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THE BIOLOGICAL CYCLE OF SUNFLOWER BROOMRAPE

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Abstract: *Orobanchaceae* is a dicot family, which consists of annual and perennial plants distributing from tropical to subarctic regions, predominately in temperate regions. Broomrape (*Orobanche cumana* Wallr. = *Orobanche cernua* Loefl.) is a parasitic angiosperm that has been causing a great deal of damage to sunflower production in many countries, including Republic of Moldova. This parasitic angiosperm depends entirely on the host for its supply of water and nutrients. A thorough understanding of its biology, including detailed knowledge of the specific mechanisms of parasitism, is needed in order to develop novel control methods. Some main developmental steps are described for the root parasites: seed conditioning and germination, haustorium formation, penetration into host tissues, maturation of the parasite plant, and seed production. All these stages were studied in artificial and natural conditions.

Key words: *Orobanche cumana* Wallr., holoparasite, host, exudate, appressorium, haustorium, attachments, tubercles, shoots, maturation, seeds.

Introduction

Broomrapes are holoparasitic members of the *Orobanchaceae* family. Of the 100 species belonging to the genus *Orobanche*, only a few parasitize crop plants. Amongst these, *Orobanche cumana* Wallr., *O. ramosa* L., *O. aegyptiaca* L. and *O. crenata* Forssk. are of economic importance since they cause severe yield losses in numerous commercial crops [LINKE & al. 1989]. This is particularly true of *O. cumana* whose hosts include sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum* Mill.), aubergine (*Solanum melongena* L.) and tobacco (*Nicotiana tabacum* L.). This parasitic weed has caused heavy yield losses in sunflower grain, oil and proteins in many countries such as Turkey, Romania, Ukraine, Bulgaria, China, the Black Sea countries and ex-URSS countries [GARCIA-TORRES, 1994; PARKER, 2009; MELERO, 2000; KAYA & al. 2004; SHINDROVA, 2006; MASIREVIC & MALIDZA, 2006; FERNANDEZ-ESCOBAR & al. 2009; GLIJIN, 2012].

Root parasites like broomrape are difficult to control, partly due to their complex life cycle which has been summarized conclusively by PAKER & RICHES (1993). The growth of this pathogen includes a series of developmental and metabolic steps, each crucial for the establishment of a direct connection with the host and for the survival of the parasite. Some developments, like the formation of a haustorium, are common only to parasitic plants.

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The *Orobanche cumana* Wallr. biological cycle comprises well-defined steps, separated both spatially and temporally, that are potential targets for host defense strategies. Individual seed in the soil require a conditioning period of 1 to 2 weeks to become imbibed, and temperature is important during that stage (15 °C to 20 °C). Following the conditioning process, each individual seed must receive a chemical stimulant from the host root, to alert the seed that host is in close proximity [PARKER & RICHES, 1993; KROSCHEL, 2001].

The first stage of the infection process is the germination and chemical guidance of the seedling (chemotropism) towards the host root. Because *Orobanche* species require the presence of germination stimulants exuded from the host root to germinate and locate the host root [WORSHAM, 1987], low stimulant producing plants could be a suitable option to reduce *O. cumana* infection. Most of these substances belong to the strigolactones and are active in extremely low concentrations (1 ppb to 1 ppm).

Upon germination, stimulated by exogenous chemical signals, broomrape seed develops a small seedling that attaches to the host root and differentiates in the attachment organ – the appressorium. On contact with the host root, the radical adheres to the surface by sticky papillae and penetration is facilitated by separation of the host cells, caused by enzymatic activity. Subsequently a connective organ, the haustorium, develops between host and parasite, with cells from each species playing part in the junction [STEWART & PRESS, 1990].

Once the haustorium has reached the host stele, haustorial cells at the interface penetrate host vessel members through their pits. These cells then open at their tips and lose their cytoplasm [DÖRR, 1997]. Adjacent cortical cells progressively differentiate into xylem elements until a continuous water conducting system is established linking the host and parasite vascular systems [MUSSELMAN & DICKISON, 1975]. The development of the xylem bridge is absolutely dependent upon direct contact of the haustorium with the host stele [RIOPEL & MUSSELMAN, 1979; HASSAN, 2004].

The nature of the host signal(s) that triggers xylem differentiation is currently uncharacterized but the phytohormones auxin and cytokinin are good candidates since these are known to trigger vascular regeneration in wounded tissues [ALONI, 1995]. After the parasite has developed a haustorium and established vascular connections with the host, it becomes one with the plant, acting as a sink for water and nutrients. This connective structure swells and forms a nodule that after one to two weeks differentiates into a tubercle with shoot bud, and eventually a flowering shoot.

After four to five weeks the flowering spike emerges from the soil, and grows to heights between 10 and 65 cm with slender stems slightly thickened at the base [PUJADAS-SALVA & VELASCO, 2000]. Stem color is variable, and it has been reported to be whitish [PUJADAS-SALVA & VELASCO, 2000], yellowish or brownish and bluish-violet. Stem color in *Orobanche* spp. is mainly determined by the accumulation of anthocyanins, which are more conspicuous because of the absence of chlorophyll [WHELDALE, 1916]. The bisexual flowers [MOLAU, 1995] are insect pollinated and the resulting seeds are produced in capsules with 1000 to 10000 seed per capsule. The seeds can remain viable in the soil for more than 10 years [LINKE &. al. 1989]. This ability to produce a prodigious number of seed per plant is the forte of these and similar parasitic agricultural weeds. Depending on environmental conditions the underground phase of the life-cycle of *Orobanche cumana* ranges from 30 to over 100 days. The whole life-cycle from seed germination to seed production lasts about 3-5 months [KROSCHEL, 2001].

This study is focused on observation and understanding of *O. cumana* stages of development in dynamics on the sunflower roots.

Materials and methods

Host and parasitic plant material

The seeds of genotype FS-6 of *Helianthus annuus* L., which is susceptible to broomrape, were used as a host plant. The seeds of *O. cumana* were collected in 2011 from inflorescences of broomrape that was parasitizing sunflower fields in Singera (municipality Chisinau). The inflorescences were dried for 60 days at temperatures ranging from 20 to 34 °C, after which the seeds were separated with 300-mm sieves and were stored in darkness at 4 °C. Seeds of sunflower and *O. cumana* were surface sterilized by soaking them in sodium hypochlorite (1%) for 15 minutes and washed twice with sterilized water before use.

Subterranean stages on development of *Orobanche*

Conditioning of broomrape seeds

Batches of 30 seeds of broomrape were placed on 5 discs of 2 cm diameter glass fiber filter paper moistened with 250 µl of sterile distilled water and incubated in the dark at 24 °C for 12 days in order to promote the necessary conditioning for germination [FERNANDEZ-APARICIO & al. 2008].

Germination bioassay

The broomrape seeds germination potential was tested in the presence of sunflower root exudates from susceptible genotype FS-6. Sunflower seeds were sown on glass balls soaked in sterile distilled water. Between first to the fifth week after sunflower germination, to collect root exudate, two seedlings were placed in 40 ml sterile distilled water for 2 days. This solution, which contained root exudates with germination stimulants, was then sterilized by filtration and could was stored at -20 °C. Preconditioned seeds of *O. cumana* on glass fiber filter paper were transferred to a small Petri dish containing 250 µl of root exudate solution and incubated at 25 °C. Germination was determined after radicle emergence.

Root chamber technique. The device (Fig. 1) was used to evaluate the underground development of root parasitic weeds such as appressorium and haustorium formation and further growth stages since such evaluation is impossible in the field.

The technique was described by KROSCHEL (2001) and slightly modified during this study. It is based on the use of a 3 sides-wood frame (30 x 15 x 2 cm) covered by glass covers on its two faces. Space between the two transparent covers was filled with sterilized sand. In the frontal face, white filter paper was put between sand and the glass cover. *O. cumana* seeds were conditioned with sterilized distilled water for 14 days in a growth chamber at 23 °C. Ten-days-old sunflower seedlings were transplanted to chamber and put in contact with *Orobanche* seeds (Fig. 1). The germination of broomrape seeds and the tubercles setting were determined at the different levels under optic microscope at different times.

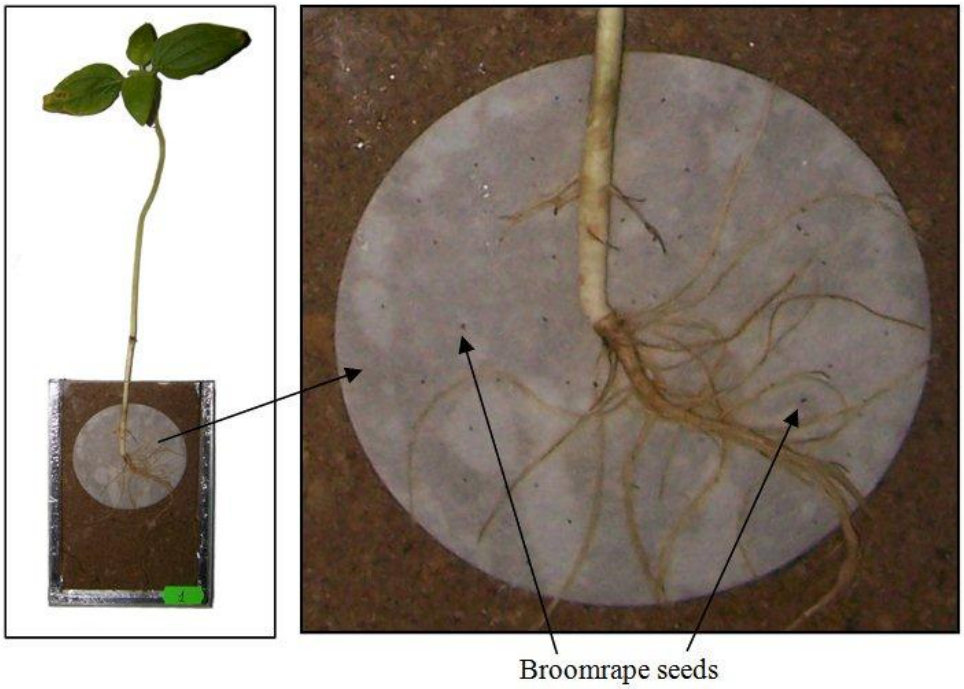

Fig. 1. The root chamber used in the assay. The root chambers were maintained in a growth chamber adjusted at 25 °C and 12 hours of photoperiod, and placed side by side to protect the roots from light. When necessary, plants were watered.

Aerial stages of *Orobanche* development

The evaluation of broomrape emergence, flowering and seed dispersal was made in a separate experiment, using small pots 5.5 x 6 x 16 cm were filled with a mixture of sand and peat (1 : 1 by wt). The soil mixture was carefully mixed with 25 mg of *O. cumana* seed (equivalent to around 5000 seeds) to obtain a homogeneously infested substrate. Sunflower seeds of the genotype FS-6 were germinated on moistened filter paper in Petri dishes and 2-day-old seedlings were planted in the pots. The plants were maintained in a growth chamber for 60 days at 25 °C / 20 °C (day / night) with a 16-h photoperiod for incubation.

Results and discussion

Results of these investigations showed a high affinity of *O. cumana* to host plant and sunflower tolerance to that agent. The analysis of ontogenetic development of host - parasite, made within sixty days, with periodic evaluation of 10 in 10 days and thoroughly study of the root system at the end of experience, revealed the several developmental stages

of sunflower broomrape: appressorium and haustorium formation, attachments, tubercles, underground and aerial shoots, maturation and seed production (Fig. 2).

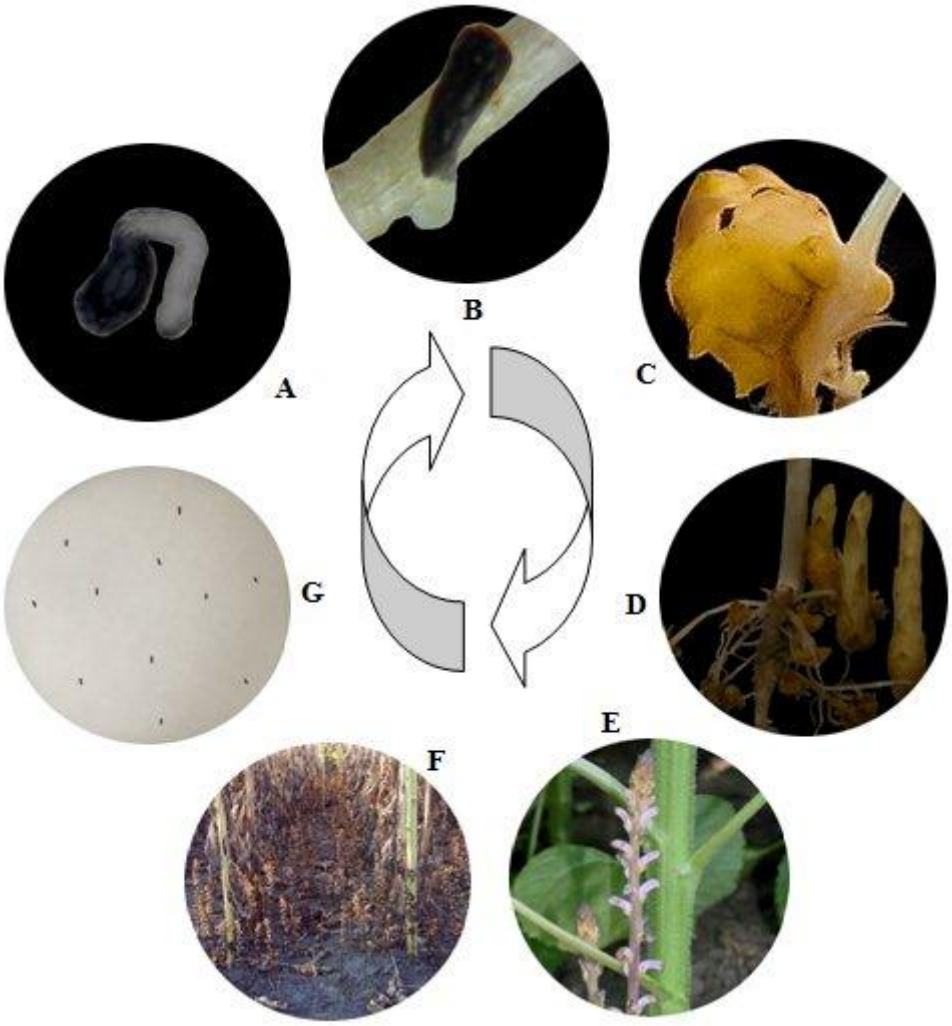

Fig. 2. *Orobanche cumana* life cycle
(A – seed germination and appressorium development; B – haustorium development;
C – nodule development; D – nodule differentiation into tubercles;
E – broomrape flowering; F – broomrape seed maturation; G – broomrape seeds)

Root exudates obtained from 1- to 5-week-old plantlets triggered *germination* of broomrape seed; germination reached approx. 90% in the second week (Fig. 2 A). Conditioned *Orobanche* seeds respond to very low concentrations of germination stimulants that are normally released from host roots [WIGCHERT & al. 1999]. Stimulant

concentrations that are higher than the optimal, inhibit *Orobanche* seed germination [JOEL & al. 1995]. An understanding of the biochemical basis of germination stimulation in root parasites may lead to the development of new practical control measures. These can be based either on the promotion of suicidal germination in the absence of a host [OSWALD & al. 2002], depleting the soil of parasitic weed seeds, or on inhibiting germination.

The germination of *O. cumana* seeds, induced by host-root exudates, leads to the development of a root-like organ, known as the germ tube or appresorium (Fig. 2 A).

The root chamber method proved useful also for studying the dynamics of the first ontogenetic stages of *Orobanche* attachments and haustorium development (Fig. 2 B). Attachment of the parasite to host root surface takes place as soon as the parasite meets a host root. This is facilitated by the secretion of an adhesive substance by the parasite [BAIRD & RIOPEL, 1985; JOEL & LOSNER-GOSHEN, 1994]. Potentially, this step may be vulnerable for control, but so far nothing is known about its control.

The next parasitism stage, the penetration into the host tissue, occurred 21 days after planting (DAP). Penetration is the first stage of intimate contact between cells of host and parasite. This is also the beginning of the true parasitic phase in which the parasite takes nutrients and water from the host. Therefore, it is crucial to any further development of the parasite. After the establishment of a conductive connection between host and parasite, at 35 DAP the parasite developed a tubercle (Fig. 2 C, D).This tubercle is the juvenile parasite. From tubercles, inside of host plant was formed a specialized structure known as haustorium, which is a connective tissue that active the junction between host and parasite [ECHEVARRÍA-ZOMEÑO & al. 2006]. At this stage one can physiologically regard the parasite as an integral part of the host, competing for host resources like a host organ. The growth of the parasite occurs at the expense of water, mineral and organic compounds from the host. The tubercle and underground shoots accumulate carbohydrates and thereby become a strong sink for all plant nutrients.

Beginning with 55 DAP on the explored sunflower roots were observed aerial shoots emerging above the soil surface (Fig. 1 D) and starts to flower (Fig. 2 E) and to produce seeds after another short period of time -70 DAP (Fig. 2 F, G).

Starting from obtained results, we can affirm that in the period 15 - 70 days from the moment of sunflower planting have been attested all *Orobanche cumana* ontogenetic stages. We can therefore conclude that after seed maturation *Orobanche* exhibits two main life phases: (a) the independent life phase, (b) the parasitic life phase. The independent phase begins with seed conditioning. It has been widely reported that for germination under chemical stimulation *Orobanchaceae* seeds required conditioning for several days in a wet environment and at suitable temperatures [PIETERSE, 1979; PRESS & al. 1990; CHAE & al. 2003]. However, the recent results of PLAKHINE & al. 2009 and PLAKHINE & JOEL, 2010, showed that non-conditioned seeds of both *Orobanche cumana* Wallr. and *O. aegyptiaca* Pers. were able to germinate in response to chemical stimulation by GR24 even without prior conditioning. Germination lasts a few days until the parasite finds a host, and attaches to it. This life phase is facilitated by the consumption of material stored in the seed. According to OKONKWO & NWOKE (1978), the parasitic life phase starts as soon as a haustorium has developed. At this point the parasite becomes dependent on nutrients derived from the host. JOEL & PORTNOY (1998) demonstrated that intrusive cells of the haustorium penetrate the host root, eventually forming a physiological bridge between the

vascular system of the host and that of the parasite. Subsequently, the parasite develops a shoot that emerges from the soil, flowers and sets seeds.

The understanding of metabolic and developmental aspects of root parasites is essential for any effort to develop effective control measures that will specifically prevent the damage it causes in agricultural fields. This is true because each developmental stage is crucial for the growth and dispersal of these root parasites. Thus, each step can potentially serve as a target for their control.

Conclusions

According to the present results, using the root chambers enables detection of *Orobanche* seeds, observation of the parasitism stages of germination, penetration into the host root tissue, establishment, tubercle production and apex production without disturbance, under natural conditions. These conditions are essential for studying major aspects of the host–parasite relationship to gain knowledge for use in modeling the parasitism process, optimizing chemical control and studying the resistance mechanism of resistant cultivars.

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