NICHE PARTITION OF TWO INVASIVE INSECT SPECIES, *PARECTOPA ROBINIELLA* (LEPIDOPTERA; GRACILLARIIDAE) AND *PHYLLONORYCTER ROBINIELLA* (CLEM.) (LEPIDOPTERA: GRACILLARIIDAE)

PARTIȚIA NIȘEI LA DOUĂ SPECII INVAZIVE, *PARECTOPA ROBINIELLA* (LEPIDOPTERA; GRACILLARIIDAE) ŞI *PHYLLONORYCTER ROBINIELLA* (CLEM.) (LEPIDOPTERA: GRACILLARIIDAE)

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Abstract: *Invasive organisms are among major threats to biodiversity. Insects, due to their mobility and short life cycle represent a vast category of expanding organisms. The black locust (Robinia pseudacacia) is also an alien species to Europe but due to the relatively long history of naturalization, it is integrated in most of central Europe landscapes. A process of natural enemy acquisitions is taking place and two new pests affect stands, hedgerows and windbreaks where R. pseudacacia vegetates: Parectopa robiniella and Phyllonorycter robiniella (Lepidoptera Gracillariidae). A heavy infestation mostly with the newly introduced Phyllonorycter robiniella was observed in western and central Romania during 2003-2004. In many cases, leaves presented also mines of Parectopa robiniella. The mines of Phyllonorycter robiniella were attacked by several natural enemies: birds predated pupae, Beauveria bassiana was isolated from mines where larvae mortality was reported and also pupae of parasitoids where found in several cases. Having the same target, the foliage of the host and being members of the same guild, mining insects utilize the same niche. The study is interested in separating the niche components, mostly spatial of the two species based on the analysis of the results of C score of association. The shape of mines and their extension on the leaflet blade were assessed using image analysis.*

Rezumat*: Organismele invazive sunt printre ameninţările majore la adresa biodiversităţii. Insectele, datorită mobilităţii şi ciclului scurt de viaţă reprezintă o categorie vastă de organisme aflate în expansiune. Salcâmul (Robina pseudacacia) este de asemenea o specie exotică pentru Europa dar datorită istoriei îndelungate de naturalizare, s-a integrat in cele mai multe din peisajele central europene. Un proces de achiziţie de duşmani naturali are loc in prezent şi doi noi dăunători afectează arboretele, aliniamentele şi perdelele forestiere în care vegetează R. pseudacacia: Parectopa robiniella şi Phyllonorycte robiniella (Lepidoptera:Gracillariidae). Între anii 2003-2004 a fost observată o infestare puternică în vestul şi centrul României cu specia nou introdusă, Phyllonorycter robiniella. În multe cazuri, frunzele prezentau şi mine de Paretopa robiniella. Minele de Phyllonorycter robiniella au fost atacate de câţiva duşmani naturali: păsările au prădat minele, Beauveria bassiana a fost izolată din din mine în care a fost observată mortalitatea larvelor şi de asemenea, pupe de parazitoizi au fost găsite în câteva cazuri. Având aceeaşi ţintă, foliajul plantei gazdă şi fiind membrii ai aceleaşi bresle, insectele miniere utilizează aceeaşi nişă. Studiul este interesat de separarea componentelor nişei spaţiale a celor două specii pe baza analizei rezultatelor aplicării testului C de asociere. Forma minelor şi extinderea pe foliole a fost determinată prin analiză de imagine.*

Key words*: Robinia pseudacacia, Parectopa robiniella, Phyllonorycter robiniella, niche partition, co-occurrence, C-score, Pianka index, shape index.*
Cuvinte cheie*: Robinia pseudacacia, Parectopa robiniella, Phyllonorycter robiniella, partiţia nişei, co-prezenţă,indicele C, indicele Pianka, indice de formă.*

INTRODUCTION

One of central concepts in ecology, the niche is still a battle field for debates. Behind the idea of species assemblages is the presumed connection between their abundances and their functional role in the community is the way they interact (SUGIHARA et al., 2003). The classical idea of competition as driving force of limiting the hyper volume of fundamental niche to a lesser extent of the realized niche was contested from the position of the neutral model (HUBBELL, 2001). However, most of experimental and observational facts do not confirm the assumption of ecological equivalence of the species which form neutral communities predicted by stochastic processes that are not influenced by species identity but may be influenced by priority effects and dispersal limitations (GILBERT at al., 2008). The coexistence of species with similar demands in what regards the resources and abiotic factors, otherwise potentially competing species is better assessed by niche partition or differentiation theory. Host plant-herbivore system as well as host plant-pathogen system represents one useful model for nutritional niche study and the way it is partitioned among several herbivores /pathogens. The same plant harbours several types of resources and habitats which are partitioned or alternatively, become the object of competition. The fundamental niche encompasses environmental favorability in terms of abiotic factors and plant susceptible to be attacked (AL-NAIMI et al., 2005). The resource partitioning decreases the like hood of interspecific competition among herbivorous insects, including leaf miners (KAGATA and OHGUSHI, 2001).

However, same plant may harbour many consumers, a fact interpreted by some authors as being the expression of food abundance which limits the competition (BEGON et al., 2006). As insect population grows, competition may appear.

Robinia pseudacacia L. is native to North America and was introduced to Europe in early 17 th century as an ornamental tree. It is considered as an invasive exotic species in Europe (RICE at al., 2004) although in several countries from central Europe including Romania it became an important landscape element being used in rehabilitation programs of degraded sites.

Phyllonorycter robiniella (CLEMENS, 1859) is one of the main pests of the black locust, a mining insect originating from North America. The species was first reported in Europe in 1983 at Basel in Switzerland (WHITEBREAD, 1989). Gradually it expanded over central Europe. *Parectopa robiniella* (Clemens, 1863) was first reported close to Milan International Airport in 1970 (WHITEBREAD, 1989). It is an oligophagous mining species feeding on black locust and originating from North America spreading over Europe during the last decades and causing important damages to cultivated *R. pseudacacia*. Both species were reported for Romania by Neţoiu and Tomescu (NEŢOIU and TOMESCU, 2006). *Ph. robiniella* and *P. robiniella* became established in Europe during the same period and occur at outbreak densities. These species apparently occur together but the mines of the first generation of *P. robiniella* appear a couple of weeks later than those of *Ph. robiniella* (IVINSKIS and ŘIMSAITÉ, 2008). Species with similar origin are characterized by dissimilar cycles: *Phyllonorycter robiniella* pupates inside the mine while *Parectopa robiniella* pupates in a cocoon, outside the mine.

In Romania, *Parectopa robiniella* was found for the first time in a black locust stand, on Danube strand at the border with Yugoslavia (NEŢOIU, 1994) and *Phyllonorycter robiniella* was first time observed in a black locust grove on the left strand of the river Olt, near Călimăneşti (NEŢOIU, 2003).

Population control is accomplished by autochthonous parasitoids (over 30 European species) entomopathogens and predators mainly birds and spiders. High parasitism rates were reported in Serbia, over 50% and Italy, over 60% (GIBOGINI et al., 1996; STOJANOVIČ and

MARCOVIĆ, 2005). Both species are attacked by same guild of parasitoids in Europe with a strong effect on larvae and pupae.

The species cause premature leaf drop and the reduction of photosynthetic active area of leaves. In central Europe *Ph. robiniella* develop 2-3 generations during a vegetation season (ŠEFROVA, 2002) and *P. robiniella* 2 generations.

MATERIAL AND METHODS

Collection sites; Leaves of *Robinia pseudacacia* were collected at random in packages of 25-50 leaves from different locations from central and eastern Transsylvania during the vegetation season (2003-2004).Those locations were: windshield plantation along the railway at Jebuc (Sălaj county) in September 2004, forest edge at Gilău (near the city of Cluj) during August 2003, recreational forest Hoia and hedgerows near Cluj during August 2004, hedgerows near the city of Sîngeorz-Băi (Bistrița county) during August 2004, Zimbru Forestry Production Unit, Forestry District Gurahonț (Arad county) during July 2003. All locations are placed in hilly landscape with broadleaved mixed forests and black locust patches disseminated near forests, along roads or railways. The main characteristic of all these locations is the existence of high connectivity due artificial corridors which are important for species' dispersion, namely for *Phyllonorycter robiniella* and *Parectopa robiniella*

Co-occurrence analysis; To compare co-occurrence patterns of the two mining microlepidopterans, C-score (Stone and Roberts, 1990) based on presence-absence data. To assess co-occurrence, the samples from and Cluj Zimbru were employed due to higher representation of *Parectopa robiniella* mines.

C-score measures the degree of co-occurrence, it quantifies the number of checkerboard unit that correspond to each pair of associations. It is also a measure of the extent to which species co-occur less frequent than randomly assembled (Sanders et al., 2003). For each species the number of checkerboard units is $(R_i\text{-}S)(R_j\text{-}S)$, where R_i is the number of occurrences of species I, R_j is the number of occurrences of species j and s is the number of sample plots, in our case of black locust leaflets. The C-score is the average number of checkerboard units for each species pair. If the index is large compared with null distribution, there is less segregation than expected by chance. If the index is small, there more aggregation of species than expected.

To compare the results of observed data with the results of randomization test we calculated also the standardized effect size (SES). SES measures the number of standard deviations that the observed index is above or below the mean index of the simulated data (randomized). We used the standard deviation of 5000 simulated indices to calculate SES according to the equation: $SES=(I_{obs}\text{-}I_{sim})/S_{sim}$. Assuming a normal distribution, SES values fall between -2 and 2 (below -2 suggesting aggregation, between -2 and 2 random co-occurrence and above 2, segregated occurrence of the species).

The calculations were conducted using ECOSIM software 7.0.

The assessment of the microfungi inside the mines and parasitoids affecting the viability of the larvae; Mining insects are exposed to entomopathogenic fungi inside the mines and interact with other fungal residents such as species coming from the phylloplane or endophytic species. We employed a qualitative isolation technique to assess the diversity of microfungi inside mines with feeding larvae.

Leaflets of mined leaves (the assessment was performed on leaves sampled at Cluj) were detached and serially washed in tap water, last washings in sterile water. The mines were opened with a sterile needle and fragments of leaflets' upper epidermis placed on Malt-Agar medium supplemented with streptomycin, in Petri dishes of 9 mm diameter. The developing colonies were subsequently cultured on Malt-Agar slants for identification.

Several leaflets with mines from Jebuc were placed in vials and watched for the emerging adults of *Phyllonorycter robiniella* and parasitoids. To assess mortality all mines were dissected and the larvae observed at the stereomicroscope.

The assessment of mine area: The area inside a mine quantifies in a precise manner the feeding performance of the larvae. In order to estimate the niches partition of the two species, mines were scanned at 600 dpi (Scanner Mustek 1200 CP) and digitally measured in order to estimate the area occupied by mines and the proportion of leaflet area occupied by mines. Samples from Cluj and Zimbru were used to assess mine areas and other morphometric measurements such as shape index (areas divided by perimeters) and elongation (long axis divided by short axis, if the value is close to 1, the object is close to circular shape). Shape index and elongation are shape descriptors and they reflect the differences in shape between the two types of mines. Shape index describes the complexity of the outline while elongation describes the departure from a circular shape (which is around 1).

Descriptive statistics were calculated using KyPlot software while shape analysis was performed using ImageTool software.

Niche overlap: The parameter niche overlap measures the degree to which two different species overlap in the use of a particular resource meaning how species partition the resource in the community. If overlap in niches is great they influence the population growth through interspecific competition. The equation proposed by MacArthur and Levins (MACARTHUR and LEVINS, 1967) initially for the calculation of α and β competition coefficients, was reconsidered by Pianka (Pianka, 1973) to estimate niche overlap.

$$O_{jk} = O_{kj} = \frac{\sum_{i}^{n} p_{ij} p_{ik}}{\sqrt{\sum {p_{ij}}^{2} \cdot {p_{ik}}^{k}}}$$

O_{jk} and O_{kj} represent the overlap of the species j on k and the symmetrical overlap of species k on species j, p_{ij} and p_{ik} represent the proportions of the resource I utilized by each species. The equation takes into account the situation of polyphagous consumers. This measure ranges from 0 (no overlap) to 1 (complete overlap). For monophagous consumers as in the case of *Phyllonorycter robiniella* and *Parectopa robiniella* consuming only black locust foliage, we considered each leaflet containing both types of mines as a distinct food resource.

The two proportions p_{ij} and p_{kj} correspond to the proportion of leaflet areas occupied by mines, considering the parenchyma from inside as the food resource.

RESULTS AND DISCUSSIONS

*Relative abundance of Parectopa robiniella and Phyllonorycter robiniella mines, fungal species isolated from mines and parasitoids emerged :*The average number of larvae per compound leaf was found to be 12,44±8.04, with a maximum of 31 mines per leaf and a minimum of 2 (data collected at Cluj). The average number of leaflets per compound leaf varied between locations: 15.82± 3.49 at Gilău, 14.29±4.11 at Cluj, 20.63±3.23 at Jebuc.

Table 1 summarizes the relative frequencies of mines corresponding to *P. robiniella* and *Ph. robiniella*. For samples collected from Cluj, those used for the digital assessment of mine areas contain both species of mining moths; however the table depicts the different situation of infestation produced only by *Ph. robiniella.*

The maximum number of *Ph. robiniella* larvae inside one mine we found to be 7 with an average of 1.38±0.87. There are citations of 8-12 caterpillars per mine [29]. We found an average number of 1.68±0.97 mines per leaflet of *Ph. robiniella* (data correspond to Cluj

sample). The most frequent number of mines per leaflet is 1 in few occasions, more than one mines of *Phyllonorycter robiniella* being found (the maximum in our observation was of 7 mines per leaflet).

Table 1.

Relative abundance of *Phylonorycter robiniella* and *Parectopa robiniella* mines on *Robinia pseudacacia* leaflets, at different locations during the vegetation season 2003-2004

	Parectopa robiniella			*Phyllonorycter robiniella*		
Location	Mean±SD	Min	Max	Mean±SD	Min	Max
Singeorz-Băi	0.14±0.08	0	0.35	0.01±0.03	0	0.75
FPU Zimbru	0.31±0.23	0	1.06	0.44±0.95	0	1
Gilău	0.75±1.36	0,1	10	0.03±0.14	0	1
Cluj	0.54±0.24	0.07	1	-	-	-
Jebuc	0.63±0.13	0.38	1.06	0.04±0.05	0.19	0.92

Fungal species isolated from mines were: *Beauveria bassiana, Verticillium lecani, Cladosporium cladosporioides, Penicillium sp, Aspergilus versicolor, Alternaria alternata.* Two species, *Beauveria bassiana* and *Verticillium lecani* are entomopathogenic, other species are saprotrophic inhabitants of the phylloplane. Direct observation at stereomicroscope put in evidence the development on cocoons and pupae of *Penicillium sp.* and *Aspergillus sp.* Microscopic observations revealed spores of *Cladosporium sp*. and *Alternaria sp*. clinging to the threads of cocoons.

At Singeorz-Băi in august 2004, 40% of the investigated mines presented active *Phyllonorycter robiniella* larvae, 43% of mines were destroyed by predators (birds and spiders), 6.6% of mines contained parasitoids: we identified adults of *Pholetesor sp*. (Braconidae), *Pediobius saulius* Walker, *Sympiesis sericeicornis* Nees and *Cirrospilus lyncus* Walker (Eulophidae).

In samples collected at Cluj in August 2004, 73% of larvae were alive. At Zimbru, in July 2003, 98% of *Phyllonorycter robiniella* larvae were alive. Some of the mines contained individuals from different developmental stages (larvae of different stages). Mortality was induced by parasitoids, spiders, birds and probably, entomopathogens as we observed the larvae which were inactive and have lost the body turgescence.

The assessment of mine areas: The analysis of scanned leaflets permitted the estimation of areas occupied by mines and the corresponding proportions.

Generally, the leaflet area occupied by mines is small, a greater proportion corresponding to *Phyllonorycter robiniella* mines observed in samples collected from Cluj, where the proportion was nearly 31% (table 2).

Table 2.

Mine areas and proportions of *Robinia pseudacacia* leaflets occupied by mines of *Parectopa robiniella* and *Phyllonorycter robiniella* at Cluj and FPU. Zimbru (Arad County) in July 2003

Location	Mean±SD leaflet area (cm^2)	Mean±SD mine area *Parectopa robiniella* (cm^2)	Mean±SD mine area *Phyllonorycter robiniella* (cm^2)	%area occupied by *Parectopa robiniella* mines (Mean±SD)	% occupied by *Phyllonorycter robiniela* mines (Mean±SD)
Cluj	7.18±2.02	0.14±0.10	1.25±0.73	2.27±2.09	30.97±15.55
FPU Zimbru	9.22±2.31	1.24±0.02	0.79±0.69	1.59±1.11	0.97±0.69

Shape descriptors as shape index and elongation are alternative quantifiers of taxonomic value. The shapes of mines are species-specific and are generally described in qualitative terms. *Phyllonorycter robiniella* mines are described as blotched, developing on the underside of the leaflet while mines of *Parectopa robiniella* develop on the upper side of the leaflet and are serpentine, amoeba-like mines (CSÓKA, 1997). In both species, mining activity

of the larvae avoid the midrib. Table 3 presents morphometric data on sampled mines of both species.

Values of average areas and perimeters are presented in table 3 to demonstrate how values of shape index were obtained. The more complicated the outline of the mine, the lesser is the index value (in comparable areas). In Cluj samples, *Ph robiniella* areas are relatively large compared to leaflet areas and *P. robiniella* areas, also fully developed. Instead, in FPU Zimbru samples, larger areas and longer outlines are observed at *P. robiniella.* The mines were fully developed, amoeba like, compared to those observed in Cluj samples where *P. robiniella* mines were simple, slightly serpentine and not fully developed, looking more as short galleries on the upper side of the leaflets.

Table 3

Morphometric data on mines of *Phyllonorycter robiniella* and *Parectopa robiniella* at Cluj and FPU. Zimbru (Arad county) in July 2003

Location	Shape index (mean±SD)		Elongation (mean±SD)	
	Ph .robiniella	*P. robiniella*	*Ph. robiniella*	*P. robiniella*
Cluj	0.21±0.06	0.05±0.02	2.57±1.18	3.39±1.92
FPU Zimbru	0.17±0.10	0.16±0.06	1.89±0.82	1.88±0.90
	Perimeters in cm (mean±SD)		Areas in cm^2 (mean±SD)	
Cluj	5.22±2.35	2.29±1.11	1.25±0.73	0.14±0.10
FPU Zimbru	3.62±1.77	6.69±3.08	0.79±0.69	1.24±0.68

Co-occurence estimation: The results of co-occurrence C-score test revealed a random association of *Ph. robiniella* and *P. robiniella.* C score at FPU Zimbru was 108 (0 variance) and the corresponding SES = 0 indicating random association the calculated C score at Singeorz-Băi was 39 (0 variance) and the corresponding SES=0 indicating also, random association. Taking into consideration that in many cases there were more than one mine of the same species on the same leaflet, mostly in *Phyllonorycter robiniella*, this observation confirmed earlier studies on the tendency of conspecific mines to occur on the same leaf [20].

Niche overlap: Considering each mine area proportion as a proxi for food resource, the calculation of the Pianka index for niche overlap of *Phyllonorycter robiniella* and *Parectopa robiniella* resulted in low index values, showing almost complete niche segregation or partition for both species: at Zimbru, the value was 0,003, at Cluj, the value was 0,009 (locations where the samples were analyzed also for mine areas and other morphometric measurements).

CONCLUSIONS

Co-occurrence of *Parectopa robiniella* and *Phyllonorycter robiniella* was relatively high only at FPU Zimbru, in July 2003 (see table 1) and one location at Cluj. Other locations were characterized by low infestations in case of *P. robiniella* but relatively high in the case of *Ph. robiniella*. We consider that one facilitative factor for pest outbreak in case of *Ph. robiniella* was the drought characterizing the summers of 2003 and 2004 considering also that both mining moth are thermo-xerophilous (CSÓKA, 1997).

Species interaction represents one important axis of the realized niche hyper volume. Despite the opinion that presented mining moths associate during the attacks, the co-occurrence analysis showed a random association of the two. The apparent association is merely due to the utilization of the same food plant species as others also observed (BULTMAN and FAETH, 1985).

The maximum infestation with *Ph. obiniella* was found at Jebuc, in September 63% but observations performed by other authors mention a level of 92.8% for autumn infestation (WOJCIECHOWICZ-ŻYTKO, and JANKOWSKA, 2004).

One of the most important components of the fundamental niche is the presence of the food plant for herbivorous insects. It is almost obvious that the spread of the species is facilitated by the proximity of roads and railways, *Robinia pseudacacia* is synanthropogenic species either cultivated, either spreading naturally, this mechanism of spread being mentioned also in other European countries (IVINSKIS and ŘIMSAITÉ, 2008). Being a non-native plant, *R. pseudacacia* is common along roadsides which are habitats of particular type; roads enhance the spread on long and short range of invasive plants (CHRISTEN and MATLACK, 2006) as well of pathogens and herbivorous insects.

The parasitoids emerged from *Ph. robiniella* mines are members of the parasitoid guild described by other authors in Europe comprising 19 species of native, European, generalist parasitoids associated with oaks and other native woody species of which we found four species, apparently most frequently encountered (MELIKA et al., 2006). Parasitism is considered a major mortality factor for mining insects (HAWKINS et al., 1997) but according to our data at time the study was carried out, the level of parasitism was low. Instead, during the autumn, preliminary observations revealed that predators, mostly birds reduced the populations of *Ph. robiniella*. However, parasitoids are in a process of insertion on newly introduced mining moths and are generating one of the realized niche dimension of these species. Entomopathogens penetrate the mines from leaflet surface where they are transient members of the phylloplane fungal communities. Saprotrophic fungal species usually isolated from leaf surfaces also penetrate the inner space of the mines, taking advantage of rich nutrients concentrated in larval excrements. *Phyllonorycter robiniella* is characterized by persistence of faeces inside the mines.

The parenchyma consumed by larvae of the two species is situated on opposite sides of the leaflets leading to spatial partition of the resource represented by the leaflet inside the mines. The position of mines is different on the leaflet blade, contributing to niche partition (SATO, 1991). The niche overlap is minimal and developing larvae do not interfere for food. It is likely to presume that competition for food appears inside same mine among cohabiting larvae. The differences in phenology of the two species also contribute to niche partition, a phenomenon observed in other mining species too (KAGATA et OHGUSHI, 2001).

Shape is a valuable morphometric descriptor largely used in taxonomy and eco-morphology. Shape index and elongation give a supplementary, numerical description of differences in what concerns shapes of mines. The longer the outline of the mine compared to a similar area, the smaller shape index is. Also elongation approximates in numerical terms nearly round to long and thin mines: for instance, blotched mines of *Phyllonorycter robiniella* are long and cover almost one half of the leaflet (in samples collected from Cluj), following the long axis of the leaflet. The shapes of mines are adapted to leaf morphology and feeding strategies. A preliminary observation shows that the presence of mines on *Robinia pseudacacia* leaflets induces a fluctuating asymmetry of the leaflets, a phenomenon also observed by other authors (CONNOR and TAVERNER, 1997).

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