# PHYSIOLOGICAL ASPECTS OF *OROBANCHE* SPECIES IN THE SPONTANEOUS FLORA OF ROMANIA

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**ABSTRACT.** In contrast to the few weedy species most Orobanche species have remained wild flowers in the spontaneous flora. We have set up a research program considering occurrence and distribution of non-weedy Orobanche species in Romania, soil properties and climatical conditions for conditioning Orobanche seeds, germination stimulants exuded by the host plants, e.g. low or sufficient germination stimulants. The associated flora around each Orobanche spike is recorded and root exudates of the associated plants are tested for allelopathic effects, e.g. germination inhibition or inhibition of radicle exoenzymes, which are required for penetrating into the host root. We are also testing possible reasons for low viable seed production or loss of seed by insects or fungi. The results will contribute to understand ecosystems, in which non-weedy Orobanche species grow, they may deliver information, under which conditions Orobanche species may turn into weedy forms, when ecological conditions change. Moreover, they should provide a basis for fostering rare Orobanche species in Botanical Gardens for the future. Methodological approaches and observations in Romania during the past two years will be presented.

**Keywords:** *Orobanche*, Romanian habitats, germination, length of radiculae

## INTRODUCTION

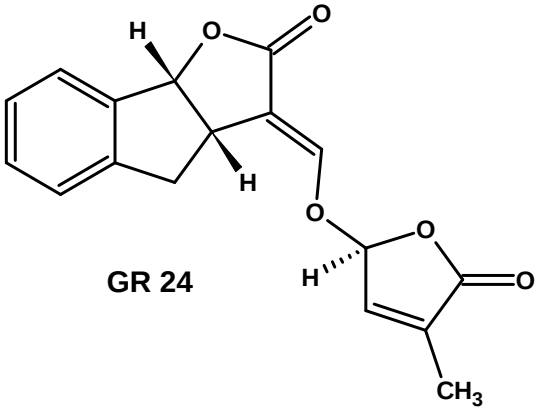
Parasites and their host plants are subjected to a complex system of chemical relations during their life cycles. As a rule parasites do not possess roots by which they can absorb water and mineral nutrients from the soil; instead they are connected by haustoria to the vascular transport systems of the host plant, from which they draw water and minerals, and also organic matter for their nutrition. For that reason it is biologically logic that parasites do only germinate if they have a chance for connecting to a host plant root. Germination is initiated by stimulants, which are exuded by the roots of host plants, but curiously by many non-host plants, too. As the seeds are tiny, the contents in their storage products allow the growth of the radicula only for a limited length, and supports its nutrition only for a short time. During that time the radicula must find and penetrate a host root to connect a haustorium to the vascular system of the host. From there on it lives on the account of the host plant, develops to mature state, produces large numbers of tiny seeds, which then rest in the soil for many years, until again a host root stimulates them for germination.

## MATERIALS AND METHODS

The seeds have been obtained from the Botanical Gardens of Macea and Iasi, and also from our own harvest in the observation fields. Seeds from Orobanche hederae were collected in the Botanical Garden of the Eberhard-Karls-University Tuebingen.

The germination was induced by root exudates of the host root, or with a synthetic analogue (GR 24) of strigol. GR 24 was kindly provided by Prof. Zwanenburg from the University of Nijmegen.

Root exudates were isolated from host plants in hydroculture, after shaking them in distilled water for two hours on a horizontal shaker.




**GR 24**

**$C_{17}H_{13}O_5$ Molecular weight = 297**
**1 M = 300 g / L**
**1 µM = 300 µg / L = 300 ppm**

Stock solution contained 3 mg GR 24 in 10 mL methanol, kept in the freezer. The stock solution was diluted 1:100 with Hoagland solution for germination experiments.

***Application protocol***

All working steps were carried out under a cleanbench. Solutions and glass ware were autoclaved 20 min at 120 oC (2 bar). After the experiment all

residues and glass ware were autoclaved 60 min at 120 oC and then discarded.

***Seed sterilisation***

If the seeds are contaminated with soil, plant or insect debris, the batch should be purified by sieving or flotation in water.

As a rule, surface sterilisation will be required, because the seed coat is contaminated by fungi and bacteria. The seeds are sterilised 10 min in a 1 % solution of calcium hypochlorite. For better wettening the addition of a drop Tween 20 (or Triton X-100) is advisable. The tube with the seeds can be put for 1 min into an ultrasound bath. After sterilisation the seeds were filtered off and washed with 500 ml sterile bidistilled water.

***Conditioning***

The sterile seeds are put on a filter paper in a Petri dish and wettened with bidistilled water. The seeds are supposed to stay wet but not to swim in the water. The lid of the Petri dish is tightened by a plastic stripe. The Petri dishes were kept in the dark in an incubator at 20 oC for 10-15 days.

***Germination stimulation***

An aliquot of the GR 24 stock solution is diluted with Hoagland solution. Final concentration is 3 ppm (= 3 mg L-1). 10 or 20 mL are sufficient for an experiment. Handling the small volumes of stock solution were carried out with a microliter syringe. Since GR 24 cannot be autoclaved, the aliquot was added to the Hoagland solution, then sterile filtered.

After the conditioning period the Petri dishes with the seeds were opened, and the Hoagland solution containing 3 ppm GR 24 was added. The Petri dishes were are again closed and sealed, and kept in the dark in the incubator at 20 oC.

Germination was observed under a binocular. Seeds were considered germinated, when the radicle is longer than the seed coat. *Orobanche ramosa* needs about 5-10 days for germination (Wegmann, 2006).

## RESULTS AND DISCUSSION

In contrast to the few weedy species most Orobanche spp. have remained wild flowers in the spontaneous flora. Most of them become rarer and belong to the endangered species. We have set up a research program on non-weedy Orobanche species in Romania (22 species known, 3 of them on the red list of Romania).

Areas of Orobanche occurrence have been searched. The finding sites have been recorded by GPS. Most studies were made in the Natural Reservation "Hill of Zakel" near Sibiu, in Covăsinţ near Arad and near Carei with the following species: Orobanche caryophyllacea, Orobanche arenaria, Orobanche teucrii, O. alba var. alba, f. communis , O. minor, O. gracilis, O. lutea. For comparison with a weedy species we used Orobanche ramosa from a tomato field in Macea, and from Orobanche hederae collected in the Botanical Garden of the Eberhard-Karls-University Tuebingen.

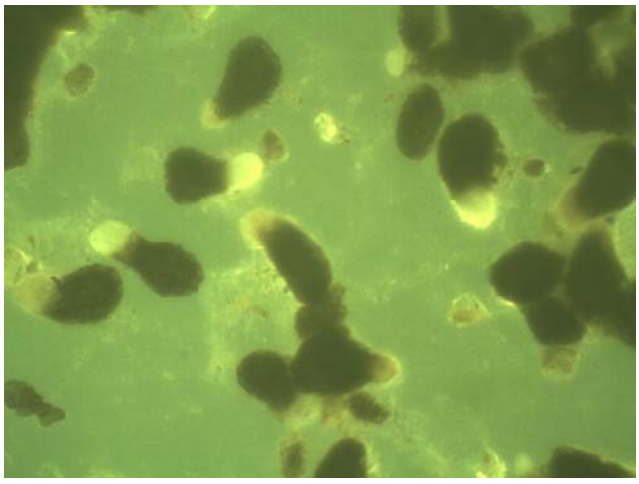


**Fig. 1** *O. arenaria* stimulated with root exudate of Artemisia campestris, after 13 days x140

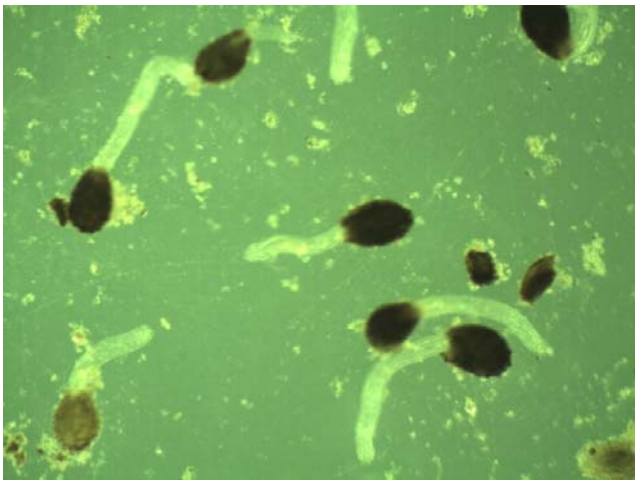


**Fig. 2** *O. ramosa* stimulated with GR24, after 9 days x45

Soil properties, climatical conditions and monthly actual weather dates were recorded and considered in respect to Orobanche seed conditioning. The weather data show that during the past years the precipitation in Spring, during the germination time, was lower than the optimal condions. This can be a reason for reducing the frequency of the endangered species. Germination stimulants exuded by the host plants, e.g. low or sufficient activity were studied. Germination studies were carried out under laboratory conditions.

The associated flora around each Orobanche spike was recorded. Root exudates of the associated plants were tested for allelopathic effects, e.g. germination inhibition or inhibition of radicle exoenzymes.

Possible reasons for low viable seed production or loss of seed by insects or fungi were investigated. We have observed serious damage of Orobanche plants by insects, however, this can not be the main reason for declining of rare Orobanche.

Orobanche ramosa seeds after stimulation need 5–10 days to germinate, while the seeds of wild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Orobanche spp. need much longer, generally 10–15 days.

Preliminary data show that the germination rates of the wild Orobanche species are distinctly lower than that of the weedy species.

Orobanche arenaria only develops short radiculae, not longer than the seed coat. In contrast the radiculae of O. ramosa reach up to the twenty-fold length of the seed coat. Therefore the probability for finding a host root for O. arenaria is very low.

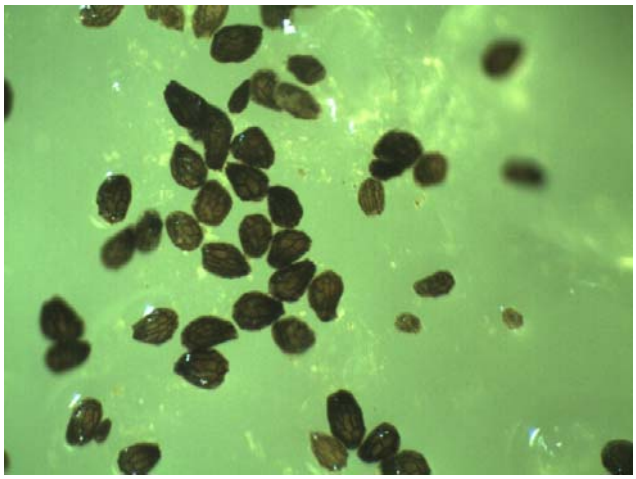

**Fig. 3** *O. hederae* stimulated with GR 24, after 7 days x36

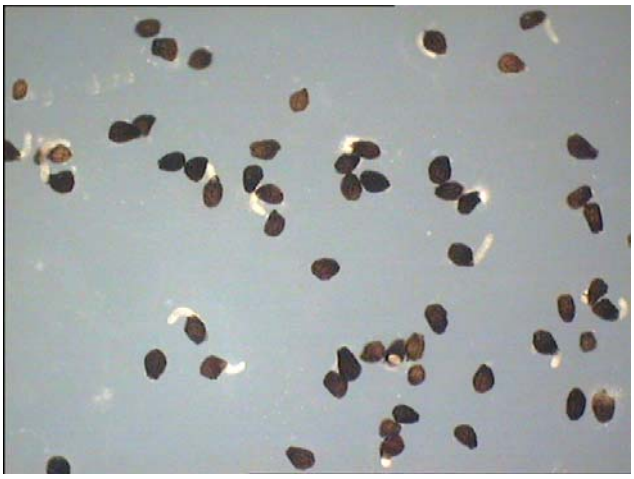

**Fig. 4** *O. hederae* stimulated with root exudate of *Hedera helix*, after 7 days x18

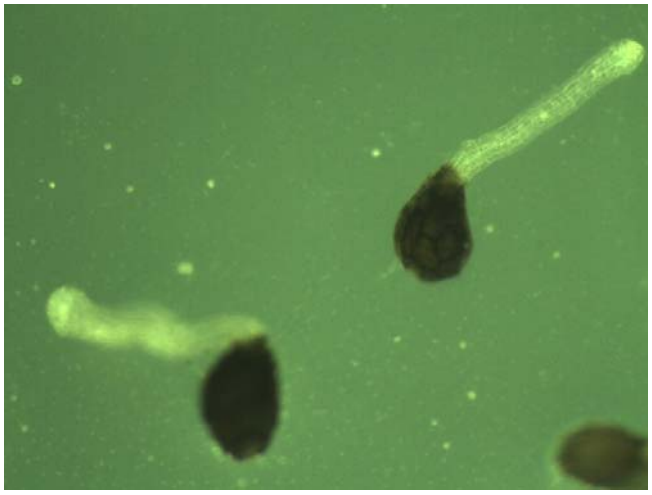

**Fig. 5** *O. hederae* stimulated with root exudate of Hedera helix, after 7 days x86

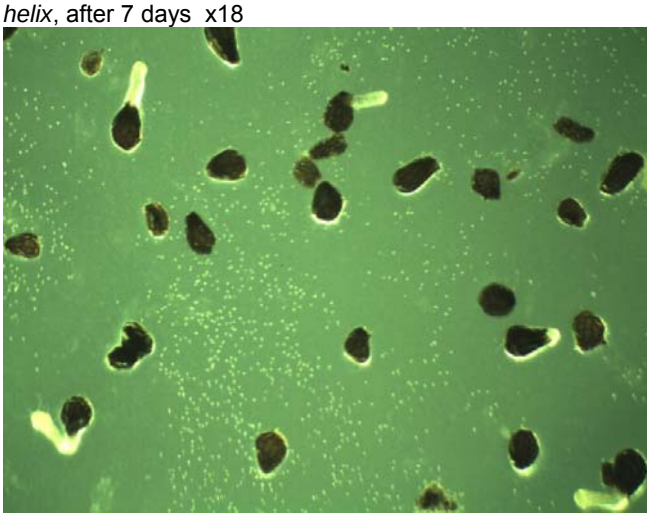

**Fig. 6** *Orobanche hederae* stimulated with root exudate of *Hedera helix* in the presence of root exudate of grasses, after 7 days x30

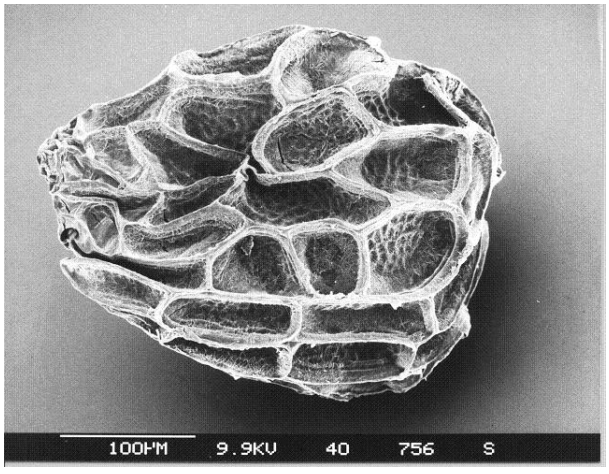


**Fig. 7** REM view of the seed coat of *Orobanche ramosa*

Orobanche species have narrow interrelations with their host plants (germination physiology, host specificity). Since the environmental conditions frequently are not advantageous, Orobanche spp. produce a huge number of seeds. The germination rates of Orobanche spp. in the spontaneous flora are generally low, frequently less than 30 %.

The Orobanche seed coat is extremely structured. For that reason careful surface sterilization is very important. Together with the sterilant a detergent needs to be applied for complete wetting of the surface.

## CONCLUSIONS

In the literature 72 species of the genus Orobanche are described, which in Europe were identified. In contrast to the weedy species of Orobanche, the species in the spontaneous flora occur in different places, most of them in limited populations. These populations do not grow regularly every year. We observe a regress of

these populations, and some are already on the red list. The spontaneous flora includes species which, when ecological conditions change, may turn into weedy forms. In Romania and generally in Europe, the problem of biodiversity conservations of these plants should be reconsidered and the Botanical Gardens can contribute to it. We consider the region of Sibiu where 16 species of Orobanche are found, especially in Natural Reservation Hill of Stepa Zakel (near Slimnic), on Guşteriţa Hill and on Ritoi Hill (near Slimnic) where we observed O. caryophyllacea, O. arenaria, O. teucrii, O. alba var. alba, f. communis, O. minor, O. gracilis an important place for our research. Hypotheses about different behavior of wild Orobanche populations in the natural ecosystems compared with those from anthropogenic ecosystems are verified. Until now, the problem of germination stimulants exuded by the host plants from the non-weedy species of Orobanche in wild flora is not clear yet and our work will contribute to understanding this problem.

Our research work will contribute to understand ecosystems, in which non-weedy Orobanche species grow, they may deliver information, under which conditions Orobanche species may turn into weedy forms, when ecological conditions change.

Moreover, a basis for fostering rare Orobanche species in Botanical Gardens will be provided to rescue them for the future.

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