PRE-EMPTIVE DEVELOPMENT OF MANAGEMENT STRATEGIES FOR BRANCHED BROOMRAPE IN CA TOMATO SYSTEMS – 2020 UPDATE

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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 (2019) 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.

The specific objectives addressed in the second year (2020) of the project were:

- Rapid identification of broomrape infected tomato plants ;
- Continue field evaluation of DSS-PICKIT technique to support herbicide registration efforts;
- Field validation opportunity for remote sensing efforts.

In addition to the above PICKIT adaptation plan, studies have continued in 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**: Tomato safety trials to evaluate the crop safety of the *DSS-PICKIT* system have been 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 was 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. The plantback experiment suggested some rotational crop issues with sulfosulfuron will need to be addressed if the herbicide programs are registered. In 2020, a third crop safety trial was conducted at the UCD campus and a broomrape control study conducted in a grower field. Thus far, the DSS-PICKIT techniques for broomrape control in tomato appear safe under California production conditions. The sulfosulfuron component of this program was prioritized for herbicide residue testing in the IR4 program with first field trials conducted during 2020 and continuing in 2021. Unfortunately, the grower field trial results indicated that broomrape control with the DSS-PICKIT programs was not as effective as anticipated, possibly due to differences between the phenology of branched broomrape in California and Egyptian broomrape in Israel where the program was developed. Additionally, potential barriers to registration of one of the herbicide components (imazapic) in California became apparent after multiple discussions with the registrant. For 2021, changes to the proposed treatment regime will be made in an effort to address broomrape growth and emergence patterns in California as well as a change in chemical focus from imazapic to imazamox which does not have the same regulatory barriers in the state.
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 (HOBO, Bourne, MA) was buried at the depth of 10 cm within four of the six rhizotrons to record hourly and daily 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.

Results from First Study: Emergence of shoots over time, flowering and maturity were also observed. The observation lasted for about 14 weeks (96 days) after tomato emergence. 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.

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

Results from Second Study: A second set of rhizotron study commenced in late May 2020, when ~ 10 cm tall tomato seedlings were transplanted into the rhizotrons. The aim of the second trial is for repeatability of first trial and to detect the attachment and development of the tubercle on tomato root. Early tubercle were first found at 504 GDD (equivalent to 37 days after transplanting tomato [DAT]). The tubercles were still being formed until 826 GDD (61 DAT). Shoot started emerging from the tubercle at 746 GDD (equivalent to 55 DAT). The emergence of multiple shoots from a single tubercle commenced at 773 GDD (equivalent to 57 DAT). The first above ground emergence of branched broomrape was about 840 GDD (62 DAT) and coincided with the early blossom stage of the tomato plants. Above ground emergence of broomrape lasted up to 1416 GDD (105 DAT). Broomrape flowering commenced about 3 days after above ground emergence and mature (black) seeds were formed about 4 weeks after emergence (~ 3 weeks after flowering).

Developmental stages: From early tubercle formation to shoot above soil level

Starlike early tubercles ~35 days after transplanting tomato

Advanced tubercle ~42 days after transplanting tomato

First shoot (~2.5 cm long) from tubercle ~55 days after transplanting tomato

Multiple ~ 57 days after transplanting tomato

Above soil emergence ~ 60 days after transplanting tomato

Flowering ~ 65 days after transplanting tomato

***Results from Third Study*:** The rhizotron study was further conducted for the third time. This study commenced in late August 2020, when ~ 10 cm tall tomato seedlings were transplanted into the rhizotrons. This is to confirm results from previous studies. The tubercle were detected earlier at 430 GDD (equivalent to 32 days after transplanting tomato [DAT]) than in the previous study. Tubercles were detected on tomato roots until 693 GDD (60 DAT). Shoot started emerging from the tubercle at 690 GDD (equivalent to 52 DAT). The emergence of multiple shoots from a single tubercle commenced at 754 GDD (equivalent to 52 DAT). The branched broomrapes started emerging above ground at about 933 GDD (71 DAT). The relatively delayed emergence above the ground was due to depth of tubercle below the ground, which was up 10 cm depth, compared to a maximum of 5 cm depth in the previous study. Broomrape flowering commenced about 5 days after above ground emergence and the first set of mature (black) seeds were being formed at 5 weeks after emergence (~ 4 weeks after flowering). The growing degree days (GDD) of the observed phenological dates in these studies were calculated based on air temperature in the greenhouse, and the GDD based on soil temperature may be slightly different from above.

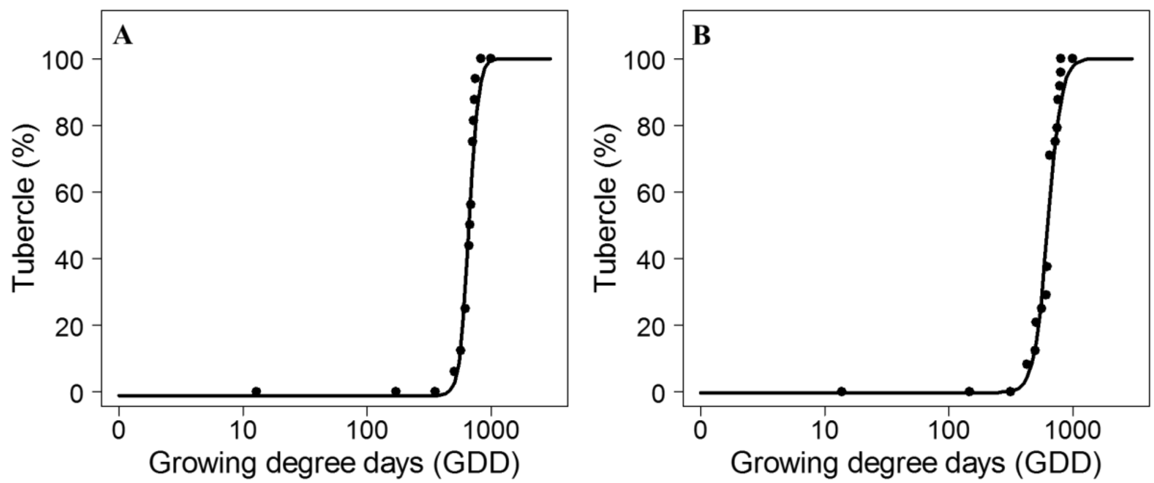
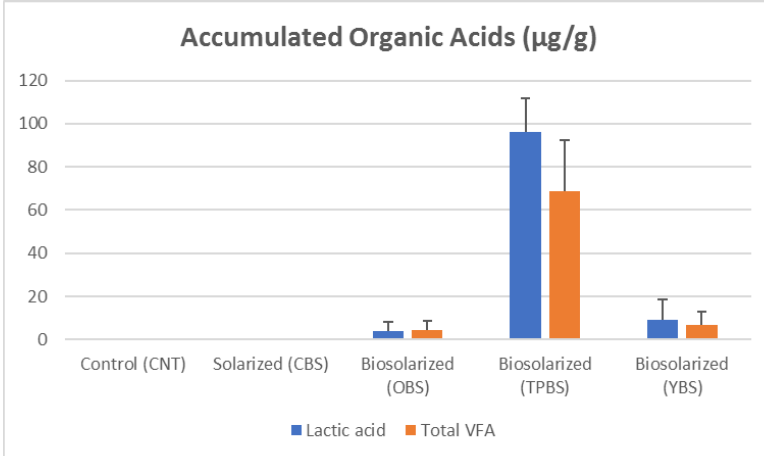
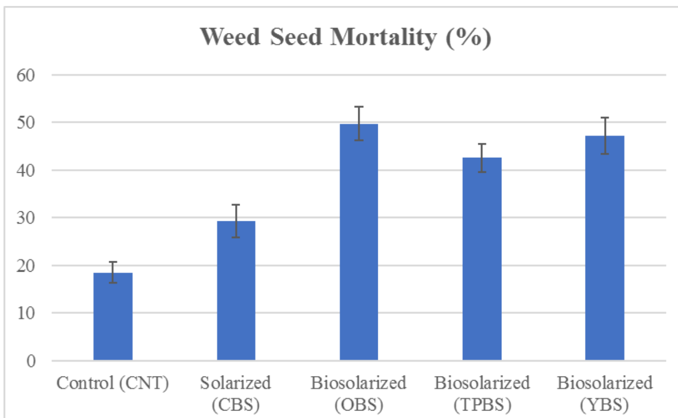


Figure: Branched broomrape tubercle attachment over thermal time, during two different studies (A and B)

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 conditions. A field-level evaluation 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. Efforts to develop this thermal time model for California condition is ongoing, however, preliminary results suggest that branched broomrape can have increased germination under 20 C temperature and its emergence aboveground is almost at the same time with early flowering stage of tomato. Our preliminary study suggests biosolarization as a potential tool for weed seedbank depletion. Efforts are 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 an integrated approach to managing broomrape in tomato.

What's next for this project: Efforts to develop a hyperspectral technology for rapid detection of broomrape parasitized tomato plants is still ongoing. The 2020 field location, with more than a thousand marked individual broomrape clumps, was used as a field validation site for a drone-based imaging systems; these data are being analyzed and will be used to supplement other rapid-detection aspects of the research. The field efficacy research will continue in 2021 with some changes to the chemigation protocol to adapt the DSS-PICKIT treatments in response to observations in 2020 and to refocus on herbicide components with greater likelihood of registration in California. The 2020 location will also be used for a field evaluation of soil fumigation research trial supported by separate research funding. Initial research on the seed mortality efficacy of ammonium compounds as equipment disinfestation techniques suggests that ammonium compounds such as alkyl dimethyl benzyl ammonium chloride (ADAC), didecyl dimethyl ammonium bromide (DDAB) and didecyl dimethyl ammonium chloride (DDAC) effectively killed branched broomrape seeds with 1% solution concentration when let sit for 20 minutes on seeds. A further study suggested the required concentration of the compounds for effective broomrape seed mortality depends on the duration of exposure of the seeds. With one hour exposure, 0.5% solution concentration of the compounds was sufficient to cause 100% seed mortality. In addition, 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. Furthermore, 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 a blog post (https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=43342), several cooperating personnel have given extension presentations on the topic during 2020, and we have submitted an extension-type article about the biology and management of branch broomrape the journal California Agriculture (Osipitan et al.).

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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