PHOTOSYNTHETIC PIGMENTS (CHLOROPHYLL a, CHLOROPHYLL b) DINAMICS IN SILVER FIR TREES NEEDLES INFECTED BY MISTLETOE

DINAMICA PIGMENȚILOR ASIMILATORI (CLOROFILA a ȘI CLOROFILA b) ÎN ACELE ARBORILOR DE BRAD DIN DIFERITE CLASE DE PARAZITARE CU VÂSC

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Abstract. *In silver fir stands of Bucovina located on the eastern border of silver fir distribution area, the presence of a pathogen agent with an important role in fir decline - mistletoe (Viscum album ssp. Abietis) – has been noticed. Mistletoe infections are located in mature and aged stands wherein the proportion of silver fir exceeds 40%, located below the elevation of 700 meters. Our objective was to evaluate the intimate transformation that occurs in silver fir needles because of mistletoe infection. In order to assess the differences of photosynthetic pigments (chlorophyll a and b) in needles of trees from different infection classes we collected samples for analysis from three trees in each infection class. Photosynthetic pigments were extracted from needle material with 80% acetone. The amount of chlorophyll increases with needle age but decreases with infection degree. The chlorophyll a/ chlorophyll b ratio decreases from the healthy to the very infected trees..*
Key words: Chlorophyll a, chlorophyll b, silver fir, mistletoe, infection class

Rezumat. *In brădetele din Bucovina situate la limita estică a arealului de distribuție se semnalează prezența unui agent patogen cu un rol important în declinul bradului – vâscul. Atacurile de vâsc sunt localizate in arboretele mature in care proportia bradului depaşeşte 40%, situate la altitudini mai mici de 700 de metri. Cercetările efectuate urmăresc să surprindă transformările ce apar la nivelul aparatului foliar ca urmare a parazitării cu vâsc și vizează determinarea conținutului de pigmenți asimilatori (clorofilă a și clorofilă) precum și a raportului clorofilă a/ clorofilă b in acele brazilor din diferite clase de parazitare. Pigmentii asimilatori au fost extrasi din materialul foliar cu acetona 80%. Am concluzionat ca raportul clorofilă a/clorofilă b descreşte de la arborii sănătoşi la arborii puternic parazitați. In acelasi timp cantitatea de clorofilă creşte cu vârsta frunzelor, dar scade cu gradul de parazitare.*
Cuvinte cheie: Clorofila a, clorofila b, brad, vâsc, clase de parazitare

INTRODUCTION

Like in many European countries, coniferous forests in Romania are also declining (Barbu, 1991; Bîndiu, 1996). The silver fir stands of Bucovina are located on the eastern border of silver fir distribution area (Barbu et Barbu, 2005).

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The mistletoe infections are located in mature and aged stands wherein the proportion of silver fir exceeds 40%, located below the elevation of 700 meters. At altitudes higher than 700 m, the infection is incipient.

Studies and investigations regarding the transformation that occurred in needles of damaged firs are numerous (Ianculescu et Tissescu, 1992; Bačic et al., 2003; Peguero-Pina et al., 2007; Rigling et al., 2010). The main objective of this paper is to underline the intimate transformation that occurs in silver fir needles because of mistletoe infection. The research aimed the determination of photosynthetic pigments - chlorophyll a and chlorophyll b and chlorophyll a/ chlorophyll b ratio.

MATERIAL AND METHODS

We selected one stand mainly of silver fir trees in the Northern part of Romania in the forest district Solca (Eastern Carpathians). This stand site was selected because 70 % of the trees are affected by mistletoe attack.

The sample was made of silver fir trees gathered into infection classes using 4 class rating system (Barbu, 2009) (fig. 1). To determine whether there were differences of photosynthetic pigments in needles of trees from different infection classes, we colected samples for analysis from three trees in each infection class.

Each tree crown was devided in 4 sections (section 1 – upper third of the crown, section 2 – middle third, section 3 – lower third and section 4 – compensation crown made of epicormic branches). In each section, 5 needle samples were collected as follows: a sample of 1 year needles, a sample of 2 years needles, a sample of 3 years needles, a sample of 4 years needles, a sample of 5 years needles. In the end, a total of 240 needle samples were collected and analyzed (4 infection classes * 3 trees/class * 4 section/tree * 5 samples/section=240).

a b c d

Fig. 1 - Examples of trees from each infection class:
a) class 0 of infection; b) 1st class of infection; c) 2nd class of infection, d) 3rd class of infection

Photosynthetic pigments were extracted from needle material with 80% acetone by grinding in the presence of quartz sand (Arnon, 1949; Holden, 1965; Ianculescu et Tissescu, 1992, Porra, 2002; Bačic et al., 2003). For quantitative determination of chlorophyll a and b as well as of total chloroplyll formulas proposed by Porra (2002) were used:

Chlorophyll a (chl a) (µg/ml) = 12.25 ($A_{663,6}$) - 2.55 ($A_{646,6}$)
Chlorophyll b (chl b) (µg/ml) = 20.31 ($A_{646,6}$) - 4.91 ($A_{663,6}$)
Total chl (µg/ml) = 17.76 ($A_{646,6}$) + 7.34 ($A_{663,6}$)
$A_{646,6}$ and $A_{663,6}$ are the absorbances of the solution at 646,6 nm and 663,6 nm.

RESULTS AND DISCUSSIONS

In healthy trees, (class 0 of infection) total clorophyll in 1 to 5 years needles harvested from different parts of the crown varies between 13.888 µg/ml for 1 year needles of the compensation crown and 20.765 µg/ml in those of 4 years of middle third of the crown (fig. 2). The amount of chlorophyll a varies between 10.514 µg/ml for 1 year needles of the compensation crown and 15.882 µg/ml in those of 4 years for the middle third of the crown. Ralated to chlorophyll b, its amount varies between 3.377 µg/ml for 1 year needles of the compensation crown and 5.001 µg/ml for 3 years old needles of the upper third of the crown.

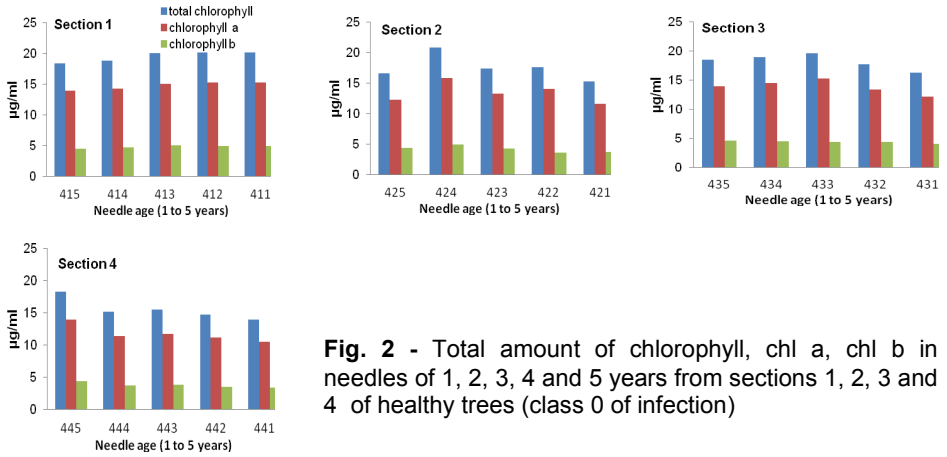

Fig. 2 - Total amount of chlorophyll, chl a, chl b in needles of 1, 2, 3, 4 and 5 years from sections 1, 2, 3 and 4 of healthy trees (class 0 of infection)

For trees of 1st infection class, (low infection) the total amount of chlorophyll in 1 to 5 years old needles harvested form different crown sections varies between 12.146 µg/ml - for 2 years needles of middle third of the crown – and 22.229 µg/ml – for 4 years needles from lower third of the crown (figure 3).

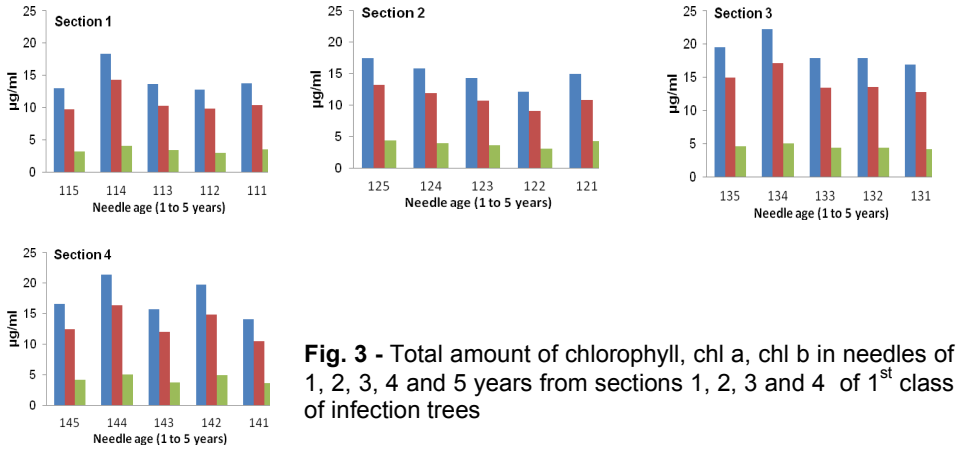

Fig. 3 - Total amount of chlorophyll, chl a, chl b in needles of 1, 2, 3, 4 and 5 years from sections 1, 2, 3 and 4 of 1st class of infection trees

The amount of chlorophyll a varies between 9.039 µg/ml for 2 years needles of the middle third of the crown and 17.169 µg/ml in those of 4 years of the lower third of the crown. Related to chlorophyll b, its amount varies between 2.958 µg/ml for 2 years needles of the upper third of the crown and 5.066 µg/ml for 4 years old needles of the lower third of the crown.

For trees of 2nd infection class (moderate infection) total amount of chlorophyll varies between 8.106 µg/ml - for 1 year needles of upper third of the crown – and 17.562 µg/ml – for 5 years needles of the compensation crown (figure 4). The amount of chlorophyll a varies between 5.970 µg/ml for 1 year needles of the upper third of the crown and 13.315 µg/ml in those of 5 years of the compensation crown.

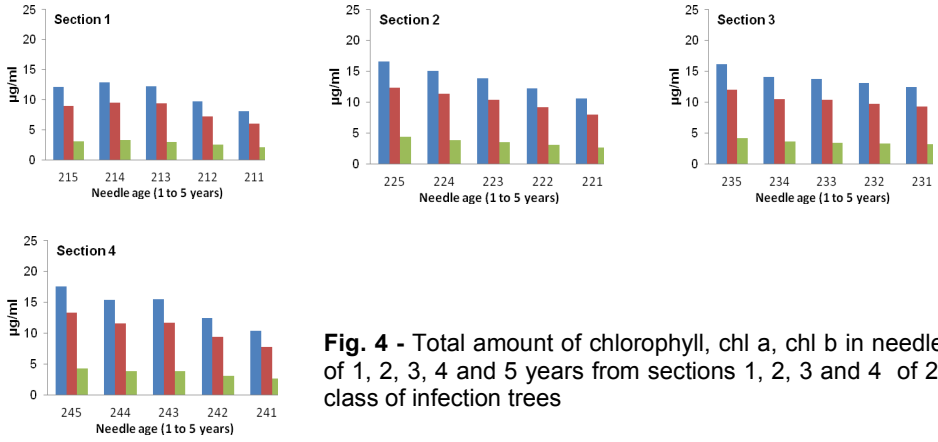

Fig. 4 - Total amount of chlorophyll, chl a, chl b in needles of 1, 2, 3, 4 and 5 years from sections 1, 2, 3 and 4 of 2nd class of infection trees

For trees of 3rd infection class (heavy infection) total amount of chlorophyll varies between 8.134 µg/ml - for 1 year needles of upper third of the crown – and 14.291 µg/ml – for 4 years needles of the compensation crown (figure 5).

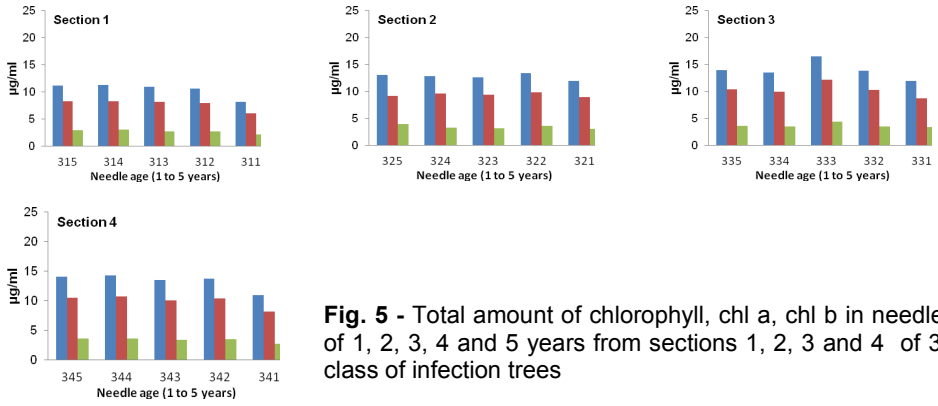

Fig. 5 - Total amount of chlorophyll, chl a, chl b in needles of 1, 2, 3, 4 and 5 years from sections 1, 2, 3 and 4 of 3rd class of infection trees

The amount of chlorophyll a varies between 6.049 µg/ml for 1-year needles of the upper third of the crown and 12.149 µg/ml in those of 3 years of the compensation crown. Related to chlorophyll b, its amount varies between 2.087 µg/ml for 1-year needles of the upper third of the crown and 4.352 µg/ml for 3 years old needles of the lower third of the crown.

Table 1

Photosinthetic pigment (Chl a and Chl b) concentration (µg/ml) in young and mature (1, 3 and 5 years) needles of silver firs from different infection classes

Needle age	class 0 of infection	1st class of infection	2nd class of infection	3rd class of infection
	Chlorophyll a (Chl a)			
1	12,40±1,78	11,08±1,12	11,08±1,12	7,94±1,78
3	13,81±1,42	11,61±1,43	11,61±1,43	9,56±0,47
5	13,50±0,71	13,10±2,16	13,10±2,16	9,94±1,34
	Chlorophyll b (Chl b)			
1	3,98±0,67	3,82±0,37	2,64±0,42	2,78±0,74
3	4,32±0,45	3,79±0,08	3,30±0,36	3,41±0,38
5	4,45±0,11	4,63±0,27	3,94±0,58	3,51±1,48
	Total chlorophyll			
1	16,39±2,46	14,95±1,22	10,39±1,79	10,54±1,79
3	18,14±1,86	15,41±1,60	10,44±1,31	12,53±2,35
5	17,95±0,76	18,51±3,10	15,60±2,09	13,07±1,31
	Chl a/Chl b			
1	3,11±0,06	2,87±0,21	2,93±0,10	2,86±0,1
3	3,19±0,20	3,05±0,08	3,06±0,11	2,92±0,08
5	3,03±0,13	3,28±0,27	2,95±0,13	2,73±1,26

Table 2

Photosinthetic pigment (Chl a and Chl b) loss (%) in silver fir needles of 1,3 and 5 years

Infection class	Chlorophyll					
	Chl a		Chl b		total	
	µg/ml	%	µg/ml	%	µg/ml	%
1 year needles						
Class 0	12,40	100	3,98	100	16,39	100
Class 1	11,08	89	3,82	96	14,95	91
Class 2	7,75	64	2,64	66	10,39	64
Class 3	7,94	62	2,78	70	10,54	63
3 years needles						
Class 0	13,81	100	4,32	100	18,14	100
Class 1	11,61	84	3,79	87	15,41	85
Class 2	10,44	76	3,30	76	10,44	69
Class 3	9,56	69	3,41	79	12,53	57
5 years needles						
Class 0	13,50	100	4,45	100	17,95	100
Class 1	13,10	97	4,63	-	18,51	-
Class 2	11,66	86	3,94	88	15,60	87
Class 3	9,94	73	3,51	79	13,07	73

The amout of chlorophyll increased with leaf age (Peguero-Pina et al., 2007; Bačic et al. 2003), but decrease with the degree of infection. Chlorophyll a/chlorophyll b ratio decreases from healthy trees (average of 3.1) to heavy infected trees (average 2.8) (tab. 1).

Decline of photosynthesis results in substantial reduction of growth. Most pronounced quantitative reduction of chlorophyll (-35-40 %) is observed at 1 and 3 years needles of moderate and heavy infected trees (class 2 and 3 of infection). For trees of 1st class of infection (low infection) quantitative reduction of chlorophyll is on average 10-15 % for 1 and 3 years old needles (tab. 2).

CONCLUSIONS

1. The chlorophyll a/chlorophyll b ratio decreases from the healthy to heavy infected trees.
2. Most pronounced quantitative reduction of chlorophyll is observed in needles of moderate and heavy infected trees (class 2 and 3 of infection).

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