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Tobacco (*Nicotiana tabaccum*) Infection by Branched Broomrape (*Orobanche ramosa*) as Influenced by Irrigation System and Fertilization, under East Mediterranean Conditions

A. Karkanis, D. Bilalis and A. Efthimiadou
Laboratory of Crop Production, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

Abstract: In field trials conducted in central Greece during two growing seasons, effects of irrigation system and fertilization on the host-parasite association tobacco-*Orobanche ramosa* were quantified. The experiment was designed as split plot design with four replicates, two main plots (drip and sprinkler irrigation) and three sub-plots (vetch as green manure, red clover as green manure and control (without fertilization)). Drip irrigation was the only treatment that significantly reduced broomrape infestation throughout the growing season. Also, drip irrigation reduced *Orobanche* dry weight by 70-76%. Finally, the use of drip irrigation system appears to be a promising cultural practice in the management of *Orobanche ramosa* in tobacco.

Key words: Drip irrigation, sprinkler irrigation, green manure, *Orobanche ramosa*

INTRODUCTION

Orobanche ramosa is a root holoparasite in the family Orobanchaceae. *Orobanche* infestations can be devastating to crops and remove otherwise productive land from effective use for very long periods of time (Rubiales *et al*., 2003a). *Orobanche* sp. parasitize crops such as sunflower (*Helianthus annuus*; *Orobanche cumara*), tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*), tobacco (*Nicotiana tabaccum*), rapeseed (*Brassica napus*), (*Orobanche ramosa*) and legumes (*Orobanche crenata* and *Orobanche aegyptiaca*) (Matusova *et al*., 2005). The broomrapes (*Orobanche* sp.) are widespread in Mediterranean areas, in Asia and Southern Europe, attacking dicotyledonous crops and depend entirely on their hosts for all their nutritional requirements (Fernandez-Aparicio *et al*., 2006).

Broomrape is very common in Mediterranean region and maintained by the fact that it has a broad host range; second, broomrape seeds can be dispersed short distances by wind; third, soil seed-bank can increase rapidly, as a single broomrape plant can produce hundred thousands of seeds and fourh, the seeds remain viable in the soil for many years and germinate only after stimulation by root exudates of the host (Rubiales *et al*., 2003a). In the last decade, broomrape has become the most important weed in tobacco production in East Mediterranean countries.

Many efforts have been development to fighting this pest, but to date there are no completely satisfactory control measures. Some success has obtained using chemical control (Qasem, 1998; Goldwasser *et al*., 2003), resistant varietes (Perez-de-Luque *et al*., 2004; Rubiales *et al*., 2003a) and agronomical practices (Acharya *et al*., 2002; Grenz *et al*., 2005; Haidar and Sidahmed, 2000). In recent years, the attention of researchers has shifted more towards cultural ways to alleviate the parasitic problems. The concept of integrated management is gaining importance to overcome broomrape problem and use of different irrigation system and organic fertilizers are some of its important components.

Organic fertilization are commonly used in cropping systems to increase soil organic matter, structural stability, water holding and cation exchange capacities and as a good sources of nutrients (Mandal *et al*., 2003). Many researchers investigated the effect of organic fertilizers on the seed germination of many plants (Haidar *et al*., 1999; Haidar and Sidahmand, 2000). For example, Haidar *et al*. (2003) reported that goat manure significantly reduced broomrape infestation.

Another control method is the use of drip irrigation system. In regions where summers are hot and dry and rainfall is infrequent, irrigation practices in row crops that reduce soil wetting would reduce the germination of weed seeds. Drip irrigation significantly reduced the weed density and dry weed compared to furrow or sprinkler irrigation systems (Sabra, 2000; Veeraputhiran and Kandasmy, 2001). The aim of present study was to study the effects of irrigation systems and fertilization on the establishment and development of *Orobanche ramosa* in tobacco (*Nicotiana tabaccum*).

Corresponding Author: D. Bilalis, Department of Crop Production, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece Fax: +32105294494

MATERIALS AND METHODS

Experimental design: A field experiment was conducted during 2004-2005 and 2005-2006. A Virginia (flue-cured) tobacco crop (*Nicotiana tabaccum* cv. NC71) was established in field in the Domokos (22.3381E, 39.0329N) district 200 km to the north of Athens. A naturally heavily infested area with *O. ramosa* was selected. The soil in the experimental area was a clay (50.7% clay, 23.3% silt and 26% sand) with a pH of 7.6, 2.2% organic matter and EC of 0.32 mS cm^{-1}. The crops before tobacco were vetch (*Vicia sativa* cv. Alexandros) and red clover (*Trifollium pratense* cv. Nemaro) which was incorporated into the soil. Prior to this study the field was under wheat cultivation. The experiment was set up on an area of 1200 m^2 according to the split plot design with four replicates, two main plots (irrigation systems) and the three sub-plots (vetch as green manure, red clover as green manure, control (without fertilization)). The plot size for each irrigation systems was 15×10 m and the sub-plot size was 5×10 m.

Irrigation systems: Overhead sprinkler and drip irrigation systems (Table 1) were set up on adjacent blocks within the same field, but far enough apart to avoid cross irrigation. A water meter was used to measure the amount of water applied.

The drip system consisted of PE laterals of 20 mm in diameter, laterals have in line drippers and at 0.40 cm distance. The drippers had a discharge rate of 4 L h^{-1} under an operation pressure of 1 atm. The sprinkler irrigation systems consisted mainly pipes of 40 mm diameter, laterals pipes of 40 mm diameter and rotor type sprinklers (radius: 5 m, discharge rate: 16.35 L min^{-1}) with 2 m risers. Laterals pipes were 15 m long with sprinklers spaced at 5 m in each lateral.

Planting and weed control: Vetch and red clover were sown by hand in rows 20 cm apart at a depth of 1.5 cm. The field was sown on 5 October 2004 and 1 October 2005 at a rate 100 and 20 kg ha^{-1} for vetch and red clover, respectively. Vetch and red clover was incorporated into the soil at the beginning of May.

Seeds of tobacco were sown on the 5th of March in a seedbed under plastic house conditions (to protect seedling from cold weather). After hardening, seeding transplanting on 22 May 2005 and 16 May 2006. Seedling was transplanted by hand in rows 90 cm apart. Transplants were set at 30 cm between each other. The weed were controlled by hand.

Table 1: Irrigation details of the experiment

Irrigation system	Year	Total quantity of water applied (mm)	No. of irrigation
Drip irrigation	2005	200	8
	2006	210	8
Sprinkler irrigation	2005	348	8
	2006	357	8

Sampling, measurements

Legumes: Biomass samples were taken at the end of April. To define biomass it was cut 1 m in 2 different places of each plot. The total N from biomass were investigated with the Kjeldahl method (Bremer, 1960).

Tobacco: The sampling was made 110 days after transplanting date of tobacco seedlings. To define biomass it was cut 1 m in 2 different places of each plot. Root samples were taken 110 days after transplanting date of tobacco. Samples were collected for each plot at soil depth 0-30 cm with a sampler 30×30 cm. In each sample roots were separated from soil after standing for 24 h in water.

Weed: Broomrape was measured using 1×1 m quadrates, 3 per subplot, on two dates (80 and 110 Days After Transplanting, DAT). All weeds were collected from a measured area and weighed to determine the weed dry matter.

Statistical analysis: For calculating analysis of variance and means comparisons the software Statshoft was used (Anonymous, 1996).

RESULTS AND DISCUSSION

Tobacco growth: Green manures increased tobacco roots dry weight (Table 2) and the most significant impact was when vetch was applied to soil. This species which has a high N content (Table 3) stimulates root growth to a greater extent than red clover. Also, the yields of tobacco crops (Table 4) were higher in 2005 than those in 2006. The main reasons for higher yields in Domokos may be attributed to precipitation. The precipitation during growing season in 2005 (97 mm) was higher than those in 2006 (42 mm). Moreover, there was found irrigation × green manure interaction (Table 4, Analysis of Variance) in yield of tobacco. Data obtained by others researchers (Mandal *et al*., 2003; Sangakkara *et al*., 2004; Stopes *et al*., 1996) clearly demonstrated the beneficial effects of legumes on the root growth of the following crops (wheat, maize, rice). The reduced biomass of the root system caused a reduction in the supply of nutritional elements to the plant, which resulted in the development of smaller plants.

Drip irrigation was characterised by a smaller amount of water (Table 1) applied to the soil. The tobacco yield (dry weight of leaves) was not affected by the reduced

Table 2: Influence of irrigation system (Drip irrigation: D; Sprinkler irrigation: S) and fertilization (control, vetch, red clover) on dry weight of roots (g plant^{-1})

Fertilization	2005		2006	
	Irrigation system		Irrigation system	
Dry weight of roots	D	S	D	S
Vetch	34.06	39.95	45.16	26.14
Control	16.59	7.70	11.78	11.84
Red clover	19.90	26.91	27.58	23.68
$LSD_{5\%}$ (Irrigation)	10.21		3.66	
$LSD_{5\%}$ (Fertilization)	5.81		5.08	
$LSD_{5\%}$ (Year)	13.26			

Analysis of variance

Source	F-ratio	p-value
Main effects		
Green manure	74.30**	0.01
Irrigation system	3.61^{ns}	0.06
Year	0.05^{ns}	0.83
Interactions		
Green manure × Irrigation system	5.34**	0.01
Green manure × Year	0.46^{ns}	0.63
Irrigation system × Year	6.90**	0.01

**Significant at p = 0.01; ns: Not significant

Table 3: Amount of N (kg ha^{-1}) that accumulated in the roots and in above-ground parts of the vetch and red clover plants (multiplying dry weight kg ha^{-1} with N%)

Accumulated N in different plant parts	2005	2006
N roots-Vetch	76.5	70.1
N biomass-Vetch	170.8	121.3
N total-Vetch	247.3	191.4
N roots-Red clover	82.8	67.8
N biomass-Red clover	78.4	90.3
N total-Red clover	161.2	158.1

Table 4: Influence of irrigation system (Drip irrigation: D; Sprinkler irrigation: S) and fertilization (control, vetch, red clover) on dry weight of leaves (g plant^{-1})

Fertilization	2005		2006	
	Irrigation system		Irrigation system	
Dry weight of leaves	D	S	D	S
Vetch	168.33	139.79	164.05	116.99
Control	91.25	81.67	77.10	50.89
Red clover	122.71	107.71	117.48	94.03
$LSD_{5\%}$ (Irrigation)	6.49		20.93	
$LSD_{5\%}$ (Fertilization)	6.67		13.44	
$LSD_{5\%}$ (Year)	5.70			

Analysis of variance

Source	F-ratio	p-value
Main effects		
Green manure	218.25**	0.01
Irrigation system	77.46**	0.01
Year	29.63**	0.01
Interactions		
Green manure × Irrigation system	5.34**	0.01
Green manure × Year	1.73^{ns}	0.19
Irrigation system × Year	6.32**	0.01

**Significant at p = 0.01; ns: Not significant

water application. The yields of tobacco crops under drip irrigation were higher than those with sprinkler irrigation (Table 4). The yield difference is statistically significant. In a similar study with cotton Cetin and Bilgel (2002) also observed that drip irrigation method produced 30% more seed-cotton than the sprinkler method. Other investigators have also reported high yields for crops under drip irrigation (Malash *et al*., 2005; Tiwari *et al*., 2003; Yohannes and Tadesse, 1998). Also, the higher tobacco yield in drip irrigation may be attributed to higher infection by broomrape in sprinkler irrigation (Table 7 and 8). For the first year, there were no significant differences between the drip and sprinkler irrigation for root biomass.

Tobacco infection by broomrape: Green manure had no effect on broomrape number and dry weight (Table 5-8). In contrast, Haidar *et al*. (2003) reported that organic soil amendments reduced broomrape infestation. Also, Westwood and Foy (1999) observed that nitrogen inhibit the germination of broomrape seeds.

Moreover, the drip irrigation reduced broomrape number and dry weight (at harvest, 110 DAT). Also, at 80 DAT, the combined analysis of variance (Table 5 and 6) of the two years data indicated that the tobacco infection by broomrape was higher in 2005 than those in 2006. The main reasons for higher infection in 2005 may be attributed to precipitation. The precipitation during growing season in 2005 (97 mm) was higher than those in 2006 (42 mm). The infection of tobacco by broomrape was not dependent on tobacco root biomass. In addition, Manschadi *et al*. (2001) indicated that the positive effect of irrigation on the numbers and dry weights of parasites can be attributed to a better development of the faba bean root system.

Moreover, Sabra (2000) and Veeraputhiran and Kandasmy (2001) reported that drip irrigation significantly reduced the weed density and dry weed compared to furrow or sprinkler irrigation system. High correlation (R^2 = 0.97; p = 0.001, Fig. 1) between the number of broomrape plants and total quantity of water was registered. R^2 was calculated using the linear equation as follow:

$$\text{No. broomrape m}^{-2} = 0.0538\ (\text{mm water}) - 9.43$$

St. Error	0.00309	0.87084
p-level	0.00001	0.00002

It is well known that water-stressed roots synthesize ABA (Zhang and Outlaw, 2001). Moreover, Matusova *et al*. (2005) reported that for *Striga* and broomrape the strigolactone germination stimulants are

Table 5: Influence of irrigation system (Drip irrigation: D; Sprinkler irrigation: S) and fertilization(control, vetch, red clover) on number of broomrape plants m^{-2}, 80 DAT

Fertilization	2005 Irrigation system		2006 Irrigation system	
No. of plant	D	S	D	S
Vetch	10.0	2.25	0.75	1.25
Control	1.5	3.50	1.50	0.50
Red clover	0.5	1.75	0.50	0.50
$LSD_{5\%}$ (Irrigation)	3.13		0.91	
$LSD_{5\%}$ (Fertilization)	1.76		1.27	
$LSD_{5\%}$ (Year)		0.91		

Analysis of variance

Source	F-ratio	p-value
Main effects		
Green manure	1.44^{ns}	0.24
Irrigation system	2.18^{ns}	0.14
Year	4.13*	0.04
Interactions		
Green manure × Irrigation system	0.06^{ns}	0.94
Green manure × Year	0.42^{ns}	0.66
Irrigation System × Year	3.41^{ns}	0.07

*Significant at p = 0.05; ns: Not significant

Table 6: Influence of irrigation system (Drip irrigation: D; Sprinkler irrigation: S) and fertilization (control, vetch, red clover) on dry weight of broomrape (kg ha^{-1}), 80 DAT

Fertilization	2005 Irrigation system		2006 Irrigation system	
Dry weight	D	S	D	S
Vetch	109.4	232.3	52.7	93.0
Control	107.7	246.0	134.7	54.9
Red clover	34.5	344.9	58.8	43.5
$LSD_{5\%}$ (Irrigation)	352.5		61.7	
$LSD_{5\%}$ (Fertilization)	165.7		99.8	
$LSD_{5\%}$ (Year)		90.87		

Analysis of variance

Source	F-ratio	p-value
Main effects		
Green manure	0.05^{ns}	0.95
Irrigation system	3.68^{ns}	0.06
Year	5.60**	0.02
Interactions		
Green manure × Irrigation system	0.58^{ns}	0.56
Green manure × Year	0.14^{ns}	0.86
Irrigation system × Year	5.41*	0.02

*, **Significant at p = 0.05, p = 0.01, respectively; ns: Not significant

Table 7: Influence of irrigation system (Drip irrigation: D; Sprinkler irrigation: S) and fertilization (control, vetch, red clover) on number of plants m^{-2}, 110 DAT

Fertilization	2005 Irrigation system		2006 Irrigation system	
Dry weight	D	S	D	S
Vetch	2.00	10.00	1.50	9.75
Control	1.75	8.25	1.75	9.00
Red clover	2.00	9.75	1.50	10.50
$LSD_{5\%}$ (Irrigation)	2.81		5.24	
$LSD_{5\%}$ (Fertilization)	2.71		2.46	
$LSD_{5\%}$ (Year)		1.47		

Analysis of variance

Source	F-ratio	p-value
Main effects		
Green manure	0.42^{ns}	0.65
Irrigation system	118.69**	0.01
Year	0.01^{ns}	0.95
Interactions		
Green manure × irrigation system	0.42^{ns}	0.65
Green manure × Year	0.10^{ns}	0.90
Irrigation system × Year	0.27^{ns}	0.60

**Significant at p = 0.01; ns: Not significant

Table 8: Influence of irrigation system (Drip irrigation: D; Sprinkler irrigation: S) and fertilization (control, vetch, red clover) on dry weight of broomrape (kg ha^{-1}), 110 DAT

Fertilization	2005 Irrigation system		2006 Irrigation system	
Dry weight	D	S	D	S
Vetch	208.5	530.5	125.0	466.8
Control	90.8	442.0	130.4	517.9
Red clover	153.8	543.3	94.7	471.8
$LSD_{5\%}$ (Irrigation)	222.1		187.3	
$LSD_{5\%}$ (Fertilization)	140.8		125.7	
$LSD_{5\%}$ (Year)		77.06		

Analysis of variance

Source	F-ratio	p-value
Main effects		
Green manure	0.32^{ns}	0.72
Irrigation system	90.17**	0.01
Year	0.50^{ns}	0.48
Interactions		
Green manure × irrigation system	0.16^{ns}	0.85
Green manure × Year	1.24^{ns}	0.25
Irrigation system × Year	0.04^{ns}	0.85

**Significant at p = 0.01; ns: Not significant

derived from the carotenoid pathway and the abscisic acid strongly reduced root exudates induced parasite germination. However, the significant effect of drip irrigation on broomrape growth was not possible to attribute to ABA accumulation. Additional studies on the relationship between ABA accumulation in tobacco roots and Orobanche infestation was recommended.

Moreover, Manschadi *et al.* (2001) reported that *O. crenata* infestation was higher under sufficient than under limiting water conditions. Also, the level of infection depends on soil moisture (El-Far, 2001; Perez-de-Luque *et al.*, 2004; Grenz *et al.*, 2005). For example, Rubiales *et al.* (2003b) reported that the infection of chickpea by broomrape was favoured by rainy autumns and springs.

In contrast, in *Striga*, an evolutionary related species, Bukar *et al.* (1996) observed that the number of *Striga hermothica* haustoria attached to millet roots increased with water deficit stress. Also, the number of haustoria per unit root length increased with water stress.

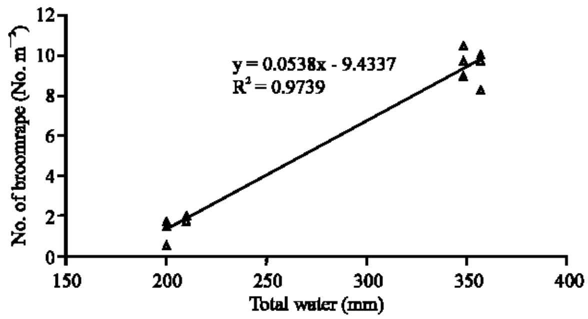

Fig. 1: Correlation between the number of broomrape plants and total quantity of water (n = 12)

also Dugje *et al.* (2006) and Gbehounou *et al.* (2004) reported that the infection of crops by *Striga* was suppressed by heavy showers.

CONCLUSIONS

The irrigation system greatly affected the infection of tobacco by broomrape. The drip irrigation system reduced broomrape number and dry weight. The highest yield of tobacco was measured under drip irrigation. Parasitism had influence on yield of the host. Also, all fertilization treatments had no effect on broomrape number and dry weight.

Present results indicate that broomrape infestation in tobacco can be suppressed by drip irrigation.

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