
3. Demonstration that the gall induction triggered oxidative stress in oak leaves, which was manifested by an increase in the level of hydrogen peroxide (H_2O_2) and guaiacol peroxidase (GPX) activity, as well as increased lipid metabolism

4. Demonstration the ambiguous role of H_2O_2 in gall cells, which may be associated with biotic stress, as well as with involvement in the process of morphogenesis, and consequently in the formation of galls.
5. Demonstration that gall formation leads to disintegration of cell membranes, and enhanced electrolyte leakage from cells exposed to biotic stress.
6. Confirmation that gall inducing species control the supply of nutrients (proteins) and the synthesis of defense compounds (phenols, polyamines, antioxidant enzymes) in the host plants.
7. Demonstration that the presence of galls induced by Cynipidae results in accelerated degradation or disturbances in the biosynthesis of chlorophyll *a* and *b* as well as carotenoids.
8. Confirmation that the presence of galls triggers oxidative stress reflected in the accumulation of anthocyanins and changes in the chlorophyll *a* fluorescence parameters.

The series of publications submitted as my *achievement* is a compilation of issues related to the physiological and biochemical interactions between gall-inducing Hymenoptera insects and oaks as their host plants. The mechanisms underlying the induction and development of galls are largely unresolved to date. The results provide new data and extend the existing knowledge of the mechanisms triggered in plants by gall-inducing insects. They can also facilitate inference about plant-influencing stimuli and processes involved in gall induction. Simultaneously, the results generate new questions, which can be the basis for further research on the molecular background of the manipulation of gall inducers with the host plant.

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4. DESCRIPTION OF OTHER SCIENTIFIC AND RESEARCH ACHIEVEMENTS

4.1. PROFESSIONAL AND SCIENTIFIC DEVELOPMENT BEFORE OBTAINING THE PHD DEGREE IN AGRICULTURAL SCIENCES

I graduated in 1998 at the Faculty of Zootechnology, Agricultural Academy in Lublin (at present: the Faculty of Biology, Animal Sciences and Bioeconomy, University of Life Sciences in Lublin). I completed my Master thesis in environmental protection at the Institute of Soil Science and Environment Shaping under the supervision from prof. dr hab. Halina Smal. In the last year of study (1997/98), I graduated from the Interfaculty Pedagogy College of Agricultural Academy in Lublin with qualifications for the teacher's profession. I started my scientific work in October 1998 as a doctoral student at the Department of Entomology, Agricultural Academy (at present: University of Life Sciences) in Lublin. During my doctoral studies, I was involved in one of the research topics investigated in the Department in the field of plant pests, with particular emphasis on pests of horticultural plants. As part of this activity, I carried out observations focused on the emergence and assessment of the harmfulness of selected pests in apple orchards in the Lubelszczyzna region. To identify the actual threats posed by phytophages, I conducted monitoring based on various methods. The investigations resulted in the identification of the dynamics of flight of *Archips rosana* L., i.e. a species occurring massively in the orchard cultivations in the Lublin Province [App. 3, publ. II.D.12]. The intensity of planting, cultivation system, and plant protection methods can affect the phenology and development of harmful arthropods, including moths from the family Tortricidae. The description of the population dynamics and accurate prediction of the appearance of the individual developmental stages helps orchard keepers to understand and control local populations of these insects. Additionally, I monitored the overwintering stages of pests in apple orchards, as the knowledge of the average number of the overwintering stages of individual species and the critical numbers for the harmfulness thresholds can be used for prediction of the degree of probable threat to plants [App. 3, publ. II.D.11].

Until 2001, I cooperated with prof. dr hab. Halina Smal. This experience offered me an opportunity to continue my Master thesis research in the field of estimation of the

load of selected heavy metals in agricultural soils in Poland in individual provinces in the 1955-1995 [App. 3, publ. II.D.10, II.D.27].

Simultaneously, in 1999, I started field research on the occurrence and harmful effects of phytophages in apple orchards, with particular focus on Lepidoptera from the family Tortricidae. The investigations were included in the doctoral dissertation "Occurrence of leafroller (Lepidoptera, Tortricidae) in apple orchards at varying intensity of plant protection treatments" supervised by dr hab. Władysław Huszcza. The main research aim was to determine the species composition and abundance of leafroller moths in apple orchards subjected to varied intensity of plant protection treatments, identify the flight dynamics in selected Tortricidae species, determine the species composition of moths parasitoids, and determine the effect of the plant cultivation and protection system on the abundance and harmfulness of the codling moth (*Cydia pomonella* L.). On June 3, 2003, I defended my doctoral thesis and was granted the doctoral degree in horticulture - plant protection, entomology by the Council of the Horticultural Department of the Agricultural Academy in Lublin on June 13.

The results included in the doctoral dissertation were also published in 8 original research papers [App. 3, publ. II.D.1, II.D.2, II.D.4, II.D.5, II.D.6, II.D.7, II.D.8, II.D.9] and presented at an international conference in Minsk in 2006 (International Scientific Conference „Strategy and Tactics of Plant Protection", Minsk, Belarus, 28 February-2 March 2006).

4.2. PROFESSIONAL AND SCIENTIFIC DEVELOPMENT AFTER OBTAINING THE PHD DEGREE IN AGRICULTURAL SCIENCES

After graduation from the doctoral studies, between August and December 2003, I completed an occupational internship at the Department of Entomology, Agricultural Academy in Lublin, which was financed by the Municipal Labour Office in Lublin. From November 2004 to February 2005, I was on contract agreement at the Agricultural Academy in Lublin to realise classes at the Department of Entomology.

I started my career as an assistant at the Department of Entomology on April 1, 2005 and a year later (on April 1, 2006) I was promoted for the position of an assistant

professor. My research interests in the postdoctoral period are focused on four main thematic issues:

1. Occurrence, biology, and harmfulness of phytophagous insects in horticultural crops;
2. Pest monitoring as an element of effective crop protection;
3. Potential application of non-chemical methods of pest control;
4. Investigations of insect-host plant interactions.

Occurrence, biology, and harmfulness of phytophagous insects in horticultural crops

The research in this area was mainly focused on aphids (Hemiptera, Sternorrhyncha, Aphidoidea), scale insects (Hemiptera, Sternorrhyncha, Coccoidea), and leafrollers (Lepidoptera, Tortricidae). I carried out observations of the occurrence of aphids in an urbanised environment. The species composition, population size, development rate, number of generations, and insect migrations in cities are influenced by the abundance of the food base and various aspects of anthropopressure. Moreover, the city climate is characterised by higher temperature in relation to the surrounding areas. In such conditions, the number of zoophages, saprophages, and phytophages with a biting mouth parts decreases, whereas the number of herbivores with piercing-sucking mouth parts is on the rise. Therefore, the aim of the research was to analyse the population of aphids infesting ornamental shrubs relative to weather conditions. In collaboration with Prof. dr hab. Bożenna Jaśkiewicz, the observations were carried out on *Cinara juniper* De Geer, i.e. a species feeding on juniper bushes (*Juniperus communis* L.) [App. 3, publ. II.D.3] and *Aphis pomi* De Geer inhabiting *Cotoneaster divaricatus* Rehd. Et Wils. [App. 3, publ. II.D.21]. The results of the research conducted in street and park sites showed greater abundance of the colonies of these species on plants growing in the street localities. Additionally, the dynamics of the population of these insects was influenced by the weather conditions. A delayed vegetation period, temperatures above 30°C, and storm type precipitation significantly limited the number of aphids. There was also a clear effect of aphid feeding on the decorative values of the shrubs.

Together with dr Katarzyna Kmieć, I conducted study of the occurrence of gall-inducing aphids from the subfamily Eriosomatinae on field elm (*Ulmus minor* Mill.) and Scots elm (*Ulmus glabra* Huds) trees [App. 3, publ. II.D.17, II.D.28]. The observations demonstrated very high abundance of two species: *Tetraneura ulmi* L. and *Schizoneura*

ulmi L., which was a contribution to the development of the bionomics of one of these species, i.e. *Tetraneura ulmi* (L.), and to assessment of the effects of the species feeding on the field elm as a primary host. The results of these studies were published as an original research in a chapter of the monograph "Aphids and Other Hemipterous Insects" [App. 3, publ. II.D.22]. It presents the results of investigations of the length of development of individual generations, the fertility of fundatrix, the effect of the gall size on aphid population, and the degree of gall occurrence on leaves. Some of the results were presented at the 20th National Hemipterological Conference "Aphids and other Hemipterous Insects" in Janów Lubelski [App. 4, publ. I.B].

The result of the cooperation established with the staff from the Department of Vegetable Crop Cultivation, University of Life Sciences in Lublin, was the analysis and publication of the species composition and number of insects inhabiting the garden rhubarb (*Rheum rhaponticum* L.) [App. 3, publ. II.D.13]. Harmful insects were represented by species from the orders Hemiptera, Coleoptera, and Diptera; nevertheless, *Aphis fabae* (Scop.) was the dominant species in each year of the study. The 'Wczesny Hosera' cultivar turned out to be most susceptible to the feeding of the harmful phytophages. The results of these studies were presented at the 46th Institute of Plant Protection Scientific Session in Poznań [App. 3, publ. II.D.30].

My important research accomplishments include the presentation, together with dr Katarzyna Kmieć, of the biology of the woolly beech aphid (*Phyllaphis fagi* L.) (Hemiptera, Sternorrhyncha, Aphidoidea), which is abundant and frequent on beech trees in artificial and natural plantings [App. 3, publ. II.A.1]. Additionally, this species is a dangerous pest in forest beech nurseries in Europe. It feeds in large colonies on the lower side of leaves, causing curling along the midrib and, consequently, leaf shedding. The description of the bionomics of the woolly beech aphid has not only a scientific and theoretical value but also a practical character. The effectiveness of pest control largely depends on the developmental stage and date of pest emergence. Hence, the knowledge of the species biology is a fundamental element of short-term signalling and prediction.

The collaborative research with dr Małgorzata Janiuk and dr hab. Magdalena Gantner focused on the occurrence of leafrollers on highbush blueberry (*Vaccinium corymbosum* L.) was prompted by the intensive increase in the cultivation area of this new fruit plant during the study years (2003-2006) in Poland. Concurrently, no research centre conducted investigations on the entomofauna of this plant in the conditions in

Poland. Preliminary research carried out on blueberry plantations located near Lublin showed a high number of leafroller caterpillars, which posed a potential threat to the blueberry shrubs. Therefore, determination of the size and dynamics of the populations of these insects in the individual study years was advisable [App. 3, publ. II.D.19].

A very important element of my scientific activity is the cooperation with Prof. dr hab. Bożena Łagowska, in which I was involved to describe the phenology of *Pulvinaria floccifera* Westwood (Hemiptera, Coccomorpha, Coccidae). In Poland, this is an invasive species, alien to the fauna inhabiting ornamental plants. Until recently, this polyphagous scale insect was only found in greenhouses in our country, but recently it has also been observed in home gardens. This implies the possibility of survival of this species during the winter in our climatic conditions. Hence, it was advisable to analyse the biology of *P. floccifera*, which is the basis for effective methods of the species control. The investigations demonstrated the terms of emergence of subsequent developmental stages on host plants in natural conditions and the overwintering stages of this pest species in our climatic conditions. The results of our research indicate that the first treatments aimed at limitation of the number of the insects should be applied at the beginning of July [App. 3, publ. II.A.12].

The same research team developed an idea of compilation of a full list of scale insect species, which are alien to the fauna of Poland but colonise and pose a threat to native plants. This list comprises 51 species dominated by representatives of the family Diaspididae. The list contains data on the first report of the species in Poland together with literature references, species origin, category and time of introduction, environment, major host plants in Poland, invasiveness status, and degree of threat to native plants [App. 3, publ. II.A.8].

Pest monitoring as an element of effective crop protection

Monitoring, i.e. systematic observation of the occurrence of harmful phytophages, is the basis for understanding the threats that they pose to plants and the key for making a decision concerning application of plant protection treatments. An effective method for monitoring and prognosis of the occurrence of harmful arthropods is winter inspection of plants. As part of the cooperation with dr hab. Katarzyna Golan from the Department of Entomology, University of Life Sciences in Lublin, I undertook the assessment of the intensity of occurrence of the overwintering stages of arthropods on

ornamental trees and shrubs in the urban conditions of Lublin [App. 3, publ. II.D.15]. The observations were carried out on five species from the family Rosaceae (*Rosa rugosa* Thunb., *Prunus domestica* subsp. syriaca Janch., *Crataegus laevigata* (Poir.), *Sorbus aucuparia* L., *Malus* x *purpurea* Rehder). They indicated that the analysis of the number of the overwintering stages of phytophages may be highly important in cities, where the use of typical measures to limit their abundance is impossible due to the immediate proximity of humans. The results of these studies were presented at the 49th Institute of Plant Protection Scientific Session in Poznań [App. 3, publ. II.D.32].

To acquire information about the populations of the peach twig borer (*Anarsia lineatella* Zeller) (Lepidoptera, Gelechiidae) and the currant clearwing (*Synanthedon tipuliformis* Cl.) (Lepidoptera, Sesiidae) in orchard crops near Lublin, the method of imago trapping in Delta type pheromone traps was used. The peach twig borer is one of the most dangerous pests of peaches, whose cultivation area and interest in production in Poland are constantly growing. It was found that the population size of this species depends mainly on the presence of host plants, i.e. peaches, plums, and blackthorn. In turn, the currant clearwing is one of the most dangerous pests of currants and gooseberries and occurs in both commercial and amateur plantations as well as plant nurseries. The research results indicate that only two generations of the peach twig borer occur in our climatic conditions, while this pest in Turkey and Italy produces from 3 to 4 generations. Additionally, the abundance of the first generation in the peach and apricot orchards near Lublin was over two-fold higher than that of the second generation [App. 3, publ. II.D.18]. In turn, the flight of the current clearwing was long and lasted from the end of May to the beginning of August. The trapping of the moths in the pheromone traps and the analysis of shoots damaged by larvae demonstrated that the economic threshold of the harmfulness of this species was exceeded. Therefore, it is essential to control this pest in both commercial and amateur plantations [App. 3, publ. II.D.16]. The results of these studies were presented at the 49th and 50th Institute of Plant Protection-National Research Institute Scientific Sessions in Poznań [App. 3, publ. II.D.33, II.D.34].

Potential application of non-chemical methods of pest control

A highly valuable result of the cooperation established with Prof. dr hab. Jerzy Księżak and dr Zbigniew Biały (Institute for Soil Science and Plant Cultivation - National

Research Institute in Puławy) was the research on the potential application of alfalfa (*Medicago* ssp.) saponins as a biological preparation for *Aphis fabae* Scop. control in faba bean. The investigations demonstrated that the use of 1 and 1.5% of total saponins from the aboveground parts and roots from three alfalfa species only slightly limited the occurrence of aphids on the faba bean plants. The average effectiveness of the treatment at day 7 after the application of saponins reached approximately 16%, but was substantially higher, i.e. approximately 75% after 14 days. The total saponins from the roots exhibited slightly higher efficiency than those from the above-ground parts [App. 3, publ. II.D.14]. The results of this study, which have a high application value, were presented at the 48th Institute of Plant Protection Scientific Session in Poznań [App. 3, publ. II.D.31].

An important factor limiting the number of plant pests are parasitoid insects. I carried out quantitative and qualitative analyses of a complex of leaf miner parasitoids together with other employees of the Department of Entomology, University of Life Sciences in Lublin, with dr hab. Edyta Górska-Drabik as the main coordinator. The study was focused on identification of the species composition of the parasitoids and determination of the degree of their parasitism in moth's larvae. The study involved larvae from the overwintering generation of *Swammerdamia pyrella* (Villers) (Lepidoptera, Yponomeutidae) feeding on apple trees [App. 3, publ. II.A.3] and two species from the genus *Phyllonorycter* (Hbn.) associated trophically with hazel bushes [App. 3, publ. II.A.10]. The Renkonen DR coefficient was used to analyse the similarity of the structure of the *Phyllonorycter coryli* and *Ph. nicellii* parasitoid complex. The parasitoid complex of these species comprised only parasitic Hymenoptera from the families Ichneumonidae, Braconidae, Eulophidae, Pteromalidae, and Eupelmidae. These were I and II degree parasitoids, endo- and ectoparasitoids, as well as solitary and gregarious parasitoids. An important finding in these investigations was the information that as many as 7 out of the 11 parasitoid species reared from *Ph. corylli* had not been previously reported from this host. In turn, 13 species were reared from the *Ph. nicelli* larvae and pupae, with 8 found for the first time in this host. A summary of the results from this research series was presented in two publications in journals from Journal Citation Reports (JCR): Journal of Insect Sciences and Turkish Journal of Zoology.

Investigations of insect-host plant interactions

Since 2011, I have been involved in a joint project implemented by a research team from the Department of Entomology, University of Life Sciences in Lublin, which is focused on analysis of the insect-host plant interaction. As part of these investigations, our team determined the biochemical response of *Aronia melanocarpa*, *Crataegus* sp., and *Sorbus aucuparia* plants to infestations by *Acrobasis advenella* (Lepidoptera, Pyralidae). The study results indicate that secondary metabolites, especially tannins, are synthesised by plants for protection against phytophagous attacks [App. 3, publ. II.A.2]. Additionally, together with the research staff from the Department of Plant Physiology, University of Life Sciences in Lublin, we analysed changes in the content of photosynthetic pigments and photosynthetic activity induced by *Coccus hesperidum* L. (Hemiptera, Coccoidea, Coccidae) infestation of *Citrus limon* var. Ponderosa and *Nephrolepis biserrata* (Swartz) Schott [App. 3, publ. II.A.6]. The investigations involved plants with varying degrees of soft scale attack, and changes in the content of chlorophyll *a* and *b* and carotenoids as well as changes in chlorophyll *a* fluorescence parameters were analysed. These studies are innovative and not only theoretical but also practical, as they provide important information suitable for development of strategies for crop pest control.

Another very important aspect in this research field was the determination of the impact of feeding of two species of scale insects *Pseudoccocus longispinus* (Targioni Tozzetti) and *P. maritimus* (Ehrhorn) on the physiological and biochemical status of host plants, as illustrated in *Phalaenopsis* × hybridum 'Innocence' orchids. As part of the physiological analyses, we assessed the impact of the insects on the stability of cytoplasmic membranes, the content of hydrogen peroxide (H_2O_2), the activity of the antioxidant system (catalase, guaiacol peroxidase, proline), and the photosynthetic activity of host plants [App. 3, publ. II.A.4, II.A.7, II.A.9]. The biochemical investigations conducted together with Prof. dr hab. Cezary Sempruch from the Department of Biochemistry and Molecular Biology, Siedlce University of Natural Sciences and Humanities, were focused on the analysis of the activity of plant enzymes involved in the decarboxylation of nitrogen compounds. These include ornithine decarboxylase (ODC), lysine decarboxylase (LDC), and tyrosine decarboxylase (TyDC) [App. 3, publ. II.A.5]. Additionally, we studied changes in the content of amino acids and phenolic compounds as well as the activity of L-phenylalanine ammonia lyase (PAL) and tyrosine ammonia

lyase (TAL), i.e. enzymes involved in the synthesis of polyphenols and their metabolism [App. 3, publ. II.A.11]. The results of the research on the physiological and biochemical changes induced in plants by insect feeding are highly valuable. They are in line with the research trend focused on elucidation of the mechanisms of natural resistance of plants against phytophagous infestations. This is important for integrated pest control of crop plants based on cultivation and introduction of resistant varieties. The results of these studies were disseminated in a series of 5 original research papers published in scientific journals indexed in the Journal Citation Reports (JCR) database (I was the first author of one of these papers [App. 3, publ. II.A.7]) and presented at the National Scientific Conference "Aphids and other Hemipterous Insects" in Białowieża [App. 3, publ. II.D.45] and 5 international conferences (with 3 abroad: Bulgaria, Italy, Greece) [App. 3, publ. II.D.35, II.D.40, II.D.41, II.D.43, II.D.47, II. D.48].

A highly important achievement is my cooperation with dr Rosario Nicoletti and Prof. Gennaro Viggiani from the University of Naples ('Federico II', Italy) and with dr hab. Beata Zimowska (Department of Phytopathology and Mycology, University of Life Sciences in Lublin), which I started in 2015. As part of the project "Fungal inquilines of *Asphondylia* galls", we investigated mutualistic interactions between gall-inducing species from the genus *Asphondylia* (Diptera, Cecidomyiidae) and fungi developing on the inner gall walls. It was found that the galls were colonised by a fungal complex, mainly *Cladosporium* spp., *Alternaria* spp., and *Botryosphaeria dothidea* (Moug.) Ces. & De Not. Particularly interesting was the presence of *B. dothidea*, as it is a dangerous pathogen of many woody plants. Therefore, it is important to assess the potential role of *Asphondylia* flies as a vector of this fungus. Additionally, the presence of *Asphondylia serpylli* Kieffer was reported for the first time from *Thymus vulgaris* L., and its life cycle and parasitoid complex were described. The result of the cooperation is my co-authorship in a paper published by Annals of Applied Biology [App. 3, publ. II.A.13.].

5. SUMMARY OF SCIENTIFIC, DIDACTIC AND SCIENCE-PROMOTING ACHIEVEMENTS

My total research accomplishments, including the series of thematically related papers submitted as the *achievement*, include 70 different studies. I am an author or co-author of:

- 17 original research papers published in journals from the Journal Citation Reports (JCR) list,

- 9 original research papers in English published in journals other than those specified in the Journal Citation Reports list,
- 11 original research papers in Polish published in journals other than those specified in the Journal Citation Reports list,
- 2 chapters in scientific monographs in English,
- 1 chapter in an academic handbook,
- 2 published research reports,
- 23 summaries and abstracts,
- 3 popular science articles,
- 1 collective elaboration.

All detailed information on the list of published scientific papers and a summary of published papers are provided in Appendix No. 3.

- The total MNiSW score for all published papers in accordance with the year of publication is 538 points, including 107 points for publications documenting the *scientific achievement*.
- The total *impact factor* (IF) in the year of publication of all papers is 19.165, including IF of 4.677 for publications documenting the *scientific achievement.*
- The number of publication citations according to the Web of Science (WoS) database is in total: 41; without self-citations: 24.
- The Hirsch Index in accordance with the Web of Science (WoS) database is: 4.

I presented the results of my research at 18 scientific conferences and symposia, including 7 international conferences (5 abroad) and 11 national conferences. At the conferences, I was the co-author of 5 lectures, 2 of which I referred in person, and presented 24 posters.

Since the beginning of my work, I have been delivering lectures and conducting classes in 20 courses [App. 4, publ. I.I.1]. I am an author of class modules in 6 subjects [App. 4, publ. I.I.2]. As part of the European Union Erasmus+ program, I taught English classes in four subjects for students from Turkey, Spain, and Italy [App. 4, publ. I.A]. In 2010, together with other staff of the Department of Entomology, I developed and published a handbook for *entomology* classes for full-time and extramural students of horticulture. In this handbook, I am an author or co-author of 4 chapters on nematodes (Nematoda), molluscs (Molusca), mites (Acari), and hemiptera (Hemiptera) [App. 3,

publ. II.E.1]. My scientific output is also a chapter on the characterisation and methods for diagnosis of edible mushrooms pests ("Pests of edible mushrooms"), which is part of the academic textbook "Industrial Mycology" edited by Prof. dr hab. Janusz Kalbarczyk [App. 3 publ. II.D.23].

In 2006-2009 and 2018-2019, I was involved in scientific supervision over students of the Faculty of Horticulture and Landscape Architecture. In turn, in 2008-2009, I was the guardian of the Horticultural Group of the Lublin University of the Third Age. Until now, I have been a promoter of 14 Master diploma theses and 21 Engineer diploma theses. In addition, I have reviewed 16 Master diploma and 13 Engineer diploma theses. Currently, I am the scientific supervisor of a PhD student, Magdalena Kryska, who conducts her doctoral thesis at the Department of Biochemistry and Molecular Biology at the Siedlce University of Natural Sciences and Humanities.

I have twice been a member of the Examination Commission in professional practice at the Faculty of Horticulture and Landscape Architecture, University of Life Sciences in Lublin.

Since 2008, I have been a member of the Polish Entomological Society. In 2013-2016, I was a secretary of the Lublin Branch of the Polish Entomological Society; since 2016, I have been its president.

In 2018, I was elected a member of the Council of the Faculty of Horticulture and Landscape Architecture, University of Life Sciences in Lublin and I have been holding this function until now.

In 2008-2018, I took part (as a project manager or co-organiser) in the implementation of 10 projects as part of consecutive editions of the Lublin Science Festival. In 2014 and 2015, I was the coordinator from the Faculty of Horticulture and Landscape Architecture at the 11th and 12th Lublin Science Festival. In turn, in 2016, as a coordinator from the University of Life Sciences in Lublin at the 13th Lublin Festival of Science, I became a member of the Organising Committee of the festival. For this activity, I was granted Rector's 3rd degree individual award for organisational achievements. Additionally, in 2018, I was a co-organiser of popular science workshops carried out as part of the Open Days of the University of Life Sciences in Lublin

I have given interviews in radio programs and television programs, as well as in local press on several occasions. The interviews addressed current problems related to the occurrence of various insect species [App. 4 publ. I.I].

In 2015, I established cooperation with the Nature Department of the Vistula River Museum in Kazimierz Dolny. I was asked to give a lecture during the Night of Museums on "Night life of insects". Additionally, I took part in substantive preparation of the exhibition "Insect – friend or foe?" and delivered a speech with the same title at the opening of the exhibition. I also prepared a publication on this exhibition on request.

Since 2016 I have been a member of the Commission for Organisation and Development of the Faculty of Horticulture and Landscape Architecture, a member of the Team for Promotion of the Faculty of Horticulture and Landscape Architecture, and a member of the Expert Team of the University of Life Sciences in Lublin (expertise domain: entomology, plant protection).

I am the co-author of an expert opinion on the assessment of pest infestation in the production line for sunflower dehulling and the possibility of their spread and contamination of stored food. The opinion was commissioned by the District Court in Poznań.

Particularly important to me is my collaboration with Universal Leaf Tobacco Poland sp. z o.o. in Jędrzejów, for which I carried out a comprehensive development of methods for monitoring aphids and cutworms in tobacco cultivation in 2007. Since 2007, I have been the leader of the project on the analysis of occurrence, harmful effects, and reduction of the number of tobacco thrips (*Thrips tabaci* Lind.) on tobacco plantations in Poland coordinated by this company. In this project, I am responsible for development of methods for monitoring tobacco thrips in selected plantations in Poland, determination of the thrips population dynamics throughout the season based on trapping insects on blue tick boards, and analysis of results to determine the control dates.

Since 2005, I have been cooperating with the Fruit and Vegetable Producer Group in Dziuchów near Lublin. The cooperation is aimed at advisory activities offered to producers in the field of plant protection, based on my research. The result of the cooperation is the authorship of the application of the product "Optimization of monitoring and terms for the control of apple pests, with regard to the habitat conditions of plantations".

I have reviewed publication in journals from the JCR list (Acta Scientiarum Polonorum, Series Hortorum Cultus) and others (Acta Entomologica Serbica, Annales UMCS Sec. Agricultura, Herba Polonica).

Thanks to the cooperation with Professor Rimantas Rakauskas, I had a month-long scientific internship at the Department of Zoology at the University of Vilnius in 2016. During my internship, I carried out research in the molecular taxonomy and phylogenetic analysis of scale insects and aphids (Hemiptera, Sternorrhyncha) occurring in Poland and Lithuania. In addition, I had two internships in national research centres, and I participated in several trainings to upgrade my professional qualifications [App. 4, publ. I.L].

I have applied three times for external sources for funding scientific research, including twice attempts to finance the research presented as the *scientific achievement*. The failure of external financing prompted me to establish cooperation and joint research with scientists from other centres. I analysed and published the results obtained in the form of a series of original scientific papers [App. 3, publ. I.B.H.1-H.5], which I consider to be my most important achievement in my current scientific activity. I submit the series as a basis in the application for the habilitated doctor degree.

Izabela Kot