Innovative solutions for broomrape management in the Mediterranean region

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Based on: Scientific outcomes and evidence-based practices,
from the PRIMA "ZeroParasitic" project
"Innovative sustainable solutions for broomrapes: prevention and integrated pest management approaches to overcome parasitism in Mediterranean cropping systems"

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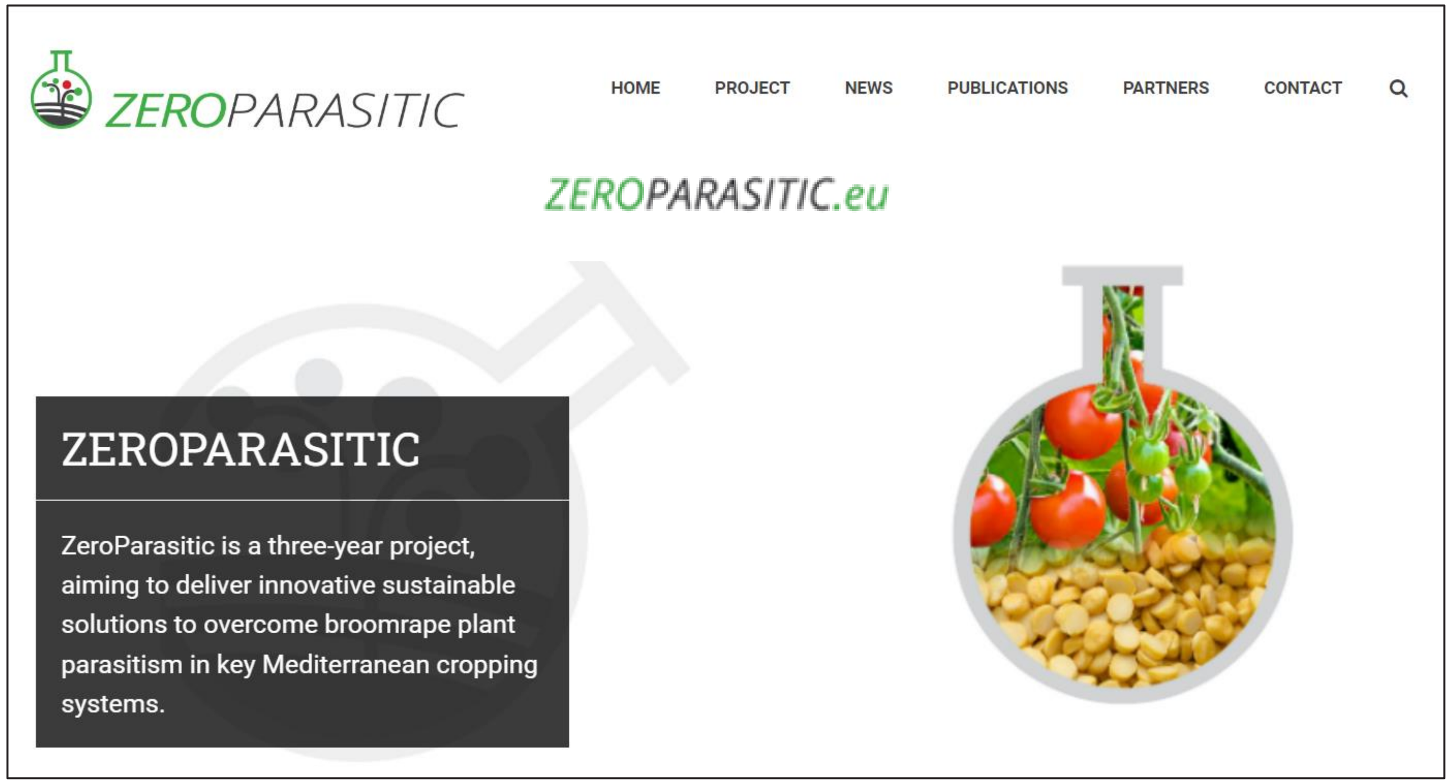

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EXECUTIVE SUMMARY

ZeroParasitic is a three-year project, aiming to deliver innovative sustainable solutions to overcome broomrape plant parasitism in key Mediterranean cropping systems. The research project targets two of the most important crops in the Mediterranean (i.e. industrial tomato and faba beans).

The project facilitated a wide diverse number of actions, interventions were tested, demonstrated and validated in order to derive actionable recommendations for supporting broomrape management in the Med region. Research shows that many of the solutions derived from the below actions are very promising and frequently sufficiently effective. However, their impact is boosted when implemented in a holistic manner, and not as a stand-alone tactics.

The actions could be divided in the following pillars.

• **Agronomic-Level actions**; those provided IPM solutions such as:
a) grafting
b) application of parasite seed germination stimulants
c) biological control using flies and soil micro-organisms
d) Intercropping as an agronomic tool

• **Technical-Level actions**; those provided the following:
a) identification of new genetic variability in different species for broomrape tolerance/resistance;
b) identification of molecular genetic markers in mapping populations;
c) identification of new hormones and metabolites involved in broomrape germination, haustoria development, and plant infection during host-plant interaction;
d) identification of the molecular basis of resistance based on pattern recognition receptors (PRRs) to enhance capabilities for targeted breeding of resistance;
e) tools for remote and satellite mapping and surveillance

• **Socioeconmic-Level actions**; those provided the following:
a) insights on behavioral drivers of farmers
b) indexes for farmer's economic outlook

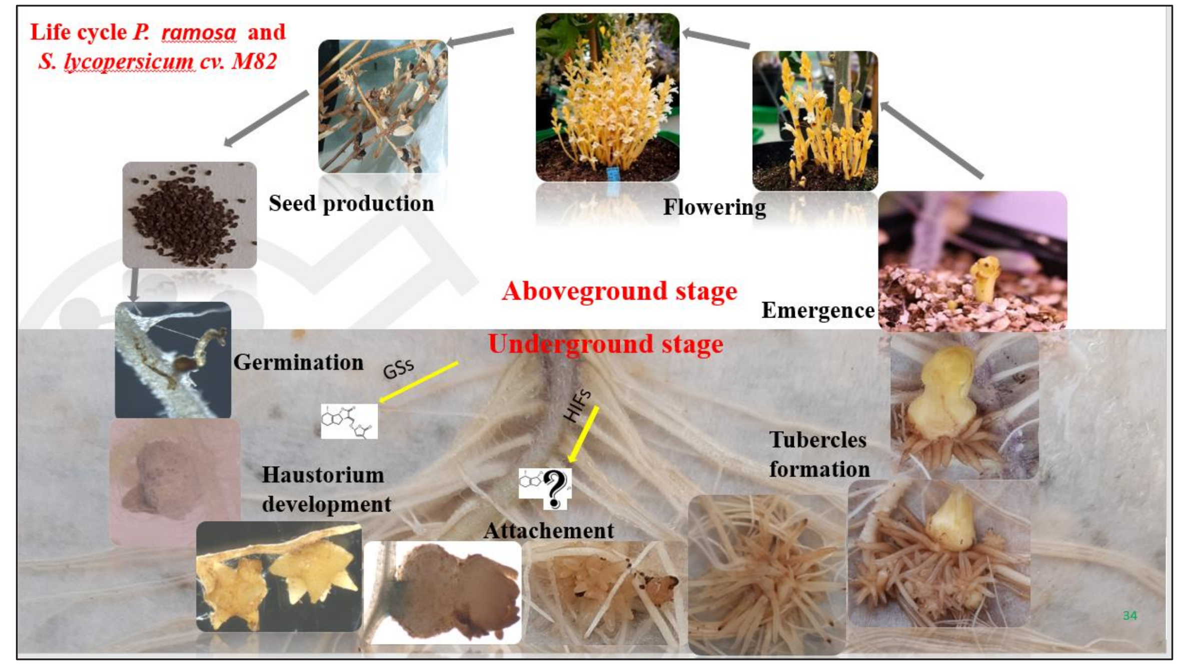
• **Policy-Level actions**; those provided the following:
a) insights for counteracting lock-ins for adoption
b) policies and programmes that facilitate a new paradigm in broomrape management.

The main goal of ZeroParasitic was to integrate innovative solutions into a realistic framework through a trans-disciplinary, multi-actor effort targeting broomrapes, which is one of the most problematic weed in the Mediterranean cropping systems.

INTRODUCTION: MANAGING BROOMRAPES – THE FRAMEWORK

Phelipanche and *Orobanche* species (broomrapes) are root holoparasitic plants that cause severe damage to economically important crops. *Phelipanche* and *Orobanche* spp. are widespread in Mediterranean areas, in Asia and in Southern and Eastern Europe, attacking dicotyledonous crops and depending entirely on their hosts for all nutritional requirements (Hershenhorn et al., 2009). Due to their physical and metabolic overlap with the crop, their underground parasitism, their achlorophyllous nature, and hardly destructible seed bank, broomrape weeds are usually not controlled by management strategies designed for non-parasitic weeds (Fernández-Aparicio et al., 2016).

SIDEBAR 1. Broomrape cycle of parasitism



Contribution: Markus Albert (FAU)

In the Mediterranean region, broomrapes attack two of the most important crops: legumes and tomato. In particular, faba beans, being one of the most important winter pulse crops in several Med countries, is a host for broomrapes (Orobanche crenata) and yield losses could range from 40 – 100%, depending on the severity of infestation. In addition, there are no herbicides registered to control broomrapes, and farmers depend largely on the non-chemical strategies. However, most of these non-chemical management strategies fail to provide satisfactory control or are not economical, or feasible on a large scale (Goldwasser & Kleifeld, 2004; Joel et al., 2007).

The current **non-chemical** most important **strategies** are listed below:

- Intercropping with parasite catch-crops or crops that inhibit the establishment of the parasite.
- Using tolerant cultivars that have been proposed for faba bens; no tomato cultivar/hybrid is known
- Application of soil micro-organisms that would inhibit parasitism; mainly available for tomatos.
- Application of various agronomics tactics (i.e. late sowing, deep tillage, summer tillage).
- Implementing long rotations with non-host crops

Regarding the biological control methods, the most important tool is the utilization of a fly [*Phytomyza orobanchia* (Diptera, Agromyzidae)]. The main impact of this biocontrol agent is the reduction of the seed production in the mother broomrape plant, resulting in lower amounts of seed dispersal in soil and leading to a reduction of the soil seed bank (Mihajlovic, L. 1986). Research data have shown that the *P. orobanchia* fly is lacking the capacity to significantly reduce natural infestation of *Orobanche* spp. in faba bens fields. As such, there is an urgent need to document the factors affecting the population dynamics of the fly, and optimize its control capacity by the following:

a) To investigate the infestation level and the distribution patterns of the Phytomyza orobanchia on different parts of O. crenata
b) To investigate the effect of infestation level by Phytomyza orobanchia on the seeds production of O. crenata

Surveillance and mapping of parasitism aiming to develop tools to map parasitism, at local, regional and national scale is essential. Various sensing approaches have been so far proposed; RGB imaging, the thermal and the multi-spectral imaging being the most important ones (Hutcher and Froud-Williams, 2017). Satellite images, particularly those with high resolution (0.5-2mresolution multispectral and/or panchromatic) could be taken to compute the region crop (i.e. industrial tomato or legumes) and the extent of broomrape infestation at regional/national level; coupled with a GIS database that processes crop historical management data, parasitism historical data, and herbicides inputs and attributes of the field sites.

Breeding for resistance is still one of the most effective, feasible and environmentally friendly management strategies against this weed and considerable efforts have been invested in many crops (Rubiales et al., 2009). Breeding-resistant varieties remains an on-going struggle because a genetic broomrape-resistance background is scarce (Fernandez-Martınez et al., 2000). In tomato, as the genetic variability available to support extensive resistance breeding programmes in existing tomato cultivars is small, several approaches have been taken to improve the situation: (i) search for resistance in wild tomato relatives and (ii) application of different mutagenesis methods to create a mutated population that extends the natural genetic variation. In this context, the replacement of large genomic regions of *S. lycopersicum* with the corresponding chromosome sections from *S. pennellii*, superior agronomic performances could be achieved. In faba beans, identifying molecular and genetic markers in mapping populations/contrasting genotypes at different phases of parasite-plant interaction is needed. During the last decades, several partial faba bean resistant varieties and breeding lines to *Orobanche* spp. were recently selected and developed by breeding programmes in several Mediterranean countries (i.e. Tunisia, Egypt, Morocco).

In both crops, industrial tomato and faba beans, the analysis of the expression of host genes associated with high tolerance/resistance of the host crop to the broomrapes needs to be performed. Additionally, greenhouse and field screening of germplasm for resistance to broomrapes is needed. As such, all promising genetic material that showed partial or full resistance to Orobanche species could be used in breeding programs, and finally new improved varieties would be registered across the Med region.

Several hormones that are major players in signaling networks during other plant defense responses have been demonstrated to also play a role in the host–parasite interaction. A better understanding in the roles of major hormones in the process of broomrape germination would facilitate the design of feasible control strategies. In tomato, strigolactones (SLs) are the main germination stimulants for *P. ramosa;* the development of low SL-producing lines may be an approach to combat the parasitic weed problem. However, since SLs are also a plant hormone controlling many aspects of plant development, SL deficiency may also have an effect on post-germination stages of the infection process, during the parasite-host interaction (Cheng et al., 2017).

Resistance of plants against pathogens is mainly based on perception systems such as **pattern recognition receptors (PRRs)** that detect molecular patterns of pathogens and consequently induce defense-related responses that lead to the rejection of the pathogen. Such resistance receptors are encoded by resistance genes, and we recently identified a resistance gene in tomato encoding a PRR that confers resistance to a parasitic plant (Hegenauer et al. 2016). In an extended genetic screen using tomato introgression lines, additional PRRs that also seem to be required to confer resistance against parasitic plants (Albert, unpublished data).

Measures to increase adoption of novel solutions by end-users is needed by characterization of groups of end-users (focusing on farmers) who demonstrate specific interest for such adoption. Examination of the motivations, perceptions, drivers and the underpinnings of farmers' decision-making (farmer-specific, farm-specific, exogenous) and their profiles needs to be done. Also, a typology of farmers across the Med region needs to be constructed according (a) to their innovation adoption profile and (b) to the practices they actually follow. The farmers typology should be dynamic aiming to incorporate adjustments, modifications, and fine tuning to adequately reflect the Mediterranean conditions and stakeholders' perceptions.

Collection of data for a proper **socioeconomic assessment** in an integrated manner that accommodates the diversity of social and economic conditions of targeted production systems across the Mediterranean is needed. This activity could be needs to address the following:

a) An economic approach of the farm management practices followed by farmers under various production systems in each country. Technical and economic data indicators of farm management (land acreage and rent, labor requirements and wages, fixed capital investments (buildings, machinery, irrigation infrastructure, vineyards, etc) and annual expenses, variable capital expenses (seeds, phytosanitary measures, energy, other purchased inputs etc) need to be collected.
b) Depiction of the profile of farms and of farmers (sociodemographic variables) and of their attitudes relating to innovation adoption and acceptance of new farming practices
b) Specific questions will record in detail their management practices relating to broomrapes (preventive or curative, biological or chemical) and the resource requirements of each practice (capital and labour).

Assessment of economic effectiveness is needed. Novel solutions need to demonstrate their economic sustainability under various scenarios (e.g. under various production volumes) by calculating investment performance indicators. Important issues need to be addressed as answers to the following questions:

(a) What is the economic importance of broomrape parasitism for each country, and in the Mediterranean region as a total?
(b) How easy is to introduce new solutions against parasitism without reducing the economic performance of Mediterranean production systems?
(c) How is the economic performance of farms influenced by the use of novel solutions?
(d) Are the proposed changes cost-effective?

Using survey data, the economic effectiveness of the novel solutions could be assessed, addressing the following issues:

a) how parasitism affects the economic performance of various farm types in Mediterranean countries?

b) how exactly the proposed methods can benefit the economic performance of farms, what might be the additional efforts or inputs to implement them and how these additional resources will be rewarded?
c) how the expected economic performance of the new solutions can be optimized under a set of constraints, considering crops, typical for the Mediterranean territories?

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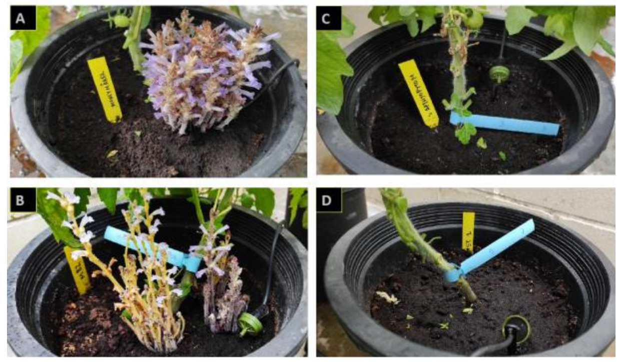
WHAT ACTIONS ARE NEEDED TO SUPPORT NOVEL SOLUTIONS?

Agronomic-Level actions

a) **Grafting** as a tool to manage broomrapes

Tomato varieties/hybrids could be grafted onto rootstocks that confer tolerance to stress factors, and that grafting is a useful tool to manage broomrapes. This line of research deals with empirical (to identify resistant rootstocks) and scientific (to identify the underlying mechanisms) approaches, not only in tomato and other graftable crop species. The hypothesis was to test if the roots of genotypes identified as resistant could transfer that level of resistance to a sensitive tomato cultivar. The results showed no resistance in the self-grafted variety “Moneymaker”, and in the plants grafted on the rootstock of the lines identified as highly sensitive. However, the percentage of resistance increased between 40% and 100% when Moneymaker was grafted onto species identified as moderately or highly resistant to *Phelipanche* Spp. These results verified the ability of the rootstock to transfer traits of resistance to broomrapes, which opens an interesting perspective in the search for new sources of resistance against holoparasitism.

SIDEBAR 3. Use of rootstocks to improve parasitism tolerance in tomato



Contribution: P. A. Martínez-Melgarejo, M. Ormazabal, C. Martínez-Andújar, F. P. Alfocea.

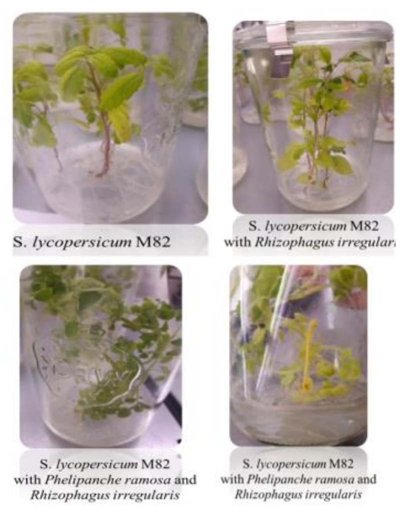
b) Application of **arbuscular mycorrhizas (AMFs)** to control parasitism

Host plants colonized by AMF could repress soil pathogens attracted by the same signal molecules. Targeting molecular and biochemical processes involved in the establishment of the connection between the parasite and the host (tomato) may offer a new perspective for the control of parasitism using AMF.

Preliminary experiments were done using *Phelipanche ramosa* and tomato *S. lycopersicum*/*S. pennellii* host plants inoculated or not with Rhizophagus spp at a development stage of 12 weeks after inoculation. Plants (tomato and faba beans) were inoculated with AMF (Rhizophagus spp.) at the seedling stage. Their ability to inhibit the germination and/or establishment of broomrapes per se was documented.

Visual observations were carried out for the following 8 weeks that proved the efficacity of mycorrhizal inoculation on root exudates. However, the effect of mycorrhizal inoculation on root exudates needs to be analyzed in future projects.

SIDEBAR 3. AMF inoculation



Contribution: FAU team

c) **Stimulants** aiming to understand broomrape seed germination; possible tools of management?

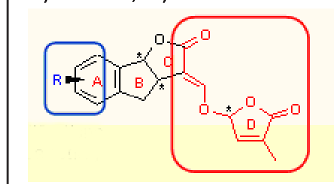
Seed germination

Using petri-dishes, under laboratory conditions, experiments were performed to measure the effect of environmental conditions on two different stimulants (i.e. GR-24, NE-1), during the two discrete phases of broomrapes (i.e. pre-conditioning, germination phase) on seed germination as follows (Picture 1):

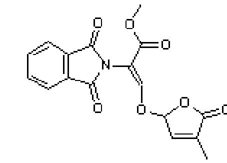
A) During the pre-conditioning phase: the effect of three temperature levels (17, 25, 25-35°C), at three different duration periods (1, 2, 3 weeks), in seeds of three broomrape species (*P. ramosa*, *P. aegyptiaca*, *O.cumana*), using two different stimulants (GR24, NE-1), at two different concentrations (10^{-6}, 10^{-8} M). The germination was done under optimal conditions (20/25 °C, 12/12h; no light) for seeds kept and examined after 2 weeks.

B) During the germination phase: the effect of three temperature levels (17, 25, 25-35°C), in seeds of three broomrape species (*P. ramosa*, *P. aegyptiaca*, *O.cumana*), using two different stimulants (GR24, NE-1), at four different concentrations (10^{-4}, 10^{-6}, 10^{-8} , 10^{-10}M), The pre-conditioning of the seeds was optimal (20/25 °C, 12/12h; no light) for a duration of 3 weeks.

SIDEBAR 2. Types of stimulants; a) GR-24; b) NE-1



a)



b)

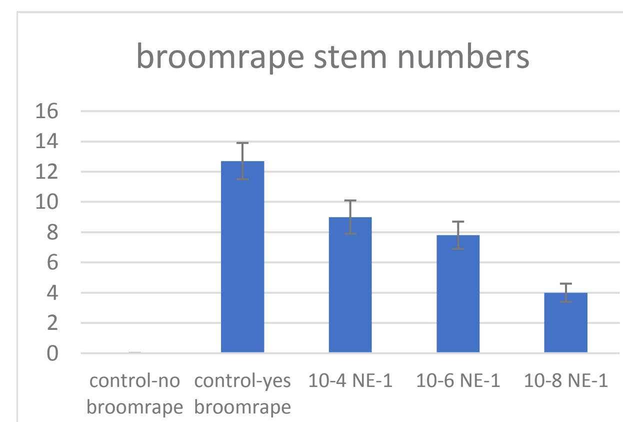
Contribution: BPI team

Depletion studies using stimulants

Utilizing new stimulants (such as NE-1) that are cheaper, produced in larger quantities and in aquatic formulations would be an interesting strategy for seed soil depletion through suicidal germination. A micro-trial experiment, based at BPI, was established to measure the effectiveness of the application of the stimulant NE-1 to deplete broomrape soil seed bank. The stimulant was in aqueous solution, three different doses of the stimulant were applied as follows: (10^{-4},10^{-6},10^{-8} M. During the tomato crop development, the establishment of broomrape plants was monitored; final yields (fruit g/plant) of tomato plants were also measured.

Results showed that increased NE-1 concentrations reduced the broomrape parasitism; optimum concentration: 10^{-8} M.

SIDEBAR 2. Depletion studies using the aqueous stimulant NE-1.



Contribution: BPI team

SIDEBAR 2. Micr-trial depletion studies.

Contribution: BPI team

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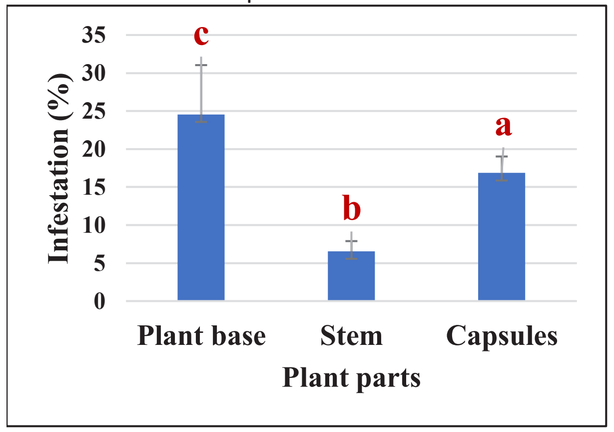
d) **Biological control** of broomrapes; utilizing **flies**

Broomrape fly, Phytomyza orobanchia Kalt. (Diptera: Agromyzidae) is oligophagous feeding only on *Orobanche* species. It feeds mainly on the immature seeds destroying between 11% and 90% of seeds. Also, it causes reduction in the length of shoots, number of capsules/shoot. One larva can destroy all seeds in the small capsule of broompare, but the big capsule need more than one larvae for complete reduction of seeds.

SIDEBAR 3. Distribution pattern of *P. orobanchia* on different parts of infested *O. crenata* plants



Contribution: Esmat Hegazi & Alexandria University team, Egypt.

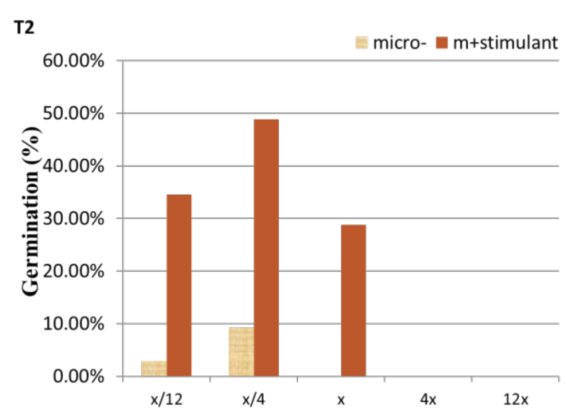
Results measured various characteristics of the P. orobanchia such as: the distribution pattrens in plant parts, the distribution of fly larvae and puparia in *O.crenata* shoots, the influence of capsule level on infestation rate. In addition, several aspects related to the *P. orobanchia* were studied such as: the longevity and dynamics of egg production, its diapause and Dynamics of egg production in honey-fed non-diapausing and diapausing females.

e) **Biological control** of broomrapes; utilizing **soil micro-organisms**

Utilizing beneficial microorganisms could be an attractive organic control method of parasitism. Treatments with beneficial micro-organisms were several commercially available products that consist of mixtures such as AMF, bacillus, bacteria and various types of fungies.

In Greece, in petri-dishes, pre-conditioned seeds of *Orobanche ramosa* were placed together with the specific product. In addition, the effective concentration of the stimulant (NE-1; 10^{-6}M) was added to measure the effect of the treatment on seed germination. Products such as Mycosym; Robur; Micosat; Trianum; Serenade; at 5 rates were tested.

SIDEBAR 3. Inhibitory effect of Robur



Contribution: BPI team.

In Tunisia, a field trial was established using different products were used as seed coating such as: Panoramix (a mixture of Bacillus subtilis + Trichoderma + Mycorhize + polysaccharide, Serenade (Bacillus subtilis), and Trianum-P (Trichoderma harzianum T-22).
Results showed that treatments by Serenad, SA (0.1mM) decreased the number of O. crenata. For O. foetida, only Panoramix treatment reduced Orobanche number.

f) Utilization of **herbicides**: Field trials in Egypt, Tunisia and Greece

In Egypt, in faba beans, experiments were done using glyphosate at very low rates (i.e. 80 g a.i./ha). Results showed a complete control of broomrape parasitism.
In Tunisia, in faba beans, experiments were done (cv. Bachaar; susceptible) using methazachlor + Imazamox, glyphosate, Clethodim and pendimethalin.

SIDEBAR 3. Herbicide treatments in a field, Egypt.

Contribution: Esmat Hegazi team, Egypt.

In Greece, in tomato, a field trial was done utilizing the combination of herbigation with foliage application (depending on their mode of action) of several herbicides such as: s-metolachlor, rimsulfuron, rimsulfuron plus s-metolachlor, fluazifop plus rimsulfuron, pinoxaden plus clodinafop plus rimsulfuron, imazamox, fluometuron, s-metolachlor plus rimsulfuron plus fluometuron.

Results showed that application of rimsufluron plus s-metolachlor, and imazamox effectively eliminated the broomrape parasitism.

In contrast, the combination of s-metolachlor plus rimsulfuron plus fluometuron, although eliminated the parasitism proved to be highly phytotoxic to tomato plants.

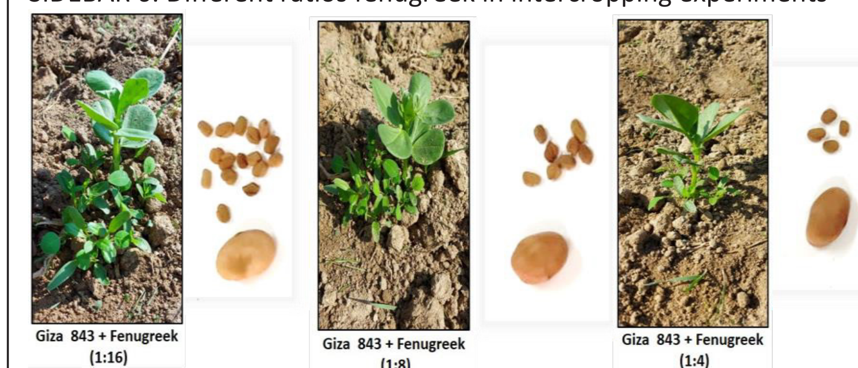
SIDEBAR 3. Herbigation treatments in tomato, Greece.

Contribution: BPI team

g) Utilization of **intercropping**: Field studies in Tunisia & Egypt

In Tunisia, a field trial using two faba bean varieties as follows: the resistant cv. Chourouk and the susceptible cv. Bachaar was established. The two faba bean varieties were grown as a mono-crop (20 seeds per m^2) or inter-cropped in the same row with fenugreek (local landrace) at various plant densities in a field naturally infested with *O. foetida*. Fenugreek proved effective to reduce broomrape parasitism.

SIDEBAR 6. Different ratios fenugreek in intercropping experiments



Contribution: Esmat Hegazi & Alexandria University team, Egypt.

In Egypt, seeds of two faba bean cultivars, Misr 3 (susceptible) and Giza 843 (resistant) were selected. Evaluation of intercropping effect using three crops (i.e. fenugreek, radish and flax) was made. Results showed that flax was more effective in reducing infestation rate by *O. crenata* than fenugreek.

Technical Science-Level actions

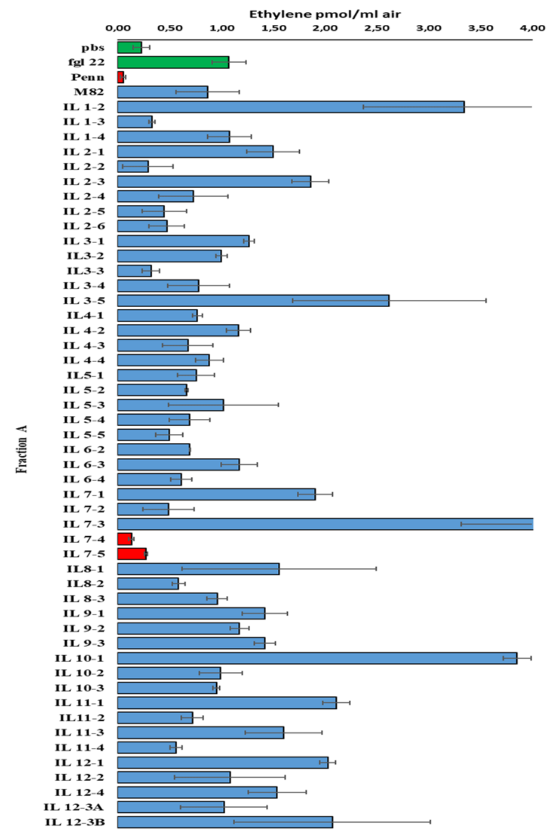
a) Hormonal interactions between host and parasite

The aim of this activity was to gain insights in particular molecular patterns involved in plant responses to holoparasitism. For that purpose, changes in the major classes of plant hormones (cytokinins, CKs; auxins, IAA; abscisic acid, ABA; jasmonic acid, JA; salicylic acid, SA; gibberellins, GAs; strigolactones, STGs; and the ethylene precursor 1-aminocyclopropane-1-carboxylate, ACC) were evaluated using UHPLC-MS (Orbitrap technology).

Moreover, other primary metabolites acting as photoassimilates that includes amino acids, organic acids and sugars, were also analysed in samples of roots and leaves of the host, and in tissues from the parasitic plants. Some of the most conclusive metabolomic data indicate that high levels of ACC, GAs and SA are associated with resistance to broomrape, while an increase in ABA and JA and a decrease in SA in response to infection are common in susceptible plants.

In this context, a decrease in the concentration of the bioactive CK trans-zeatin (t-Z) in the leaves and the increase in the roots of infected (susceptible) plants, can induce sink activity (i.e., accumulation of sugars in the root) for the benefit of the parasitic plant, and the strong induction of STGs levels in the infected roots. On the other hand, the tissues of holoparasitic plants accumulated photoassimilates and hormones mainly in the host-parasite interaction zone and at the base of the broomrape, suggesting an active transport from the host plant.

SIDEBAR 3. Mapping of responsiveness to *P. ramosa* extracts in tomato Introgression Lines (ILs).



Contribution: CSIC team

Finally, it is worth noting that correlation analysis based on sensitive and resistant rootstocks showed that STGs, GAs and t-Z clustered with plant photoassimilates only in the sensitive rootstocks, suggesting an important role of these hormones in the induction of sink activity on the root of the host for further infection and development of broomrapes. However, other metabolic and hormonal factors such as citric acid, ethylene precursor (ACC), salicylic and jasmonic acids, were grouped with the biomass parameters and negatively with those traits that favour parasitism, suggesting a role for these hormones in the defence against infections and the broomrape development. The metabolomic profiles analysed contribute to the progress in the identification of metabolic traits of resistance and sensitivity in host species, and in understanding host-parasite communication through haustorial connections.

b) Role of **Pattern Recognition Receptors (PRRs)**

Solanum lycopersicum is known to be highly susceptible to broomrape infestation whereas some wild tomato species (i.e. *Solanum pennellii*) has been determined to be resistant. In this context, the replacement of large genomic regions of

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S. lycopersicum with the corresponding chromosome sections from *S. pennellii*, superior agronomic performances could be achieved. How and if this could be related to resistance against *Phelipanche* ssp. was part of our investigations.

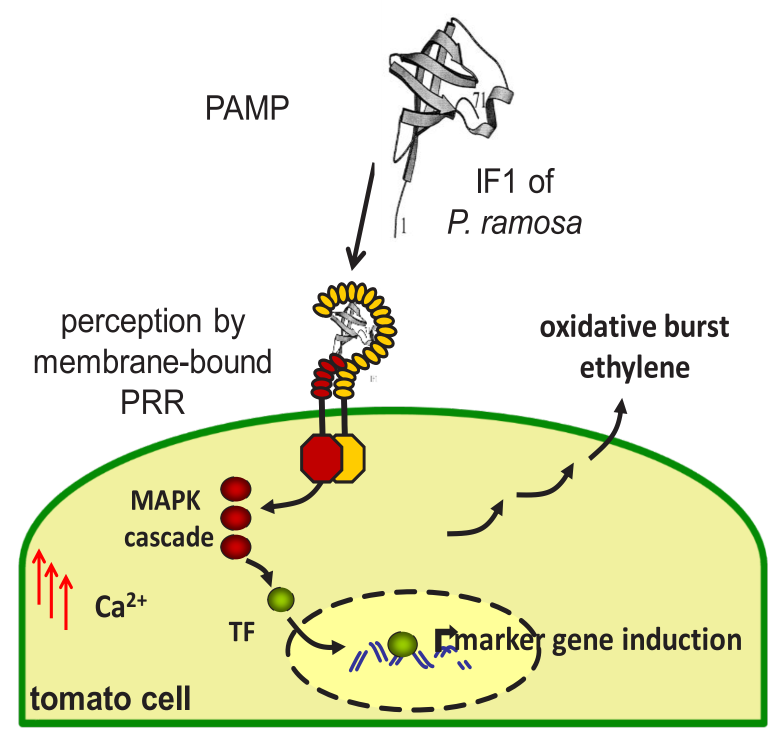
Also, if this kind of resistance is the result of a common principle such as described for pattern-triggered immunity (PTI) are yet unanswered questions. Commonly, the two-layered plant innate immune system is involved in the detection of microbial phytopathogens as well as plant parasites and essentially participates in fending off the invaders. To trigger plant immunity, a recognition of pathogen- or damage-associated molecular patterns (PAMPs/DAMPs) by pattern-recognition receptors (PRRs) is an indispensable requirement.

In our project, we aimed to identify PAMPs of *Phelipanche* spp. that are able to initiate plant defense responses and their corresponding PRRs of tomato host plants. We exploited the 55 Introgression lines (ILs) of the susceptible *S. lycopersicum* and the resistant *S. pennellii* and screened these plants for their capability to respond in well-known plant patho-bioassays (ethylene emission, reactive oxygen production) when treated with *Phelipanche* spp. extracts or isolated, heterologously produced *Phelipanche* spp. molecules.

We identified a protein, translation initiation factor 1 (IF1), of *P. ramosa* as a potential PAMP. After cloning and heterologous expression of *P. ramosa IF1* we successfully tested this protein for its capability to induce PTI responses in tomato plants, like the production of ethylene or reactive oxygen species (ROS).

A potential PRR-encoding gene was suggested to be present in the tomato genome and could be mapped to chromosome 7 by screening the tomato ILs; the cloning of the IF-receptor candidate from tomato, however, failed. In addition to the tomato IL collection, transgenic tobacco plants expressing the **Cu**scuta **Re**ceptor **1** (**CuRe1**) have been tested for ethylene production after treatment with *P. aegyptiaca* extracts. The obtained results suggest, that CuRe1 also can recognize a *P. aegyptiaca* PAMP and can induce ethylene emission in a CuRe1-dependent manner, but does not mediate resistance to a sufficient extent. Obviously, tomato CuRe1 detects a PAMP of *P. aegyptiaca*, that likely resembles a homologue of the *Cuscuta reflexa* Glycine-rich protein, which has been described as ligand for CuRe1.

SIDEBAR 3. Tomato pattern recognition receptors (PRRs) recognizing potential pathogen-assosciated molecular patterns (PAMPs), like IF1 of *Phelipanche* spp.



Contribution: Mouna Khalloufi and Markus Albert (FAU)

Taken together, in our work we could demonstrate that a yet unknown tomato PRR recognizes the *P. ramosa* IF1. In turn, the well-characterized tomato PRR CuRe1 seems to detect a yet unknown PAMP that is present in extracts of *P. aegyptiaca*. Future experiments will be necessary to identify the corresponding partner to commit defense reactions.

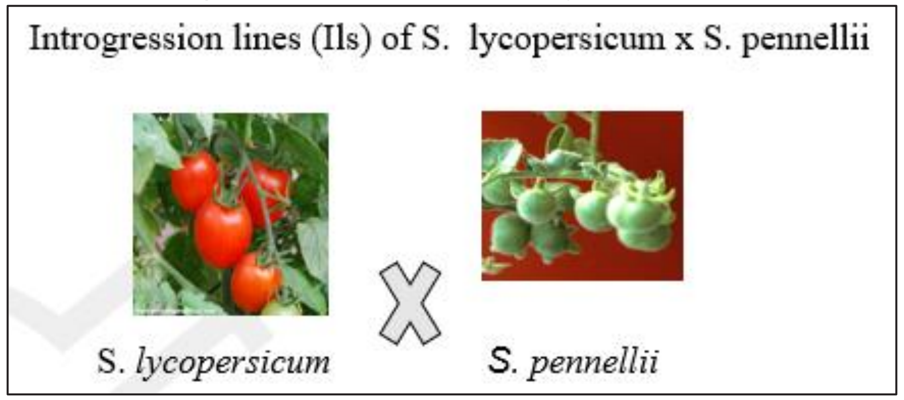
The PRIMA programme is supported and funded under Horizon 2020, the Framework European Union's Programme for Research and Innovation

c) Resistance development in host plants

New germplasm resistant to broomrapes

In Zeroparasitic, a main objective was to identify new germplasm resistant to broomrapes. A germplasm collection was evaluated, including: wild tomato species Solanum galapagense, S. peruvianum, S. chilense, S. chmielewskii, S. pennellii (acc. PE - 47); introgression lines (ILs) 6-2 and 6-3 and their parental lines S. lycopersicum (cv M82) x S. pennellii (LA716); and the hormonal mutants affected in strigolactone production CCD7 and ST1.

SIDEBAR 3. Introgression Lines used in the Zeroparasitic



Contribution: P. A. Martínez-Melgarejo, M. Ormazabal, C. Martínez-Andújar, F. P. Alfocea (SCIC)

In vitro screening experiments based on the germination and infection capacities of P. ramosa identified five genotypes as fully resistant, four with medium-high resistance and the rest as highly susceptible species. Those results were further confirmed under greenhouse conditions.

Field screening: Tunisia

In Tunisia, field screening was performed during the cropping season 2019/2020; one hundred twelve faba bean entries was screened for the detection of more genetic material carrying resistance to *Orobanche* Spp. The screening was done for *O. foetida* in the field at Oued Beja Research Unit and to *O. crenata* in a farmer field at Mateur. The tested entries (breeding lines, varieties, and landraces) were from different origin (Tunisia and ICARDA multiple origin).

SIDEBAR 3. Field screening for *O. crenata* resistance in a farmer field at Mateur and *O. foetida* resistance at Beja experimental unit, in Tunisia

Contribution: INRAT team members

The sowing was done in naturally infested field on late November at Mateur 2019 (Fig. 13) and early December 2019 at Oued Beja according to a non-replicated design in which each test entry was surrounded by a susceptible check (cv. Bachaar). Several parameters were recorded (number of emerged faba bean plant in each row, incidence and severity of Orobanche infestation, Orobanche number and dry weight and faba bean grain yield) for each entry including the susceptible check in Oued Beja field. Among the 112 tested entries at Oued Beja, eleven showed good performance (grain production and less infested by orobanche) were selected for tolerance to broomrape parasitism.

Field screening: Morocco

A trial of 48 genotypes of faba bean was led under both treatments of the presence and absence of *orobanche crenata* in the station of Merchouch in two years 2021-2022 and 2022-2023. The 48 genotypes include the susceptible checks Aguadulce.

Among the genotypes tested, four distinct groups were identified.

(1) Group 1 is the most susceptible to orobanche crenata with a particular high level of parasitism. Within this group, there are three genotypes: G33, G40 and G43.

(2) Group 2 exhibits high productivity and grain yield, as well as early pods setting. This group includes the G18 genotype, as well as the G21, G36 and G45 genotypes.

(3) Group 3 is constituted of genotypes displaying a relatively low level of infestation and registering minimal yield loss when subjected to the presence of Orobanche. This group includes two genotypes, namely G3 and G27.

(4) Group 4 represents genotypes that have a late fruit setting date. This group is made up of genotypes G2, G12, G17 and G22. These results confirm that the G3 and G27 genotypes have less sensitivity to Orobanche, thus demonstrating tolerance.

These results are consistent with the previously conducted analysis of yield loss rate, which revealed that these genotypes maintained a significant portion of their yield despite the presence of Orobanche.

SIDEBAR 4. Fiel trial in Merchouch (2022-2023), in Morocco; a) infested; b) non-infested

Contribution: Lamiae Ghaouti (IAV)

Plastic dish screening

Experiments were undertaken as a co-culture host plants with broomrape seeds. In Tunisia, the best faba bean entries in resistance were further studied in a mini-rhizotron experiment to assess their ability to reduce the germination and tubercles formation of *O. crenata* and *O. foetida in vitro*. Experiment was carried out in plastic square dishes. The whole was covered with aluminum foil and maintained under controlled conditions at 20±2°C. Several populations were identified as tolerant/resistant to broomrape infestation.

In Greece, similar experiments were undertaken to screen the performance of a large number of Introgression Lines (ILs). Results indicated their differential response in parasitism.

SIDEBAR 4. Mini-rhizotron studies.

Contribution: INRAT team. Tunisia.

Genetic markers of broomrape resistance

A) In this topic we tried to detect and quickly identify the different broomrape species, in real field conditions, utilizing that knowledge for quick management of the parasite infestation. For that perspective, potential molecular markers using High Resolution Melting analysis (HRM), revealed for the trustworthy detection of several different broomrape species. Furthermore, the analysis of genetic diversity and structuring by dominant RAPD type markers allowed us to structure the Tunisian population of broomrapes clearly. These techniques can be successfully used identify in a quick manner the different broomrape species and manage the parasitism on time (Figure A).

SIDEBAR 4. Discrimination of broomrape species

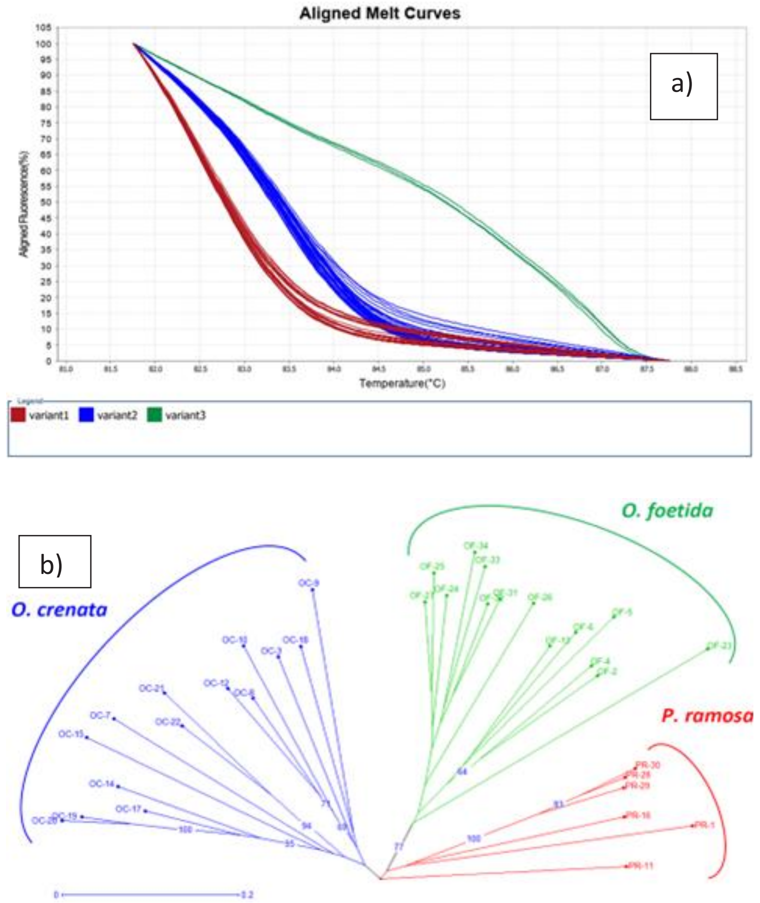


Figure A. a) HRM analysis and clear discrimination of 3 different broomrape species. b) Broomrape species discrimination using RAPD molecular markers.

B) Differences in gene expression between parasitized and non-parasitized tomato plants were revealed. These findings are interesting and can be used for the identification of key genes, involved in tomato tolerance against broomrapes. Utilizing these results, we can study the role of "key" genes and use them in future tomato breeding programs, creating commercial hybrids resistant/tolerant to the holoparasite.

Regarding faba bean, experimental results confirm that several genotypes are the best in terms of growth and yield under broomrape infestation.
These results can be utilized to unravel tolerant genotypes and mechanisms that are involved in broomrape tolerance of different host-plant species (Figure B).

SIDEBAR 4. Differences in gene expression between parasitized and non-parasitized plants.

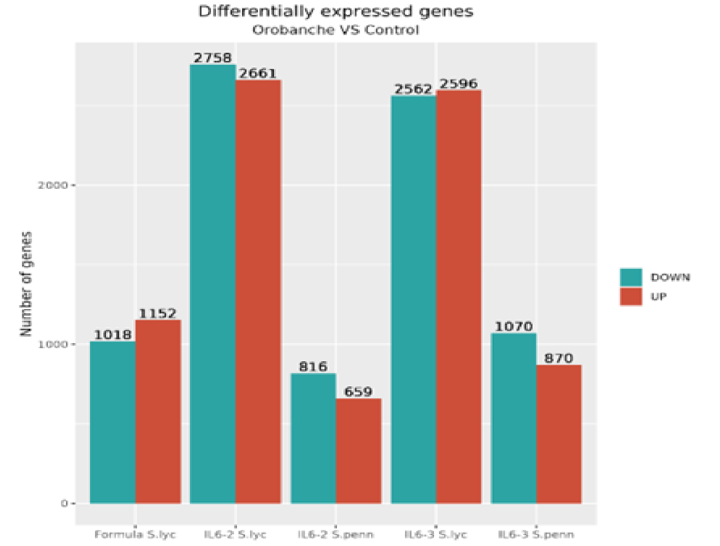


Figure B. a) DEGs revealed in parasitized and non-parasitized tomato genotypes.
Contribution: Vasiliki Kotsira, Maria Gerakari, Eleni Tani

Assessment of Genetic Diversity with SSRs Markers: Jordanian tomato populations

In this study, three landraces of Jordanian tomatoes (*Solanum lycopersicum* L.) were investigated. Two genes regulating pivotal enzymes (CCD7 and CCD8) in the biosynthesis of strigolactones were examined. We found that both of these genes were downregulated in the tomato land races 4, 5, and 6 respectively compared to non- infected tomato control.

Additionally, we investigated the expression patterns of Plant Defensin (PDF), a gene associated with the plant's innate immune response. Plant defensins are known for their multifaceted role in defense against various pathogens. The unexpected downregulation of PDF in our study prompts a reevaluation of its role in the context of broomrape resistance.

Furthermore, we explore the expression of Protease Inhibitors 1 (PI-1) and Protease Inhibitors 2 (PI-2), key components of plant defense mechanisms against herbivores and pathogens. The observed upregulation of PI-1 and PI-2 in our study suggests a robust defense response in the tomato landraces resistant to broomrape, indicating their potential role in impeding parasitic infestation.

SIDEBAR 4. Best faba bean genotypes, regarding their yield.

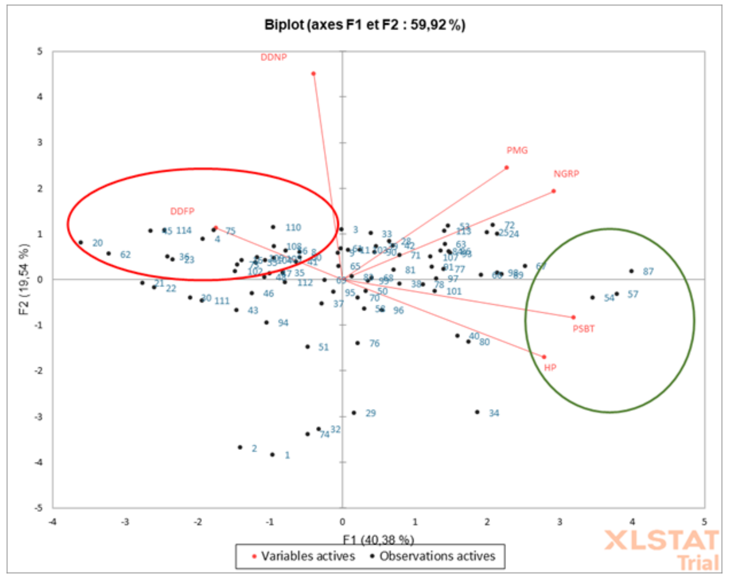


Figure B. b) PCA analysis regarding the best yiels of different faba bean genotypes.
Contribution: Lamiae Ghaouti

The PRIMA programme is supported and funded under Horizon 2020, the Framework European Union's Programme for Research and Innovation
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d) Remote and satellite technologies to map parasitism

Developing tools to map parasitism, at local, regional and national scale is essential. Various sensing approaches have been so far proposed, the thermal and the multi-spectral imaging being the most important ones (Hutcher and Froud-Williams, 2017). Thermal sensing could be achieved by the use of a thermal camera that detects canopy leaf temperature variations due to parasitism. Multi-spectra imaging could produce NDVI values that would be associated with parasitism through lower host plant growth, and could be particularly useful for early detection (prior to broomrape emergence).

Use of Unmanned Aerial Vehicles and multispectral sensors

On an industrial tomato crop of Central Greece with a recorded history of broomrape (*O. ramosa*) infestation, three data acquisition missions were carried out.

The data consisted of ground data and of aerial images captured with the use of a small tetra-copter drone and multispectral sensors (Green, Red, Red-Edge, Near InfraRed and Thermal bands) of the spectrum.

The aerial multispectral imagery data was photogrammetrically processed and vegetation indices (NDVI, Chlorophyll Index and SAVI) and thermal orthophotomaps were produced; mean values were calculated within the boundaries of 139 delimited square plots (1.5m^2) of a grid.

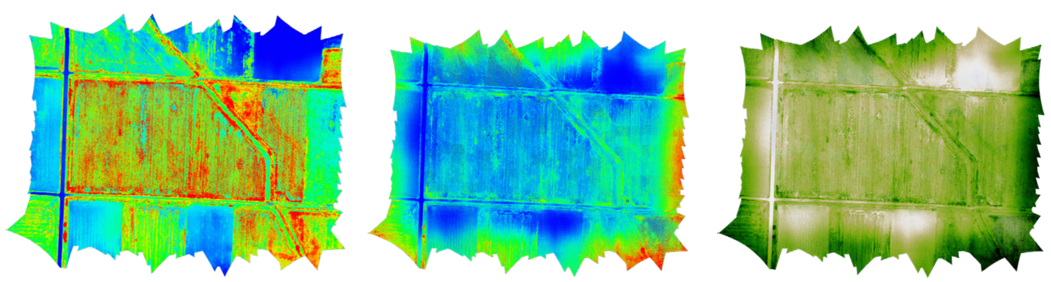
On the ground, hand-held SPAD (leaf Chlorophyll concentration) measurements and visual observations of broomrape infestation were acquired in each plot.

In this study, we analyzed the relationship between the variables for the three summer dates to assess the parasitism effect during the growth period of tomato plants.

SIDEBAR 5. Utilization of UAVs equipped with sensors, in Thessaly region, Greece.

Contribution: Antonis Kavvadias & BPI team

SIDEBAR 5. NDVI, CI & SAVI indexes from the tomato field.



Contribution: Antonis Kavvadias & BPI team

Satellite imaging for remote sensing of broomrape parasitism in industrial tomato in Greece

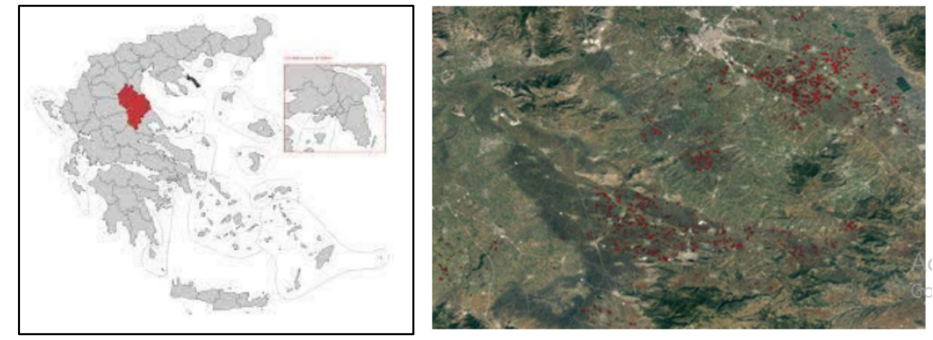
Developing tools to map parasitism, at local, regional and national scale is essential.

In this study, imagery data were acquired from the United States Geological survey (USGS) utilizing Landsat 8 satellite images from Thessaly region (i.e. 4327Km^2), were taken. In this area, filed data (including polygons) of the industrial tomato fields from the year 2018 to 2021 were taken through the local collaborating partners of the project.

For each year, 3 satellite images that correspond to June, July and August were collected. Standard procedures were used (atmospheric correction, clip of multiple rasters, contrast enhancement and GIS methodologies) prior to calculations of the NDVI and NDMI values. Based on those values, a generic model for prediction of risk for broomrape parasitism was developed. The model accounts the two most critical stages of parasitism that relates to the onset of parasitism and the full-scale parasitism in the crop.

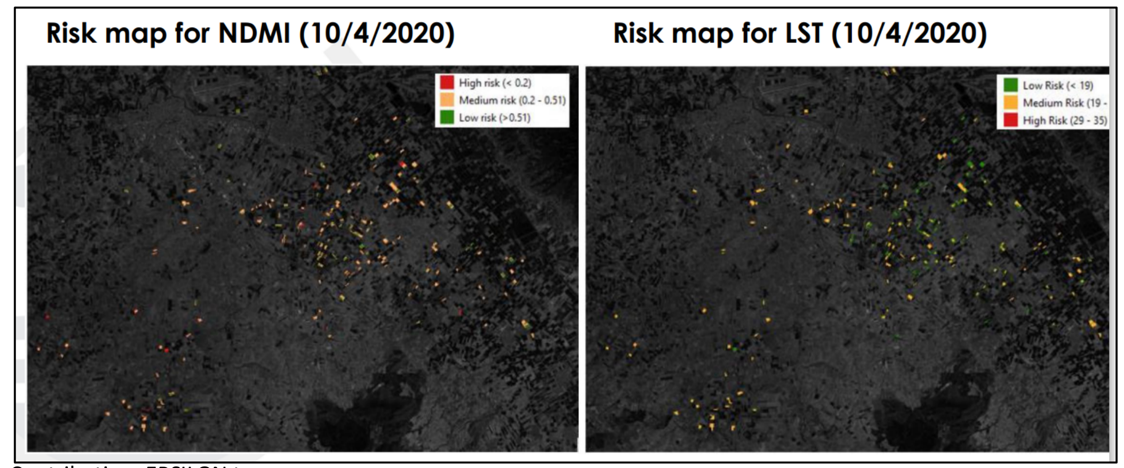
The risk of broomrape parasitism (as low, medium, or high), is set accordingly, based on a range of values for the two parameters that represent the worst-case scenario design. Finally, the usefulness of the model for prediction of parasitism is discussed and management strategies could be developed for the industrial tomato broomrape problems in Thessaly region.

SIDEBAR 5. Satellite image of Thessaly region, Greece, with tomato fields



Contribution: EPSILON team

SIDEBAR 5. Broomrape emergence risk of parasitism maps of Thessaly region, Greece.



Contribution: EPSILON team

ZEROPARASITIC ZEROPARASITIC.eu PRIMA PARTNERSHIP FOR RESEARCH AND INNOVATION IN THE MEDITERRANEAN AREA The PRIMA programme is supported and funded under Horizon 2020, the Framework European Union's Programme for Research and Innovation

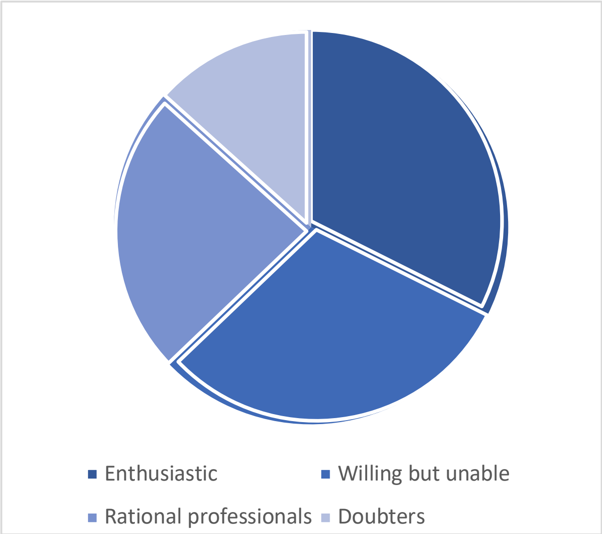
Socioeconomic-Level actions

One of the most central objectives of ZeroParasitic was to shed light on the socioeconomic potential of the proposed solutions against broomrape parasitism. By combining economic analysis for various farm types across the Mediterrane-an and combining it with the deployment of a farmer typology, ZeroParasitic has achieved a contribution towards under-standing the specific practices, needs, opinions and aspirations of Mediterranean farmers not only with regards to com-batting broomrape but towards Sustainable Weed Management Practices (SWMP) in general.

a) Identifying **behavioural drivers** of farmers

Another significant contribution of ZeroParasitic was to demonstrate that farmers are not homogeneous when it comes to innovation adoption. A typology that was developed in the project showed that about one third of farmers were “Enthusiastic” and prone to adopt easily, while a significant percentage were “Willing but unable” to adopt due to their high age or due to inertia and risk-averse behaviour. Only a small part of farmers were “Rational professionals” will-ing to adopt innovations with proven economic performance and compatibility. The smallest group comprised “Doubt-ers” against innovation. This typology showed in an illustrative way that generic measures cannot guarantee adoption and Mediterranean farmers are quite heterogenous. While farmers themselves did not proclaim the design of tailored-made measures as a priority, it has been made evident that only through such measures can SWMP be promoted. Measures to mobilise “Willing but unable” farmers – for example - can have a significant impact for older farmers with low self-confidence while income support can induce adoption for “Rational professionals”. Therefore, strategies and policies towards SWMP need to incorporate social, economic and also geographical considerations in order to approach more farmers and “not leave anyone behind”, which is one of the main characteristics of the European Green Deal.

SIDEBAR 8. Mediterranean farmer typology against adoption of innovation

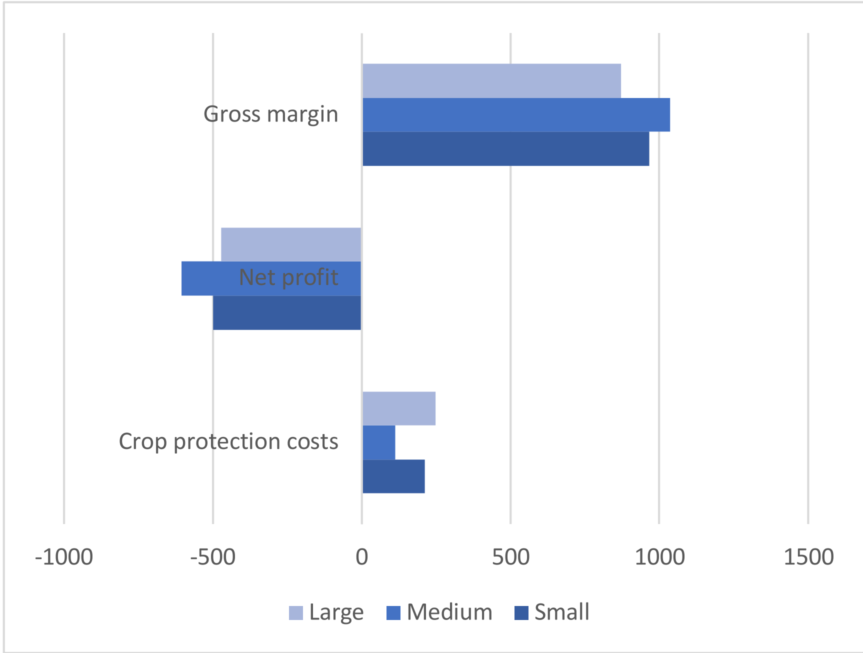


Contribution: Efstratios Michalis, Athanasios Ragkos

b) Improving **farmers’ economic outlook**

With regards to economic performance, the financial analysis showed that SWMP can have a negative impact on rev-enues and farm income. This is due to the higher labor requirements that SWMP entail while these increased costs are not offset by higher revenues. Medium-sized farms were found to be keener to adopt SWMP in industrial tomato and although they saved costs from reduced agrochemical expenses, they experienced significant losses due to excessive hired labor and machinery requirements. Solutions against broomrape parasitism such as smart farming applications can have a positive impact if acquisition and implementation costs can be born collectively at the level of Cooperatives or Producer Groups. On the other hand, differences in the revenues of farms were not significant, however revenues from industrial tomato were the lowest for medium-sized farms adopting SWMP. This was due to the lower yields of these farms which were not balanced by increased market prices. Therefore, the necessity for policy support emerges from this analysis.

SIDEBAR 9. Financial aspects of SWMP in industrial tomato (€/ha)



Contribution: Efstratios Michalis, Athanasios Ragkos

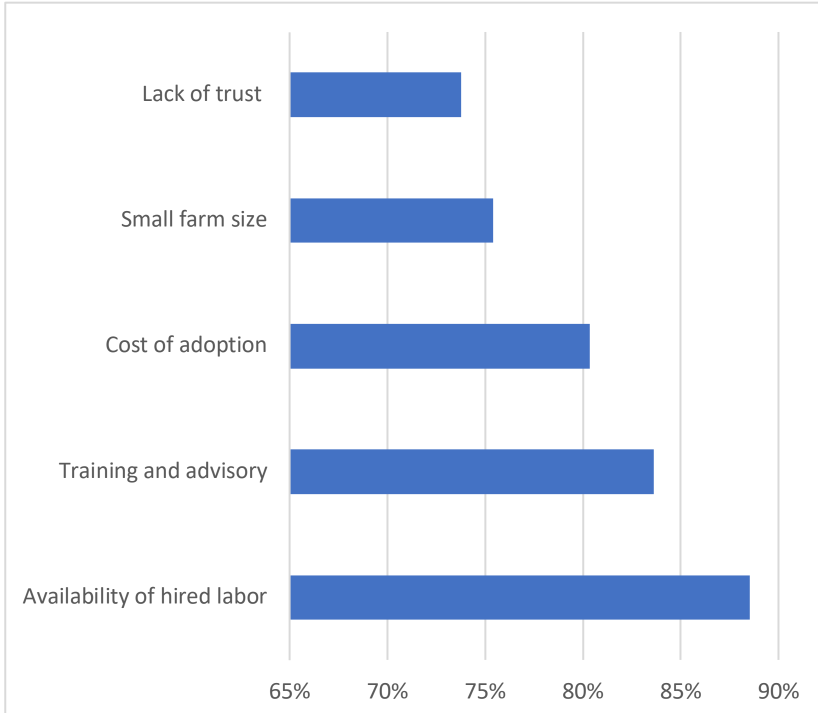
Policy-Level actions

First, identification of policies in-depth that have influences on the current state, development and uptake of IPM solutions. The goal of these actions is a) to discover the potential barriers and bottlenecks, reduce overlaps and to come up with solutions to counter them, and b) to propose adequate and corresponding policy recommendations, best practices and solutions.

a) Framing the **lock-ins for adoption** of IPM strategies

The ZeroParasitic survey delved into the different barriers hindering the adoption of SWMP. As it has been witnessed that farmers in different countries may reject (or adopt at very low levels) solutions and innovations that are otherwise quite promising, it is a prerequisite for effective policy making to understand the reasons behind this lag. Barriers relate to the mindset of farmers – such as lack of trust – and the characteristics of their farms – such as size – but also to external factors. The (un)availability of hired labor to accommodate mechanical or manual weed treatments was – quite surprisingly – proclaimed as the most important barrier to adopt SWMP. The mix of policies and strategies towards the promotion of SWMP should take effectively into account the magnitude of these barriers and target them effectively

SIDEBAR 10. Top-5 barriers to adopt SWMP



Contribution: Efstratios Michalis, Athanasios Ragkos

b) **Policies and strategies** towards SWMP in the Mediterranean

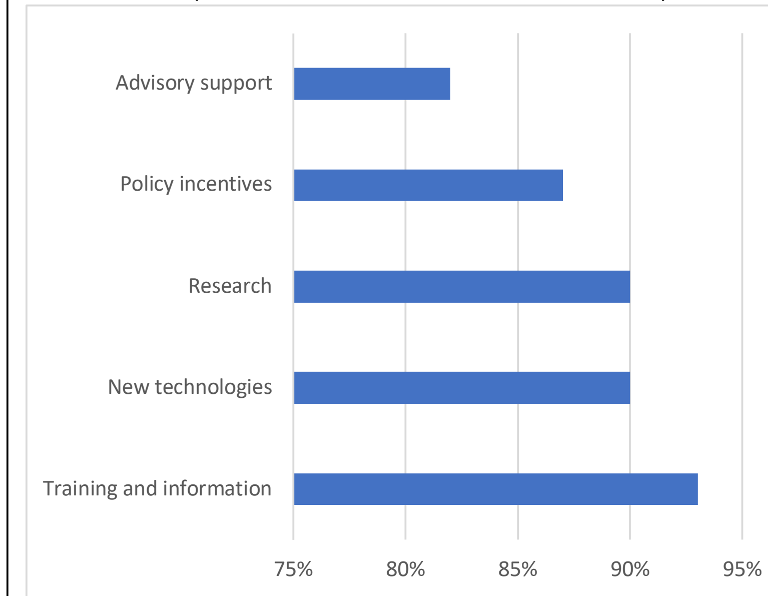
The diversity of farmer profiles combined with the economic losses that relate to SWMP highlight the necessity of policies and strategies to support farmers throughout the adoption and transition processes. According to a survey of farmers, training, information and advisory support as well as new research topics and effective technologies were proclaimed among the most preferred measures.

All these policy elements are effectively comprised in the Agricultural Knowledge and Innovation system (AKIS) that constitutes on the 10 objectives of the CAP 2023-2027.

The integration of research findings related with SWMP within the advisory and farm education systems as well as the training of skilled and specialized advisors to support the adoption and transition of farmers towards SWMP constitute main challenges for AKIS but also opportunities for the current CAP period.

Policy support was one of the highest-ranked measures to overcome barriers hindering the adoption of SWMP, while measures to increase awareness in the general public were not as much appreciated. Policies such as the newly-introduced ecoschemes in the European Union which focus on non-chemical solutions can therefore have a positive impact on SWMP adoption.

SIDEBAR 11. Top-5 solutions to overcome barriers to adopt SWMP



Contribution: Efstratios Michalis, Athanasios Ragkos

RECOMMENDATIONS

The recommendations below provide information for enhancement of research to support novel solution development and implementation in broomrape management in the Med region. The ZeroParasitic project aims to provide an after-life agenda, the consortium members have developed a capacity-building network and frequent meetings (either physical or remote) are taking place aiming to further progress the knowledge and experience acquired in the project. Based on the experience gained through this project, the following recommendations could be drawn:

- **Demonstration is a key issue.** Funding for pilot testing of emerging best practices is highly needed to boost the reach and effectiveness of the novel solutions. Key benefits need to be visualized. Demonstration at the field level, living-labs employment and participatory evaluation of results would enhance the impact and actively disseminate novel solutions into the Med farmers and cropping systems.

- **Training is essential.** The Med region is generally lacking of IPM training for the farmers whereas the new challenges (i.e. Green Deal, that is the minimization of chemical inputs) are exerting severe pressure on producers/advisors. As such, a new paradigm of dissemination of knowledge is needed (farmers & advisors are key players).

• **State-of-the-art solutions.** This could include molecular breeding on resistant cultivars and biological control agents; development of user-friendly digital technologies such as drone monitoring, remote forecasting, weed species identification through DNA barcoding.

• **Utilizing digital tools and Precision Agriculture technologies.** Minor crops (such as most legume crops) or pests (such as broomrapes) frequently have minimal options for weed control using herbicides. However, these minor crops are vital for both food and nutritional at global, european and more importantly in the Mediterranean re-gions. Site-specific broomrape management using Precision Agriculture technologies (i.e. promixal or remote sen-sors, satellite imaging and cloud platforms) are, therefore, highly needed.

• Developing capacity-building networks and appropriate Med broomrape hubs. Collection of all critical information on broomrape management, risks, IPM tools and funding, by launching databases and other information services.

• Align with existing sustainability initiatives, particularly targeting the drought tolerance and high added value of leguminous crops that are needed for the green transition of the cropping systems in the Med region, which often include best practices related to broomrape management and IPM strategies.

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