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nejczer

warsztaty metodyczne – ekologia ewolucyjna

Development of a photosynthetic area due to a different sun exposure on the example of *Taraxacum*

Invasive species are more attractive for pollinators. A case study on two species of *Solidago*

Spis treści

Warsztaty Metodyczne – Ekologia Ewolucyjna

31.08-5.09.2015, Łazy

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Tematy zaproponowane przez uczestników:

1. Czy gatunki inwazyjne są bardziej atrakcyjne dla zapylaczy – przykład nawłoci (EJ)

2. Czy chwasty segetalne preferują obrzeża upraw? (JD)

3. Wpływ siedliska na atrakcyjność kwiatów mięty dla zapylaczy. (KJ)

4. Czy szybkość reakcji rozwielitki na drapieżnika zależy od temperatury wody? (BZ)

5. Zróżnicowanie alokacji zasobów w nasiona i sytemu korzeniowy u soi w zależności od odległości od źródła wody (PK)

6. Czy występuje związek pomiędzy nasłonecznieniem siedliska a wielkością i liczbą liściu u rodzaju mniszek? (EJ)

7. Czy odległość od drogi wpływa na wielkość szyszek chmielu? (KJ)

8. Czas nasłonecznienia a powierzchnia liści u rzęsy wodnej (PK)

9. Czy reakcja pająka sieciowego jest zależna od wielkości ofiary? (BZ)

10. Zależność zapasożycenia owoców jarzębiny od ich wielkości (JD)

11. Wpływ przewodności wody na turgor roślin (EJ)

12. Czy zainfekowanie liści dębu czerwcami zależy od odległości od drogi? (BZ)

13. Jak położenie liścia w stosunku do pnia dębu wpływa na poziom zapasożycenia? (KJ)

14. Wpływ masowego pojawu rzęsy wodnej na bioróżnorodność zbiorników wodnych (PK)

15. Czy częstotliwość użytkowania drogi wpływa na bioróżnorodność przydroży (JD)

Tematy wybrane i zespoły badawcze

1.Wpływ nasłonecznienia na powierzchnie asymilacyjną liści *Taraxacum*

Ela Jędrzejczak, Jakub Dębowski

2. Czy gatunki inwazyjne są bardziej atrakcyjne dla zapylaczy – przykład nawłoci

Katarzyna Janas, Paulina Kosztyła, Bartłomiej Zając

Galeria

Rysunek 1Wyprawa w poszukiwaniu inspiracji…

Rysunek 2 Uczestnicy warsztatów z dr Klimkiem przy rzece Gróbce.

Rysunek 3 Zespół mniszkowy przy pracy.

Rysunek 4 Zespół nawłociowy w skupieniu:)

Rysunek 5 Zasłużona pizza :)

Wpływ nasłonecznienia na powierzchnie asymilacyjną liści *Taraxacum*

Projekt

Ela Jędrzejczak, Jakub Dębowski

Instytut Botaniki, Uniwersytet Jagielloński

Summary

Celem projektu jest zbadanie czy występuje związek pomiędzy nasłonecznieniem stanowiska, a powierzchnią asymilacyjną liści *Taraxacum*. Plastyczność fenotypową *Taraxacum* obserwowano już wielokrotnie, koncentrując się głownie na kwiatostanach. Wykorzystując metody komputerowe zmierzona zostanie powierzchnia asymilacyjna u osobników rosnących w dwóch różnych typach siedlisk. Przewidywanym rezultatem badań jest znacząca różnica powierzchni blaszki liściowej u dwóch grup eksperymentalnych. Świadczyłoby to o konieczności większej inwestycji w organy wegetatywne u roślin występujących w miejscach o mniejszym nasłonecznieniu.

Aim/Hypothesis

Wiadomo że rodzaj *Taraxacum* ma dużą plastyczność fenotypową objawiającą się wzrostem liczby kwiatostanów wytwarzanych przez jednego osobnika w trakcie całego sezonu wegetacyjnego. Rozrzut tych wartości wynosi od 1 do 200 kwiatostanów w zależności od stopnia nasłonecznienia siedliska. W związku z powyższym można zadać sobie pytanie czy podobna zależność dotyczy również organów wegetacyjnych. Można przypuszczać, że rośliny rosnące na stanowiskach zacienionych wytwarzać będą większą powierzchnię asymilacyjną w stosunku do roślin ze stanowisk słonecznych. Celem naszych badań jest sprawdzenie czy całkowita powierzchnia asymilacyjna wszystkich liści u osobników rosnących w cieniu jest większa od powierzchni asymilacyjnej liści osobników rosnących na stanowiskach o dużym nasłonecznieniu.

Methods

Badania prowadzone będą na terenie stacji badawczej Łazy należącej do Uniwersytetu Jagiellońskiego. Wytypowanych zostanie pięć stanowisk w miejscach, które przez większość dnia są nasłonecznione oraz pięć stanowisk w miejscach, które przez większą część dnia są zacienione. Następnie na obszarze stanowisk wybranych zostanie losowo, za pomocą rzutu okręgiem, pięć osobników, z których pobrane zostaną liście. Wykonane zostaną wyskalowane zdjęcia wszystkich zebranych liści, a ich całkowita powierzchnia zostanie policzona za pomocą programu komputerowego Gimp. Uzyskane dane zostaną poddane analizie statystycznej za pomocą hierarchicznej analizy wariancji.

Impact of results

Przewidujemy, że rośliny ze stanowisk zacienionych przeznaczają większe zasoby na organy wegetatywne dzięki czemu mogą zrekompensować mniejszą ilość słońca na stanowisku. Większa powierzchnia liści, a co za tym idzie większa powierzchnia dla miękiszu asymilacyjnego, umożliwi wyprodukowanie porównywalnej w stosunku do roślin ze słonecznych stanowisk ilości cukrów. Biorąc pod uwagę, że rośliny z zacienionych stanowisk potrzebują inwestować dużo w organy wegetatywne, uzasadniałoby to mniejszą liczbę kwiatostanów u osobników z takich siedlisk. Uzyskane wyniki można porównać z przeprowadzanymi wcześniej badaniami i obserwacjami porównującymi liczbę kwiatostanów *Taraxacum* na stanowiskach o różnym nasłonecznieniu.

Raport - pierwsza wersja

Development of a photosynthetic area due to a different sun exposure on the example of *Taraxacum*

ELA JĘDRZEJCZAK, JAKUB DĘBOWSKI

INSTYTUT BOTANIKI, UNIWERSYTET JAGIELLOŃSKI

Summary

Variation of fenotype in the genus *Taraxacum* was observed multiple times but researchers focus mainly on generative parts of a plant. Aim of a project was to investigate connection between sun exposure and growth of a photosynthetic area. Specimens from the morfotype *Taraxacum officinale* were drew and photographed. Using computer methods there was measured a photosynthetic surface of every specimen. Results were checked with analysis of variance. Study showed that there is a significant difference in development of vegetative parts of plants that grow in different light condition. Specimens that grow in a shade develop larger photosynthetic surface.

Introduction

It was observed before that *Taraxacum* is a genus that show a big variation of phenotype. It refers to the number of composite flower heads per year of vegetation but also to the shape and size of leafs. Specimens of genus *Taraxacum* were noticed to develop more flower heads while growing in a full light. Researchers point out that different habitats can strongly affect on the plant's morphological aspects (HOLM ET AL., 1997; LONGYEAR, 1918). However they see it from a wide angle. Considering all factors at once (shade, nutrition, moisture, exposure etc.) it is impossible to see which exactly cause the difference in phenotype. Jordan and Smith compared development of a leaf surface to attitude above sea level. As a result they suggest that specimens from higher attitude were smaller due to higher sun radiation (JORDAN & SMITH, 1995). We assumed that specimens that grow in a shade will have more developed photosynthetic area than those that grow in a good light conditions. Variety of research samples help to eliminate other factors.

Our study aimed to examine is it a correlation between sun exposure and growth of a photosynthetic area. *Taraxacum* as a very common and variable genus of plant become a perfect model for such research. We ask a question if there is a difference in a development of a photosynthetic area between specimens from the morfotype of *Taraxacum officinale* that grow in a shade and those that grow in full sun light. According to earlier research it show if a plant is forced to focus on growing vegetative parts instead of generative due to shade.

We test following hypothesis:
i) Specimens of *Taraxacum* develop larger photosynthetic area in a shade
ii) Specimens of *Taraxacum* develop more leafs in a shade

Materials and Methods

Our research take place in Field Research Station at Łazy near Bochnia during september. At first there were chosen 10 test stands divided into two types. One half was characterised by high level of sun radiation and other half as a contrast stay in the shade. For the first type of test stands direct sun exposure last at least ten hours per day. Second type of test stands were most of the time in a shade and were exposed on direct sunlight less than five hours a day. Other factors (e.g. humidity, distance from road or building wall, soil richness) were diversified in both types of test stands. However the height of a sod was about 10 cm with a cover about 90% in every test stand.

At every test stand five specimens of *Taraxacum officinale* morfotype were drawn. All living leafs were collected from every specimen and transferred to laboratory. Than leafs were flattened on a white sheet of paper and photographed using scaled, stationery camera. All photos were processed using computer program 'Gimp 2.8' which made it possible to measure whole leafs surface for specimens. Collected data was than analysed by computer program 'Statistica 12.5' and tested by analysis of variance.

Fig. 1
Example of photographs used to measure combined surface of leafs example taraxacum *Taraxacum officinale.* We can see variety of leafs shapes and sizes among specimens.

Results

Relationship between the photosynthetic area and the sun radiation is statistically significant ($F = 9.823$, $df = 1$, $p = 0.003$). Specimens from habitats with lower sun radiation have bigger leaf surface and therefore bigger photosynthetic area.

Analysis of variance has shown that number of leafs is not a statistically significant parameter ($F = 1.6145$, $df = 1$, $p= 0.211$).

All result were shown on following figures:

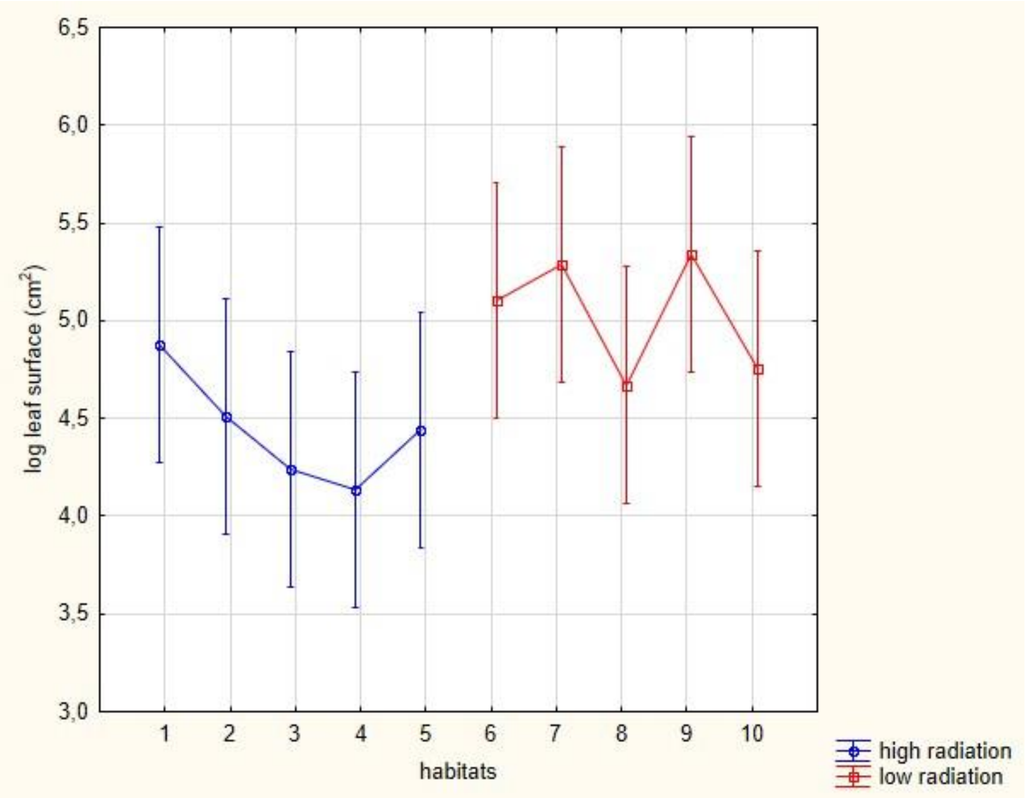

Fig. 2
Analysis of variance for values of leaf surface compared to different habitats.
F(8, 40) = 0,98695, p = 0,46052 vertical lines - 0,95 degrees of freedom.

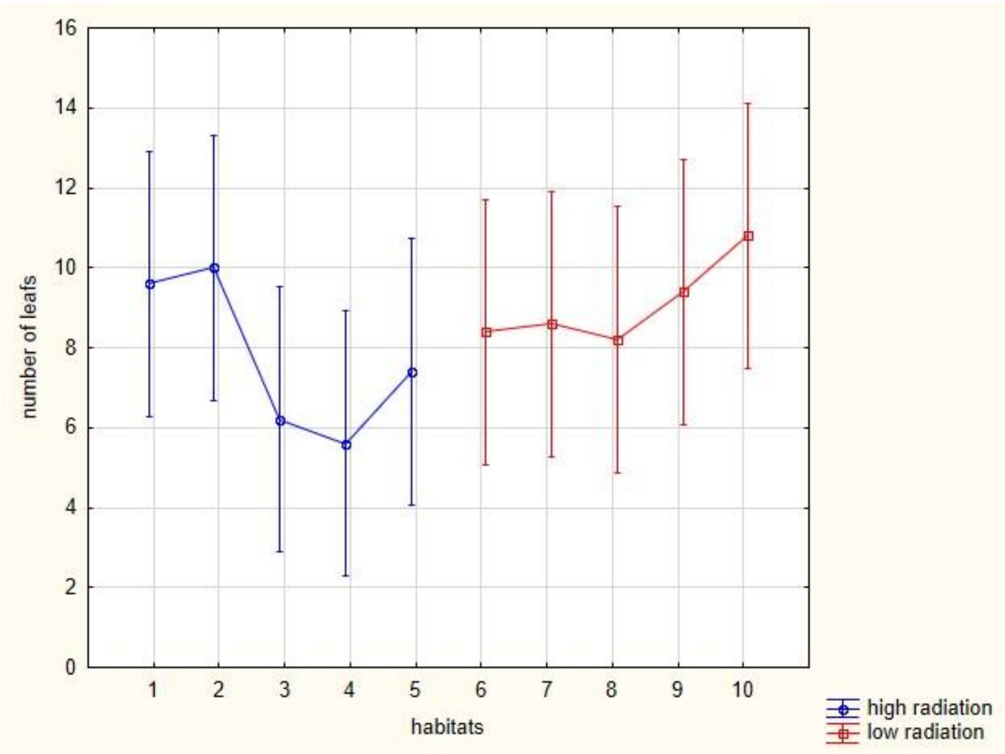

Fig. 3
Analysis of variance for number of leafs compared to different habitats.
F(8, 40) = 0,93403, p=0,49969
vertical lines - 0,95 degrees of freedom

Discussion

Research showed difference in leaf size of specimens from habitats with different sun exposure. On the areas with shadow dominance we observed bigger leafs of *Taraxacum*. Results tested with statistics showed us that this difference is significant and light factor could be much more important for growth of vegetative parts than other (e.g. humidity or soil factors). Our research is corresponding well with researchers claiming that light factor could have big influence on leaf shape (SLABNIK, E. 1981). Higher radiation will cause higher degree of lobbing and decrease length of leafs (SÁNCHEZ 1967; SLABNIK 1981). Our research show that despite genetic and morphological diversity inside and outside the population of *Taraxacum officinale* morfotype (TAYLOR, 1987) environmental factors (on the example of sun radiation) can show patterns of growth. Reassuming variability may be more connected with environmental than genetic factors.

Acknowledgments

We would like to thank Bartosz Zając for borrowing professional camera and Joanna Rutkowska for help in statistics and flattening leafs. We are also very grateful for their attitude and kind word.

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Cover Letters

03 September 2015

Bernhard Schmid
Editor-in-Chief
University of Zürich, Switzerland

Dear Professor:

Attached is a manuscript "Development of a photosynthetic area due to a different sun exposure on the example of *Taraxacum*" by Jakub Dębowski and myself. We would like to submit for consideration of publication in Journal of Plant Ecology. There are 3 figures and 4 pages with double space in the text.

Variation of fenotype in the genus *Taraxacum* was observed multiple times but researchers focus mainly on generative parts of a plant. The aim of our study was to investigate connection between sun exposure and growth of a photosynthetic area. We used a novel method of measuring leaf surface. Our study showed that there is a significant difference in development of vegetative parts of plants that grow in different light condition. Specimens that grow in a shade develop larger photosynthetic surface.

Data and findings presented in this manuscript have not been published nor are under consideration for publication in anywhere else. The submission for publication has been approved by all relevant authors. All persons entitled to authorship have been so named and all authors have seen and agreed to the submitted version of the manuscript.

Thank You for considering of our manuscript.

With Best Regards,

Sincerely,

Elżbieta Jędrzejczak
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3 September 2015

The Editor
Oecologia

Dear Sir or Madame,

Please find enclosed a manuscript of our paper entitled *Development of a photosynthetic area due to a different sun exposure on the example of Taraxacum* accompanying this letter.

We find our findings significant and novelty and highly appropriate in general topic for their publication in Oecologia. This manuscript describes original work and is not under consideration by any other journal. All authors approved the manuscript and this submission.

Author Contributions: EJ originally formulated the idea. JD and EJ conceived and designed the experiments. JD and EJ performed the experiments. JD and EJ analyzed the data. JD and EJ wrote the manuscript.

Thank you for receiving our manuscript and considering it for review. We appreciate your time and look forward to your response.

Yours sincerly,
Jakub Dębowski

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Recenzje

Mariusz Cichoń

Badania przedstawione w raporcie miały na celu wykazanie, że liście mniszka lekarskiego są większe jeśli roślina rośnie w miejscu zacienionym w porównani do roślin rosnących w miejscach dobrze nasłonecznionych. Hipotezę tą badania przedstawione w raporcie potwierdziły.

Poniżej kilka krytycznych uwag.

1) Tytuł raportu jest trochę mylący. Nie rozumiem dlaczego używać tak skomplikowanego określenia tak prostego słowa jak liść. Tak naprawdę powierzchni liści była badana, a nie powierzchnia fotosyntetyczna, ta druga rożni się zapewne od tego co była mierzone. Ponadto słowo „due to” sugeruje zależność przyczynowo skutkową, którą dałoby się jedynie zbadać przy pomocy eksperymentu a nie obserwacji jaka tu miała miejsce.

2) Metody badawcze przedstawiono zbyt lakonicznie i przez to niejasno. Nie wiem co to są opisane w metodach „stands”, jak je ustalono i jak były położone w stosunku do siebie. Ile było właściwie roślin? W jakich warunkach siedliskowych rosły zbierane rośliny. Mniej istotne jest podanie nazw programów używanych do analiz niż opisy samych analiz. Nie wiadomo jak mierzono powierzchnie liści, w jakich jednostkach i z jaką dokładnością. Zapewne program Gimp 2.8 sam tego nie zmierzył, a tylko ułatwił te pomiary. Podobnie info o użyciu programy Statistica nic nie wnosi, natomiast kluczowym byłoby napisać jak wyglądał model analizy wariancji, który użyli autorzy oraz informacja czy spełniony były założenia tej analizy.

3) Rysunki są kompletnie mylące. Celem pracy było wykazanie zależności między poziomem nasłonecznienia a wielkością i liczbą liści. Na rysunkach przedstawiono analizę różnic między poszczególnymi miejscami zbioru roślin, a nie miejscami nasłonecznionymi i tymi w cieniu. Przy okazji tego rysunku domyślam się, ze analiza była 2 czynnikowa. Ta informacja koniecznie powinna była znaleźć się w metodach. Podobnie jak informacja o transformacji danych w przypadku powierzchni liści. Linie pionowe to na pewno nie stopnie swobody, ale przedziały ufności.

4) Przy analizach przedstawianych w tekście brakuje stopni swobody dla zmienności niewyjaśnionej. Dodatkowo zdanie „all results were shown on following figures” jest nieuprawnione, bo nie wszystkie wyniki są na wykresach. Ponadto wszystkie wyniki powinny znaleźć się tekście z odniesieniami do wykresów.

5) Dyskusja jest stanowczo mało wyczerpująca. Można sobie wyobrazić wiele czynników, które w sposób pośredni mogłyby tłumaczyć występowanie zależności wielkości liści od natężenia światła. Wymagałoby to szerszej dyskusji. Na jakiej podstawie np. autorzy twierdzą, że efekty środowiskowe mogą być bardziej istotnym determinantem zmienności wielkości liści niż efekty genetyczne. Wyniki przedstawione w raporcie nie uprawniają do takich stanowczych stwierdzeń.

Agata Plesnar-Bielak

The study was aimed at testing if *Taraxacum* plans grow larger/more leafs in shadowed vs. sunny habitats which might be of interest to botanists that work on plant ecology. However, if and why it should be interesting for a broader audience is not clear for me. Neither is it clarified in the text. Both introduction and discussion are very short and the reader has a problem with placing the study in the context of the existing literature.

The methods used in the study are clear and suitable for the problem. However, I had some problems with the statistics. First, authors mention in the introduction that they checked for “a correlation between sun exposure and growth of a photosynthetic area”, but from further text (Materials and Methods) it seems that they used the analysis of variance with just two levels of exposure. And indeed, in the results section, the results of ANOVA are provided. The F statistics (together with df and p values) are doubled – they are presented in the text and in the figure legends - and, more surprisingly, they indicate two different results from the same test. The figures will need further improvement. It is not clear what 95% degrees of freedom should be – perhaps confidence intervals. And what do habitat numbers mean? Are they somehow ordered?

Bartłomiej Zając

Review of "Development of a photosynthetic area due to a different sun exposure on the example of *Taraxacum*" by Elżbieta Jędrzejczak and Jakub Dębowski.

Manuscript is 4 pages long and contains all expected parts, namely: summary, introduction, material and methods, results, discussion with conclusions and literature references. It includes three figures – one being example of used photographic method and two being graphs of ANOVA results.

In my opinion, paper will be very interesting for people who are concerned with phenotypical variability of organisms, especially of plants. I would also suggest to read this paper to everyone whose scientific work is plant taxonomy based only on morphological characteristics. It is amazing that specimens of one species (ok, I know it is species sensu lato, but still) inhabiting so closely situated sites could be so different just because of one (although crucial) environmental characteristics.

Introduction, in my opinion, is little unclear. What authors mean by "However they see it from a wide angle"? It is not explained. Also, I think the potential mechanism of sun radiation on leaf surface size should be a little more elaborated. Both hypotheses are clear, besides phrase "photosynthetic area". I understand that authors meant surface of the leafs combined, but I think it should be mentioned that authors define "photosynthetic area" as surface of leafs.

I have some remarks about materials and methods. First, the difference in sunshine duration in two sets of plots is not very well defined. Second, do authors checked all plots if they really meets assumed duration of sunshine? Third, are authors 100% sure that such difference in sunshine duration is retained during whole vegetation period?

Results are clear, although I would avoid expression "statistically significant"– statistical test results proved that difference is significant/not significant, and that is all. In my opinion, graphs of ANOVA results should contain only mean values and standard error instead of values of all repetitions, because it shows results more clearly.

Discussion is little short to my taste. I would expect a little more information about influence of sun radiation not only on leaf size, but also shape.

I think manuscript has two quite large drawbacks. First, I think comparing effect of sun radiation in both leaf size and leaf shape would make this study even more attractive. Second, is language – there is many mistakes in spelling, some word or expressions do not fit in context in which they were used, etc.

In my opinion, after corrections, manuscript should be accepted.

Paulina Kosztyła

This study tries to examine difference in a development of a photosynthetic area and numer of leafs between specimens from the morfotype of *Taraxacum officinale* that grow in a shade and those that grow in full sun light.

In Summary the sentences are short and clear, but sometimes lack coherence. Nothing is mentioned about the number of leaves. I would add one sentence of general introduction of the topic, not directly go to methods.

Introduction clearly inform about what we know about genus *Taraxacum*, but there is a lack of explenation why it is worth to check this pattern for leafs. The reason for using that specific taxa is quite broadly described, however there are several places where you started repeating your arguments and information. There is unclear use of naming which sometimes can be confusing- ones you write about *Taraxacum*, next about morphotype *Taraxacum officinale*.

You presented two hypotheses- connected with area and numer of leafs. The first one (about the leaf surface) was widely discussed in context of literature. You explain very well why you expect differences in leaf photosynthetic area, but why you expect differences in number of leafs? It was not explained.

There are no clearly formulated predictions and explain what exactly the project brings to what is already known. Why is this investigation needed? I agree but I would like to read why.

Methods of material collecting are clearly described and leaves no questions as to how to carry out the experiment.

Statistic methods are chosen well and clear and the data was correctly analyzed. Hovewer, graphical representation of data should be improved. For example:

- Text around the figures look very messy
- I would use the legend close - it is much easier to follow
- do not use color where there is no clear need- different types of lines on the graphs are enough
- Description and schedule should be on the same page (Fig. 3)
- Notation with „ ,”instead of „ .” is incorrect

Discussion is the one of the most importandt part of article and unfortunately, the part of your article which need to be improoved. Some parts in here is not quite clear and they required additional explanations. For example, there is no explanation why we should expect such and not other trends in the number of leaves.

Literature cited in this paragraph is from 60s' and 80's. I'm not saying that because of the date of publication of the data is questionable, but it is worth checking also some more recent sources. Unfortunately, there is no justification for the performance such project and its broader meaning. Are the results of this research can be extrapolated into other groups of plants?

My last remark relates to a method of cited items in the list of literature which is not consistent.

Despite these comments I think it is interesting study, it should be further developed the justifications for these studies and discussion of results.

Katarzyna Janas

Dear researchers,

I am aware of unplanned logistic obstacles that robbed you of time for preparing this report, however I must point out some of the most important problems.

In introduction you didn't clearly defined the gap in existing knowledge. Moreover, first of your research question have been answered already, at least twice (Haugland et al., 1993, Brock et al. 2004). Probably you should focus more on the second research question that seems to be less explored or think of a new one. Sentence like: "Variety of research samples help to eliminate other factors" should be in methods section rather than in introduction.

When you are describing methods try to avoid imprecise statements like: "about 10 cm with a cover about 90%". It gives the impression that you don't care for exactness in your research. While characterising your test stands, you write that other factors were diverse in both of the types, but you don't give any details.

In your description of statistic, we have to guess what kind of ANOVA you have used exactly. You give results for each of the tests twice (in text and below figures), with slightly different results and different degrees of freedom, which is really confusing.

In discussion you write that "light factor could be much more important for growth of vegetative parts than other (e.g. humidity or soil factors)" but in this experiment, you didn't checked any other factor, so you cannot formulate such conclusions. You also wrote anything about broader impact of your research.

I have also a few comment on the text itself. In whole summary and introduction there is no single coma, apart from the text in brackets. It may seriously disrupt the reading fluency and even discourage potential reviewer from accepting your article. Apart from that, you have made several mistakes like: “examine is it a correlation”, “than” instead of then, “september” without capital letter and many others.

Raport – wersja ostateczna

Development of the leaf surface in various light conditions on the example of *Taraxacum*

ELŻBIETA JĘDRZEJCZAK, JAKUB DĘBOWSKI

INSTYTUT BOTANIKI, UNIWERSYTET JAGIELLOŃSKI

Summary

A phenotype variation of the *Taraxacum* genus has been observed multiple times so far, however, researchers tend to focus mainly on generative parts of the plant and perform research on a divided populations. The aim of the project was to investigate a relationship between sun exposure and growth of the leaf surface. Specimens from the *Taraxacum officinale* morphotype were drawn and photographed. The photosynthetic surface of every specimen was measured using computer methods. Results were checked by analysis of variance. Study showed that there is a significant difference in the development of vegetative parts of plants that grow in different light condition. Specimens that grow in shaded areas develop larger photosynthetic surface. However, the project did not prove any significant difference in the number of leaves.

Introduction

Understanding the factors that may affect the phenotypic variability of selected plant species is important for plant taxonomy, especially for critical taxa. Due to a wide range of phenotypic plasticity, the classification is difficult and the collected material is frequently marked incorrectly (BRANDSHAW, 1965, FALTYN & JAKUBSKA-BUSSE, 2008). The number of publications concerning phenotypic plasticity has increased in recent years (SCHLICHTING, 2002). *Taraxacum officinale* is a good example, as it is characterized by high genetic and phenotypic variability (TAILOR, 1987), and it has been put to numerous tests so far. In his works of 1967 and 1971, Sanchez observed a relationship between the shape of leaves and sunlight, while in 1995 Jordan & Smith carried out a study about the size of *Taraxacum officinale* leaf at different altitudes. Research study about impact of light on the development of leaf surface was conducted in laboratory conditions (SLABNIK, 1981). However, we have no data from any research conducted in situ within one population that would help us eliminate the external genetic variation. In addition, measurement methods applied previously (ratio of leaf length to width) may not fully reflect the actual leaf surface, since *Taraxacum* leaves have a variety of shapes, as shown in an earlier research conducted by Sanchez. Recent study did not focus on the problem of differences in number of leaves among specimens exposed to different sun radiation.

The aim of our study was to investigate a relationship between the sun exposure and the leaf surface on one native population of Taraxacum.

We tested the following hypotheses: i) Specimens of *Taraxacum* develop larger leaves in a shaded area. ii) Specimens of *Taraxacum* develop more leaves in a shaded area.

Materials and Methods used

Our research took place in the Field Research Station in Łazy near Bochnia in September 2015. At first, 10 study sites were selected and divided into two types. Half of them were characterised by high level of sun radiation, and the other half were located in the shaded areas. In the first group, direct sun exposure lasted at least ten hours per day. In the second group, the sites were shaded for most of the time, and were exposed to direct sunlight for less than five hours a day. Other factors (e.g. humidity, distance from road or building wall, soil richness) were diversified but same for the pare of both types of sites. The sod was about 10 cm high, and covered about 90% of each study site.

At every study site, five specimens of the *Taraxacum officinale* morphotype were drawn (summing up to a total of 50 plants). All living leaves were collected from every specimen and transferred to laboratory. Then leaves were flattened on a white sheet of paper and photographed using scaled, stationery camera (Fig. 1). All photos were processed using a computer program ‘Gimp 2.8’ which made it possible to measure the whole leaf surface of each specimen. The pixels on the photographed leaf surface were counted and, and the results were converted into square centimetres and then logarithmized. Collected data was then analysed by a computer program ‘Statistica 12.5’ and tested with nested analysis of variance.

Fig. 1
Examples of photographs used to measure combined leaf surface of the *Taraxacum officinale.* We can see a difference in sizes of specimens.

Results

The difference between the leaf surface and the sun radiation is significant:

(F(1, 40) = 9,8234, p = 0,00322, Fig.2)

Specimens from habitats with lower sun radiation have larger leaf surface and therefore larger photosynthetic area.

Analysis of variance has shown that the number of leaves is not a significant parameter:

(F(1, 40) = 0,93403, p = 0,49969 , Fig.3)

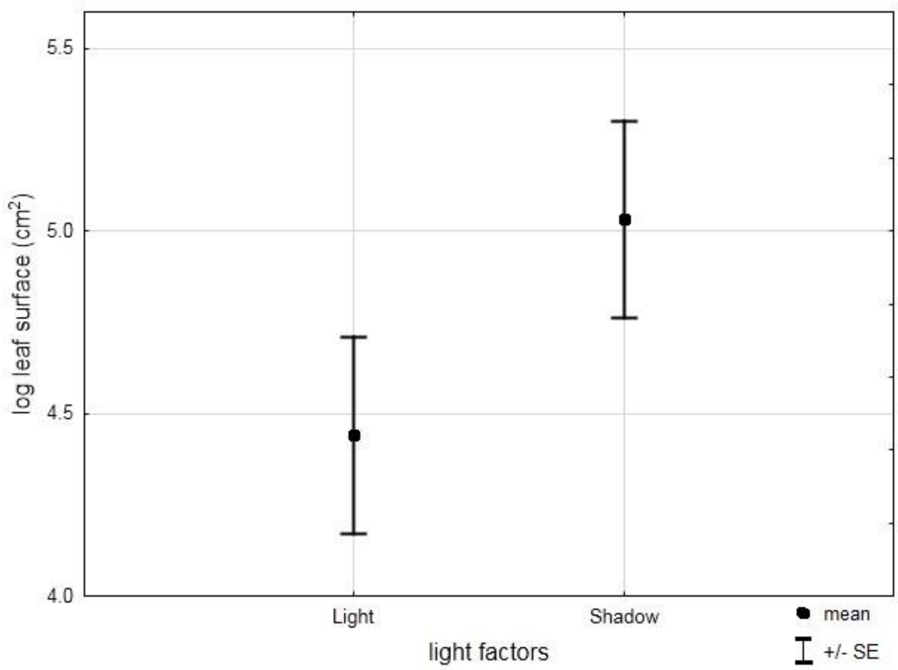

Fig. 2 Leaf surface of *Taraxacum officinale* different study sites.

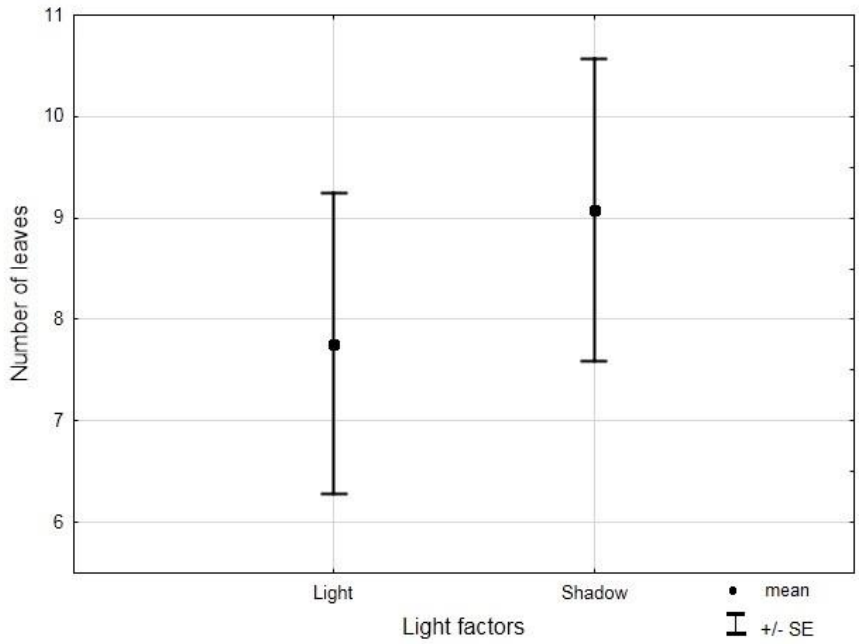

Fig. 3 Number of *Taraxacum officinale* leaves at different study sites.

Discussion

Our Research shows that leaf surface of specimens that grew in a shaded areas is greater than the leaf surface of those specimens that grew in places with good access to sunlight. Therefore, we confirmed the first hypothesis, but we did not observe any relationship between the number of leaves and the sun radiation. Similar results about leaf size were observed in the laboratory tests and studies conducted on different populations of *Taraxacum* (SLABNIK, 1981, JORDAN & SMITH, 1995). Research has shown that light is an important environmental factor affecting the *Taraxacum* phenotypic variation even within one population, and may be more important than other factors, e.g. moisture and soil fertility.

In 2002, Stewart-Wade and others reported that, during the vegetative season, particular specimens of *Taraxacum* may produce different number of inflorescences, from one inflorescence in shaded areas to fifty in the sunny environment. If we compare these data with our results, we may assume that individuals of *Taraxacum* that grew in areas characterized by low radiation invested more resources in their vegetative organs, rather than generative ones. Similar results were obtained in research projects concerning phenotypic variation costs, conducted on the model species *Sinapis arvensis* (STEINGER, 2003).

Our research shows how environmental factors like light can affect the morphology of a specimen, even within a single population. As a conclusion, all research projects concerning taxonomy, and carried out on a critical taxa, should pay attention to environmental factors and include genetic tests for morphometric measurements.

Acknowledgments

We would like express our gratitude to Bartłomiej Zając for borrowing a professional camera, to Joanna Rutkowska for her assistance in calculating statistics and pressing leaves, to Katarzyna Janas for useful references, and Paulina Kosztyła for evaluation. We are also very grateful to our supporters for their kind attitude. We are also immensely grateful to our reviewers who supported our work with good ideas and necessary corrections.

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Czy gatunki inwazyjne są bardziej atrakcyjne dla zapylaczy – przykład nawłoci.

Projekt

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Summary:

Nasilający się problem gatunków inwazyjnych, powodujących wypieranie gatunków rodzimych i obniżenie lokalnej bioróżnorodności czyni z nich obiekt intensywnych badań. Dotychczas opisano wiele przykładów konkurencji o różne zasoby, jednak w dalszym ciągu niewiele wiadomo o znaczeniu konkurencji o owady zapylające. Celem naszych badań jest odpowiedzenie na pytanie czy gatunki inwazyjne takie jak nawłoć kanadyjska są bardziej atrakcyjne dla zapylaczy niż gatunek rodzimy - nawłoć pospolita. W ramach badań sprawdzimy na którym z gatunków owady zapylające chętniej i dłużej przebywają, oraz jak kształtuje się ich rozpiętość taksonomiczna. Wyniki naszych badań mogą mieć znaczenie zarówno w kontekście ochrony bioróżnorodności jak i lepszego poznania mechanizmów ekspansji gatunków inwazyjnych.

Aim/ hypothesis

Rozprzestrzenianie się roślin inwazyjnych jest obecnie jednym z najpoważniejszych zagrożeń dla bioróżnorodności. Istnieje wiele teorii tłumaczących przewagę gatunków inwazyjnych nad rodzimymi, a wśród nich jedną z najczęściej wymienianych jest konkurencja z gatunkami rodzimymi. Dotychczas stwierdzono, że rośliny inwazyjne mogą wygrywać konkurencję z rodzimymi m. in ze względu na szybszy wzrost, produkcję substancji allelopatycznych, czy mniejsze wymagania siedliskowe. Wciąż jednak słabo poznane pozostaje zjawisko konkurencji o zapylaczy, które może mieć kluczowe znaczenie dla rozprzestrzeniania się gatunków inwazyjnych. W planowanych badaniach sprawdzimy czy rośliny inwazyjne są bardziej atrakcyjne dla owadów zapylających niż gatunki rodzime, w szczególności zaś spróbujemy odpowiedzieć na następujące pytania:

-Czy kwiaty gatunków inwazyjnych będą częściej odwiedzane przez owady zapylające?

-Czy różnorodność gatunkowa zapylaczy odwiedzających kwiaty roślin inwazyjnych będzie wyższa niż u gatunków rodzimych?

-Czy czas przebywania zapylaczy na kwiatach roślin inwazyjnych będzie dłuższy niż na roślinach rodzimych?

Jednym z najbardziej ekspansywnych gatunków inwazyjnych w Europie (w tym w Polsce) jest nawłoć kanadyjska *(Solidago canadensis),*wypierająca między innymi rodzimy gatunek nawłoć pospolitą *(Solidago virgaurea).* Współwystępowanie tych gatunków na tym samym siedlisku w miejscowości Łazy umożliwi nam eksperymentalne sprawdzenie powyższych hipotez.

Methods

Planowany eksperyment będzie polegał na obserwacji kwiatostanów obydwu gatunków, pod kątem częstotliwości i czasu odwiedzania kwiatów przez zapylaczy oraz ich różnorodności gatunkowej.

Aby wyeliminować potencjalny wpływ innych czynników, w eksperymencie użyjemy roślin występujących na jednym stanowisku. Wyznaczymy po 20 par złożonych z osobników obydwu gatunków. Każda para będzie obserwowany przez 10 minut, pod katem obecności zapylaczy i czasu ich przebywania na kwiatach. Przed obserwacją zapylacze obecne na kwiecie zostaną z niego strącone. Zanotowane zostanie każde lądownie oraz czas przebywania owada na kwiecie a osobniki zostaną przypisane do morfogatunków. Następnie porównamy średnią liczbę odwiedzin oraz czas przebywania owadów na kwiatach obydwu gatunków. W celu porównania różnorodności zapylaczy na podstawie liczby obserwowanych morfogatunków obliczony zostanie współczynnik Margalefa. Otrzymane wyniki zostaną przeanalizowane za pomocą testu t studenta dla par wiązanych, lub w wypadku braku rozkładu normalnego lub zbyt małej liczby powtórzeń - testu Wilcoxona.

Impact of results

Gatunki rodzime powinny być lepiej przystosowane do lokalnych warunków siedliskowych w których ewoluowały, mimo to rośliny inwazyjne wydają się mieć nad nimi przewagę. Wyniki naszych badań, dzięki zastosowaniu blisko spokrewnionych gatunków, mogą przyczynić się do wyjaśnienia mechanizmu dającego przewagę roślinom inwazyjnym. Poza znaczeniem w kontekście ochrony przyrody, nasze badania mogą rzucić światło na rolę konkurencji o zapylaczy w kształtowaniu się zbiorowisk roślinnych

Raport – pierwsza wersja

Invasive species are more attractive for pollinators. A case study on two species of *Solidago*.

Katarzyna Janas[1], Paulina Kosztyła[1], Bartłomiej Zając[1],

[1]Institute of Environmental Sciences, Jagiellonian University

Summary

One of the biggest difficulties in maintaining the global biodiversity is a problem of invasive species. Dozens of studies have been devoted to this subject, especially to explain success of invasive plants in their new habitats. We already know many mechanisms allowing them to supersede local species, for example by allelopathy, faster growth or bigger resistance to environmental stressors. Nevertheless, we still do not understand all aspects of competition of pollinators, which might be crucial for their success. Here we show that inflorescences of invasive *Solidago canadensis* are more attractive for pollinators (bigger number of visitors and wider range of pollinators) than those of native *Solidago virgaurea*. This result can be explained by the difference in size of inflorescencesof *S. canadensis*,which are significantly bigger than those of *S. virgaurea*, what help them to attract bigger amount, and wider variety of pollinators. Although we did not managed to show the same pattern with the duration of pollinators visit, we think that bigger inflorescence is important element of their successful strategy which allowed them to colony so many habitats. This is another step to understand not only success of *S. canadensis*, but also other flowering invasive species, and thus it might important for creating strategies counteracting their uncontrolled dispersal.

Introduction

The spread of invasive plant species is currently one of the most serious threats to biodiversity of plants and their pollinators (Moroń et al., 2009). There are many mechanisms proposed to explain the advantage of invasive species over native, including: allelopathy (Butcko and Jensen, 2002), faster growth (Fenesi et al., 2015), lower habitat selectivity and higher resistance to environmental condition (Weber et al., 2008). However, still little is known about the competition for pollinators between invasive and native species of plants. Pollination is a key factor, allowing plants for generative reproduction and in greater evolutionary scale to adapt to local conditions. Therefore elucidating the mechanisms of competition for pollinators can shed light on the reasons of their unprecedented success.

Here, we attempt to assess whether invasive plants are more attractive to pollinators than the native species. In our research we will try to answer the following questions:

1) Do flowers of invasive species are more frequently visited by pollinators?

2) Does the duration of visit of pollinators on flowers of invasive plants is longer then on native species?

3) Do the diversity of pollinators visiting the flowers is higher on invasive plants than on local species?

One of the most expansive invasive species in Europe (including Poland) is originating from North America, Canadian goldenrod (*Solidago canadensis*). It supersede closely related, native species of goldenrod (*Solidago virgaurea*). Here we take advantage of their coexistence in one habitat, which allow us to test our hypothesis in natural rather than artificially created conditions.

Materials and methods

Study area

Study was performed in Łazy, small village situated near Bochnia town, Lesser Poland Voivodeship. Fieldwork was conducted near Field Research Station of Institute of Geography and Spatial Management, Jagiellonian University, on meadow placed on southern slope of small hill, where both species occur.

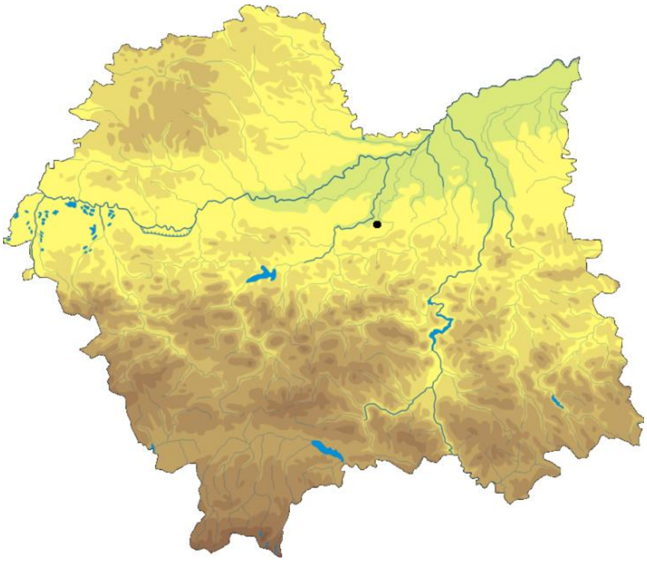

Figure 1. Localization of Łazy on Lesser Poland Voivodeship map.

Figure 2. Satellite image of fieldwork area.

Fieldwork protocol

Fieldwork was conducted 01.09.2015, between 7 and 12 AM. During that time atmospheric conditions changed gradually from clear sky and 20°C to full overcast, strong wind and temperatures under the 15°C.

To check if *S. canadensis* inflorescencesare more attractive to pollinators than these of *S.virgaurea*, we performed observations, during which we collected data of three different parameters. To exclude influence of changing atmospheric conditions, observations were performed simultaneously for one, randomly chosen specimen of each species. Every observation lasted 10 minutes. After end of observation period, plant was tagged with small sheet of white paper to avoid using same specimen more than one time. We used surface of flattened inflorescence as indicator of its size, using height and width as diagonals of rhombus, which shape is resembled by flattened inflorescence. A total number of 20 plant (10 of each species) have been used in experiment.

To avoid observer bias, we conducted observation in rotation system – two researchers worked with *S. canadensis* – one observed plant and second noted observed pollinators and duration of visits. Third researcher worked on both of this tasks with *S. virgaurea*, as its inflorescences were smaller and easier to observe. On each pair, observation were conducted with one of three possible researcher combination.

Collected parameters were:

- number of visits of pollinating insects on inflorescence during time of observation,

- duration of visit on inflorescence of each specimen of pollinator (in seconds),

- number of morphospecies identified on each inflorescence during time of observation.

We decided that number of visits is good indicator of visual attractiveness, of inflorescence to pollinators. We are aware that some individuals might return to the inflorescence many times, but nevertheless in this case we are specifically interested in number of visits, not number of individuals visiting inflorescences. We treated time spent on inflorescence as a indicator of nectar attractiveness.

We used morphospecies (defined as group of organisms sharing morphological similarities, that are not possible to differentiate during short eye-sight observation by non specialist) instead of exact identification to species level because simplicity of this approach allowed us to quickly asses the diversity of possible pollinator types of each species.

Statistical analysis

We used paired samples t-test to check significance of differences in pollinator visit number and pollinator morphospecies number between two species of *Solidago*. Because of lack of normal distribution, we used Wilcoxon signed-rank test to check significance of differences in duration of pollinator visit on inflorescence. To check if the size of inflorescence has stronger effect on results than species of plant, we ran GLM with number of visits as a dependent variable and species as a categorical variable and size of inflorescence as continuous variable. All statistical analyses were performed using Statistica 10 software.

Results

Number of pollinators visits
Mean numbers of visits on inflorescence for *S. canadensis* and *S. virgaurea*, were 7,0 and 2,2 respectively. Paired samples t-test showed that number of visits between two study species is significant (t=2,967, df=9, $\boldsymbol{p}$**=0,016**).

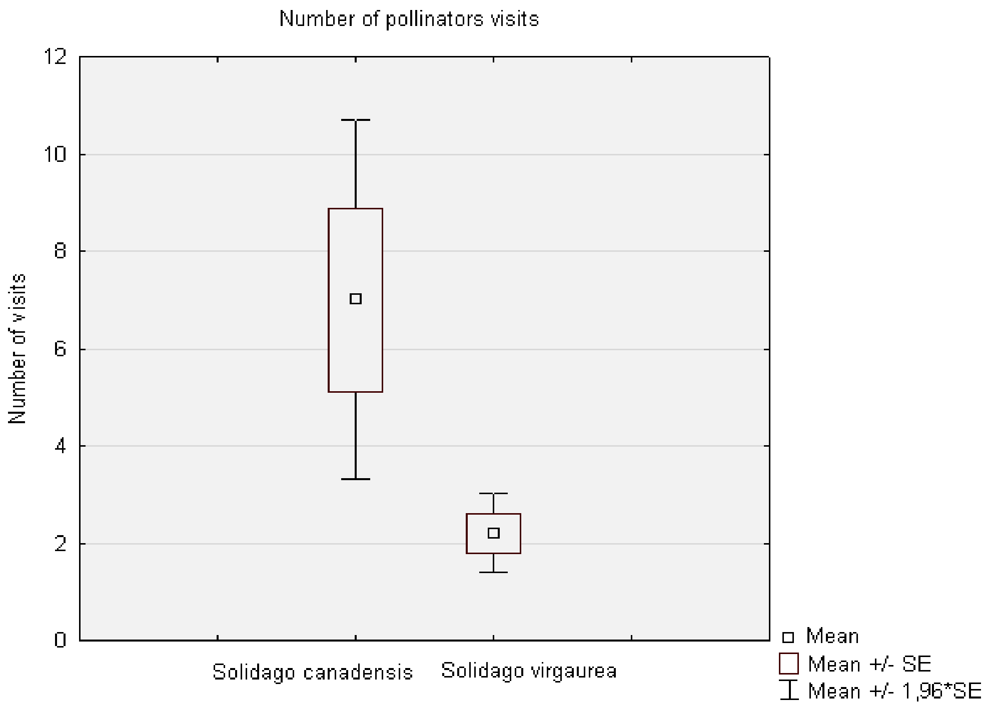

Figure 3. Mean value and standard error for number of pollinator visits in *S. canadensis* and *S. virgaurea*.

Duration of pollinators visits

Wilcoxon signed-rank test showed that difference between duration of pollinator visit on study species is not significant (*Z*=0,296, *p*=0,767).

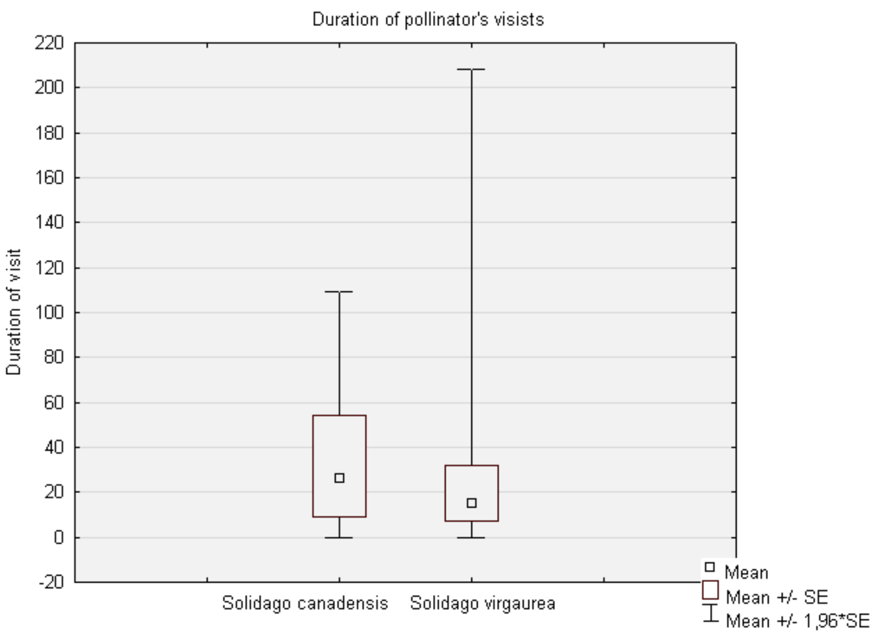

Figure 4. Mean value and standard error for pollinator visit duration in *S. canadensis* and *S. virgaurea*.

Variety of pollinators

In total, we observed eleven morphospecies of pollinators, one of which was exclusive to *S. canadensis*, and three was exclusive to *S. virgaurea*.

Table 1. List of observed morphospecies and their occurrence on study plants

Morphospecies	Occurrence on *S. canadensis*	Occurrence on *S. virgaurea*
"small fly"	Y	
"medium fly"	Y	Y
"big fly"	Y	Y
"metallic green fly"		Y
"small thin fly"	Y	Y
"thin fly"	Y	Y
"small hoverfly"	Y	Y
"medium hoverfly"	Y	Y
"big hoverfly"	Y	Y
"wasp"		Y
"honey bee"		Y

Despite the total number of morphospecies was higher for *S. virgaurea* (10) than *S. canadensis (*8), mean number of morphospecies in *S. canadensis* was 2,9 and in *S. virgaurea* 1,7. Paired t-test does not show significant difference in number of pollinator morphospecies between study species(t=2,197, df=9, p=0,058).

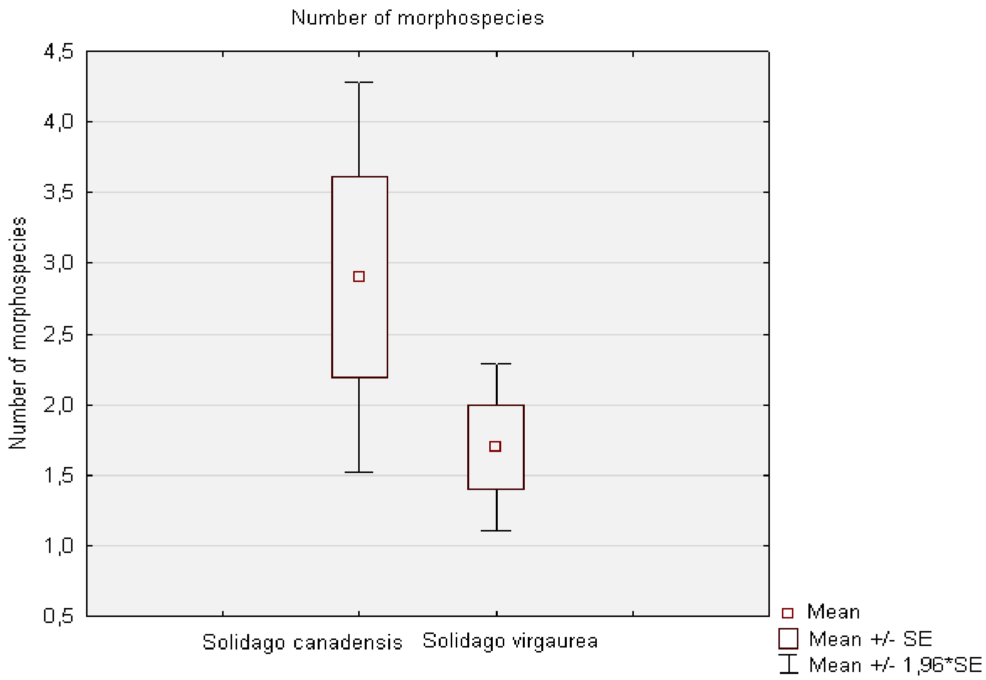

Figure 5. Mean value and standard error for number of pollinator morphospecies in *S. canadensis* and *S. virgaurea*.

Influence of size of inflorescences

Model considering interaction was insignificant, so we removed interaction from the model and obtain significant result (F_1=5,038, ***p*=0,038**) for size of inflorescences but not for species (F_1=0,305, *p*=0,588).

Discussion

We demonstrated that inflorescences of *S. canadensis* were visited more often by pollinators than those of *S. virgaurea*. However, we did not find similar pattern for duration of pollinator's visits and pollinators diversity. After checking the influence of size of inflorescences on visit frequency, it occurred that it is more important factor affecting attractiveness to pollinators than taxonomic identity. According to this result the size of inflorescences is a dominant factor deciding about attractiveness of *S. canadensis* for pollinators

Result of investigation conducted on similar problem, and performed on plants from the genus *Lythrum* shown that invasive species decreased number of pollinators visits and seed production of related native species (Brown et al., 2002). Similar results were obtained also in other studies (Grabas and Lavert ,1999, Chittka and Schurkens, 2003)

We are aware that our number of observation is relatively low, what might be a serious objections against our results. Unfortunately unfavorable weather condition forced us to finish experiment before making optimal number of observations (at least 20 pairs). By conducting experiment with larger number of individuals we would be able to obtain more reliable results, nevertheless we still manage to show significant relations described above.

In contrast to our study, Carrion-Tacuri et al., in 2014, showed that on Galapagos islands, pollinators remained longer on flowers of invasive species of the genus *Lantana*, than on native relative. In our study we did not managed to show such relation. It might be caused by differences in size and details of flower anatomy as well as nectar composition and its abundance.

We did not found any difference in variety of pollinators, between the two species although our experiment was performed o small amount of individuals and only during one day in the end of the summer season so we did not cover full seasonal variety of pollinators. For this reason further studies are necessary to check if our observations were reliable.

Although our research were conducted on plants from genus *Solidago*, while combined with results of different investigation performed on other plants, might contribute to the understanding the advantage of invasive species over the native competitors. This knowledge may be useful in preparing efficient strategies of mitigating biodiversity loss and habitat changes caused by invasive species.

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Cover Letters

3 September 2015

The Editor
Elsevier: Biological Conservation

Dear Sir or Madame,

Please find enclosed a manuscript entitled *Invasive species are more attractive for pollinators. A case study on two species of Solidago.*
Main aim of our research was to elucidate the mechanisms standing behind the advantage of invasive over native plant species in concurrence of pollinators. Here we show that

inflorescences of invasive *S. canadensis* are significantly often visited by pollinating insects than native *S. virgaurea* and this relation is explained by bigger size of inflorescences of *S. canadensis*. As invasive plant are serious threat not only for biodiversity of native plant but also for biodiversity of pollinating insects, we believe that our finding might be useful in creating efficient strategies counteracting their dispersal.
We would like to declare that the work is our original research and that all authors agreed with the contents of the manuscript and its submission to this journal. No part of this research have been published or submitted in this and any other scientific journal. Currently our manuscript is not consider for publication in any other journal.
In acknowledgements of our manuscript we clearly stated the contributions of people, that are not authors of this research, and the sources of funding. We declare that we will not receive any kind of direct financial benefits that could result from publication of this research.
None of the procedures performed during experiment, were in any way invasive for pollinating insects.

Yours sincerely,
Katarzyna Janas

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Phone: 48 660261520
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Kraków, 3.09.2015

The Editor
Biological Invasions

Dear Sir,

We would be very grateful if you could kindly consider our manuscript titled “ Invasive species are more attractive for pollinators. A case study on two species of Solidago” for publication in Biological Invasions.
We would like to ask you to consider the submission of our manuscript, because we think there is strong reason why it would be beneficial – both to the journal and to the scientific community – to publish our study in Biological Invasions.
While surveying the literature on problem of invasive species, we found that there still little is known about about the competition for pollinators between invasive and native species of plants. a unified approach to the subject. That is why we decided to address this problem in our manuscript and provide recommendations that would encourage future researchers to pay more attention to this problem. Given that Biological Invasions is a prestigious journal read by many researcher, our appeal would have better chances to reach a greater number of scientists than if it was published in a lower rank journal.
Thank you in advance for you time to read the manuscript itself. We hope you will evaluate

our work as worth to be published in Biological Invasions.

Yours sincerely,
The Authors

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03.09.2015

The Editor
Ecology

Dear Sir/Madam,

Here I present you a brief description of our paper entitled "**Invasive species are more attractive for pollinators. A case study on two species of *Solidago***." along with abstract attached in another file.

Invasive species is one of the two most dangerous threats to worldwide biodiversity, along with habitat destruction. In case of invasive plants, these two threats literally merge in one, as often along with alien plants invasion, native habitats are subjected to often irreversible changes. These changes includes also trophic niches and connections shifts or breakings, which possibly could change whole ecosystems.

Despite of these radical effects, mechanisms that gives invasive plants advantage over native plant species is not very well known. In our paper, we present data suggesting that one of the crucial mechanisms of invasion is superiority in competing for pollinators. We found that inflorescences of *S. canadensis* attract pollinators more efficiently than inflorescences of related native species, *S. virgaurea.* Additional analysis showed that main characteristic that provide advantage of inflorescence of *S. canadensis*, is size of inflorescence alone.

In our opinion, these findings are important for both ecologists working on applications against invasive species, and for plant ecologist, whose works focusing on plant competition for pollinators and its effects on ecosystem and plant communities.

Our paper is not in the consideration in any other journal, data was not published in the past, and all coauthors, which are Katarzyna Janas and Paulina Kosztyła, are aware of it being sent to publish in your journal.

Yours sincerely,
Bartłomiej Zając

Recenzje

Mariusz Cichoń

Przedstawione w raporcie badania miały na celu sprawdzenie na ile rośliny inwazyjne mogą wygrywać konkurencję o zapylaczy z roślinami natywnymi. Roślinami modelowymi były 2 blisko spokrewnione gatunki: nawłoć kanadyjska jako roślina inwazyjna i nawłoć pospolita jako roślina rodzima. Obie rośliny są silnie miododajne. Badano częstość odwiedzin kwiatostanów przez owady, długość przebywania na kwiatostanach, a także skład gatunkowy odwiedzających. Okazało się, że zgodnie z przewidywaniami okazy rośliny inwazyjnej były częściej odwiedzane przez owady, jednak autorzy wykazali, że to nie przynależność taksonomiczna, ale wielkość rośliny była głównym determinantem częstości odwiedzin.

Podejmowany w raporcie temat jest bardzo interesujący i przykuwa uwagę badaczy ze względu na ważne znaczenie aplikacyjne w ochronie przyrody. Częstsze wizyty zapylaczy na roślinach inwazyjnych mogą mieć poważne konsekwencje dla sukcesu roślin rodzimych. Badania zostały poprawnie zaprojektowane, ale warunki atmosferyczne nie były sprzyjające ze względu na niskie temperatury i zachmurzenie. Mimo to udało się zebrać materiał, który pozwolił na skuteczna analizę i wyciąganie wniosków.

Mam jednak kilka uwag krytycznych.

1) Lista gatunków owadów obserwowanych na nawłociach przedstawiona w tabeli 1 wskazuje, że raczej nie wszystkie gatunki są zapylaczami. Czy zatem tytuł tego raportu, postawione cele i wnioski są prawidłowe?

2) Wstęp generalnie nie przekonuje, dlaczego należałoby oczekiwać konkurencji między gatunkami roślin o zapylaczy? Skąd zatem pomysł na postawienie pytań badawczych (na jakiej podstawie je postawiono)? Brakuje też hipotez i przewidywań.

3) W rozdziale Wyniki niepotrzebne sa podtytuły jeśli zawierają tylko jedno zdanie.

4) Rys 3. wskazuje, że zmienność liczby wizyt bardzo różni się między gatunkami. Czy na pewno zatem było spełnione założenie normaności różnic?

5) Rys 4. ma niesymetryczne przedziały ufności. Dodatkowo jest spora różnica w zmienności obserwowanej dla obu gatunków, a zastosowany test jest parametryczny. Czy na pewno sprawdzono tu normalność rozkładu?

6) Figury i tabela nie mają odniesień w tekście.

7) Nie do końca jasne są analizy różnorodności gatunkowej owadów. Nie ma formalnej analizy, która miałaby na celu sprawdzenie, który z gatunków przywabia więcej owadów i czy być może skład gatunkowy owadów odwiedzających oba gatunki roślin jest różny. Nie bardzo wiem co wnosi porównywanie liczby gatunków odwiedzających osobno dla każdej rośliny.

8) W tekście znalazłem drobne potknięcia językowe, głównie w postaci literówek.

Agata Plesnar-Bielak

The study considers a very interesting and important problem of invasive plant species that might be of interest not only to ecologists and botanists but also to people responsible for environment management and protection. The authors tested the hypothesis that invasive species are more attractive to pollinators than native species are. They used two species of goldenrod that are found in Lesser Poland. They found out that invasive Canadian goldenrod is more frequently visited by pollinators than a native goldenrod species and they propose this difference to be the result of inflorescence size differentiation. At the same time, the authors found no difference in the time of visit or diversity of visitors between the species.

The paper is well written and is easy to follow, although the text needs some grammar corrections. The methods are correct and described in sufficient detail. However, it is not clear to me how the authors measured a visit duration. It seems that they used a mean time period spent by a pollinator on each flower, but it should be stated more explicitly. The data are analyzed properly, although they might have been presented in a more attractive way. The results are discussed in a broad context of previous research that enables to draw more general conclusions. Summing up, the manuscript may need further editorial work, but is undoubtedly very interesting and sound.

Elżbieta Jędrzejczak

Manuscript *"Invasive species are more attractive for pollinators. A case study on two species of Solidago"* concerns very important and interesting topic: relations between plant and pollinators. Authors suggested that evolutionary success of invasive plants may dependent on more attractive flowers than those of natives species. The objects of reaches were two species of *Soligago* genus: *Solidago canadensis* as an invader and *Soligago virgaurea* as an native species. The topic is not new, but I find two strong points in that work: 1) objects of the study is very well selected – *Solidago canadensis* is one of the most invasive species in Poland; 2) as an control authors used the other species with the same genus, it's a great advantage and innovation in that kind of research. Generally, I thing that the conception of the study is very good.

All the report is clear and contains all necessary elements. The title and abstract accurately describe the content of the article, the hypothesis is stated clearly, the methods used to test it are well designed and the results are correctly analysed.

I have a few additional comments:

1) Observations were conducted only in the morning. Some genus of pollinators has a high activity at noon or in the evening. Complete research on observations from the other times of day could make some changes in the results.

2) The name of morphospecies in Tab.1 are very colloquial and I think it's not necessary to add this table in the report. In addition, I think, it is possible that "small fly" is the same species as "medium fly" etc.

3) I very like figure with satellite image of fieldwork area but it can be more readable. The graphs should have white background and bigger type.

4) I get the impression, authors criticize your study too much instead of emphasizing strong points. In the discussions is written: "*We are aware that our number of observation is relatively low, what might be a serious objections against our results. Unfortunately unfavorable weather condition forced us to finish experiment before making optimal number of observations (at least 20 pairs).*" – In my opinion the second sentence is not adequate to scientific publication.

Jakub Dębowski

Study aims to check if inflorescences of invasive species are more attractive for potential pollinators than flowers of native species. The whole topic seems to be novelty and interesting. General question is corresponding with important and popular nowadays problem of invasive organisms. Very positive aspect of this paper is broad reference in literature and widely presented background. Main failure of this study is lack of prepositions and spaces in the text. There are some minor grammatical or stylistic mistakes.

Summary

This part is providing short introduction and presentation of a topic. The whole paragraph seems to long because it contains to many conclusions from discussion section although the language used in text is good and clear.

Introduction

In introduction authors suggest that pollination lead to the most important factor for invasiveness – generative reproduction. Examples from many different invasive plants show us that more effective form of fast expansion is when plant is using vegetative reproduction. This make a huge difference between kingdom of animals and kingdom of plants. Reynoutria japonica is a invasive plant that settle in similar habitats. Its factor of invasiveness is high but Reynoutria never produce seeds in European conditions. Therefore fight with this species is harder due to its possibility to recreate a whole plant from scratch.

Materials and methods

Everything in this section is clear except one. Why authors measured the size of inflorescences? It was not explained how they are going to use those measurements. Except this, section seems to be well organized and precise.

Results

Results were presented on graphs with proper subtitles and explanation. Text maybe to much shattered and because of that less readable.

Discussion

Authors conclude that they gathered to small data for better analysis. Maybe there were to many factors and different

Magdalena Lenda

The authors studied a very interesting problem: how invasive alien flowering plant species affect pollinators community and their behavior in comparison with native plants. Studies like this may deliver very important knowledge about ecology of invasive species, in particular about direct and indirect competition between invasive and native species. In most of studies where scientists investigated how invasive species influence pollinators, different genera of plants were compared. The advantage of this study is that invasive species and native counterparts belong to the same plant genus. In my opinion authors should underline this more in the introduction. The study was made during one day, without help of specialist who could identify pollinating species and that was a challenge, because flies, bees and butterflies are sometimes very difficult to identify. However, authors cleverly classified pollinating insects according to their morphology, which helped them to estimate and compare the (functional) diversity of insects visiting plant species. The idea to test if size of inflorescences affects the visiting rate is very good, maybe it is worth to add this as a one of hypotheses.

Although the general idea of authors was very good, I see some weak points of the study and report. They should be addressed in the revised text, discussed in the report or changed in the methodology if authors would like to continue this study.

1. In the introduction authors should write 2-3 sentences describing the theoretical background why they expect different effect of invasive and native plant species belonging to the same genus.

2. Authors did not take into consideration the location of plants on the meadow what is important to control for example the edge effect (the number and variety of insects can be different at the edge and in the inside, or for the part of the meadow that is close to the road or a forest). This, as well as other methodological shortcomings should be mentioned and discussed in the special paragraph of the discussion e.g. "constrains of the study", to show that authors are aware of potential confounding variables.

3. Statistical analysis also could have been better. Authors stated that they used GLM (the abbreviation should be explained anyway) to estimate relative effects of inflorescence size

and plant species. In my opinion this test is meaningless. Generally, it is known from the literature that inflorescences of *S. canadensis* are larger than that of *S. virgaurea*. Thus, the size of inflorescences is redundant with the effect of species. The interaction between species and size of inflorescences might have been introduced in GLM to partially cope with this problem.

Authors also did not explain why they used paired t-tests? How observation on both species were connected in pairs?

4. Second problem with interpretation of these results is that pollinator visitation rate may be dependent on density of other flowers around the observed inflorescences. Optimal foraging theory states that number of visits should be larger but foraging time shorter in locations with large density of food resources (inflorescences). It seems authors did not take density of other flowers into account in this analysis. Thus, ideal study should be an experiment where confounding factors are controlled. For example, authors might have cut inflorescences of two goldenrod species, put them into bottles and place in part of the meadow with grass (and no other flowering plants) and observe pollinator visitation rate. Also, authors should choose inflorescences of similar size to find out if nectar amount or flower features plays a role in the observed pattern of pollinator visitation rate.

5. In my opinion the effect of the inflorescences size can not be tested in the way Authors did, because they did not measure other important plants' features such as amount of nectar and pollen. These features are usually different among different species, and there is no clear relationship between size of inflorescences and amount of nectar or pollen (both are costly to produce). Thus, if authors do not control for the effect of the amount of potential food produced by plants, they can not say if the size of flowers is the main factor that attracts pollinators. However, the idea to check it was very good.

6. Another problem with this study is that it was performed only in one meadow. In field studies good replicates are crucial for receiving meaningful estimates because many ecological processes undergo at large spatial scales. This is especially true for pollination processes. Observations of pollinators visiting flowers should be performed on several (a few) meadows to show that the observed pattern in visitation rate is general rather than site-specific. This should also be discussed in the report.

Raport - wersja ostateczna

Invasive species are more attractive for pollinators. A case study on two species of *Solidago*

Katarzyna Janas, Paulina Kosztyła, Bartłomiej Zając,

Institute of Environmental Sciences, Jagiellonian University

Summary

One of the biggest difficulties in maintaining the global biodiversity is a problem of invasive species. Dozens of studies have been devoted to this subject, especially to explain success of invasive plants in their new habitats. We already know many mechanisms allowing them to supersede local species, for example by allelopathy, faster growth or bigger resistance to environmental stressors. Nevertheless, we still do not understand all aspects of competition for pollinators, which might be crucial for their success. Here we show that inflorescences of invasive *Solidago canadensis* are more attractive for pollinators (bigger number of visitors and wider range of pollinators) than those of native *Solidago virgaurea*. This result can be explained by the difference in size of inflorescencesof *S. canadensis*, which are significantly bigger than those of *S. virgaurea*, what help them to attract higher amount, and wider variety of pollinators. Although we did not managed to show the same pattern for the duration of pollinators visit, we think that bigger inflorescence is an important element of their successful strategy which allowed them to colony so many habitats. This is another step to understand not only success of *S. canadensis*, but also other flowering invasive species, and thus it might be important for creating strategies counteracting their uncontrolled dispersal.

Introduction

The spread of invasive plant species is currently one of the most serious threats to biodiversity of plants and their pollinators (Moroń et al., 2009). There are many mechanisms proposed to explain the advantage of invasive species over native, including: allelopathy (Butcko and Jensen, 2002), faster growth (Fenesi et al., 2015), lower habitat selectivity and higher resistance to environmental condition (Weber et al., 2008). However, still little is known about the competition for pollinators between invasive and native species of plants. Pollination is a key factor, allowing plants for generative reproduction and in greater evolutionary scale to adapt to local conditions. Therefore, elucidating the mechanisms of competition for pollinators can shed light on the reasons of their unprecedented success.

Here, we attempt to assess whether invasive plants are more attractive to pollinators than the native species. In our research we will try to answer the following questions:

1) Are flowers of invasive species more frequently visited by pollinators?

2) Is the duration of visit of pollinators on flowers of invasive plants longer than on native species?

3) Is the diversity of pollinators visiting the flowers higher on invasive plants than on local species?

One of the most expansive invasive species in Europe (including Poland) originating from North America, is the Canadian goldenrod (*Solidago canadensis*). It supersede closely related, native species of goldenrod (*Solidago virgaurea*). Here we take advantage of their coexistence in one habitat, which allows us to test our hypothesis in natural rather than artificially created conditions. Thus, our predictions are that *S. canadensis*, due to more attractive inflorescences, will lure pollinators more efficiently than *S. virgaurea*.

Materials and methods

Study area

Study was performed in Łazy, small village situated near Bochnia town, Lesser Poland Voivodeship (49.96^{0}N, 20.49^{0}E, Fig. 1). Fieldwork was conducted near Field Research Station of the Institute of Geography and Spatial Management, Jagiellonian University, on meadow placed on southern slope of small hill, where both species occur (Fig. 2).

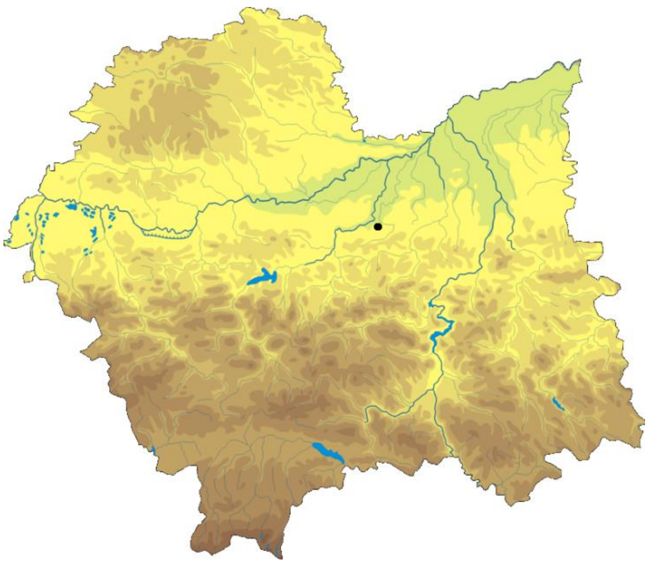

Figure 1. Localization of Łazy on Lesser Poland Voivodeship map.

Figure 2. Satellite image of fieldwork area.

Fieldwork protocol

Fieldwork was conducted 01.09.2015 between 8 and 12 AM and 04.09.2015 between 16 and 17 PM. During the first day atmospheric conditions changed gradually from clear sky and 20°C to full overcast, strong wind and temperatures under the 15°C. On the second day the weather was stable, with temperature around 18°C.

To check if *S. canadensis* inflorescences are more attractive to pollinators than those of *S. virgaurea*, we performed observations, during which we collected data of three different parameters. To exclude influence of changing atmospheric conditions, observations were performed simultaneously for pair of randomly chosen individuals of each species. Every observation lasted 10 minutes. After end of observation period, plant was tagged with small sheet of white paper to avoid using same specimen more than one time. We used surface of flattened inflorescence as indicator of its size, using height and width as diagonals of rhombus, which shape is resembled by flattened inflorescence. A total number of 30 plant (15 of each species) have been used in experiment.

To avoid observer bias, we conducted observation in rotation system – two researchers worked with *S. canadensis* – one observed plant and second noted observed pollinators and duration of visits. Third researcher worked on both of this tasks with *S. virgaurea*, as its inflorescences were smaller and easier to observe. In each pair, observation were conducted with one of three possible researcher combination.

Collected parameters were:

- number of visits of pollinating insects on inflorescence during time of observation,

- duration of visit on inflorescence of each specimen of pollinator (in seconds),

- number of morpho-species identified on each inflorescence during time of observation.

We are convinced that number of visits is a good indicator of visual attractiveness of inflorescence to pollinators. We are aware that some individuals might return to the inflorescence many times, but nevertheless in this case we are specifically interested in number of visits, not number of individuals visiting inflorescences. We treated time spent on inflorescence as an indicator of nectar attractiveness.

We used morpho-species (defined as group of organisms sharing morphological similarities, that are not possible to differentiate during short eye-sight observation by non-specialist) instead of exact identification to species level because simplicity of this approach allowed us to quickly asses the diversity of possible pollinator types of each species.

Statistical analysis

We used paired samples t-test to check if number and duration of pollinators visit and number of pollinators morpho-species differ significantly between two the species of *Solidago*. In each case we performed Shapiro-Wilk test to check if differences have normal distribution and log-transformed the data in necessary. To check if the size of inflorescence has stronger effect on results than species of plant, we ran two General Linear Models (GLM) with number of visits and number of morphospecies as a dependent variable and *Solidago* species as a categorical variable and size of inflorescence as a continuous variable. In both cases we had to perform transformation (log(variable+1)) to obtain normal distribution. All statistical analyses were performed using Statistica 10 software.

Results

Mean numbers of visits on inflorescence for *S. canadensis* and *S. virgaurea*, were 6.3 and 2.1 respectively and the number of visits was significantly higher for *S. canadensis* (t=3.600, df=14, p=0.003; Fig. 3).

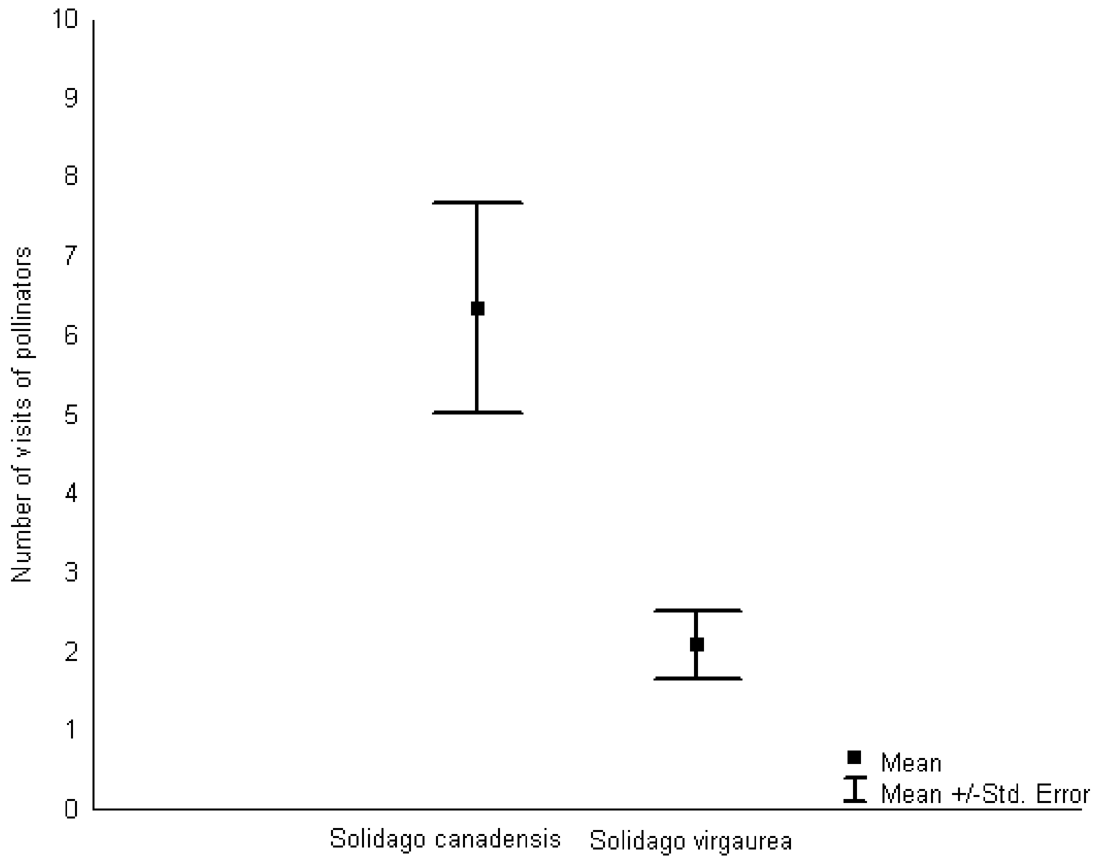

Figure 3. Mean value and standard error for number of pollinator visits in *S. canadensis* and *S. virgaurea*.

There were no differences between mean log-transformed duration of visits of pollinators (t=0.861, df=14, p=0.403; Fig. 4).

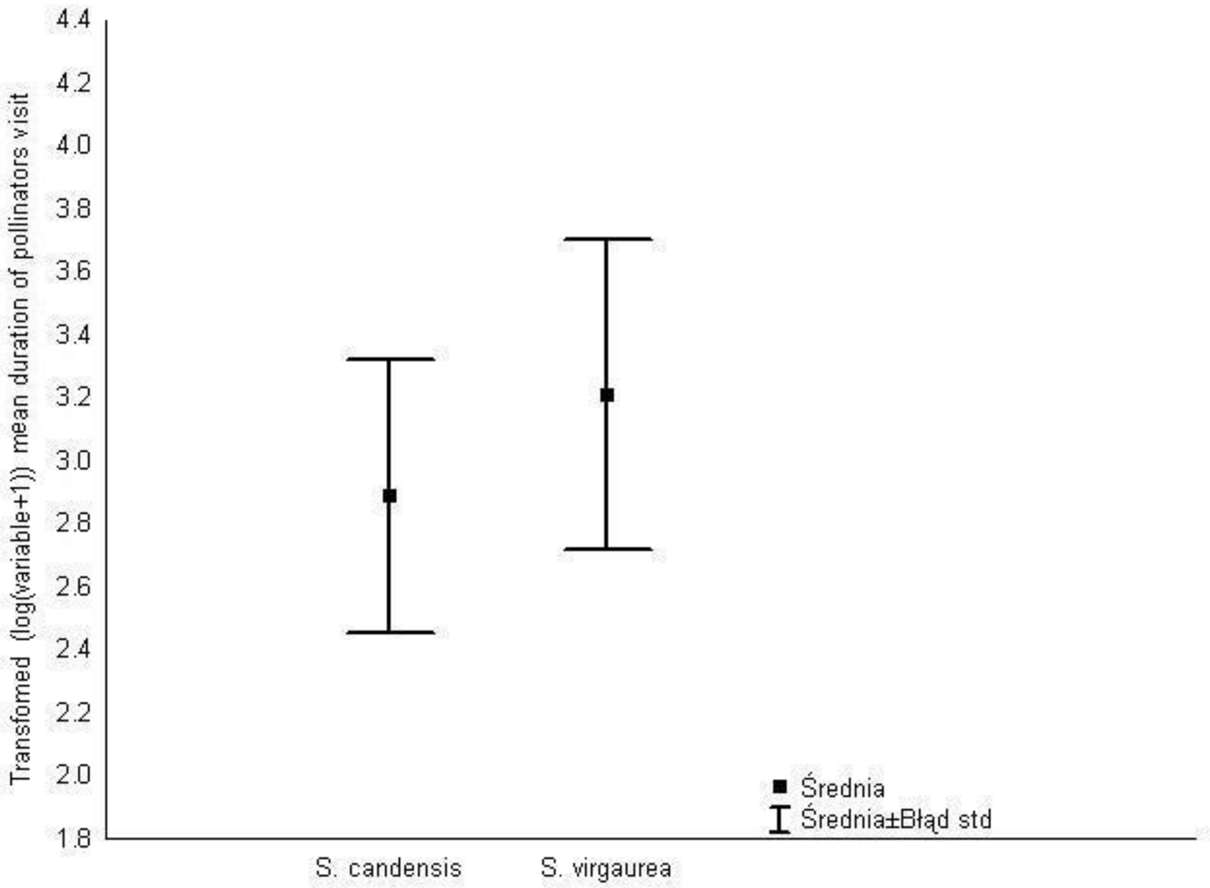

Figure 4. Mean value and standard error for pollinator transformed (log(duration[s]+1)visit duration [s] in *S. canadensis* and *S. virgaurea*.

In total, we observed eleven morphospecies of pollinators (honey bee, wasp, big hoverfly, medium hoverfly, small hoverfly, thin fly, small thin fly, metallic green fly, big fly, medium fly, small fly), one of which was exclusive to *S. canadensis*, and three were exclusive to *S. virgaurea*.

The total number of morphospecies was higher for *S. virgaurea* (10) than *S. canadensis (*8) and mean number (in *S. canadensis* was 2.9 and in *S. virgaurea* 1.7) differed significantly between the study species (t=2.415 , df=14, p=0.030; Fig. 5).

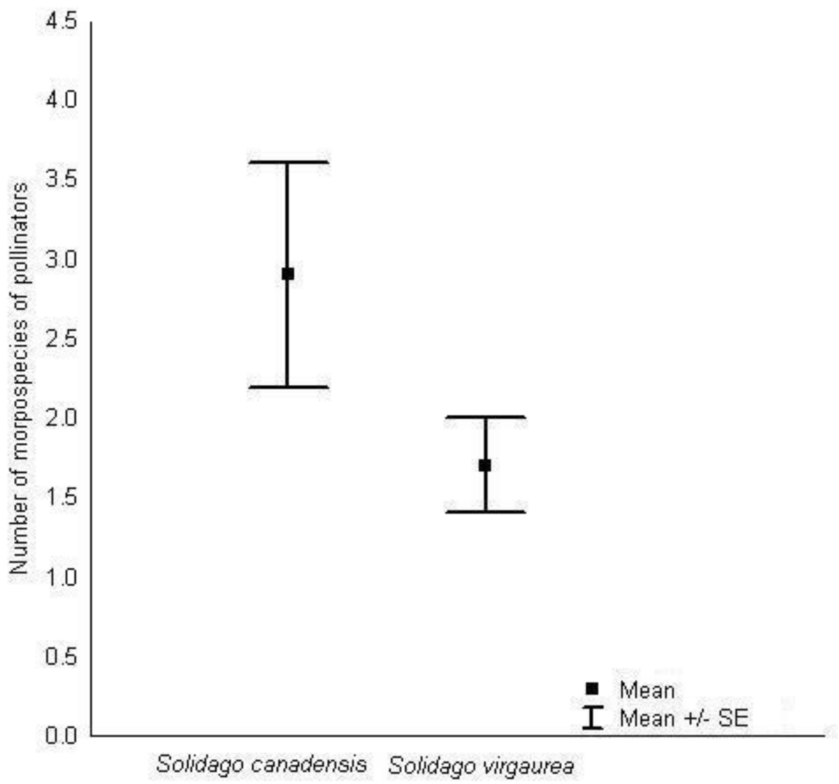

Figure 5. Mean value and standard error for number of pollinator morphospecies in *S. canadensis* and *S. virgaurea*.

Finally we have performed GLMto control if size of inflorescences does not affect the number and variety of pollinators visits stronger than the *Solidago* species. Model for number of pollinators considering interaction was insignificant, so we removed interaction from the model and obtain significant result ($F_{1,27}$=5.501, p=0.027) for size of inflorescences but not for the species ($F_{1,27}$=0.022, p=0.882). In another GLM, for variety of morphospecies, we also removed insignificant interaction, and obtained ($F_{1,27}$=10.213, p=0.003) for size of inflorescences and ($F_{1,27}$=1.605, p=0.216) for species of *Solidago*.

Discussion

We demonstrated that inflorescences of *S. canadensis* were visited by bigger number and wider variety of pollinators than those of *S. virgaurea*. However, we did not find similar pattern for duration of pollinator's visits. After checking the influence of size of inflorescences on visit frequency and variety of pollinators, it occurred that it is more important factor affecting attractiveness to pollinators than taxonomic identity. According to this result, the size of inflorescences is a dominant factor deciding about attractiveness of *S. canadensis* for pollinators

Result of investigation conducted on similar problem, and performed on plants from the genus *Lythrum* shown that invasive species decreased number of pollinators visits and seed production of related native species (Brown et al., 2002). Similar results were obtained also in other studies performed on purple loosestrife and impatiens glandulifera (Grabas and Lavert, 1999, Chittka and Schurkens, 2003).

In contrast to our study, Carrion-Tacuri et al., in 2014, showed that on Galapagos islands, pollinators remained longer on flowers of invasive species of the genus *Lantana*, than on native relative. In our study we did not managed to show such relation. It might be caused by differences in size and details of flower anatomy as well as nectar composition and its abundance.

We are aware that our experimental design have some constraints. For example, we had only one study site, and we had performed experiments during only two days at the beginning of September, so we did not cover full seasonal variety of pollinators. By conducting experiment with larger number of individuals we would be able to obtain more reliable results, nevertheless we still manage to show significant relations described above.

Our research, conducted on plants from genus *Solidago*, combined with results of different investigation performed on other plants, might contribute to the understanding the advantage of invasive species over the native competitors. This knowledge may be useful in preparing efficient strategies of mitigating biodiversity loss and habitat changes caused by invasive species.

Acknowledgements

We would like to thank Elżbieta Jędrzejczak for the inspiration, Joanna Rutkowska for useful comments on statistics and writing and Jakub Dębowski for his help and assistance during field part of the study.

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