REPORT ON RESEARCH VISIT

Grant body	British Scholarship Trust
Subject	Two months research visit
Grantee	Jovana Raičević
Home Institution	University of Belgrade – Faculty of Biology, Belgrade, Serbia
Host Institution	Rothamsted Research, Harpenden, United Kingdom
Visit period	14th February – 17th April 2022.

First impressions

Due to the Covid-19 pandemic, there were logistical and technical issues to complete the visit to the Rothamsted Research in the 2020/2021 academic year, as initially planned. However, I owe great gratitude to the British Scholarship Trust for agreeing to postpone my research visit for the next academic year and to provide me with an extraordinary opportunity to gain knowledge from experienced Dr Sam Cook and her team from the Biointeractions and Crop Protection Department in Rothamsted Research. Likewise, Dr Cook and my mentor Dr Milan Plećaš, have agreed to organize my research activities in the manner to maximize the benefit for my future work in these changed circumstances.

There were a lot of administrative matters to complete before, as well as upon the visit, including all the forms and safety protocols for Rothamsted Research, and accommodation paperwork for Lawes Agricultural Trust. Everything went well and smoothly thanks to many kind people from Rothamsted Research and Lawes Agricultural Trust, especially Dr Sam Cook, Dr Gaetan Seimandi-Corda and Julie Merriman. Upon my arrival at Luton Airport Dr Cook's team members organized my transport to Harpenden and the Rothamsted Research campus, helped me with my accommodation and showed me the main offices and laboratory in the BCP department where I spent the next two months.

Work activities

All the activities that I was involved in were divided into two main parts - laboratory and field work. We discussed the dynamics of all forthcoming work activities in advance, as well as during the first week of my visit. Field work was reserved for one day each week until the end of the visit to maintain sampling dynamics. During the last week of March, we have added another day of field work according to the new project we have started with. Laboratory work included sorting out different kinds of samples, using identification keys, creating databases, and analyzing the obtained data. Overall, I have experienced new sampling techniques as well as sample processing, including dissection of pollen beetle larvae and work with the stereomicroscope.

Project 1 – Phenology of the cabbage steam flea beetle in oilseed rape field with different margin types

The cabbage stem flea beetle, *Psylliodes chrysocephala*, is one of the most important pests in oilseed rape (*Brassica napus*) across the Europe. Adults can cause complete crop loss via feeding on young leaf tissue while the larvae mine plant tissue, causing significant damage resulting in yield loss. Regarding this, it is important to better understand the phenology of CSFB larvae and adults through season to create the most efficient control strategies. To successfully control the CSFB population without excess pesticide use it is of great importance to understand the abundance and phenology of their natural enemies (e.g., predators and parasitoids) and to support them. The aims of study are to test the effects of different types of field margins on abundance of CSFB adult and larvae and to determine the seasonal peaks of CSFB larvae falling out of plants, ready for pupation.

The project started one week before my arrival, lasted for the next 9 weeks of my visit, and continued after my departure from the UK. The sampling of CSFB adults and larvae was done using standard entomological methods in the oilseed rape field that has four different boundaries – other arable crop, flower, hedge and woody margins. Next to each margin within oilseed rape crop, there was 30 m long transect with 10 sampling points where the traps were placed. Selected oilseed rape field was located within wider area of the Rothamsted Research campus. We used standard tray traps covered with chicken-wire for collecting falling CSFB larvae. Samples were kept in pots with 70% ethanol until processing.

Total number of the samples I have processed from the tray traps was 320. During processing material from tray traps, individuals belonging to different insect groups were sorted, identified, and counted. CSFB larvae were separated from the rest of the sample and their growth stage was determined. Colleagues from the BCP department shared their knowledge with me and provided me with a key for identification of CSFB and determining their growth stage. Preliminary results, including only the period from mid-February until mid-April, show that adults of CSFB are more abundant near the crop and flower margins, but the most CSFB larvae ready for pupation were detected near flower and hedge margins. Total number of larvae shows small variations during the sampling period. These results are not sufficient for final conclusions and will be completed at the end of the season.

Project 2 – Detection of parasitoids in pollen beetle larvae through dissection

The pollen beetle, *Brassicogethes aeneus*, is another important pest of winter oilseed rape during spring in Europe. Adults feed on the pollen and ovipositing eggs within flower buds, where larvae develop and feed on flower tissue until pupation. Both adults and larvae create damage that leads to yield reduction of oilseed rape crop. To successfully control pollen beetles, and reduce pesticide use, more attention should be paid to their natural enemies (e.g., predators and parasitoids) with developing strategies to support and promote natural enemies' abundance. According to literature, the most common parasitoids of pollen beetles are *Tersilochus heterocerus*, *Phradis interstitialis* and *Phradis morionellus*, while *Diospilus capito* is less often. These larval parasitoids can substantially reduce the population density of the pollen beetle. The aims of study are to determine parasitism rate of pollen beetle larvae through dissection and to

precisely identify the parasitoids species through DNA metabarcoding, as well as to compare detectability of parasitoids between dissection and DNA metabarcoding.

This project started in March, lasted until the end of my visit, and continued after my departure. Total number of larvae I have dissected was 400: 220 larvae from previous samples and 180 larvae from new samples after the start of the project. The previous samples are from the field season of 2019, collected from the funnel traps and stored in the ethanol. Pollen beetle larvae from these samples are not appropriate for DNA metabarcoding and I have used them to practice the dissection technique. Colleagues from the BCP department trained me how to dissect the host larvae and how to detect parasitoid eggs and larvae inside them, and prepared useful protocol, key and literature for me to use during the project. After I have become more experienced with the technique, I have started with the new samples from the field season of 2021. These pollen beetle larvae were collected directly from the flowers, transferred to the tubes, and stored in the freezer.

Preliminary results show that 40 of 180 pollen beetle larvae from new samples were parasitized. The main parasitoids that have been found were *Tersilochus* sp. and *Phradis* sp., but further identification to the species level will be possible with DNA metabarcoding. Predominantly I found eggs of *Tersilochus* sp. (probably *Tersilochus heterocerus*), some of which were cracked exposing half-hatched larva. *Tersilochus* sp. eggs are easy to spot because of their ellipsoidal shape and dark brown colour. Less abundant were fully developed larvae. Larvae found without co-presence of brown eggs are probably *Phradis* sp. This project will be completed in the next few months (including DNA metabarcoding) and the final results will be obtained.

During the last week of my visit, I had the opportunity to use the Leica fluorescence stereo microscope M205 FA to create detailed images of parasitoid larvae for this project. Colleagues from Bioimaging provided me with the short training for use of the Leica M205 FA microscope and its software.

Project 3 – Testing insecticide resistance in ground beetles

There is an ever growing need to increase crop production to satisfy demand for food and fiber, which leads to intensification of agriculture and negative pressure on the environment. The consequences include loss of ecosystem services and increased pest pressure, which leads to increased use of pesticides. Insecticides targeting pests often have a high impact on beneficiary insects that provide valuable ecosystem services such as pollination and biological control. For biological control of great importance are predators and parasitoids, and one of the main predators in oilseed rape fields are beetles in the family Carabidae. The aim of the study is to test resistance of various ground beetle species on different concentration of insecticides used to control pests in oilseed rape fields.

The project started at the end of March and continued after my departure. We have actively collected beetles using electric aspirators in the oilseed rape field. Expert colleagues have identified ground beetles to the species level. Collected beetles were kept in good condition in the laboratory before insecticide exposure. The experiment was conducted using vials sprayed with different concentrations of insecticide, including vials with 0,8 %, 4 %, 20 %, 100 % (field dose)

and 200 % dose of insecticide, as well as a control tube sprayed with acetone. Depending on the collected number of individuals of each species, we were running one or more sets of doses. The vials were kept with enough airflow to sustain beetles. Each individual was observed after 5h and 24h of exposure and its condition was recorded as mobile, affected or dead. Preliminary results show that most individuals of various species were affected or dead after 24h in the 100% (field dose) and 200% (double field dose), as expected. The final results will be obtained at the end of the field season.

Another related project

During the spare time between three main projects I helped the colleagues in the BCP department with another ongoing related project. The main focus of the project was phenology and activity of ground beetles (adults and larvae) as well as other predators in oilseed rape fields. We have placed the regular and subterranean pitfall traps near each margin type (other arable crop, flower, hedge and woody) within the oilseed rape field to collect ground beetles and spiders. The aims are to test the effect of different boundary types on diversity and abundance of those predators and to compare their phenology with the larvae phenology of the main pests of oilseed rape crop, such as flea beetles, pollen beetles and weevils.

Overall contribution to my future work

The visit to Rothamsted Research was an exceptional experience that greatly contributed to my current and future work and career. The great theoretical and practical knowledge gained from Dr Sam Cook and her team, especially Dr Gaetan Seimandi-Corda, Dr Dan Blumgart, Dr Patricia Ortega-Ramos and Dr Martin Torrance, contributed to my understanding and experience in ecosystem services and Integrated Pest Management strategies. Well-equipped laboratories in Rothamsted Research provided me with the opportunity to work with various devices and to practice novel techniques which I can use in my home institution. During the visit I have learned to precisely identify larvae and adults of different pests of oilseed rape, as well as some of ground beetles as main predators of those pests. Likewise, my knowledge about parasitoids and their identification has grown. One of the most valuable parts of the visit was learning and practicing dissection of pollen beetle larvae and detecting parasitoids within them. This technique will help me to conduct planned activities in my home institution as well as future activities with other species involved.

My main research focus is on biodiversity and ecosystem services in agroecosystems and my PhD is linked with oilseed rape. After my return to Serbia, my colleagues and I have started the activities described within Project 2 and Project 3, all under the scope of H2020 project EcoStack in which both the Faculty of Biology and Rothamsted Research are participants. Research training in the Rothamsted have prepared me to conduct those projects without delay, to disseminate obtained knowledge and skills to other colleagues, and to help my team gather valuable results regarding oilseed rape production in Serbia. Finally, collaboration between Dr Cook’s team and our team has been further strengthened and future co-participation in projects is promising.

Additional contribution and final impressions

During 1st and 2nd March, in the Fowden Hall of Rothamsted Conference Centre, PhD Symposium was taking place. Due to Covid-19 measures, I was participating in the event as a guest through online streaming. Many young researchers presented their work from various subjects, and it was a pleasure to hear a lot of interesting information and ideas. To all visiting researchers Rothamsted has provided library access and that was an opportunity for me to gather more information about certain topics. Mandatory actions for all visiting researchers in Rothamsted were completing Health and Safety training by reading protocols, participating in online courses and taking part in testing. My knowledge has strengthened on this subject, and I have obtained valid certification after the training. Finally, the visit helped me improve my English speaking and writing skills.

There are things that cannot be obviously measured but have great importance in developing one young researcher such as myself. During the two months visit I have learned more about English history and culture by interaction with English people and by visiting some historical places and museums. Likewise, Rothamsted Research is a large community with members from all around the world. I met a lot of people from different countries and learned about their culture as well and have remained in contact with some of them after my departure.

Weekends were the opportunity to visit many places. London was only half an hour from Harpenden by train, and that was a wonderful opportunity for me to visit many museums, monuments and parks. For obvious reasons, the Natural History Museum and Grant Museum of Zoology are my favorites, but I was impressed by the British Museum, National Gallery, British Library and Victoria and Albert Museum as well. Imperative for myself was to visit Oxford and Cambridge as one of the most famous university cities in the world. History and architecture of those two cities left a strong impression on me, and I have enjoyed visiting colleges and museums. Many colleges were closed for visitors due to Covid-19, but I have caught the glimpse of the overall atmosphere.

This visit has helped me to build up my research profile by providing me with remarkable knowledge, valuable contacts and everlasting memories.

Appreciations

I would like to express my deep gratitude to Mrs Celia Hawkesworth and all the Trustees for the fantastic opportunity I have been given to conduct my research in the UK and for their kind support during the whole process. Likewise, I am exceptionally grateful to Dr Sam Cook and her team in the Biointeractions and Crop Protection department for the warm welcome and valuable knowledge provided. This journey would not have been possible without my mentor Dr Milan Plećaš and his unconditional help and support, for which I am immensely grateful. I would like to extend my deep gratitude to Prof. Dr Željko Tomanović and to my colleagues from Animal Ecology and Zoogeography department and Invertebrate Zoology and Entomology department, for their sincere support.