UDC 633.71
Тутун/Tobacco, Vol.62, N^{o} 1-6, 45-48, 2012
University "St. Kliment Ohridski" - Bitola
Scientific Tobacco Institute – Prilep, R. Macedonia

ISSN 0494-3244
UDK: 633.71-157.4:632.954(497.2)"2007/08"
Original scientific paper

INFLUENCE OF OXYFLUORFEN ON SOME ANATOMIC INDICES IN THE LEAVES OF VIRGINIA TOBACCO PLANT *(NICOTIANA TABACUM L.)*

Hristo Anastasov, Shteliyana Kalinova

Agricultural University, 4000 Plovdiv, Bulgaria, E-mail: s_kalinova@yahoo.com

ABSTRACT

The oxyfluorfen was applied at a dose of 80ml/dka and 100ml/dka 72 hours before the process of tobacco planting. During the vegetation period some visible signs of phytotoxicity in the crop were observed – plant growth inhibition, deformation of leaves and vegetation tip, weak chlorosis, etc. For the purpose of determining the herbicide influence on the tobacco leaf anatomy, several fixed samples from the leaves' middle sections were taken as well. The following indices were taken into consideration – stomata number/mm2 and stomata size (μm) from the upper and lower epidermis, size of assimilation parenchyma (mesophyll) in a leaf. It was established that oxyfluorfen caused considerable changes in the tobacco leaf anatomy, which were expressed in reduction of stomata number/mm2, and increase of the thickness of leaf lamina (blade), compared to those in the non-treated control plants.

Keywords: oxyfluorfen, phytotoxicity, tobacco leaf anatomy, stomata number/mm2, mesophyll

ВЛИЈАНИЕ НА ОКСИФЛУОРФЕНОТ НА НЕКОИ АНАТОМСКИ ПОКАЗАТЕЛИ ВО ЛИСТОВИТЕ ОД ВИРЦИНИСКИОТ ТУТУН

Оксифлуорфенот е примент во доза од 80ml/декар и 100ml/декар 72 часа пред садењето на тутунот. За време на вегетациониот период забележани се некои видливи знаци на фитотоксичност– инхибирање на порастот на растението, деформации на листовите и вегетативнит врв, слаба хлороза, итн. За одредување на влијанието на хербицидот врз градбата на тутунскиот лист, земени се и неколку фиксирани лисни примероци од средните сектори на листовите. Разгледувани се следниве показатели: број на стоми/mm^2 и големина на стомите (μm) од горниот и долниот епидерм, големината на асимилациониот паренхим (мезофил) на листот. Утврдено е дека оксифлуорфенот го редуцира бројот на стоми/mm^2 и зголемување на дебелината на лисната плојка, во споредба со нетретираните контролни растенија.

Клучни зборови: оксифлуорфен, фитотоксичност, градба на тутунскиот лист, број на стоми/mm^2, мезофил

INTRODUCTION

The negative influence of herbicides on crops in modern intensive agriculture is a topic of a number of studies which reveal crop changes emerging as a result of exposure to the effects of chemicals. The in-depth analysis of these changes involves studying the modifications in their anatomy which leads to changes on biochemical and physiological level, and the visible signs of these changes are the morphological anomalies observed in the plants.

Oxyfluorfen reduces the content of chloroplasts in the cells of the assimilation parenchyma of leaves, and this has an adverse effect on the biochemical and physiological processes in plants (2, 4, 7, 8, 9, 16, 17). The photosynthesis, transpiration, and gas exchange disruptions are expressed in delayed plant growth and development, and frequently, this is a contributing factor to plant death (1, 3, 5, 6, 10, 11, 12, 13, 14, 15, 17).

The purpose of this study was to determine the influence of oxyfluorfen on some anatomic indicators in the leaves of the Virginia tobacco plant.

MATERIAL AND METHODS

In the period 2007-2008, at the Markovo village testing grounds of the Tobacco and Tobacco Products Institute in Plovdiv, in humus-carbonate soil, a field experiment was set up to determine the biological effectiveness and selectivity of some soil herbicides used in growing Virginia tobacco plants. One of them was the Goal 2E herbicide with active ingredient oxyfluorfen 24%.

Oxyfluorfen was applied in a dose of 80 ml/dka and 100 ml/dka 72 hours before the process of tobacco planting.

During the vegetation period, some visible signs of phytotoxicity in the crop were observed – plant growth inhibition, leaf and vegetation tip deformation, weak chlorosis, etc.

For the purpose of determining the effects of the herbicide on tobacco leaf anatomy, samples from the midsection of the leaves of the damaged plants and from the untreated control plants were taken and fixed in 70% Ethanol. To examine the anatomic indicators, an Amplival light microscope was used. The indicators taken into consideration were stomata number/ mm^2 and stomata size (μm) from the upper and lower epidermis, and size of the assimilation parenchyma (mesophyll) of the leaf – all at a combined magnification of 400 X (10 X ocular and 40 X objective).

RESULTS AND DISCUSSION

The leaf of tobacco plant (*Nicotiana tabacum L.*) is dorsoventral. The stomata are located on both sides of the leaf, characterizing it as amphistomatic. The basic epidermal cells are more or less isodiametric in shape, with undulated, curvy anticlinal walls. The stomatal complex is of the anomocytic type (lacking differentiated subsidiary cells), in which the stomata-surrounding cells are indistinguishable from the other epidermal cells, and the guard cells are bean-shaped.

The mesophyll is heterogeneous, represented by palisade (columnar) and loosely packed (spongy) parenchyma. The palisade parenchyma is in two rows and is located directly underneath the upper epidermis of the leaf. The spongy parenchyma consists of dispersedly situated, isodiametrically shaped parenchyma cells, interspersed with larger or smaller intercellular spaces, which are frequently connected to the stomata on lower epidermis of the leaf. Data on the influence of oxyfluorfen on some anatomic indicators in the leaves of broadleaf tobacco plant are given in Table 1. As a result of the herbicide action, the thickness of the assimilation parenchyma in the leaves of the treated plants increases. Regarding the palisade parenchyma, the highest values – (112.5)134.6±1.8(150) μm are observed in the lower dose of 80 ml/dka, in which the size of the columnar parenchyma is greater than the one measured in the 100 ml/dka dose.

Table 1. Influence of oxyfluorfen on some anatomic indices in the leaves of Virginia tobacco

Indexes		(min) % ±S % (max)	(min) v ±S % (max)	(min) x ±S x (max)
Variants		non treated	treated - 80 ml/dka	treated - 100 ml/dka
leaf parenchyma (mesophyll), um	palisade parenchyma	(100)114±1.2(125)	(1 12.5)134.6±1.8(150)	(100)124.3±2.0(145)
	spongy parenchyma	(150)165±1.3(180)	(170)188.1±1.6(202.5)	(100)190.1±3.7(212.5)
upper epidermis stomata	number/mm^2	(58.3)75±1.54(83.3)	(50)57.8±1.2(66.7)	(41.7)65.5±2.7(91.7)
	length, um	(35)38±0.5(42.5)	(27.5)37.2±0.6(42.5)	(25)31.5±0.8(40)
	width, um	(20)21.3*0.23(22.5)	(25)26.8±0.4(30)	(12.5)I9.7±0.8(30)
lower epidermis stomata	number/mm^2	(108.3)150.2±3.58(183.3)	(83.3)90.5± 1.2(100)	(108.3)137.5±2.5(166.6)
	length, um	(32.5)35.6±0.3(37.5)	(32.5)36.4±0.4(40)	(20)28.25±1.1(40)
	width, um	(20)22±0.4(25)	(20)24.3±0.4(27.5)	(20)23.3±0.5(27.5)

In the case of spongy parenchyma, the increase is the largest in the higher herbicide dose of 100 ml/dka, with recorded values of (100)190.1±3.7(212.5) μm. The degree to which the mesophyll in the leaves of treated plants increases is different in comparison to that of the control plants, and it encompasses the differences, both in terms of assimilation parenchyma and in terms of preparation dosage, and therefore, this is the cause for lamina deformation due to uneven growth of their palisade and spongy parenchyma.

Oxyfluorfen causes a reduction in the number of stomata per mm^2 of the upper (adaxial) and the lower (abaxial) leaf epidermis of the treated plants. The lowest number of stomata per mm^2 – (50)57.8±1.2(66.7) for the upper and (83.3)90.5±1.2(100) for the lower epidermis were recorded in plants treated with 80 ml/dka herbicide. In plants treated with higher dose of 100 ml/dka, some increase in the stomata number is observed. both in the adaxial epidermis – (41.7)65.5±2.7(91.7) stomata/mm^2 and in the abaxial epidermis – (108.3)137.5±2.5(166.6) stomata/mm^2, but these results are lower than those recorded in untreated plants. In plants treated with 100 ml/dka herbicide, there are deformed stomata noticeable on the upper epidermis of the leaf, as well as stomata with only one guard cell, which has an adverse effect on their transpiration and gas exchange processes. The reduction in the number of stomata in plant leaves, the atrophy of the lamina and the ineffective functioning of the stomata complex are the cause for growth and development inhibition of treated plants.

CONCLUSION

Oxyfluorfen causes an increase in thickness of the assimilation parenchyma, lamina deformation, reduction of the stomata number/mm^2, and atrophy of the guard cells of stomata in the leaves of Virginia tobacco.

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