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Some Peculiarities of the *Xanthium italicum* Moretti Germination Process

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Abstract

Fruit formations of *Xanthium italicum* were gathered from plants, sand and a river bank. They were put in germination conditions to observe how this process takes places and what the seedlings' growth rate is in the early stages, until the first true leaf appears. Data were processed by variance analysis; using the Duncan test, the correlations between germination and seedlings' growth in *X. italicum* and the place of sampling the anthodiums was highlighted. The fruit formations' harvesting site influences the germination process, the finding being statistically significant; between the length of hypocotyl and cotyledons there is a distinct correlation, statistically significant (r = 0.908) regardless of the place of harvest.

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Keywords: Xanthium italicum, fruit formations, germination, the collection site.

1. Introduction

Xanthium italicum Moretti is a species originating in North America, adventitious in S an SE Europe (Chirilă et al., 2002), with large dispersion and propagation capacity. It has been reported, since 1990, in China, where it became a quarantine weed: mathematical models based on the species' behavior in Europe, Asia and South America forecasted the possibility of it installing in most provinces where there are favorable conditions for its survival (Rui and FangHao, 2010).

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In Romania, the species is found on light textured soils, well supplied with water (mesophilic-mezohigrophilic), in meadows or irrigated land, in the steppes to oak floor (thermophilic-subtermophilic); is indifferent to the pH (euriacidophilic) but grows in areas well-stocked in nutrients, especially nitrogen (eutrophic, nitrophilic) (Ciocârlan et al., 2004; Ciocârlan, 2009; Sirbu et al., 2013). If in 1972 Anghel et al. cited the plant as being frequent in the South of Dobrogea and southern Moldova, and as rare in Transylvania, the 2002 map shows the species is expanding its area, namely in W, E central Transylvania and E Moldavia (Figure 1) (Chirilă et al., 2002).

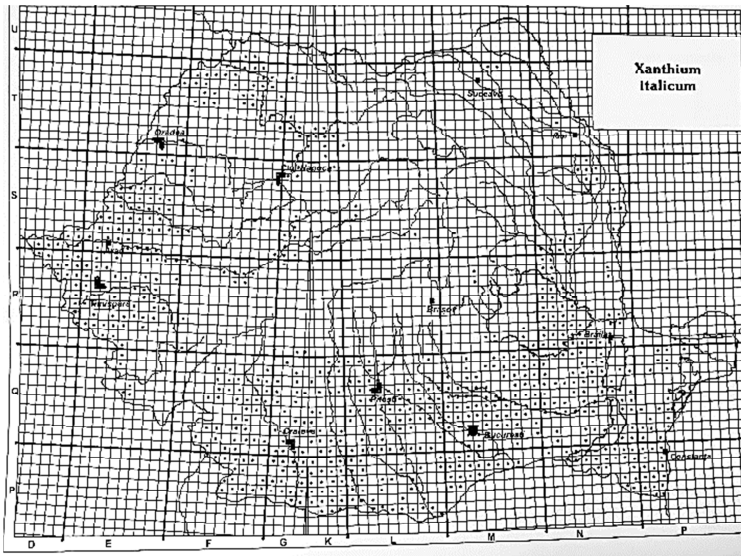

Fig. 1. Map of the *Xanthium italicum* Moretti specieis' spread (Chirilă et al., 2002).

The successful spread of the species is granted, inter alia, by morpho-anatomical peculiarities of fruit and mechanisms that determine staggered seed germination. In a thorny involucre there are two unequal achene, situated at different levels – the superior achene, smaller, germinates a year later thant the inferior one, which is larger.

The peculiarities of the germination, emergence and growth of seedlings have been extensively studied for the *Xanthium strumarium* L species. For germination, the seeds of lower achene require a rest period after ripening, although requirements vary among populations, and freshly harvested seeds can germinate in favorable conditions (Weawer & Lechowicz 1982). Staggered germination is induced by the two seeds' different skin permeability to oxygen and by embryo requirements: in the case of the lower seed, the embryo finds favorable growth conditions faster, requiring 0.6% oxygen, compared to 1.2% required by the higher embryo, at an average temperature of 21^{o}C (Champagnat, 1969; Weawer & Lechowicz, 1982).

Subsequent research showed that the higher seed's dormancy is also induced by a number of germination inhibitors (Porter and Wareing, 1974 cited by Weawer & Lechowicz, 1982). Co-germination of seeds can be stimulated by microbial decomposition, perforation of the seed's exterior coat, scarification using acids for 3-5 minutes or exposure to a temperature of 50^{o}C for 10 days (Kaul, 1965 cited by Weawer & Lechowicz, 1982). The percentage and germination rate, as well as the cotyledon leaves' active surface and the embryonic roots' length are positively correlated with the size of the fruit / seeds for the *X. strumarium* species (Zimmerman and Weis, 1983). In addition, the percentage of *X. strumarium* seedlings' emergence is not affected by the seeds' origin (from plants growing in natural habitat or in the urban ruderal one); although ruderal plants form larger fruit, the differences are not significant (Blais, Lechowicz, 1989).

Xanthium italicum Moretti species success in competition with other plants is also provided by the fruits' phytotoxic action: they contain xanthiosin, a lactozo-sesquiterpene that deeply affected plant growth in tested species - *Amaranthus mangostanus*, *Lactuca sativa*, *Triticum aestivum* or *Lolium multiflorum* (Shao and al., 2012).

This paper brings additional detail related to the process of germination and seedlings growth's specificities in the *Xanthium italicum* species.

2. Materials and Methods

Xanthium italicum's fruit formations were harvested from the Buzău river's bank, in the locality of Scorțarul Nou, Brăila County, in early March, 2013. Anthodiums (heads) were taken from plants and soil (sand and river bank) where they rested during winter.

In the laboratory, they were put to germinate in Petri dishes, each dish containing 5 anthodiums (Figure 2). The experiment was done in three variants, corresponding to the 3 places of the fruit formations' harvesting, each variant boasting three repetitions. The process of germination and seedlings' growth was followed, until the formation of the first true leaf. Another experiment tested individual capacity germination of achenes.

Anthodiums from the ground came from the vegetation period of the previous year.

They were removed from the thorny involucre and put to germinate in an incubator at 25°C (Figure 3). In both types of experiments substrate moisture was maintained through proper watering with distilled water.

Data were processed by variance analysis; using the Duncan test, the correlations between germination and seedlings' growth in *X. italicum* and the place of sampling the anthodiums was highlighted.

Fig. 2. *X. italicum* fruit formations in Petri dish.

Fig. 3. Free achene, put to germinate.

3. Results and Discussions

The germination of fruit formations took place in stages, starting on 20.03.2013, two days after the start of the experiment (Figure 4) and occurred only if the lower seeds, to over 90% of antodiums, regardless of their origin (Figure 5). This behavior is similar to that described in the literature for other species of the genus *Xanthium*.

Fig. 4. The start of fruit formations germination (20.03.2013).

Fig. 5. Formation of a single seedling on fruit formation.

Achenes from formations collected from the sand germinated in large numbers in the first part of the observation, after which the process stalled, the germination percentage being 40.5% for all fruit formations utilized for the three repetitions (Figure 6, Table 1).

For the achenes from the anthodiums harvested from the river's bank, the germination rate was in a relatively steady growth, and the proportion of anthodiums germinated on 08.04.2013 is 40.5, equal to that of the formations collected from the sand (Figure 6, Table 1). Achenes from fruit formations on the plant germinated at a rate lower throughout the observation interval, the final percentage being 34.5 (Figure 6, Table 1).

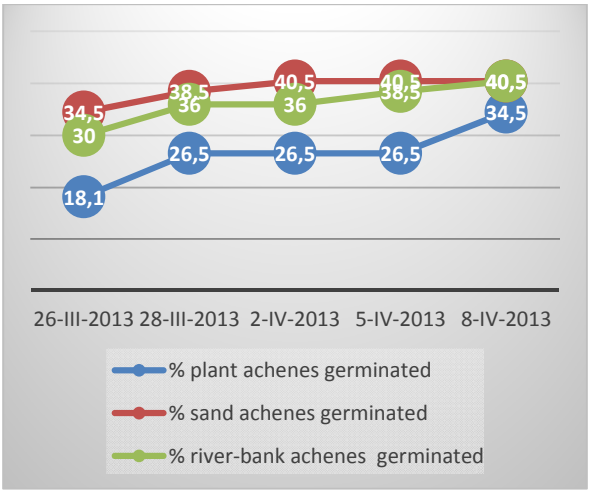

Fig. 6. Germination rythm of fruit formations depending on the place of sampling.

Table 1. The combined influence of the factors duration of germination (A factor) and anthodiums' place of harvest (B factor), on the germination percentage in *Xanthium italicum* Moretti.

A – duration of germination	B – Sampling site			Average A
	Plant	Sand	Terrace	
26-III-2013	18.1	34.5	30.0	c 27.53
28-III-2013	26.5	38.5	36.0	b 33.66
2-IV-2013	26.5	40.5	36.0	b 34.33
5-IV-2013	26.5	40.5	38.5	a 35.16
8-IV-2013	34.5	40.5	40.5	a 38.50
Average B	26.4 b	38.900 a	36.200 a	
DL	Pt A	Pt B		
5%	5.62*	7.72*		

The analysis through Duncan's test of different fruit formations' germination results, in correlation with the time and place of sampling observation, shows the following:

- Harvesting site of fruit formations influences the germination process, the finding being statistically significant (Table 1);
- The percentage of germination of harvested fruit formations from sand and river banks is statistically higher than in those taken from plants, being included in the control group (Table 1).

- Rapid germination of fruit formations collected from sand is due to the substrate favoring imbibing, sand yielding more easily water to the seeds, unlike wet or clay soils where there is competition between soil colloids and seed colloids (Champagnat, 1969).
- The germination percentage recorded for achenes from the plant indicates a lower degree of maturity due to their staggered appearance.
- The germination rate of 40.5% of fruit formations detached from the plant and placed on sand or river terrace constitutes the germination potential of achenes from the *Xanthium italicum* population selected for observation.

The germination process of achenes isolated in the thorny shell started 10 hours after placing them in the incubator. After 2 days, 90% of them were germinated, regardless of their size or the work version (Figure 7). The two achenes placed in the common involucre have equal potential to germinate.

The increase of the seedlings' hypocotyl from fruit formation harvested from plants occurred in the first period, reaching the maximum value of 77 mm (Figure 8, Table 2).

For seedling originationg from fruit formations detached from the plant, hypocotyl growth occurred consistently, the highest length being recorded for the anthodiums taken from the sand (Figure 8, Table 2).

Statistical analysis shows that the hypocotyl growth is significantly influenced by the site of harvest of the fruit formations (Table 2):

- The control group "a" anthodiums have been collected on the sand with hypocotyl length of 82.83 mm, compared to that harvested from the plant, which were classified in group "B" (hypocotyl length: 76.5 mm).
- Anthodiums harvested from the river bank were placed in Group 'C', with a value of 62.16 mm, representing 75.04% of hypocotyl length of anthodiums harvested from sand, or 81.25% of hypocotyl length of anthodiums harvested from plants.

Fig. 7. Achenes isolated germinated.

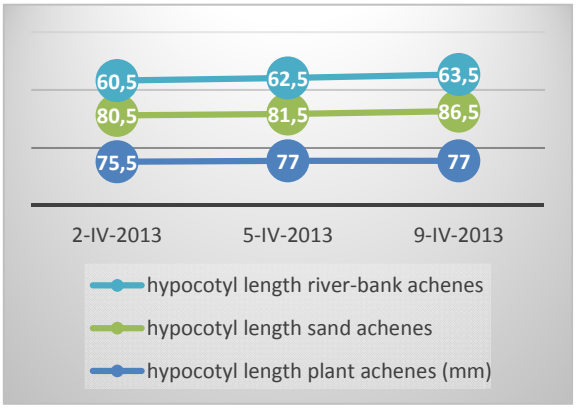

Fig. 8. Hypocotyl growth rythm depending on where sampling of fruit formations occured.

Table 2. Growth of seedlings' hypocotyl (mm) depending on the fruit formations'.

A – Germination duration	B – Place of anthodiums harvested			Average A
	Plant	Sand	Terrace	
2-IV-2013	75.5	80.5	60.5	b 72.1
5-IV-2013	77.0	81.5	62.5	a 73.6
9-IV-2013	77.0	86.5	63.5	a 75.6
Average B	76.50 b	82.83 a	62.16 c	
DL 5%	Pt A 2.45	Pt B 4.75		

Hypocotyl length in germinated seeds of formations fruit taken from sand is due, on the one hand, to the original habitat's characteristics (river's edge), and on the other hand to the sand's specificities as a substrate.

Growth of the seedlings' cotyledons, harvested from anthodium harvested on sand, took place until May-IV-2013 (for the first two determinations), after which a decrease in their length was noticed, due to their consumption of nutrients from seed reserve tissues. The process was the same for the seedlings grown from anthodiums taken from the plant (Table 3). For anthodiums from the river bank, seedlings' cotyledons had a steady growth all throughout the period analyzed (Table 3). The greatest length was reached by the cotyledons of seedlings from achenes originationg from fruit formations collected on sand (Table 3). Between the length of hypocotyl and cotyledons there is a distinct correlation, statistically significant (r = 0.908) regardless of the place of harvest (Figure 9).

Table 3. Influence of sampling site on the length of cotyledons from *Xanthium italicum*.

A – Germination duration	B – Place of anthodiums harvested			Average A
	Plant	Sand	Terrace	
2-IV-2013	37,000	43,500	31,400	a 37,300
5-IV-2013	39,500	49,000	35,000	a 41,167
9-IV-2013	38,500	47,500	36,833	a 40,944
Average B	38,333 b	46,667 a	34,411 c	
DL	Pt A	Pt B		
5%	4.5153	2.7891		

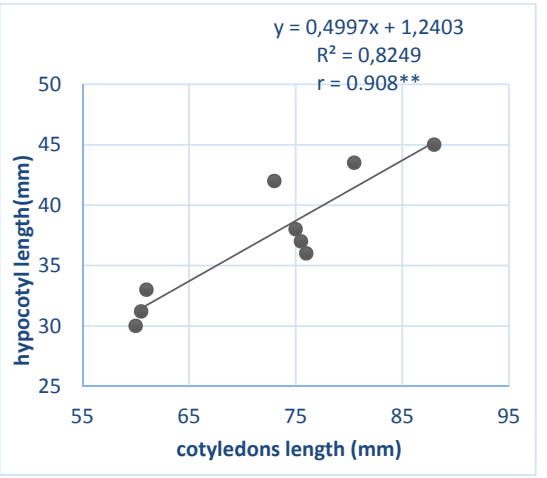

Fig. 9. Linear correlation between the hypocotyl length and the length of the cotyledons in *Xanthium italicum*.

4. Conclusions

The place of harvest of *Xanthium italicum* species fruit formations' directly influences the germination and growth of seedlings processes in the early stages.

For achenes collected from the sand, they germinated first and their hypocotyl and cotyledons recorded the highest lengths, which is explained by the original habitat of this species and soil preferences - riverbanks, coastal sands.

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