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Biological Methods to Control Parasitic Weed *Phelipanche ramosa* L. Pomel in the Field Tomato Crop

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Abstract*—Phelipanche ramosa* L. Pomel is a root holoparasitic weed plant of many cultivations, particularly of tomato (*Lycopersicum esculentum* L.) crop. In Italy, *Phelipanche* problem is increasing, both in density and in acreage. The biological control of this parasitic weed involves the use of living organisms as numerous fungi and bacteria that can infect the parasitic weed, while it may improve the crop growth. This paper deals with the biocontrol with microorganism, including Arbuscular mycorrhizal (AM) fungi and fungal pathogens as *Fusarium oxisporum* spp. Colonization of crop roots by AM fungi can provide protection of crops against parasitic weeds because of a reduction in their seed germination and attachment, while *F. oxisporum*, isolated from diseased broomrape tubercles, proved to be highly virulent on *P. ramosa*. The experimental trial was carried out in open field at Foggia province (Apulia Region, Southern Italy), during the spring-summer season 2016, in order to evaluate the effect of four biological treatments: AM fungi and *Fusarium oxisporum* applied in the soil alone or combined together, and Rizosum Max® product, compared with the untreated control, to reduce the *P. ramosa* infestation in processing tomato crop. The principal results to be drawn from this study under field condition, in contrast of those reported previously under laboratory and greenhouse conditions, show that both AM fungi and *F. oxisporum* do not provide the reduction of the number of emerged shoots of *P. ramosa*. This can arise probably from the low efficacy seedling of the agent pathogens for the control of this parasite in the field. On the contrary, the Rizosum Max® product, containing AM fungi and some rizophere bacteria combined with several minerals and organic substances, appears to be most effective for the reduction of *P. ramosa* infestation.

***Keywords*—**Arbuscular mycorrhizal fungi, biocontrol methods, *Phelipanche ramosa, F. oxisporum* spp.

I. Introduction

PHYTOPARASITIC weeds are known as destructive parasites on many agricultural crops in the Mediterranean region, Eastern Europe, and North Africa [1]. *Phelipanche ramosa* is the most widespread *Orobanche* species which is the most dangerous for field tomato (*Lycopersicon esculentum* Mill.) and for many other Dicotyledonous. Germination of *P. ramosa* seeds is stimulated by root exudates from crop hosts, but in the absence of a host, seeds can remain viable for 10 years or more, making it difficult for any crop rotation to be efficient. The persistent seed bank emphasizes the need for rigorous prevention of seed production for the successful control of parasitic weeds. The early growth stages of parasitic plant development, such as seed germination, host attachment, and tubercle development, are key phases and ideal targets for successful management of this weed.

Several methods for the control of *P. ramosa* have been investigates, including the use of physical, chemical, agronomic, biological, and biotechnological methods [2], [3]. Biological control of parasitic weed, involving the use of living organism, could be a promising tool for controlling parasitic plants. Various natural enemies with biocontrol potential have been recorded on *Phelipanche,* that interfering with above mentioned phases of parasite life cycle, could result in attractive management strategies. In recent years, biological control agents such as Arbuscular mycorrhizal (AM) fungi and soil-borne pathogens which belong to genus *Fusarium* have received more attention for the reduction of *P. ramosa* seed germination [4]-[6].

AM fungi belonging to the *Glomeromyceta phytium* [7] establish the most ancient and widespread symbiosis [8]. This symbiosis is based on the improvement of the nutritional status of both partners. The plant provides organic carbon compounds to the fungus, whereas the fungus acquires inorganic mineral nutrients, mainly phosphate, that are translocated to the arbuscular and released to the plant. Colonisation of crop root by AM fungi can provide protection against parasitic weeds, as reported in sorghum and maize, reducing infection by decreasing in germination and attachment of parasite [9], [10], in tomato against *P. ramosa*, or in soybean and in pea reducing germination of various *Orobanche* and *Phelipanche* species [11]. Moreover, isolate *Fusarium oxisporum* was host specific, highly virulent to *P. ramosa* and it was considered a good bioherbicide candidate. This fungus applied in pot in green house experiments, strongly reduced the number and weight of emerging broomrape shoots and the number of tubercles attached to the host roots. Applied by drip irrigation in field experiments, the fungus confirmed its efficacy in reducing number and weight of emerging broomrapes [12]. Due to the parasitic plant life cycle, multiple applications of *Fusarium* at the soil level would be necessary [13]. Conidial suspension of two *F. oxisporum* isolated reduced *O. crenata* and *P. ramosa* germination in vitro by 76-80%, in root chambers by 46-50%, and in polyethylene by 40-55% [14].

Both granular soil applications and conidial suspensions of

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Fusarium spp. caused extensive mortality of *P. ramosa* in pot experiments. On the contrary, in field experiments over three years, results were inconsistent as reduction *P. ramosa* shoot number and biomass [15].

This paper deals with the results of biological methods to control the *P. ramosa* parasite in field tomato crop using AM fungi, combined with *Fusarium* spp. fungal pathogens or both used alone and the application of a commercial product (Rizosum Max®).

II. Materials and Methods

The study was carried out during the spring-summer season 2016, in open field, at the private "Futuragri" farm located in an agricultural area of the Foggia district (Apulia Region, Southern Italy, 41°27'N; 15°31'56"E), where the processing tomato crop is very intensive and the infestation of *Phelipanche ramosa* is widely diffuse.

The experimental trial was carried out on the clay-loam soil (USDA classification) having the following characteristics: sand = 30.6%; silt = 21.4%; clay = 48%; total N (Kjeldahl) = 1.3‰; assimilable P_2O_5 (Olsen) = 86 ppm; exchangeable K_2O (Schollemberger) = 1430 ppm; electrical conductivity (ECe) = 0.9 dS cm^{-1}; pH (in water) = 8.2; organic matter (Walkley and Black) = 1.2%.

Four biological treatments (tomato plants colonized by AM fungi combined with *F. oxisporum* applied into the soil, *F. oxisporum* applied alone into the soil, tomato plants colonized by AM fungi alone, Rizosum Max® product applied into the soil) compared with the untreated control were carried out.

In Table I, the composition, time, and application mode of the products used in the experiment are reported. As regards the AM fungi treatment, the roots of tomato plants were inoculated with a culture of *G. intraradices* N.C. Schenck & G.S. Sm., directly by the nursery seedling farm, whereas the *F. oxysporum* was isolated from necrotic shoots and inflorescences of *P. ramosa*, collected from infested tomato crops in the area of Foggia province. The isolated of *F. oxysporum* was grown for 30 days on pieces of straw sterilized in an autoclave for 20 min. at 121 °C.

The processing tomato (*Licopersicon esculentum* Mill.) (cultivar Dres) of each treatment and the untreated control was transplanted into plots of 20 m^2 on 19 May 2016, in double rows (40 cm apart) spaced at 200 cm, with the plants at the distance of 30 cm along each single row, resulting in a theoretical plant density of 3.3 plants m^{-2}. A drip irrigation method was used with the drip lines placed between each couple of plant rows. The water volume at each irrigation varied from 100 m^3 ha^{-1} to 300 m^3 ha^{-1}, depending on the crop growth stage, with a watering interval of about 3-4 days. The agricultural management practices applied to tomato crop during the experimental trial were those commonly adopted by local farmers, such as for fertilizing and for weed and pest control.

A randomised block design with three replicates (pots) for each of the treatments was adopted.

During the entire experimental period, daily main climatic parameters (mean temperature, total rainfall and total "Class A" Pan evaporation) were recorded at the nearest meteorological station, few kilometers from the experimental area and supplied by Consorzio per la Bonifica della Capitanata of Foggia.

During the tomato cycle, at 72, 81, 93, and 103 days after transplanting (DAT), *Phelipanche* emerged shoots (branched plants) from soil surface on a sampling area of 1 m^2, were counted.

The tomato fruits were harvested at full-stage of maturity on September 12, 2016, when the marketable yield and plant biomass from each sampling area of 5 m^2 were measured. On a sample of 10 fruits from each plot, the following main quali-quantitative parameters were determined: mean weight (g); dry matter content (% fruit fresh matter), soluble solids content (Brix); pH; titratable acidity (TA; g citric acid 100 ml fresh juice) [16]. The colour parameters were also measured, using a spectrophometer a CM-700d (Minolta Camera Co. Ltd., Osaka, Japan), as the CIELAB coordinates (i.e., L*, a*, b*) on four randomly selected areas of the fruit surface. Here, only the colour coordinate L and a*/b* ratio is reported, which represent the indices that describe well the color changes of tomato fruit (Francis and Clydestable, 1975; Favati et al., 2009) [17], [18].

All data were subjected to analysis of variance (ANOVA), and the means were compared by Tukey's test at 5% probability level.

TABLE I
Composition, Time and Application Mode of the Products Used in the Experiment

Tomato plants colonized by Arbuscular mycorrhizal fungi combined with *Fusarium oxisporum* spp. applied into the soil two days before the seedling transplant. Furthermore, tomato roots were soaked in concentrated solution ($1x10^6$ ml^{-1}) at transplanting time and applied by the first two drip irrigations (at 75 kg ha^{-1} of product), on June 28, July 20, 2016.
F. oxysporum applied alone into the soil two days before the seedling transplantation. Furthermore, tomato roots were soaked in concentrated solution ($1x10^6$ ml^{-1}) at transplanting time, and applied by drip irrigation (as 75 kg ha^{-1} of product) during the first two irrigations on June 28 and July 2016.
Tomato plants colonized by Arbuscular mycorrhizal fungi alone.
Rhizosum Max® (Biosum) **+ Prosum + Push up:** Rhizosum Max® is a powder product containing mycorrhize 2%; rhizosphere bacteria (*Azotobacter vinelandii, Bacillus megaterium, Pseudomonas putido*) $1x10^{10}$ CFU g^{-1}; Prosum, solution of total nitrogen 8%, ureic nitrogen 8%, phosphorus anhydride, 4%, potassium oxide 3%, iron 0.08% and zinc 0.6%; Push up, solution of N 15%, K 5.5%; Mg 13.5%; Mn 7.2% Fe 30%, S 40%, aminoacids 60%, vitamin 30%; alga Kelp 24%; triacontanol 0,2%, Mo 5%. The product was applied into the soil two days before the seedling transplant. Furthermore, tomato root were soaked in concentrated solution (0.84 g l^{-1}) at transplanting time and applied by the first two drip irrigations (at 75 kg ha^{-1} of product), on June 28 and July 2016.
Untreated control

III. Results and Discussion

A. Climate Conditions of the Experimental Site

The 10-day (decade) mean climate parameters recorded during the 2016 tomato growing season are reported in Table II. The mean air temperature increased almost linearly through the summer, from 15.1 °C on the first decade of May to 26.6 °C on the third decade of July; then decreases up to 17.7 °C on the end of September. The summer season was very dry, with

the effective rainfall for the tomato crop, ranging from 26 to 66 mm, mainly occurring during the early stage of the cropping season (first, second decades of May, and first decade of June) and at end of tomato grown cycle (in September). Through the season, the decade total evaporation increased almost linearly from 27.4 mm on the first decade of May, to 46.9 on second decade of July, then decreases up to 20.9 at the end of September.

TABLE II
10-DAY (DECADE) MEAN TEMPERATURE, TOTAL RAINFALL AND TOTAL "CLASS A" PAN EVAPORATION DURING THE GROWING CYCLE OF TOMATO CROP (YEAR 2016)

Month	Decade	Mean temperature (°C)	Rainfall (mm)	Class A Pan-evaporation (mm)
May	I	15.1	26.2	27.4
	II	16.5	36.7	29.7
	III	21.2	0.2	45.0
June	I	21.0	63.2	33.8
	II	22.4	2.05	39.4
	III	25.2	0	45.7
July	I	26.3	0	45,8
	II	24.7	11.0	46,9
	III	26.6	0	46.8
August	I	25.0	20.5	38.8
	II	23.8	0	38.8
	III	25.2	0.7	41.4
September	I	22.4	60.0	25.8
	II	21.1	46.7	22.9
	III	17.7	35.5	20.9
Mean		22.24		
Total			330.7	549.1

B. Effects of Treatments on the Control of Phelipanche ramosa

The early parasitic branched plant emerged above the ground 60 days after transplanting (DAT) on untreated tomato crop. In Fig. 1, the number of *P. ramosa* emerged shoot from 1 m^2 soil surface, at 74, 81, 93 DAT and at the end of crop cycle (103 DAT) in all the tested treatments are reported. Despite some differences among treatments, the number of emerged shoots increased particularly from 81 to 91 and to 103 DAT. In particular, no differences among all treatments at 74 DAT were observed, while significant differences were recorded at 81, 93 and 103 DAT, when only Rhizosum Max® treatment showed significantly lower values (ranging from 1.3 and 3.0 no m^{-2}) then the others biological treatments (ranging from 1.3 to 13.0 no m^{-2}) and the untreated control (ranging from 6.6 to 14.3 no m^{-2}). Therefore, in the present trial, the use of tomato plants colonized by AM alone, AM combined with application of the straw chopped substrate actived by *F. Oxisporum,* and the *Fusarium* alone did not show any effects for reduction of *Phelipanche* infestation. These results are in contrast with the other studies carried out on tomato that showed a reduced germination of the seeds of this parasite by lowering the amounts of stigolanctones in the root exudates of the colonized plants [19], [20]. This might be due to the response of different variety [21]-[23], or for the low efficacy of seedling of the agent pathogens for the control of this parasite in the field.

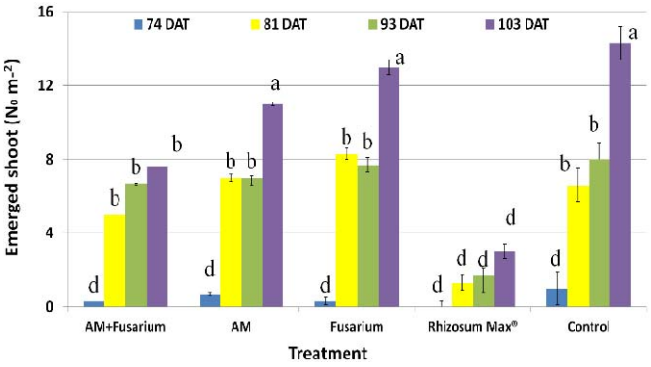

Fig. 1 Number of emerged branched shoots of *P. ramosa* at 74, 81, 93, and 103 days after transplanting (DAT) of the different treatments: data are means ± standard error, as measured from each plot (n = 3x3 replicates). Means with different letters are significantly different at $P \leq 0.05$, according to Tukey's test

As regards the Rhizosum Max® treatment, it shows a reduction of 78% of *P. ramosa* emerged shoots respect to the untreated control. This might be due to the presence in this commercial product of AM fungi, rhizosphere bacteria together with minerals and organic substances (see Table I), that include negative effects on *P. ramosa* seed germination. In this regard, it has been reported that mineral nutrients as well as organic matter introduced into a crop rhizosphere can result in severe physiological disorders of the germination of *P. ramosa* seeds, with a reduction of weed infestation.

C. Effects on Quali-Quantitative Parameters

Table III shows the results of different experimental treatments on the quanti-quantitative traits of the processing tomato crop. The Rhizosum Max® treatment, provided the highest marketable yield (106.0 t ha^{-1}) and plant biomass (15.3 t ha^{-1}), significantly different from all others treatments (on average 73.4 t ha^{-1} and 12.1 t ha^{-1} respectively) and from the untreated control (11.4 t ha^{-1} and 69.4 t ha^{-1} respectively).

Considering the relationship between tomato marketable yield and *P. ramosa* infestation at the end of tomato cycle, the results show that in Rhizosum Max® treatment, were there were small number of emerged shoot, this resulted in numerically increasing marketable yield. In comparison with the untreated control, the reduction of 78% of *Phelipanche ramosa* emerged shoots recorded for the Rhizosum Max® treatment provided an increased 54% of the tomato crop yield. Regarding the other fruit characteristics, such as mean fruit weight, dry matter, the colour coordinate, colour index, pH and the soluble solids content, no significant differences among treatments were observed.

IV. CONCLUSIONS

The principal results to be drawn from this study on biological methods to control *P. ramose,* under field conditions show that the root tomato plants mycorrhized by Arbuscular fungi (AM) alone or combined with *F. oxisporum* application, and *F. oxisporum* applied alone were ineffective

in reducing the parasitism. This can arise probability from the low efficacy seedling of the agent pathogens to control this parasite under field conditions, whereas the soil application of the Rizosum Max® product, which contains mycorrhize, some rhizosphere bacteria, and several minerals and organic substances, appears to be most effective for the reduction of infestation of *P. ramosa*. However, we wish to emphasize that further investigation needs to be carried out to obtain more data representing a broader range of infestation level and growing condition of tomato crop.

TABLE III
QUANTI-QUALITATIVE TRAITS OF THE TOMATO FRUIT UNDER THE DIFFERENT TREATMENTS

Treatment	Marketable yield (t ha^{-1})	Plant biomass (t ha^{-1})	Mean fruit weight (g)	Dry matter (%)	Colour Coordinate (L)	Colour Index (a/b)	Soluble solids content (°Brix)	pH	TA (g citric acid /100 ml juice
AM + *Fusarium*	71.9±5.9 b	12.3±1.3 b	68.9±4.2	4.39±0.9	49.5±0.3	1.25±0.05	4.1±0.3	4.5±0.3	0.14±00.2
AM	73.9±2.1 b	10.1±0.7 b	75.3±6.2	6.53±1.0	48.7±0.3	1.13±00.4	4.2±0.2	4.4±0.2	0.38±00.2
Fusarium	74.5±2.3 b	14.0±3.5 ab	76.0±5.4	5.12±0.9	50.1±0.3	1.17±0.03	4.6±0.2	4.5±0.2	0.22±0.02
Rizosum Max®	106.0±14.8 a	15.3±2.9 a	71.0±6.3	5.75±0.8	49.4±0.2	1.15±0.02	4.2±0.2	4.7±0.4	0.47±0.03
Control	69.4±3.7 b	11.4±2.3 b	70.0±5.0	5.61±1.0	49.3±0.3	1.12±0.03	4.3±0.2	4.6±0.3	0.39±0.03

† Marketable yield and plant biomass data are means±standard error, as measured from each plot (n =3 × 3 replicates)
Other data (Mean fruit weight, Dry matter, Colour coordinate, Colour index, Soluble solids content, pH and TA) are means ±standar error, as measured from 30 marketable fruit (10 fruit per pot × 3 replicates). Means followed by the same letters in each column are not significantly different (P=0.05, Tukey's tests)

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