PRE-EMPTIVE DEVELOPMENT OF MANAGEMENT STRATEGIES FOR BRANCHED BROOMRAPE IN CA TOMATO SYSTEMS

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THE MAIN *GOAL* and CORRESPONDING *OBJECTIVES*: The overarching goal of this project is to develop actionable, cost-effective solutions for rapid detection, containment (sanitation), management, and eradication of branched broomrape in both fresh and processing tomato production systems of California.

The specific objectives addressed in the first year of the project were:

- Evaluate the crop safety of *DSS-PICKIT* technique under California conditions;
- Determine the cardinal temperatures for seed germination of broomrape;
- Calibrate a thermal time model for prediction of the parasitism dynamics.

In addition to the above PICKIT adaptation plan, we initiated some preliminary studies exploring the potential effectiveness of biosolarization in seedbank depletion of small weed seeds. Another study in collaboration with a group of scientists in Germany is conducting a genetic analysis of California branched broomrape.

Introduction:

The parasitic broomrapes (*Orobanche* and *Phelipanche* spp.) are considered as one of the most disrupting weeds in many economically important crops (Jain & Foy, 1989). Lacking chlorophyll, broomrapes entirely survive on uptaking water and assimilates from the roots of their hosts and therefore can cause severe yield losses or, in case of heavy infestations, even total crop failure (Hershenhorn et al., 2009). Studies in Israel show that at high infestation levels (~100 shoots m^{-2}), Egyptian broomrape (*P. aegyptiaca*) can cause yield losses as high as 70 ton ha^{-1} in the processing tomato. In a semi-commercial field in Israel, effective management of Egyptian broomrape (~95% control) increased the tomato yield by 40 ton ha^{-1} and the net revenue by \$4,731 ha^{-1} (Eizenberg & Goldwasser, 2018). The annual losses in tomato due to broomrapes in Israel and Turkey are estimated at \$5 and \$200 million, respectively (Hershenhorn et al., 2009). Up to 80% crop loss due to branched broomrape (*P. ramosa*) has been reported in tomato in Chile. About 30% of tomato growing areas in Greece were once thought to be infested to branched broomrape, with an estimated yield loss of 25% (Parker, 2009).

The re-emergence of branched broomrape in California is particularly concerning to the tomato industry as: 1) the experience in other regions of the world has established extreme vulnerability of the tomato crop to branched broomrape parasitism (Hershenhorn et al., 2009), (2) broomrapes are highly likely to establish and spread in California because of the similarity of California's climate to the native range of species, (3) the availability of a wide range of hosts (e.g. carrot, sunflower, safflower) in California, (4) broomrapes produce copious number of minute seeds (0.2 to 0.4 mm) that can easily disperse via machinery and irrigation water in highly mechanized and irrigated cropping system of California, (5) high seed longevity (~40 years) allows the parasite to persist even in the absence of any hosts, and (6) the major part of the parasite's lifespan occur underground, making it inaccessible to conventional means of weed control such as cultivation and contact herbicides (Hershenhorn et al., 2009). The spread of broomrape in California will further constrain the export of produce out of the state (and country), severely affecting the bottomline of growers.

In the wake of the potential threats posed by this parasitic weed to California tomato industry, there is an urgent need to develop short- and long-term strategies for effective management, containment and eradication of broomrape. Fortunately, we can leverage the decisions support system, known as *PICKIT*, developed by Israeli scientists over the past 25 years, for effective management of Egyptian broomrape in tomato (Eizenberg & Goldwasser, 2018). Implementation of *PICKIT* over 33 commercial tomato fields (400 ha) in Israel, gave 95% Egyptian broomrape control with tomato yields ranging from 115 to 145 tons ha^{-1}. The *PICKIT* system uses a GDD-based phenological model to precisely time the application of PRE and POST herbicides. However, the adaptation of *PICKIT* in California tomato cropping system requires some modifications and further evaluations because: (1) the current *PICKIT* model has been optimized based on the growth and development of Egyptian broomrape and therefore needs to be re-calibrated for branched broomrape, (2) the herbicides found to be most effective in *PICKIT* (sulfosulfuron and imazapic: Eizenberg et al. 2012) have not been registered for use in tomato in California, and (3) differences in soil and climate conditions, crop variety, and management practices can affect the phenology of parasite and herbicide efficacy necessitating the reassessment of *PICKIT* under California tomato growing conditions.

Methodology and Results:

A. **Crop Safety Trials**: As scheduled, tomato safety trials to evaluate the crop safety of the *DSS-PICKIT* system were conducted with supplemental funding from the USDA-IR4 program in spring 2019. Two trials were initiated in May and June-planted tomatoes and a third rotational crop safety experiment was established in June 2019 and will be planted to rotational crops in 2020. Regular crop injury evaluations and plant vigor ratings did not reveal any visible crop injury or developmental delays. Fruit yield data did not suggest negative impact of the *DSS-PICKIT* on tomato. Thus far, the *DSS-PICKIT* techniques for broomrape control in tomato appear safe under California production conditions.
B. **Seed Collection**: Broomrape seed collection from greenhouse propagated plants was scheduled for May to July, 2019. However, the planned soil collection from an infested field site was delayed by winter soil conditions and delays in the CDFA permitting process. Soil was collected in June 14, 2019 and branched broomrape plants were propagated at the Contained Research Facility (CRF) of UC Davis. Matured seeds were collected during the last week of October 2019 and are being stored in dark at room temperature for future uses.
C. **Seed Germination:** Seed germination represents the first step in the progression of broomrape parasitism and the success of soil applied herbicides in controlling germinant (i.e. germinated but unattached seeds), to large extent, depends on the precise prediction of germination timing. The timing of germination also dictates the progression of other phenological events including attachment, emergence, flowering and maturity. Once chemo-stimulants are released from the roots of the host to the rhizosphere, the germination process is mainly governed by the soil temperature. One of our objectives in this project is therefore to model the germination responses of branched broomrape seeds to temperature as the first step in calibration of *PICKIT.* The seed germination studies were scheduled to commence by August but were delayed until first week of November as a result of delayed seed collection and permits explained above. Seed germination were tested under two temperature condition (10 and 20 C) unlike the initial plan of testing a wide range of temperatures (4, 8, 12, 16, 20, 24, 28, 32, and 36 C). This due to limited growth chamber in the CRF. About 100 seeds of branched broomrape were placed on moistened filter paper in a 5-cm diameter Petri dish and kept in dark at 20 C for a week as pre-conditioning, and then moistened with a solution (10 ppm) of GR_{24} (a synthetic germination stimulant). Using one growth chamber, the 10 and 20 C constant temperatures were tested in a sequential manner, with three replications of Petri dish containing the pre-conditioned seeds treated with germination stimulant. Observation of seed germination using a microscope lasted two weeks for each temperature. The results

showed that about 55% of the seeds germinated under 20 C compared to about 10% germination at 10 C. Relatively higher germination under 20 C was not surprising as a previous study suggested 18 to 23 C as the optimum germination temperature in a similar parasitic species, Egyptian broomrape (Kebreab and Murdoch 1999). It is important to evaluate temperature higher than 20 C in order to conclude on the optimal temperature for branched broomrape.

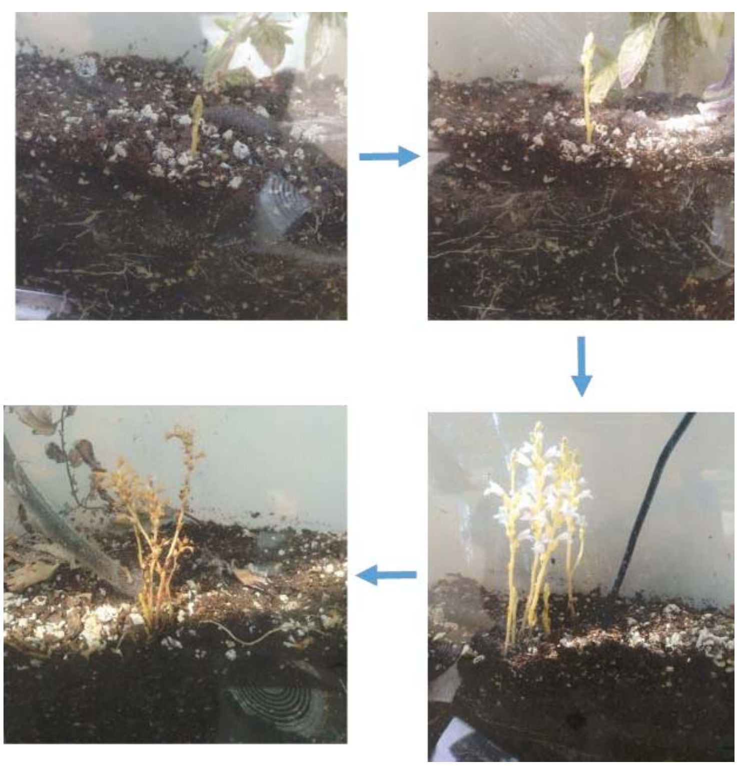
A germinating seed of branched broomrape, viewed under microscope in the CRF at UC Davis

D. Thermal time model of Parasitism: The belowground development of broomrape parasitism was aimed to be quantified nondestructively by using glass-fronted rhizotrons made of cut-in-half PVC tubes (40 cm in diameter and 65 cm in length) as shown in images below:

Liquid glue was used to fix branched broomrape seeds on the transparent glass of the six rhizotrons. Rhizotrons was then filled with soil mix (1:1:1 sand:peat:dolomite) and tomato was directly seeded directly into the soil. Rhizotrons was tilted (~30 degree angle) downward so as the glass front facing the ground to ensure the tomato root encounter with the broomrape seeds fixed on the glass. Arranged rhizotrons on a bench in the CRF greenhouse are shown below:

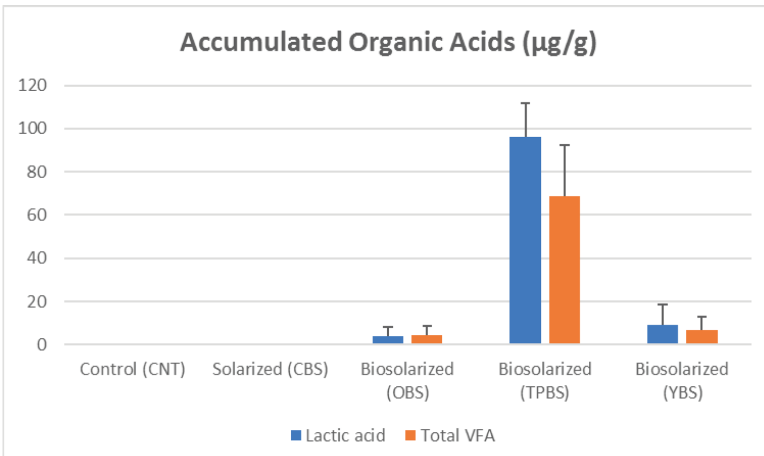
Due to limited space in the CRF, the study was conducted only with 25/18 C (day/night) temperature unlike the initial plan of four different temperature regimes of 20/12 C, 23/15 C, 26/18 C, and 29/21 C (day/night). However, a data logger (Meter Group) was buried at the depth of 10 cm within four of the six rhizotrons to record hourly temperature aimed to calculate thermal time in growing degree days (GDD). The rhizotrons were inspected visually and by taking photos of tomato roots, through the transparent glass, for the presence of broomrape attachments and development of tubercle. Emergence of shoots over time, flowering and maturity were also observed. The observation lasted for about 14 weeks (96 days) after tomato emergence. A thermal time component of the model is yet to be calculated as effort is still being made to extract the data from the logger. However, observation indicated that branched broomrape emergence coincided with early blossom stage of tomato which was about 10 weeks (70 days) after tomato emergence, and broomrape flowering commenced about 4 to 7 days after emergence. Seeds were formed about 4 weeks after emergence (2 to 3 weeks after flowering). Very few branched broomrape tubercle were observed and these were observed at the first flush of broomrape emergence. Another set of rhizotron study are being conducted in the CRF for repeatability and with the hope of detecting the development/attachment of the tubercle on tomato root.

A 1-cm long tubercle on tomato root observed at the first flush of branched broomrape emergence

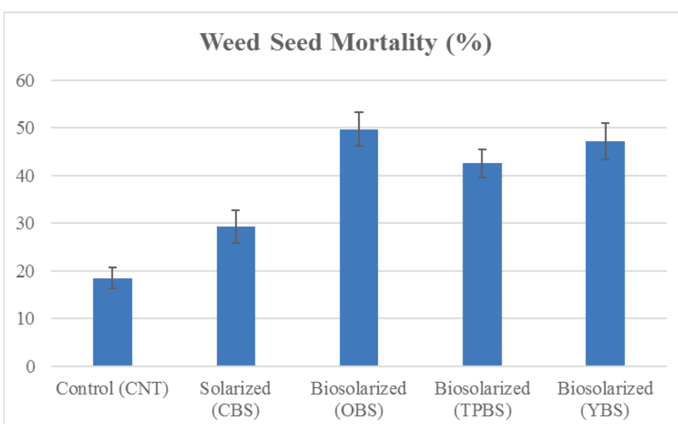


Developmental stages: From early emergence to flowering and maturity stage

E. Biosolarization Study: The biosolarization study was conducted as planned in summer 2019. Treatments were incorporated to the soil prior to the solarization: early development tomato plants (YBS), late development tomato plants (OBS) and tomato pomace (TPBS). Non-amended (CBS) and control (CNT) plots were included. The tomato pomace was spread on the soil surface and the tomato plants were first flail mowed before all plots were mechanically incorporated to a depth of 5 cm with a rototiller. Individual plots were 1.5 m wide and 3.7 m long and each treatment was replicated six times. The evaluated weed seeds were redroot pigweed (*Amaranthus retroflexus)*, common lambsquarters (*Chenopodium album*), and field bindweed (*Convolvulus arvensis*). Weed seeds were buried following incorporation of plant debris, prior to plastic tarp installation. For each weed species, there were 2 cloth bags (~ 0.3 litre in size) containing 100 seeds each, buried in a depth of about 5 cm in each plot. The buried seeds were collected after two weeks for viability test. All the tested treatments with organic amendment showed accumulation of organic acids (e.g lactic acid and total volatile fatty acid [total VFA]) that are the target bio-pesticides that are expected to have an important role in the inactivation of broomrape seeds in the soil. Results showed that the incorporation of tomato pomace generated greater amounts of lactic acid and total VFA compared to those generated by tomato plants.



Biosolarization partly killed the seeds of the evaluated weeds; the level of mortality varied with the type of organic amendment. Treatments with organic amendments provided greater weed seed mortality (42 to 50%) compared to those without organic amendments (18 to 29%), averaged across weed species. The next step would be to confirm if the detected levels do inactivate broomrape seeds.



F. **Genetic analysis of California branch broomrape:** We joined an international effort aimed at exploring the genetic diversity of broomrape species across the globe. We sent plant materials of branched broomrape obtained from our CRF experiment to Dr. Susann Wicke (Institute for Evolution and Biodiversity, University of Muenster, Germany). The genetic analysis will allow us to gain insight into the origin of California broomrape population i.e. where does California branched broomrape come from? This study was not part of our initial proposed plan.

Discussion: Parasitic broomrapes are notorious for their devastating impacts on various high value crops in many regions of the world and the re-emergence of branched broomrape (*Phelipanche ramosa*) in California is deemed as a big threat to the sustainability and profitability of tomato industry. California is lacking effective management solutions to cope with this difficult-to-control weed. The DSS-*PICKIT*, has been developed over two decades of research in Israel, which has been proven to provide successful management of "Egyptian" broomrape (*P. aegyptiaca*) in tomato. Our crop safety study suggested that the *DSS-PICKIT* system which includes a PPI application of sulfosulfuron followed by several chemigation treatments of low rates of imazapic at prescribed intervals caused no tomato injury or yield loss in California condition. A field-level evaluations of this system is ongoing in a grower's field known to be infested with branched broomrape, results from this study will provide information on whether this *PICKIT* system is effective for branched control in California. The *PICKIT* system is largely based on a thermal time model that forecast the belowground development of parasite to precisely time the application of PRE and POST herbicides. Effort to develop this thermal time model for California condition is ongoing, however, a preliminary result suggest that branched broomrape can have increased germination under 20 C temperature and it emergence aboveground is almost at the same time with early flowering stage of tomato. Our preliminary study suggest biosolarization as a potential tool for weed seedbank depletion. Efforts is being made to know the level of mortality this method can have on branched broomrape seeds; this information will help to know the contribution of biosolarization as a component of integrated approach to managing broomrape in tomato.

What's next for this project: A preliminary observation with the current seed propagation suggests that the level of broomrape parasitism in tomato may depend on the variety of the crop. We would like to conduct a study that evaluates a wide range of tomato varieties, to determine if there are differences among varieties with regard to hosting broomrape. Secondly, we only evaluated the safety of the DSS-PICKIT techniques on a single tomato variety; it may be important to determine if there is differential response of California commercial tomato varieties to these techniques. We have also prepared an extension-type article, currently under review in California Agriculture Journal, about the biology and management of branch broomrape that will be released very soon.

This project as leverage for other dollars:

CTRI funds for this project are being successfully leveraged with other sources to address this important risk to the California tomato industry. Other funding sources include CDFA-PHPPS (CTRI-Bagley lead), USDA-IR4 program (Hanson lead), and most recently a CDFA-Specialty Crop Block Grant (Mesgaran lead-PI). Those additional sources of funding will likely total nearly $500k over the next three years.

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