

## Influence of glyphosate on leaf gas exchange and photosynthetic pigments of broomrape-infested tobacco plants

Mariyan Yanev and Shteliyana Kalinova\*

Agricultural University, 4000 Plovdiv, Bulgaria

\*Corresponding author: s\_kalinova@yahoo.com

### Abstract

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Changes in the leaf gas exchange and photosynthetic pigments of Oriental tobacco plants, parasitized by *Phelipanche ramosa* L. and *Phelipanche mutelii* SCH, were determined after treatment with glyphosate. The study was carried out in two consecutive years. The plant samples were collected from the experimental field of the Institute of Tobacco and Tobacco Products in Markovo, Plovdiv region: leaves of untreated plants; leaves from the upper part of the plant, treated with 0.144 l/ha a.s. glyphosate; leaves from the lower part of the plant, treated with 0.144 l/ha a.s. glyphosate; leaves from the upper part of the plant, treated with 0.240 l/ha a.s. glyphosate; leaves from the lower part of the plant, treated with 0.240 l/ha a.s. glyphosate.

It was found that the decrease in the rate of net photosynthesis in tobacco plants treated with glyphosate, was due to the decreased content of photosynthetic pigments. Under the conditions of the present study, the total content of chlorophyll A and chlorophyll B in the plants treated with 0.240 l/ha glyphosate was reduced from 16% to 18% compared to the untreated plants. The intensity of transpiration in tobacco plants was influenced more strongly by environmental conditions and less by the treatment with glyphosate. The differences in stomatal conductance values of untreated and glyphosate-treated tobacco leaves were small and did not show statistical significance.

*Key words:* broomrape; leaf; gas exchange; photosynthetic pigments; glyphosate; tobacco

### Introduction

One of the most widely studied herbicides for broomrape control is glyphosate. It is the first promising herbicide, which successfully controls *Orobanche crenata* in *Vicia faba* L. (Schmitt et al., 1979). Kassasian (1973), Lutzeyer et al. (1994), Kharrat & Halila (1998) established the efficacy of different rates of glyphosate against *O. crenata* and *O. aegyptiaca* in *Vicia faba* L. Arjona-Berral et al. (1988) followed out the efficacy of the herbicide against *O. crenata* in peas and lentils. It should be noted that there were a large number of studies on the application of glyphosate against various species of *Orobanche* genus in tomatoes. As early as 1979, Zahran published data on the efficacy of glyphosate against

broomrape (Zahran, 1979). The control of *O. ramosa* L., *O. aegyptiaca* in *Lycopersicon esculentum* Mill., provided by glyphosate, was studied by Kotoula-Syka and Eleftherohorinos (1991) and Jain & Foy (1997). Vouzounis & Americanos (1998) reported data on the efficacy of glyphosate against two broomrape species – *O. ramosa* and *O. aegyptiaca*, both in tomato and eggplant. The effect of glyphosate against broomrape species in faba bean and tomato was also studied by Khalaf (1998). The control of *Orobanche aegyptica* in *Vicia sativa*, *Brassica napus* and mustard provided by glyphosate, was studied by Nandula et al. (1999) and Kumar (2002). Studies on the effect of glyphosate against *O. crenata* in *Vicia faba* L., *Lens esculentum* L. and *Pisum sativum* L. were carried out by Arjona-Berral & García-Torres (1984);

Mesa-García & García-Torres (1985). Results of experiments with glyphosate against *O. ramosa* in potatoes were reported by Haidar et al. (2005). Promising results about the efficacy of glyphosate against *O. cumana* and *O. cernua* in sunflower were reported by Petzoldt & Sneyd (1986) and Castejón-Muñoz et al. (1990). Current data on broomrape control in carrots were published by Ghalwash et al. (2014).

A significant number of studies related to the efficacy of glyphosate against broomrape were found in different types of tobacco. For example, (Lolas, 1994; 1998) reported excellent results of broomrape control in tobacco after treatment with glyphosate at a rate of 200 g a.s./ha. Mazaheri et al. (1991) established satisfactory control of *Orobancha* and higher tobacco yield as a result of glyphosate treatment. Kogan & Ureta (1996) also reported that there was high efficacy against *O. cernua* and selectivity to Virginia tobacco crop after treatment with the herbicide.

Labradka (1994) announced that treatment with glyphosate at a rate of 25 g a.s./ha, applied 30 days after tobacco transplanting, caused phytotoxicity in the lower leaves. In India, Raju & Nagarajan (1998) found out that low-rate glyphosate treatment could not completely eliminate broomrape but at the same time reduced yields due to phytotoxicity.

Jacobsohn & Kelman (1980), Langston et al. (1985), Lolas (1986) reported 50% to 73% reduction in the number of broomrape plants in the tobacco crop after treatment with glyphosate at the rates of 0.5 to 4 l/ha, which were not phytotoxic to the crop but killed the parasite.

However, the difference between the rates effective for broomrape control and those phytotoxic to the crop is too small (Brown et al., 1984; Janudi & Saghir, 1984; Langston et al., 1985). Therefore, when applying glyphosate against the parasitic plant, it should be used at precisely defined rates and at a specific period of the host development (Brown et al., 1984).

In Bulgaria, Bozukov (1999) found out that the percentage of broomrape plants emerging after treatment of the tobacco fields with glyphosate, significantly decreased. Following the different physiological parameters after treatment with glyphosate is motivated by the fact that glyphosate is a total systemic herbicide that despite the low rates applied for broomrape control; it could have a certain phytotoxic effect on the crop. Bozukov (2002) also announced that unlike the introduction of the herbicide on the upper tobacco leaves, when applied to the lower leaves, no phytotoxicity was observed.

In order to use the chemicals for an efficient control of the species of *Orobancha* genus and at the same time to provide safety to the crop plants, the effect of the herbicides on the major physiological processes in the crop plants should

be studied (Kalinova, 1989; Dimitrov et al., 1994; Kalinova, 2005; Ghasem, 2008; Lambers et al., 2008; Koshkin, 2010; Tonev & Vasilev, 2011;).

The basic physiological process related to the problem of herbicide selectivity, is the leaf gas exchange.

The characteristics of the leaf gas exchange are the rate of the photosynthesis, the intensity of transpiration, the stomatal conductance and the intracellular CO<sub>2</sub> concentration.

The primary toxic effects of the herbicides are mainly found in the chloroplasts, mitochondria, cytoplasm and cytoplasmic membranes.

The herbicide role for selectivity is associated mainly with its mechanism of action. Currently there are about 270 synthesized and registered chemical substances with herbicidal activity. About 170 of them have been widely applied and they are divided into 23 groups according to their mechanism of action (Fedtke & Duke, 2005).

The toxic effect of 14 of the 23 groups mentioned is associated with the chloroplasts, which makes it clear that they are the most important cellular organelle for herbicidal activity. That can be explained by the central role of the photosynthetic process for the cell metabolism contributing to the formation of energy suitable for use, as well as carbon and nitrogen sources. To some extent, that explains why most herbicides require light to realize their toxic effects.

## Materials and Methods

The study was carried out in two consecutive years. The plant samples were collected from the experimental field of the Institute of Tobacco and Tobacco Products in Markovo, Plovdiv region. The effect of glyphosate treatment on the physiological performance of broomrape-infested tobacco plants was studied by measuring the parameters of leaf gas exchange and the content of photosynthetic pigments, once in a year, in the third decade of July, when the crop is in the active growth stage and after the hypogean stage of the parasite.

Changes in the leaf gas exchange and in the photosynthetic pigments of Oriental tobacco plants, infested by *Phelipanche ramosa* and *Phelipanche mutelii* SCH, were identified. Plant samples included: leaves of untreated plants; leaves from the upper part of the plant, treated with 0.144 l/ha a.s. glyphosate; leaves from the lower part of the plant, treated with 0.144 l/ha a.s. glyphosate; leaves from the upper part of the plant, treated with 0.240 l/ha a.s. glyphosate; leaves from the lower part of the plant, treated with 0.240 l/ha a.s. glyphosate.

Leaf gas exchange and its common parameters – the rate of net photosynthesis (A) and the intensity of transpiration

(E) were determined using the LCA-4 portable photometric system [Analytical Development Company Ltd., Hoddesdon, England]. The rate of the net photosynthesis was determined by the decreasing CO<sub>2</sub> concentration in the chamber and the intensity of transpiration – by the increasing concentration of water vapor (Rubin & Artsikhovskaya, 1968; Mamonov & Kim, 1978; Berova et al., 2004; Lambers et al., 2008; Kerin et al., 2011).

The ambient conditions in the leaf chamber during the analyses were: light intensity – 750 μmol m<sup>-2</sup> s<sup>-1</sup> (PHARE), CO<sub>2</sub> concentration – 350 μmol mol<sup>-1</sup>, leaf temperature – 27°C, relative air humidity – 75%.

Physiological parameters and photosynthetic pigment values were determined once at 72<sup>nd</sup> hour after tobacco treatment. Photosynthetic pigments (chlorophyll A, chlorophyll B and common carotenoids) were extracted into 80% acetone, measured spectrophotometrically and calculated according to the formula of Lichtenthaler (1987).

Mathematical data processing was performed by a single-factor dispersion analysis (ANOVA) LSD at P = 0.05, using specialized statistical software STATGRAPHICS Plus package for Windows, Version 2.1.

## Results and Discussion

The rate of photosynthesis (A) in the plants treated once with glyphosate, was comparatively slightly reduced (6% to 18%) compared to the untreated plants. Changes in the inten-

sity of transpiration (E) and stomatal conductance (gs) were not established. There were slightly expressed tendencies towards a stronger negative effect of the herbicide applied at the higher rate – 0.240 l/ha of the active substance, similar in the leaves of the upper or lower part of the plant (Table 1 and Table 2).

Taking into consideration the fact that broomrape density was relatively low, it could be stated that the decrease in the rate of photosynthesis (A) indicated the presence of a phytotoxic effect of the herbicide to the tobacco plants.

The stronger negative effect of glyphosate on the net rate of photosynthesis after treatment of the lower leaves could be explained by its direct effect on the leaves, in which the leaf gas exchange parameters were measured, those leaves being the last fully developed from the top of the plant down.

The decrease in the rate of photosynthesis in the treated tobacco plants was mainly due to mesophilic factors, as their stomatal conductance did not change. It is known that glyphosate does not directly affect photosynthesis but inhibits the shikimate biosynthetic pathway. Due to the close relationship of the shikimate pathway with the pentose phosphate photosynthetic cycle, the biosynthesis of the chlorophylls and carotenoids in the treated plants is disturbed and a number of other disturbances in the photosynthetic process are observed. In the present case, the decrease in the rate of photosynthesis (A) in tobacco plants was probably due to a decreased content of photosynthetic pigments (Table 3 and Table 4). The total content of chlorophyll A and chlorophyll

**Table 1. Leaf gas exchange parameters in tobacco plants treated with glyphosate – first study**

Variants	A (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	E (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	gs (mol m <sup>-2</sup> s <sup>-1</sup> )
Untreated plants	18.71 c	1.92 a	0.07 a
Upper leaves 0.144 l/ha glyphosate	15.96 a	1.74 a	0.05 a
Lower leaves 0.144 l/ha glyphosate	17.54 bc	1.91 a	0.06 a
Upper leaves 0.240 l/ha glyphosate	15.40 a	1.59 a	0.05 a
Lower leaves 0.240 l/ha glyphosate	16.70 ab	1.74 a	0.05 a
	gD <sub>5%</sub> = 1.410	gD <sub>5%</sub> = 0.507	gD <sub>5%</sub> = 0,022

A – rate of photosynthesis (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>); E – intensity of transpiration (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>); gs – stomatal conductance (mol m<sup>-2</sup> s<sup>-1</sup>)

**Table 2. Leaf gas exchange parameters in tobacco plants treated with glyphosate – second study**

Variants	A (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	E (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	gs (mol m <sup>-2</sup> s <sup>-1</sup> )
Untreated plants	10.02 bc	1.39 ab	0.03 a
Upper leaves 0.144 l/ha glyphosate	16.10 d	1.69 b	0.05 b
Lower leaves 0.144 l/ha glyphosate	10.58 c	1.38 ab	0.04 ab
Upper leaves 0.240 l/ha glyphosate	7.91 a	1.30 a	0.03 a
Lower leaves 0.240 l/ha glyphosate	9.12 b	1.48 ab	0.04 ab
	gD <sub>5%</sub> = 0.935	gD <sub>5%</sub> = 0.358	gD <sub>5%</sub> = 0.012

A – rate of photosynthesis (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>); E – intensity of transpiration (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>); gs – stomatal conductance (mol m<sup>-2</sup> s<sup>-1</sup>)

**Table 3. Pigment content (mg/g fresh weight) in glyphosate-treated tobacco leaves – first study**

Variants	Chlorophyll A	Chlorophyll B	Chlorophyll A+B	Carotenoids	Chlorophyll A/ Chlorophyll B	Chlorophyll A+B/ Carotenoids
Untreated plants	0.97 c	0.28 c	1.24 c	0.41 c	3.48 b	3.03 b
Upper leaves 0.144 l/ha glyphosate	0.99 c	0.29 c	1.28 c	0.43 c	3.45 b	3.01 b
Lower leaves 0.144 l/ha glyphosate	0.95 c	0.25 b	1.20 c	0.42 c	3.75 c	2.86 ab
Upper leaves 0.240 l/ha glyphosate	0.68 a	0.21 a	0.89 a	0.31 a	3.22 a	2.86 ab
Lower leaves 0.240 l/ha glyphosate	0.77 b	0.22 a	0.99 b	0.36 b	3.57 bc	2.77 a
	$gD_{5\%} = 0.075$	$gD_{5\%} = 0.027$	$gD_{5\%} = 0.100$	$gD_{5\%} = 0.023$	$gD_{5\%} = 0.223$	$gD_{5\%} = 0.197$

**Table 4. Pigment content (mg/g fresh weight) in glyphosate-treated tobacco leaves – second study**

Variants	Chlorophyll A	Chlorophyll B	Chlorophyll A+B	Carotenoids	Chlorophyll A/ Chlorophyll B	Chlorophyll A+B/ Carotenoids
Untreated plants	0.74 a	0.31 b	1.05 ab	0.43 ab	2.38 a	2.45 a
Upper leaves 0.144 l/ha glyphosate	0.99 d	0.33 c	1.32 d	0.50 c	2.99 c	2.63 c
Lower leaves 0.144 l/ha glyphosate	0.80 b	0.30 ab	1.10 b	0.44 b	2.66 b	2.49 b
Upper leaves 0.240 l/ha glyphosate	0.74 a	0.29 a	1.03 a	0.41 a	2.59 b	2.49 b
Lower leaves 0.240 l/ha glyphosate	0.86 c	0.33 c	1.18 c	0.44 b	2.61 b	2.70 d
	$gD_{5\%} = 0.047$	$gD_{5\%} = 0.017$	$gD_{5\%} = 0.054$	$gD_{5\%} = 0.023$	$gD_{5\%} = 0.176$	$gD_{5\%} = 0.030$

B in the plants treated at the rate of 0.240 l/ha was significantly reduced – by 16 to 18% (Table 3).

Under the conditions of the first study, the intensity of transpiration (E) varied from 18.71 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in the untreated plants to 15.40 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in the upper tobacco leaves treated with 0.240 l/ha glyphosate.

Stomatal conductance (gs) varied from 0.07 mol m<sup>-2</sup> s<sup>-1</sup> in the untreated plants to 0.05 mol m<sup>-2</sup> s<sup>-1</sup> in all the other variants except for the variant, in which the tobacco leaves of the lower plant part were treated with 0.144 l/ha glyphosate.

Those results were statistically significant at a level of significance gD 5%.

In the second study the intensity of transpiration had the highest value in the upper leaves treated with 0.144 l/ha glyphosate and the lowest values were established in the upper leaves treated with the higher rate of glyphosate.

The differences in the stomatal conductance values in untreated and glyphosate-treated tobacco leaves were insignificant and did not show a clear tendency.

In the first year of the study the carotenoid content ranged from 0.43 mg/g fresh weight in the upper leaves treated with 0.144 ml/l glyphosate to 0.31 mg/g of fresh weight in the up-

per leaves treated with 0.240 l/ha glyphosate. All the differences were statistically significant at a level of significance gD 5%.

In the second year of the study data of the carotenoid content in glyphosate-treated tobacco leaves showed the same tendency as in the first study.

## Conclusions

The decrease in the rate of net photosynthesis in tobacco plants treated with glyphosate is due to the decreased content of photosynthetic pigments. Under the conditions of the study the total content of chlorophyll A and chlorophyll B in the plants treated with 0.240 l/ha glyphosate was reduced from 16 to 18% compared to the untreated plants.

The intensity of transpiration varied from 18.71 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in the untreated plants to 15.40 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in the tobacco leaves from the upper part of the plant treated with 0.240 l/ha glyphosate. The intensity of transpiration reported under other abiotic conditions was the highest in the upper leaves, treated with 0.144 l/ha glyphosate and the lowest value was established in the upper leaves treated with

the higher rate of glyphosate. That means that the intensity of transpiration in the tobacco plants was affected more strongly by the environmental conditions and less by the treatment with glyphosate.

The differences in stomatal conductance values in the untreated and glyphosate-treated tobacco leaves were insignificant and did not show a clear tendency.

The content of carotenoids in the tobacco leaves treated with glyphosate varied from 0.43 mg/g fresh mass in the upper leaves treated with 0.144 l/ha glyphosate to 0.31 mg/g fresh mass in the upper leaves treated with 0.240 l/ha glyphosate. The results obtained are sustainable with all the differences between the variants being statistically significant.

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