

POPULATION DENSITY OF PHYTONOMUS VARIABILIS HRBST. AND PHYTODECTA FORNICATA BRUGG. ON MULTIFOLIOLATE AND TRIFOLIOLATE ALFALFA IN RELATION TO ANATOMICAL CHARACTERISTICS ON THEIR LEAVES

ПОПУЛАЦИОННА ПЛЪТНОСТ НА МАЛКИЯТ ЛЮЦЕРНОВ ЛИСТОВ ХОБОТНИК PHYTONOMUS VARIABILIS HRBST. И ЛЮЦЕРНОВИЯТ ЛИСТОЯД PHYTODECTA FORNICATA BRUGG. ПРИ МНОГОЛИСТНА И ТРИЛИСТНА ЛЮЦЕРНА ВЪВ ВРЪЗКА С АНАТОМИЧНИТЕ ОСОБЕНОСТИ НА ЛИСТАТА ИМ.

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ABSTRACT

Two alfalfa cultivars - the multifoliolate “Mnogolistna 1” and the trifoliolate “Europe” were investigated for their resistance towards two main alfalfa defoliators *Phytonomus variabilis* Hrbst., 1795 and *Phytodecta fornicata* Brugg., 1873. The results of the two year study showed that the population density of both pests was higher in the “Mnogolistna 1” fields. The anatomical study of the leaves showed that multifoliolate alfalfa cultivar has narrower palisade and spongy parenchyma and thinner leaves and cuticle which make it less resistant to *Ph. variabilis* and *Ph. fornicata*.

Key words: feeding behavior, alfalfa weevil, *Phytodecta fornicata* Brugg., epidermis, mesophyll, cuticle, trichomes.

РЕЗЮМЕ

Проучени са популационната плътност и хранителните предпочитания на малкия люцернов листов хоботник (*Phytonomus variabilis* Hrbst., 1795) и на люцерновият листояд (*Phytodecta fornicata* Brugg., 1873) към два сорта люцерна – многолистният “Многолистна 1” и трилистният “Европа”. Две годишните наблюдения показват по-висока популационна плътност и на двата неприятеля при сорт “Многолистна 1”. Резултатите от анатомичните проучвания на листата показаха по-малка височина на гъбчестия и палисадния паренхим, по-тънки листни петури, а също и формирането на по-тънка кутикула при сорта “Многолистна 1” в сравнение с “Европа”, което очевидно я прави по-предпочитана от проучваните листогризещи неприятели.

Ключови думи: хранителни предпочитания, люцернов листов хоботник, люцернов листояд, мезофил, епидерма, кутикула, власинки.

DETAILED ABSTRACT

Проучването беше проведено през периода 2006-2007 г. в Учебно Опитното Поле на Аграрен Университет-Пловдив, катедра Ентомология. Бяха обследвани два сорта люцерна: многолистният – “Многолистна 1” и трилистният – “Европа”. Наблюденията бяха провеждани в интервал от 7-10 дни от март до октомври, като за отчитане плътността на неприятели беше използван метода на косене с ентомологичен сак. Констатирана беше разлика в популационната плътност и хранителните предпочитания на основните листогризеци неприятели по люцерната: малкият люцернов листов хоботник (*Ph. variabilis*.) и люцерновият листояд (*Ph. fornicata*) към проучваните сортове. Неприятелите бяха установени в по-висока плътност при многолистната люцерна, в сравнение с трилистната. Морфолого-анатомичният анализ на листата беше направен в катедра Ботаника на АУ-Пловдив като беше използвана цитологичната техника [4]. Анатомичната структура на листата на двата сорта люцерна беше проучена с цел да се установи нейната роля върху хранителните предпочитания на неприятели. Бяха взети проби от напълно развити листни петури и фиксирани в 75% етилов алкохол. От тях бяха направени напречни прерези и изготвени трайни микроскопски препарати. Измерването на височината на епидермалните клетки, мезофила, кутикулата и дебелината на листа беше извършено със светлинен микроскоп при увеличение 100x. Беше определен вида на власинките и тяхното разположение по листната петура. Данните бяха обработени по методите на вариационната статистика с изчисляване на средно квадратно отклонение. Резултатите показаха, че многолистната люцерна има по-тънки листа и по-малко власинки, в сравнение с трилистната и това е една от причините да бъде по-предпочитана от листогризеците неприятели. Беше определен типа на власинките. Те са многоклетъчни, не разклонени и при двата сорта в по-голямо количество от долната страна на листните петури.

INTRODUCTION

Plants and insects have a long time coexisted relationship. Harmful insects were suppressed by other insects – predators and parasitoids or by plant defense mechanisms, to create a balance between the insect pest population and host and to avoid serious crop losses. The use of resistant varieties is one of the most effective tools for reducing insect damage. There are three well known mechanisms of plant defense to insect damage: antixenosis, antibiosis and tolerance. Antixenosis is a

host plant mechanism which includes morphological, physical or structural qualities that interfere with insect behavior such as mating, oviposition, and feeding. As a resistance mechanism, antixenosis acts as structure barrier which affect the insect behavior in selecting their hosts [1]. Morphological and anatomical characteristics of the leaves, like length and thickness of trichomes, the substances they are secreting, thickness of cuticle, wax layer, etc. could play decisive role on the preference of the pest to the specific culture [2].

Alfalfa (*Medicago sativa* L.) is attacked by a great number of insect species that cause considerable damage and reduce forage yield. Alfalfa weevil *Phytonomus variabilis* Hrbst. is one of the most important pest that consumes foliage severely and leaves only leaf veins unfed [6]. Another serious pest of alfalfa is *Phytodecta fornicata* Bruggemann that causes significant crop loss in the Balkans peninsula [7]. Yildirim et al., (1996) describe biology and damage of alfalfa leaf beetle *Phytodecta fornicata* a serious pest of alfalfa (*Medicago sativa* L.) in Erzurum and Erzincan provinces [8]. *Phytonomus variabilis* Hrbst. and *Phytodecta fornicata* Brugg. are the main alfalfa defoliators in Bulgaria. There is no data about their density and preference to a new multifoliolate alfalfa cultivar “Mnogolistna 1” (*Medicago sativa* L.) which is the most widely spread multifoliolate alfalfa cultivar in Bulgaria. In contrast to a trifoliolate alfalfa, the multifoliolate one has from 5 to 7 leaflets per leaf and higher content of crude protein [5]. Bingham et al. [3] found that multifoliolate alfalfa has thinner leaves than the trifoliolate one and believe that this plays an important role at the pests feeding behavior. With regard to this we assessed the morphological and anatomical characteristics on the leaves of the two alfalfa cultivars – “Mnogolistna 1” and “Europe” and their role on the population density and feeding behavior of the main alfalfa defoliators – *Ph. variabilis* and *Ph. fornicata*.

MATERIAL AND METHODS

The study was conducted at the Experimental field of the Agricultural University - Plovdiv, in the Departments of Entomology and Crop Science. Two alfalfa cultivars - the multifoliolate “Mnogolistna 1” and the trifoliolate “Europe” were investigated. The population density of *Ph. variabilis* and *Ph. fornicata* was determined by collecting specimens every 7-10 days from March to October by sweep net. The population density was calculated per 1 square meter.

The anatomical analyses of the leaves were made in the Department of Botany by Cytological technics [4]. The anatomical structure of the leaves was examined in

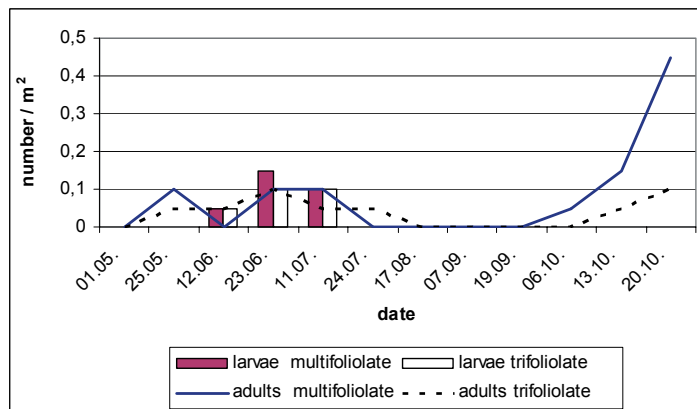


Figure 1. Dynamics of population density per 1 square meter of *Phytonomus variabilis* Herbst. in multifoliolate and trifoliolate alfalfa in 2006.

Фигура 1. Динамика на популационната плътност на *Phytonomus variabilis* Herbst. при многолистна и трилистна люцерна през 2006 година.

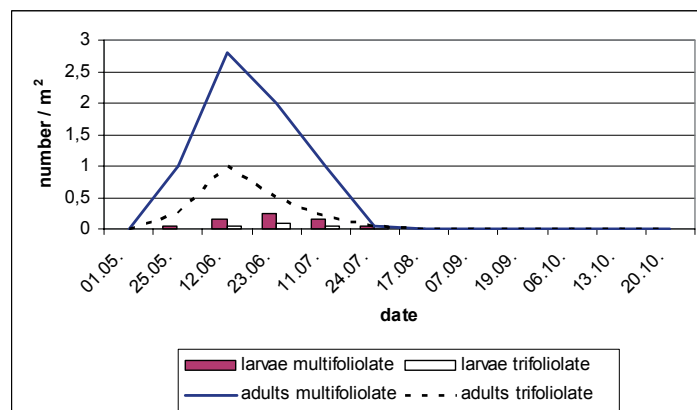


Figure 2. Dynamics of population density per 1 square meter of *Phytodecta fornicata* Brugg. in multifoliolate and trifoliolate alfalfa in 2006.

Фигура 2. Динамика на популационната плътност на *Phytodecta fornicata* Brugg. при многолистна и трилистна люцерна през 2006 година.

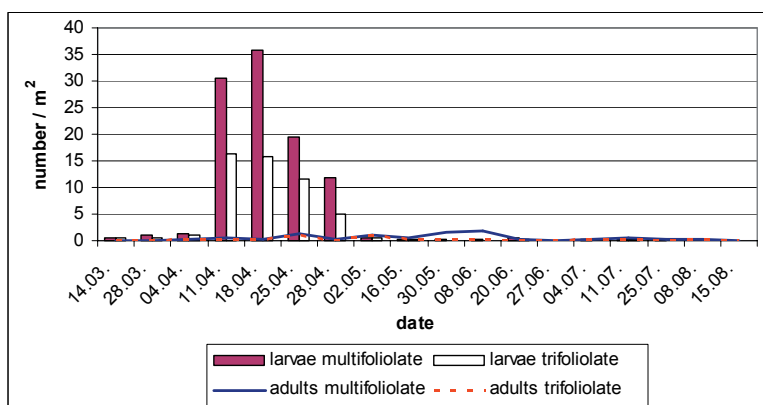


Figure 3. Dynamics of population density per 1 square meter of *Phytonomus variabilis* Hrbst. in multifoliolate and trifoliolate alfalfa in 2007.

Фигура 3. Динамика на популационната плътност на *Phytonomus variabilis* Hrbst. при многолистна и трилистна люцерна през 2007 година.

order to establish its role in the pests feeding behavior. Samples of fully developed leaves of the two alfalfa cultivars were collected from four fields in the region of Plovdiv (every field was 0,5 ha). The samples were preserved in 75% ethyl alcohol. Transverse sections were mounted in 10% glycerol and the slides were examined at X100 magnification under light microscope. The size of the cuticle and epidermal cells, palisade and spongy parenchyma and the thickness of the leaves were measured in 30 repetitions. The type of the leaf trichomes was determined.

RESULTS AND DISCUSSION

In 2006 adults of *Ph. variabilis* appeared at the end of May in alfalfa stands and the larvae in mid-June. The density of the weevil adults and larvae was low, but they predominated in multifoliolate alfalfa (Fig. 1). Adults of *Ph. fornicata* appeared at the end of April and larvae in the end of May. Both adults and larvae were observed in higher population density in multifoliolate alfalfa than in the trifoliolate (Fig. 2).

In 2007 in alfalfa stands first appeared larvae of alfalfa weevil in the middle of March and then appeared adults at the end of March. The population density of larvae was higher in multifoliolate alfalfa than in the trifoliolate one and exceeded the economy injury level in the middle of April (Fig. 3). Adults of *Ph. fornicata* were observed in the middle of March and larvae in the middle of April. The population density of adults was higher in multifoliolate alfalfa and exceeded the economic threshold (Fig. 4).

It was established that the main alfalfa defoliators were in higher population density in multifoliolate alfalfa cultivar during both years of study.

In both cultivars the epidermis is composed of a single

layer of epidermal cells with wavy anticlinal walls. The stomata are of anomocytic type (Fig.5 a, b). The anatomical study of the leaves showed significant differences in the size of the palisade and spongy parenchyma and in the thickness of the leaves, respectively 85µm / 54,86µm and 199,33µm for the multifoliolate cultivar “Mnogolistna 1” and 105,33µm / 71,33µm and 218,66µm for the trifoliolate cultivar “Europe”(table1). These results correspond with the size of the cuticle and the number of the trichomes as well. Simple, multicellular trichomes, located mainly on the lower epidermis were observed and their number is higher on the trifoliolate alfalfa leaves. The anatomical structure of the “Mnogolistna 1” cultivar characterized by smaller palisade and spongy parenchyma, thinner leaves and cuticle (Fig.6a, b) and less trichomes makes it vulnerable to *Ph. variabilis* and *Ph. fornicata*. Our results confirm previous findings reported by Bingham et al. [3].

CONCLUSIONS

The “Mnogolistna 1” is the most widely spread cultivar multifoliolate alfalfa (*Medicago sativa* L.) in Bulgaria and this is the first study about feeding behavior of the two main alfalfa defoliators, *Ph. variabilis* and *Ph. fornicata*. The results obtained in this study showed that multifoliolate alfalfa is preferred to trifoliolate by *Ph. variabilis* and *Ph. fornicata*. This could be explained with the differences in the anatomical structure of their leaves. The anatomical study of the leaves showed that multifoliolate alfalfa cultivar has narrower palisade and spongy mesophyll, thinner leaf blades and cuticle which make it more vulnerable to *Ph. variabilis* and *Ph. fornicata*.

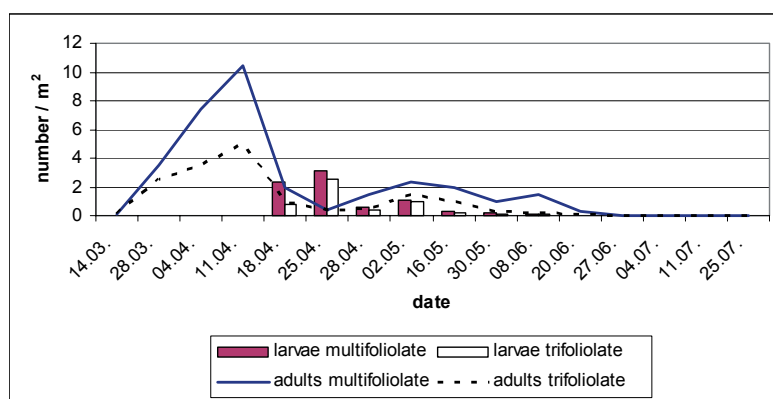


Figure 4. Dynamics of population density per 1 square meter of *Phytodecta fornicata* Brugg. in multifoliolate and trifoliolate alfalfa in 2007.

Фигура 4. Динамика на популационната плътност на *Phytodecta fornicata* Brugg. при многолистна и трилистна люцерна през 2007 година.

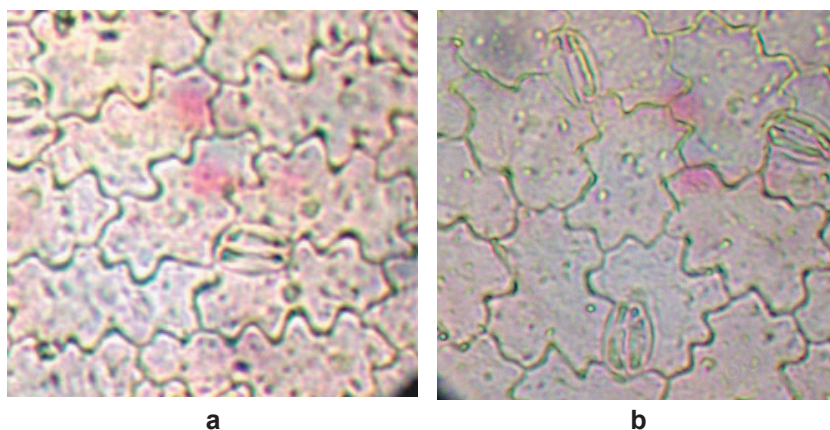


Figure 5. a Epidermal cells in lower epidermis of the “Mnogolistna 1” cultivar
 b Epidermal cells in lower epidermis of the “Europe” cultivar
 Фигура 5. а Епидермални клетки на долна епидерма на сорт “Многолистна 1”
 б Епидермални клетки на долна епидерма на сорт “Европа”

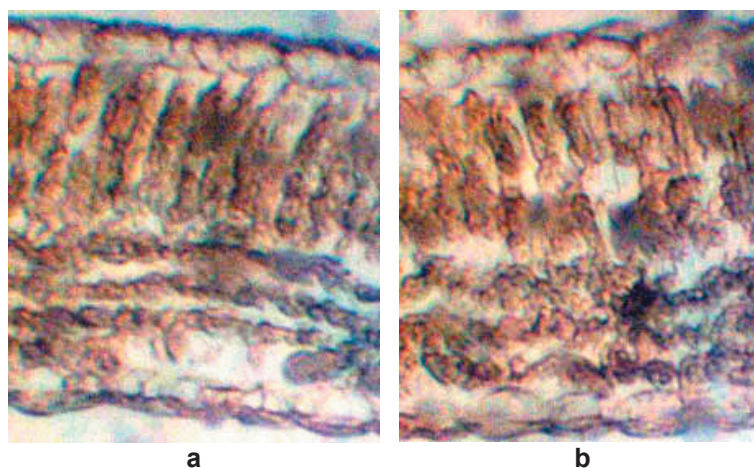


Figure 6. a Transverse section of a leaf blade of the “Mnogolistna 1” cultivar
 b Transverse section of a leaf blade of the “Europe” cultivar
 Фигура 6. а Напречен прerez на листна петура при сорт “Многолистна 1”
 б Напречен прerez на листна петура при сорт “Европа”

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