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Effect of Different Methods to Control the Parasitic Weed *Phelipanche ramosa* (L.- Pomel) in Tomato Crop

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Abstract**—***Phelipanche ramosa* is the most damaging obligate flowering parasitic weed on wide species of cultivated plants. The semi-arid regions of the world are considered the main centers of this parasitic plant that causes heavy infestation. This is due to its production of high numbers of seeds (up to 200,000) that remain viable for extended periods (up to 20 years). In this study, 13 treatments for the control of *Phelipanche* were carried out, which included agronomic, chemical, and biological treatments and the use of resistant plant methods. In 2014, a trial was performed at the Department of Agriculture, Food and Environment, University of Foggia (southern Italy), on processing tomato (cv 'Docet') grown in pots filled with soil taken from a field that was heavily infested by *P. ramosa*). The tomato seedlings were transplanted on May 8, 2014, into a sandy-clay soil (USDA). A randomized block design with 3 replicates (pots) was adopted. During the growing cycle of the tomato, at 70, 75, 81 and 88 days after transplantation, the number of *P. ramosa* shoots emerged in each pot was determined. The tomato fruit were harvested on August 8, 2014, and the quantitative and qualitative parameters were determined. All of the data were subjected to analysis of variance (ANOVA) using the JMP software (SAS Institute Inc. Cary, NC, USA), and for comparisons of means (Tukey's tests). The data show that each treatment studied did not provide complete control against *P. ramosa.* However, the virulence of the attacks was mitigated by some of the treatments tried: radicon biostimulant, compost activated with *Fusarium*, mineral fertilizer nitrogen, sulfur, enzone, and the resistant tomato genotype. It is assumed that these effects can be improved by combining some of these treatments with each other, especially for a gradual and continuing reduction of the "seed bank" of the parasite in the soil.

Keywords**—**Control methods, *Phelipanche ramosa,* tomato crop.

I. Introduction

In the Apulia region (southern Italy), the chlorophyll-lacking root holoparasite plant *Phelipanche ramosa* (L.) Pomel (syn. *Orobanche ramosa* L.) is devastating the cultivation of processing tomato crops. The heavy infestation is due to the production of high numbers of seeds by *P. ramosa* (up to 500,000 per plant) [1] that have very small dimensions (about 0.2-0.3 mm) and that in the absence of a host can remain viable in the soil for extended periods(up to 20 years) [2],[3].

The parasitized tomato plants initially show some stunted growth, and subsequently a decrease in the quantity and quality of the yield, as a consequence of the reduction in the capacity utilization of the nutrients and the absorption of water removed from the parasitic plant.

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Studies on different control methods against this parasitic weed have been tried (e.g., physical, chemical, agronomical, biological, biotechnological). However, to date, there are no practical methods that are effective, economical and protective against this parasite, though the results are dependent on environmental conditions. For this reason, an integrated approach is needed that uses different control methods, to maintain the parasite populations below the threshold levels for damage [4]-[7].

To overcome the use of chemical methods, more attention towards suitable methods to control *P. ramosa* have been proposed. These concern the use of agronomic soil management, such as the use of nitrogen, phosphorus and sulfur fertilizers [8]-[11], organic compounds [12]-[17], biological agents like *Fusarium* spp. or *arbuscular mycorrhiza* [18]-[25], and biotechnological techniques, such as crops that are resistant to the parasite [26]-[27].

The aim of the present study was to determine the effects of several methods for the control of the root-parasitic *P. ramosa* in processing tomato crops. The methods were mainly focused on agronomic techniques, with the use of some organic composts (i.e., olive-mill wastewater, biostimulants), where there is absolutely no information on the control of *Phelipanche* in the literature, and inorganic fertilizers applied in to the soil (i.e., nitrogen, sulfur). Also, biological (i.e., *Fusariumoxy sporium* and arbuscular mycorrhiza) and biotechnological (i.e., resistant tomato cultivar) methods were used. In particular, the objectives of the study were: (*i*) to determine the effects of each experimental treatment on the control of the root parasite during tomato crop stages;(*ii*) to evaluate the effects of 13 treatments on qualitative and quantitative aspects of tomato crop production.

II. Material and Methods

The experiment was performed in 2014 at the Department of Agriculture, Food and Environment, University of Foggia (southern Italy) (41°27'27''N; 15° 31'56''E; 75 m a.s.l.), on processing tomato grown in parallelepiped pots (63 cm long, 32 cm wide, 28 cm high), filled with soil taken from a field that was heavily infested with *P. ramosa.* The pots were placed in an open field.

The experimental site was in a typical semi-arid zone that is characterized by a Mediterranean climate, with a mild winter and dry-warm summer. According to long-term climatic data, the annual mean rainfall and temperature are 526 mm and 10.9 °C, respectively [28]. The crop was transplanted on May 8, 2014 (two plants per pot) in a sandy-clay soil (USDA).

Basic mineral fertilization was carried out, which was equivalent to 100 kg ha^{-1} nitrogen (N); 60 kg ha^{-1} phosphorus (P_2O_5), and 20 kg ha^{-1} sulfur (S). Afterwards, top dressing was performed with 70 kg ha^{-1} N. Irrigation scheduling (i.e., times, volumes of water supplied to the pots) was performed according to the soil water balance approach. Therefore, the gravimetric soil moisture was measured weekly by weighing each pot to determine the water depletion from the soil; the amount of water supplied with the irrigation re-established the soil water content to field capacity.

In this experiment, 12 treatments against the parasitic weed were compared with the untreated control, as reported in Table I. A randomized block design with 3 replicates (pots) for each of the treatments was adapted.

During the tomato crop cycle, the *P. ramosa* infestation was assessed by the number of emerged shoots (branched plants) in each pot at 70, 75, 81, and 88 days after transplanting (DAT).

At harvesting, on August 8, 2014, the marketable yield (MY; kg pot^{-1}) was determined. Moreover, for a sample of 10 marketable tomato fruit from each pot, the following qualitative parameters were measured: mean weight (MW; g); soluble solids content of the fresh fruit (SSC; °Brix), dry matter content of the fruit (DM; % fruit fresh matter) (AOAC 1990), and the a^*/b^* ratio (CI) [29]. The tomato fruit characteristics were measured using a CM-700d spectrophotometer (Minolta Camera Co. Ltd., Osaka, Japan), as the CIELAB coordinates (i.e., L^*, a^*, b^*) on four randomly selected areas of the fruit surface. Only the a*/b* ratio and the L coordinate are reported, which represent the parameters that describes the color modifications of the tomato fruit well [30],[31].

The whole dataset was tested related to the basic assumptions of analysis of variance (ANOVA), and the significance differences of the means were determined using Tukey's tests, at 5%.

TABLE I
DESCRIPTION OF THE TREATMENTS USED IN THIS STUDY

Treatment	Description
T1	*FUSARIUM* spp. isolated from diseased *Orobanche tubercles* were grown in solid culture (wheat seeds) and incorporate into the soil 7 days prior to tomato seedling transplantation.
T2	OLIVE-MILL WASTEWATER, incorporated into the soil at an equivalent dose of 80 m^3 ha^{-1} (amount permitted by Italian Law No 574, 1996), 60 days prior to seed ling transplantation.
T3	OLIVE-MILL WASTEWATER, incorporated into the soil at an equivalent dose of 160 m^3 ha^1, 60 days prior to the seedling transplantation.
T4	SUMUS, organic fertilizer including a manure mixture from cattle, poultry and domestic stallatic, incorporated into the soil at a dose of 3.3 t ha^{-1} at 7 days prior to the seedling transplantation.
T5	RADICON BIOSTIMULANT, a suspension–solution containing humic and fulvic acids, obtained from the compost of worms (night crawled), applied at seedling transplantation by soaking the tomato seedling roots in the concentrated solution of 1.5%, and at the three early irrigations with the solution at the same concentration.
T6	'*RED SETTER*' TOMATO cv., a processing tomato round fruit
T7	*FUSARIUM* spp. INOCULATED COMPOST, an agro-industrial sludge of husk and straw, applied at a dose 4.0t ha^{-1} at 7 days prior to the seedling transplantation.
T8	'*RED SETTER*' TILLING TOMATO, mutant resistant plant created from the cv. 'Red setter'tomato by tilling technology (targeting induced local lesions in genomes).
T9	TAYLOR TOMATO CULTIVAR, INOCULATED WITH *ARBUSCULAR MYCORRHIZAL* FUNGI (*Glomus intraradices*), performed by the seedling nursery.
T10	ELEMENTAR SULFUR, incorporated into the soil at a dose of 8 t ha^{-1} prior to the seedling transplantation.
T11	NITROGEN FERTILIZER, N applied to the soil at a dose 80 kg ha^{-1} using ammonium sulfate prior to the seedling transplantation.
T12	ENZONE soil fumigant with the active ingredient of sodium tetrathiocarbonate (402 g L^{-1} H_2O) in the form of a water-soluble concentrate, incorporated into the soil 60 days prior to the seedling transplantation.
T13	CONTROL, no treatment.

*The processing tomato cultivar used in all of the treatments was 'Docet', whereas different cultivars were used in T_6, T_8 and T_9 as indicated in the Table.

III. RESULTS AND DISCUSSION

The earliest shoots of *P. ramosa* that emerged from the soil (i.e., branched plants) appeared at 53 DAT of the tomato plants for the treatment with applied sulfur. Afterwards, at 70 DAT, the parasitic weed was detected for almost all of the treatments compared, where its numbers increased progressively throughout the tomato crop cycle, albeit with differences among the treatments.

Among the treatments compared, in general, the greatest increase in the number of shoots that emerged from the soil appeared from 75DAT to81 DAT. Only in the olive-mill wastewater (T_2), for the 'Red setter' tomato cultivar (T_6),and for the control (T_{13}), did the increase in the number of shoots emerged occur earlier than 75 DAT. There were little or no significant differences among treatments at 70 DAT and 75 DAT, whereas marked differences were observed at both 81 DAT and 88 DAT. At the end of the tomato crop cycle (88 DAT), significant variations in the parasitic shoot numbers were noted across the treatments, from 5.0 to 19.7 (Fig. 1). Significantly lower values were recorded, in increasing order, for the follow treatments: Radicon biostimulant (T_5), compost inoculated with *Fusarium* (T_7), nitrogen (T_{11}), sulfur (T_{10}), and the 'Red setter' improved tomato cultivar (T_8), which corresponded to 5.0, 6.0, 6.3, 8.0, and 8.7 emerged shoots, respectively. Therefore, the percentages of the reductions in the numbers of the *P. ramosa* shoots in each of these treatments, as compared to the untreated control, were 61.3%, 59.9%, 49.0%, and 44.5%, respectively. For the other treatments compared, including the use of *Fusarium* (T_1) and olive-mill wastewater (T_2 and T_3), the number of emerged shoots was very close to that of the untreated control (15.0) (Fig. 1).

In particular, the positive effects of the radicon biostimulant, which is a suspension-solution containing humic substances (HS) that was introduced into the soil rhizosphere, can cause severe physiological disorders of the germinating *P. ramosa* seeds, thus reducing the number of developing tubercles of the parasite, as reported also in a previous study [32].

High levels of nitrogen fertilizer (80 kg ha^{-1} N) or sulfur (8 t ha^{-}S) applied prior to the tomato seedling transplantation showed a suppressive effect on the seed germination of *Phelipanche*. Similar results were obtained in a previous study on tomato and on eggplants and potato [11].

For the biological methods, the treatment of the compost actived by *Fusarium* was efficient in reducing the infection, by minimizing the number of parasitic spikes on the host tomato plant. This might be due to additive effects on the seed germination of the parasite of the organic compound along with the soil-borne fungi. This is in agreement with earlier studies that examined *Fusarium* spp. [33],[34].

In the present trial, the use of tomato plants colonized by arbuscular mycorrhiza did not show any effects for are reduction of *Phelipanche* control. These results are in contrast with other studies on tomato that have shown reduced germination of the seeds of this parasite by lowering the amounts of stigolanctones in the root exudates of the colonized plants [35],[36]. This might be due to different types of stigolanctones and different quantities that are produced by any single plant species, and also to different varieties within the some species [37]-[39].

The resistant 'Red setter' tomato cultivar that had been improved by TILLING technology that was used in this study showed lower numbers of emerged parasitic shoots than the original cultivar.

For the marketable yield of the tomato crops, no differences among treatments were detected, probably due to interference from others factors related to the different genotypes and organic and inorganic nutritional products used in this study.

However, the treatments with the same 'Docet' tomato cultivar that corresponding to the higher numbers of emerged *P. ramosa* shoots gave a yield that was lower (by about 15%) than the other treatments compared (Fig. 2).

For the qualitative traits of the tomato fruit, only the mean weight (MW), color (L) and dry matter content (DM) showed significant differences, with higher values for T_{11} and T_{12} (41.24, 42.3, respectively) compared to the other experimental treatments.

IV. Conclusions

In view of the importance of processing tomato as a major cash crop for farmers, and the heavy losses in the field mainly due to *Phelipanche ramosa* infestation in the Apulia region (southern Italy), it is very important to select the best method to control this harmful weed. Thus the search for sustainable methods for controlling this parasite has become increasingly important.

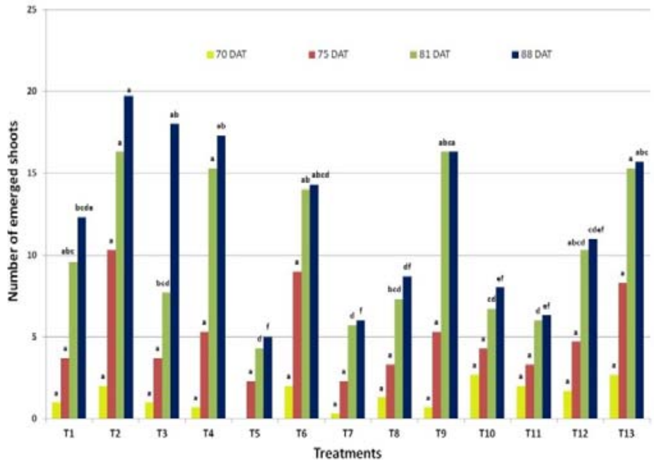

Fig. 1 Mean numbers of emerged shoots of *Phelipanche* at 70, 75, 81, and 88 DAT for each pot under the different treatments. See Material and methods for experimental details. Different letters on cumulative values for each DAT and for each treatment differ significantly at P≤0.05 (Tukey's tests). The differences among the treatments were related each DAT sample.

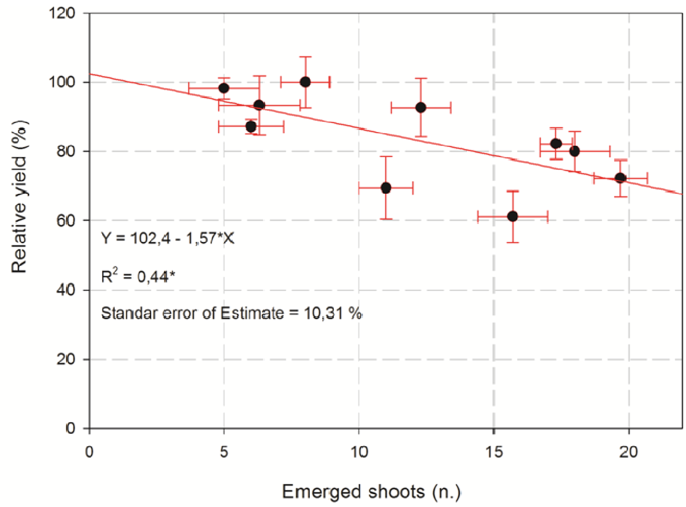

Fig. 2 Relationship between relative tomato yield and number of emerged shoots of *P. ramosa* at 88 DAT. The bars indicate vertical and horizontal standard errors.

The main conclusion to be drawn from the present study is to confirm that no single technique can provide complete control of *P. ramose*, and resorting to some of them is unavoidable.

Some of these methods appear to be more effective for reduction of the infestation of this parasitic weed in tomato crop, such as soil application of Radicon biostimulant, high levels of nitrogen or sulfur fertilizers, compost activated with *Fusarium* spp., and resistant tomato plants. It is assumed that these effects can be improved by combining some of these treatments with one another, especially for gradual and continuing reduction of the "seed bank" of the parasite in the soil.

Based on these results, we suggest that the agronomic, biological, and biotechnological methods to control *P. ramose* that can be used to preclude chemical contamination are suitable also for organic crop systems.

Therefore, more investigation should be carried out with integrated methods for the control of this parasitic in processing tomato crops.

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