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Evaluation of the virulence of *Sclerotium rolfsii* isolates on *Arachis hypogaea* and screening for resistant genotypes in greenhouse conditions

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Summary *Sclerotium rolfsii* is a soil borne pathogen responsible for root and stem rot on a wide range of crops. This study was conducted to identify the virulence of different *S. rolfsii* isolates on a susceptible local peanut germplasm and determine the resistance of 20 peanut genotypes to the most virulent isolate and also the relationship between virulence and mycelial compatibility groups (MCGs). Seventy eight isolates of this fungus from 10 host plants and six known MCGs were used in the experiment. The experiment was done in greenhouse conditions (25±5°C) using a complete randomized block design with three replications. Pots containing sterile soil (pH=6.7) were inoculated with barley seeds colonized by each isolate separately before being seeded with the peanut germplasm. Disease severity was assessed by scoring the wilting, yellowing or death of plants, mycelia or sclerotia production on the soil surface or on plant stem, stem area affected (%) and stem lesion length, at the stage of plant maturity. Also, shoot wet weight and plant height were recorded at this stage. According to the results of the pathogenicity tests, all of the isolates were virulent on the susceptible peanut germplasm and the virulence differed significantly between the isolates ($P \leq 0.01$). There was no relationship between the virulence of the five groups of isolates identified in the present study and the MCGs. The peanut genotype 140, which was better than the others based on seed size, plant height and the canopy size, was also the most resistant one.

Additional keywords: diversity, groundnut, pathogenicity, southern blight, stem rot

Introduction

Sclerotium rolfsii Sacc. (teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough) is one of those soil borne plant pathogenic fungi that are prevalent in warm temperate and subtropical regions of the world (Punja *et al*., 1984). This pathogen has a host range of over 500 plant species mostly of dicotyledonous plants. A wide range of symptoms are produced by this pathogen on its hosts including crown and root rot, stem canker and damping-off and resulting diseases called southern wilt, blight or stem rot (Punja, 1985). The pathogen is of great importance especially when the disease severity is high in the fields. The crop loss may be between 10-25% or even more than 81% in some fields (Mehan *et al*., 1995).

Groundnut or peanut (*Arachis hypogaea* L.) is an annual legume crop cultivated in more than 80 countries in the tropics, subtropics and warm temperate zones (Hammons, 1994). It is a major source of edible oil, vitamins and amino acids and is used extensively for feed and food (Savage and Keenan, 1994). Groundnut is also a main crop in Guilan province of Iran with about 3500 hectares cultivation area.

Southern blight, stem rot or white mould, caused by *S. rolfsii*, is one of the most important diseases of peanut. The disease appears in peanut growing areas and causes great yield losses when climatic conditions, such as soil temperature and humidity, are favorable for fungal development and the dis-

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ease incidence is high (Kolte, 1984; Le, 2004; Nguyen, 2004).

Sclerotium rolfsii overwinters as mycelium or sclerotia in infected plant tissues and soil. Under favorable conditions, hyphae or germinating sclerotia infect the plant and subsequently colonize and invade the root and stem tissue with typical silky white mycelium (Brewster, 2001). Infected plants become yellow and then wilt, the collar root turns brown and rots. In groundnut, *S. rolfsii* also infects the pegs and pods, leading to yield losses.

Sclerotium rolfsii is difficult to control by physical and cultural practices due to its wide host range of over 500 plant species (Aycock, 1966; Punja, 1985) and persistent sclerotia (Lakpale, 2007; Punja, 1985). To successfully implement management practices (e.g., chemical and biological) to control *S. rolfsii*, knowledge of the distribution and diversity especially in pathogenicity and virulence of the pathogen is essential.

Branch and Brenneman (1999) evaluated the resistance of mass-selected populations derived from combinations of crosses among two resistant and two susceptible peanut cultivars. Fery and Dukes sr. (2002) determined the cowpea resistance to *S. rolfsii*. There was significant variability in cowpea germplasm for resistance to southern blight. In another study (Flores-Moctezuma *et al*., 2006), two onion isolates of *S. rolfsii* were inoculated to 51 plant species and disease severity levels were determined. Subsequently, 12 out of 51 plant species were selected for the determination of pathogenic reaction to 20 isolates of *S. rolfsii* from different regions. Onion isolates produced variable levels of disease severity for half of the plants tested. Five plant species were susceptible or highly susceptible to all isolates.

Eleven sugar beet genotypes were evaluated at National Agricultural Research Center, Islamabad, Pakistan, during the year 2009 for their resistance against root rot caused by *S. rolfsii* (Farooq *et al*., 2011). Inoculation of eleven genotypes with *S. rolfsii* exhibited resistant response only in SD-PAK-09/07 and moderate resistance in SD-PAK-07/071.

The results of a recent study showed that *S. rolfsii* isolates originating from groundnut, tomato and taro were all pathogenic on groundnut, but displayed substantial diversity of various genetic and phenotypic traits, including mycelial compatibility, growth rate, and sclerotial characteristics (Le *et al*., 2012).

The aim of this study was to identify the virulence of different *S. rolfsii* isolates on a susceptible local peanut germplasm and determine the resistance of twenty peanut genotypes to the most virulent isolate and also the relationship between virulence and mycelial compatibility groups (MCGs).

Materials and Methods

Isolates virulence determination

Seventy eight isolates of *S. rolfsii* from ten different hosts in Guilan province with known MCGs (Mehri *et al*., 2013) were applied for inoculation of a local susceptible peanut germplasm in greenhouse conditions (Table 1).

Barley seeds were boiled in distilled water for twenty minutes and then 12 gr of seeds were added to each 100 ml Erlenmeyer flask and autoclaved twice at 121°C and 1.5 atmospheres for thirty minutes. For each isolate a 5 mm disk of growing fungus on PDA medium was transferred to the Erlenmeyer flask containing sterilized barley seeds and the cultures were maintained in the growth chamber (27±1°C) (Sennoi *et al*., 2010).

The applied soil (1:1:2 clay, compost, sand, pH=6.7) was autoclaved at 121°C and 1.5 atmospheres for thirty minutes and added to the pots with 500 gr soil capacity. Seeds of a local susceptible peanut germplasm were sterilized with Sodium Hypochlorite 1% solution for three minutes and rinsed with sterilized distilled water three times, then soaked in sterilized distilled water. The peanut seeds were placed in a moist chamber at 25±5°C for 72 h to germinate.

When the mycelium covered all the barley seeds and enough sclerotia were formed, each pot was inoculated with thirty infected barley seeds and the seeds were covered

Table 1. *Sclerotium rolfsii* isolates from different MCGs and host plants.

MCGs	pepper	tomato	squash	bean	sunflower	eggplant	groundnut	*Amaranthus* sp.	*Euphorbia* sp.	cowpea	Total for all hosts
MCG1	13	0	0	13	2	0	5	2	0	1	36
MCG2	1	0	0	1	0	0	0	0	0	0	2
MCG3	3	5	1	3	0	2	15	0	2	1	32
MCG4	0	0	0	3	0	1	0	0	0	0	4
MCG5	2	0	0	1	0	0	0	0	0	0	3
MCG6	0	0	0	1	0	0	0	0	0	0	1
Total for all MCGs	19	5	1	22	2	3	20	2	2	2	78

with a thin soil layer. The experiment was done as a complete randomized block design with three replications. There were two controls for each treatment including pots inoculated with thirty sterile barley seeds. After establishment of the fungus in soil, the germinated peanut seeds were cultured into pots (one seed per pot) (Toribio *et al*., 1992). The pots were maintained in greenhouse conditions at 25±5°C (Erkilic *et al.,* 2006). The pots were irrigated based on seedlings need for prevention of water stress (Flores-Moctezuma *et al*., 2006; Sennoi *et al*., 2010).

Disease symptoms were monitored daily from one week after peanut seeding, when the symptoms were observed. At the plants maturity (about 6 weeks after seeding), all the plants were uprooted at the same time and the roots were washed in running tap water to remove soil particles (Yaqub and Shahzad, 2005).

Disease severity was assessed by scoring the plant wilting, yellowing or death, mycelia or sclerotia production on the soil surface or on plant stem, stem area affected (%) and lesion length. Disease severity index was calculated for each treatment using these scores according to the Townsend-Heuberger formula (Erkilic *et al.,* 2006) as bellow:

$$DS(\%) = \frac{\sum(n \times v)}{N \times V} \times 100$$

Where,
n: degree of infection according to the scale (Le *et al*., 2012),
v: number of seedlings per category,
N: total number of seedlings were screened and
V: highest degree of infection.
Also, shoot wet weight and plant height were recorded at this stage. The data were analyzed by the one-way ANOVA followed by Tukey's multiple range test for mean comparison using SAS v. 9.0 software.

Peanut genotypes resistance evaluation

One of the most virulent isolates of *S. rolfsii* on the tested germplasm was used for evaluation of the twenty peanut genotypes resistance in greenhouse conditions as completely randomized design. The germinated peanut seeds and the infected barley seeds were prepared in the same way as mentioned in isolates virulence determination experiment. The germinated peanut seeds were cultured into pots and two weeks after seeding, the seedlings were inoculated with three infected barley seeds (Sennoi *et al*., 2010). Each cultivar was also inoculated by three sterile barley seeds as control treatment. The genotype resistance was evaluated one month after seeding, based on the scale and characteristics mentioned before (Le *et al*., 2012).

Results

S. rolfsii isolates covered the barley seeds 2-3 weeks after inoculation as white mycelia. In the isolates virulence determination experiment, two weeks after seeding of peanut germplasm, disease symptoms were observed on peanut seedling stems as water soaked spots which turned to rot soon (Figure 1). These spots resulted in wilting and death of plants during their maturation. The fungus mycelia extended around the stems and on the soil surface. Sclerotia were also observed on these mycelia.

All of the isolates were virulent on tested peanut germplasm and the virulence was significantly different within the isolates at $p \leq 0.01$ (Figures 2 and 3). There were significant differences between the shoot wet weight and plant height in the treatments ($p \leq 0.01$). The plants which were inoculated with isolates 8 and 73 were the highest (33.5 cm) and those inoculated with isolate 64 were the shortest plants (7.33 cm). There was a significant negative correlation between plant height and disease severity index (DSI). The higher was the DSI, the shorter was the plant height (Table 2).

Stem and root rot resulting from this disease reduced the shoot wet weight. There was a significant negative correlation between shoot wet weight and DSI. The higher was the DSI, the less was the shoot weight.

Variance analysis of the data related to stem lesion length showed significant difference between the isolates ($p \leq 0.01$). Isolate 73 caused the shortest lesion (2.35 mm) and isolate 53 caused the longest one (100.56 mm).

Figure 1. Stem lesion caused by a virulent isolate of *Sclerotium rolfsii* on a susceptible local peanut germplasm. The sclerotia produced on the infected stem and on the soil surface.

Stem area affected (%) shows the proportion of the lesion width to the healthy stem circumference. For the isolates 1, 6, 38, 42, 44, 47, 53, 57, 64, 69 and 70 this proportion was 100%. The least amount was for the isolates 8 (15%).

Disease symptoms occurrence in an infected plant can be compared with a healthy control plant and this criterion can be expressed as percent in an overview. Thus, the symptoms like wilting, yellowing of the entire leaves or only the lower leaves, occurrence of the lesions and crown infection were evaluated. This criterion was 100% for isolates 1, 6, 38, 42, 44, 47, 53, 57, 64, 69 and 70 and 16.66% for isolate 8 (Figure 3).

Based on the correlation analysis results, there was a significant correlation within all measured criteria at $p \leq 0.01$ (Table 2). There was a significant positive correlation between percent of symptoms occurrence, DSI, stem lesion length and stem area affected (%), but a negative correlation between these mentioned criteria and plant height and shoot wet weight. The greater was the amount of lesion length or stem area affected, the higher were the symptoms occurrence and DSI, the less the plant height and wet weight and therefore the more virulent the tested isolate. When the plants had been infected by the fungus especially in their seedling stage, the seedlings lost their normal growth and the final plant height decreased compared to uninfected control plants. The isolates which were more virulent also decreased the peanut seedlings emergence rate more.

The 78 tested isolates in this study were significantly different in their virulence on the tested peanut germplasm and were divided to five groups based on their virulence ($P \leq 0.01$). The isolates 38, 6, 1, 42, 44, 47, 53, 57, 64, 69 and 70 were the most virulent and the isolates 8 and 73 were the least virulent ones. Considering the calculated DSI for each isolate, the tested peanut germplasm reaction to these isolates was expressed as

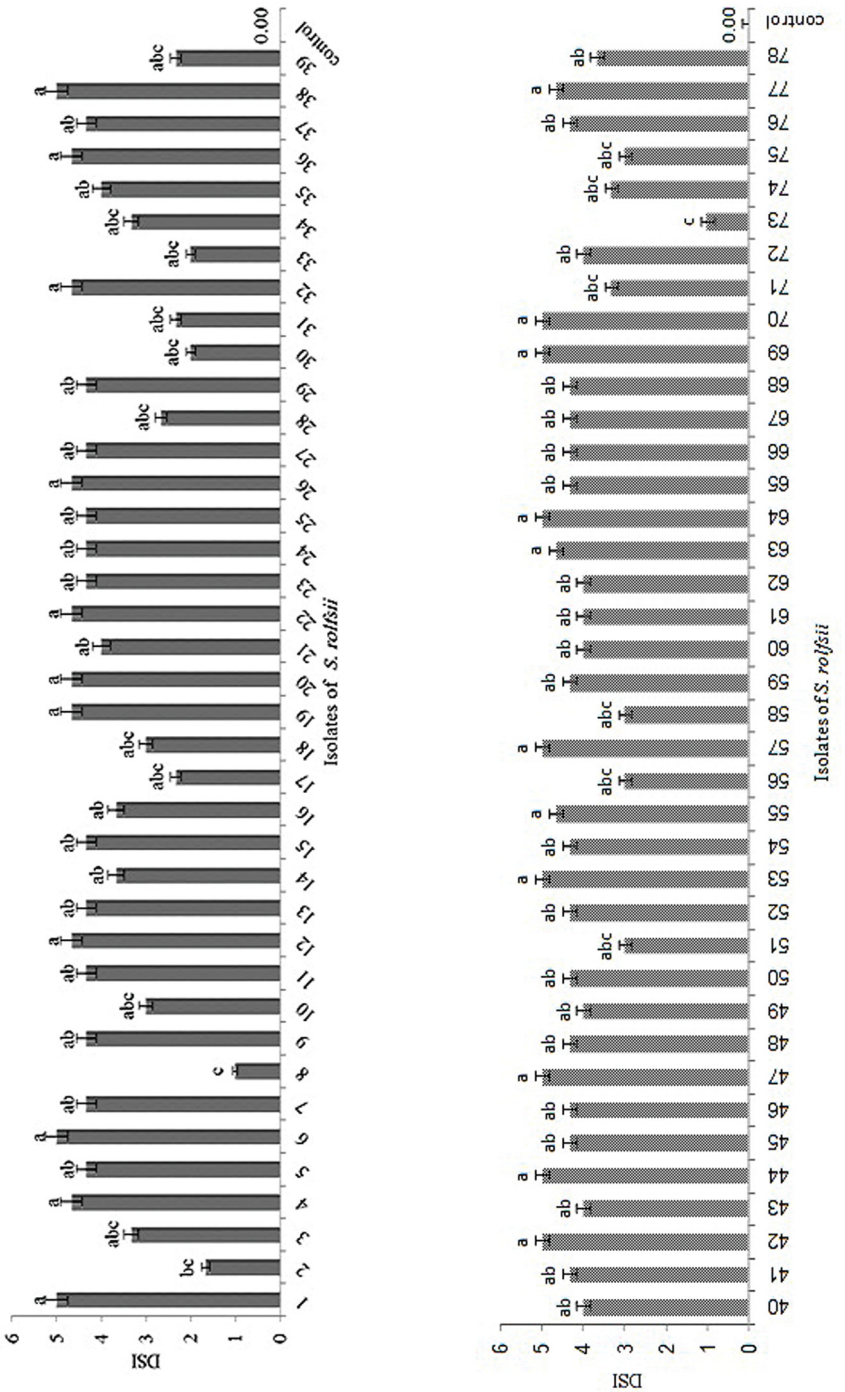

Figure 2. Disease severity index (DSI) for the peanut tested germplasm when inoculated with different isolates of *Sclerotium rolfsii*.

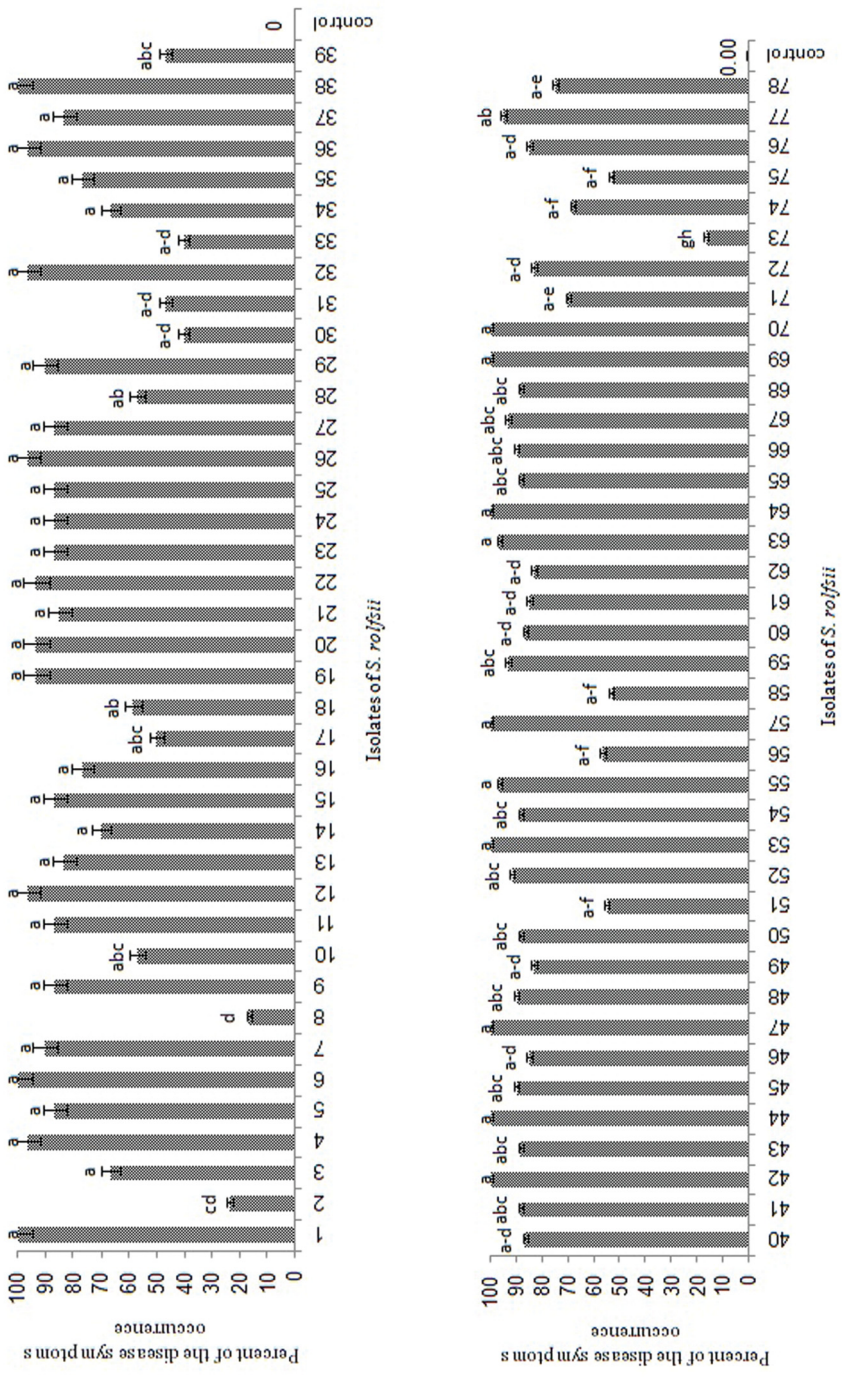

Figure 3. Percent of the disease symptoms occurrence in the peanut tested germplasm compared to the control plant when inoculated with different isolates of *Sclerotium rolfsii*.

Table 2. Correlation between the measured criteria in the *Sclerotium rolfsii* isolates virulence evaluation test.

		Lesion length	Stem area affected (%)	Symptom occurrence (%)	DSI	Plant height	Shoot wet weight
Lesion length	Pearson Correlation	1					
	N	78					
Stem area affected (%)	Pearson Correlation	0.810**	1				
	N	78	78				
Symptom occurrence (%)	Pearson Correlation	0.815**	0.981**	1			
	N	78	78	78			
DSI	Pearson Correlation	0.831**	0.989**	0.990**	1		
	N	78	78	78	78		
Plant height	Pearson Correlation	-0.730**	-0.896**	-0.894**	-0.906**	1	
	N	78	78	78	78	78	
Shoot wet weight	Pearson Correlation	-0.299**	-0.404**	-0.371**	-0.396**	0.349**	1
	N	78	78	78	78	78	78

R= resistant with scores of 0-2, MR= moderately resistant with scores 2.1-3, S= susceptible with scores 3.1-4.99 or HS= highly susceptible with score 5 (Farooq *et al*., 2011; Flores-Moctezuma *et al*., 2006). The tested peanut germplasm was resistant, moderately resistant, susceptible and highly susceptible to 6.41, 12.82, 66.67 and 14.1 percent of tested isolates, respectively.

The five identified groups in the tested isolates based on the virulence, overlapped to some extent with six MCGs identified for these isolates before (Mehri *et al*., 2013). For example, MCG4 only included 4 isolates and the tested peanut germplasm was susceptible to all of them. The MCG5 only had three members which caused susceptible or highly susceptible reaction on the tested germplasm. This overlap has not been observed for all MCGs and some of them included the isolates with different virulence.

Different peanut genotypes resistance to one of the most virulent isolates of *S. rolfsii* (isolate 53) was determined based on the scores and criteria mentioned above for isolates virulence evaluation. Variance analysis of the data showed that the genotypes were significantly different in their resistance to *S. rolfsii*. Considering the calculated DSI for each genotype (Figure 4), its reaction to the tested isolate was expressed as R= resistant with scores of 0-2, MR= moderately resistant with scores 2.1-3, S= susceptible with scores 3.1-4.99 or HS= highly susceptible with score 5 (Farooq *et al*., 2011; Flores-Moctezuma *et al*., 2006).

The genotypes 140 and 183 were resistant to the tested isolate. The genotype 129 was moderately resistant and 137, 138, 193 and 208 were highly susceptible. All the other genotypes showed susceptible reaction. The genotypes 140 and 183 had the least amount of stem lesion length, stem area affection and disease symptom occurrence

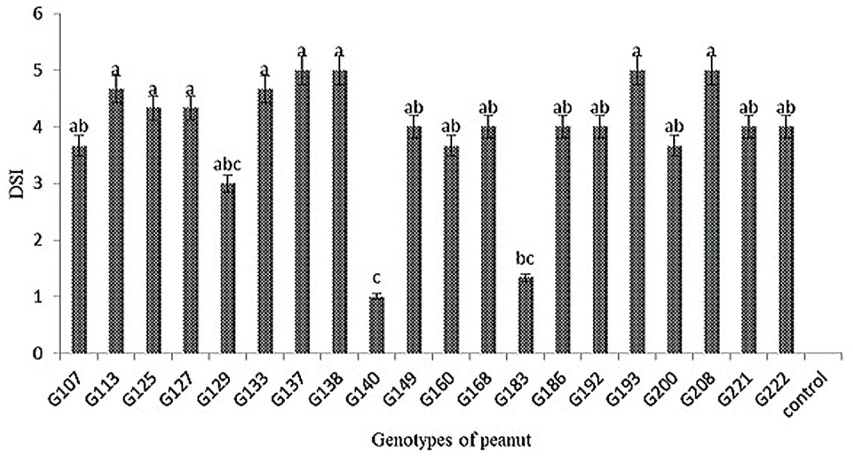

Figure 4. Disease severity index (DSI) for different peanut genotypes when inoculated with one of the most virulent isolates of *Sclerotium rolfsii*.

and the highest amount of height or shoot wet weight. No systemic infection symptoms such as yellowing or wilting were observed in these two genotypes.

Discussion

Based on our research results, the shoot wet weight and plant height decreased in different treatments related to the isolates virulence. Also, the isolates which were more virulent decreased the peanut seedlings emergence rate more. These results on isolates virulence evaluation were compatible with those achieved by Yaqub and Shahzad (2005) who evaluated the *S. rolfsii* isolates virulence on different host plants. Based on their study results, soil infestation with *S. rolfsii* caused a significant reduction in germination of sunflower, mungbean and sugar beet seeds as compared to control. Germination of tomato, sweet pumpkin, cabbage and cauliflower seeds were slightly reduced. The highest reduction in plant length, weight and shoot weight as compared to control was observed in sunflower and mungbean followed by sugar beet, tomato, sweet pumpkin and cabbage. Cauliflower plants showed no effect of *S. rolfsii* infection on plant growth. *S. rolfsii* proved to be highly pathogenic on sunflower, mungbean and sugar beet, mildly pathogenic on tomato, lentil, sweet pumpkin and cabbage, and non-pathogenic on cauliflower plants in pot experiments in their study.

Our results related to isolates virulence and genotypes resistance differences are comparable with those in the study by Flores-Moctezuma *et al*. (2006), in which two onion isolates of *S. rolfsii* from the states of Morelos and Guanajuato, Mexico were inoculated to 51 plant species and disease severity levels were determined. Subsequently, 12 out of 51 plant species were selected for the determination of pathogenic reaction to 20 isolates of *S. rolfsii* from different regions of Mexico. Onion isolates from Morelos and Guanajuato produced variable levels of disease severity for half of the plants tested. Five plant species were susceptible or highly susceptible to all isolates. The remaining plants tested showed differential reactions to individual isolates, ranging from highly resistant to highly susceptible.

As already mentioned, the five identified groups in the tested isolates based on the virulence, overlapped to some extent

with six MCGs identified for these isolates by Mehri *et al*. (2013). Harlton *et al*. (1995) found 49 MCG and 12 RFLP-ITS groups in a worldwide collection of *S. rolfsii* isolates and they did not find a correlation between MCG groups and pathogenicity. In our case, there was also no significant correlation between the isolates virulence and their geographical or host plant origin. In a random selection, not all the identified high virulent isolates had been isolated from peanut and some, which were isolated from peanut, were not virulent on the tested peanut local germplasm. These results were compatible with the results of research conducted by Flores-Moctezuma *et al*. (2006) and Le *et al*. (2012).

Regarding the peanut genotypes resistance evaluation, most of the genotypes were susceptible to the selected most virulent isolate and the resistant reaction was observed only in few genotypes which showed no systemic infection symptoms such as yellowing or wilting. The results were similar to the results reported in other investigations (Farooq *et al*., 2011; Flores-Moctezuma *et al*., 2006; Yaqub and Shahzad, 2005).

In this investigation, disease severity was evaluated using several scoring methods like the plant wilting, yellowing or death, mycelia or sclerotia production on the soil surface or on plant stem, stem area affected (%) and lesion length, shoot wet weight and plant height and also percent of the disease symptom occurrence. We finally concluded that stem area affection is a very useful criterion for the evaluation of isolates virulence or genotype resistance and stem lesion length is of second importance. The two resistant peanut genotypes to *S. rolfsii* identified in our study will be useful for the control of the white rot disease in the peanut fields and the reduction of the losses through the introduction of the genotypes in Guilan province, especially because the genotype 140 is better than the others based on seed size, plant height and the canopy.

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Literature Cited

Aycock, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*. *North Carolina Agricultural Experiment Station Technical Bulletin,* 174, 202.

Branch, W.D. and Brenneman, T.B. 1999. Stem rot disease evaluation of mass-selected peanut populations**.** *Crop Protection,* 18: 127-130.

Brewster, V. 2001. Southern blight, Southern stem blight, White mold. APS net. Online publication.

Erkilic, A., Guven, B. and Akgul, D.S. 2006. Effects of some plant activators and plant materials on stem rot disease of peanut and pepper caused by *Sclerotium rolfsii*. *Journal of Turkish Phytopathology,* 35 (1-3), 15-28.

FAO. 2010. FAO Statistics Division. Online publication.

Farooq, M.A., Iqbal, U., Rasool, A., Zubair, M., Iqbal, S.M. and Ahmad, A. 2011. Evaluation of sugar beet (*Beta vulgaris* L.) genotypes for resistance against root rot caused by *Sclerotium rolfsii*. *Mycopath,* 9(1): 13-15.

Farr, D.F., Bills, G.F., Chamuris, G.P. and Rossman, A.Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopatholgical Society, St Paul, MN, p. 1252.

Fery, R.L. and Dukes Sr.,P.D. 2002. Southern blight (Sclerotium rolfsii Sacc.) of cowpea: yield-loss estimates and sources of resistance. *Crop Protection*, 21: 403-408.

Flores-Moctezuma, H.E., Montes-Belmont, R., Jiménez-Pérez, A. and Nava-Juárez, R. 2006. Pathogenic diversity of *Sclerotium rolfsii* isolates from Mexico, and potential control of southern blight through solarization and organic amendments. *Crop Protection,* 25: 195–201.

Hammons, R.O. 1994. The origin and history of the groundnut. Pp. 24-42. In: The Groundnut Crop: A Scientific Basis for Improvement (Smartt, J., ed). Chapman & Hall, London.

Harlton, C.E., Levesque, C.A. and Punja, Z.K. 1995. Genetic diversity in *Sclerotium (Athelia) rolfsii* and related species. *Molecular Plant Pathology,* 85 (10): 1269-1281.

Kolte, S.J. 1984. Diseases of Annual Edible Oilseed Crops. Vol. I. Peanut Diseases.CRC Press, Boca Raton, FL.

Lakpale, N., Khare, N. and Thrimurty, V.S. 2007. Suppression of *Sclerotium rolfsii* Sacc.: an intergrated approach. *Soils and Crops,* 17: 241-245.

Le, C.N. 2004.Study wilt diseases on groundnut and some methods to control them in Thua Thien

Hue province. *Vietnam National Journal of Plant Protection*, 1: 9-15.

Le, C.N., Mendes, R., Kruijt, M. and Raaijmakers, J.M. 2012. Genetic and phenotypic diversity of *Sclerotium rolfsii* in groundnut fields in central Vietnam. *Plant Disease,* 96: 389-397.

Mehan, V.K., Mayee, C. D., McDonald, D., Ramakrishna, N. and Jayanthi, S. 1995. Resistance in groundnut to *Sclerotium rolfsii* caused stem and pod rots. *International Journal of Pest Mangement,* 41: 79-82.

Mehri, Z., Khodaparast, S.A. and Mousanejad, S. 2013. Genetic diversity in *Sclerotium rolfsii* population based on mycelia compatibility groups in Guilan province, Iran. *Iranian Journal of Plant Pathology,* 49 (3): 317-324.

Nguyen, T.N., Tran, V.M., Nguyen, T.T. and Le, C.N. 2004. Research on groundnut diseases in Quang Binh province. *National Agricultural Rural Development*,17: 337-342.

Punja, Z.K. 1985. The biology, ecology, and control of *Sclerotium rolfsii. Annual Review of Phytopathology,* 23: 97-127.

Punja, Z.K. and Jenkins, S.F. 1984. Influence of temperature, moisture, modified gaseous atmosphere, and depth in soil on eruptive sclerotial germination of *Sclerotium rolfsii. Phytopathology,* 74: 749-54.

Savage, G.P. and Keenan, J.I. 1994. The composition and nutritive value of groundnut kernels. Pp. 173-21. In: The Groundnut Crop: A Scientific Basis for Improvement (Smartt, J., ed). Chapman & Hall, London.

Sennoi, R., Jogloy, S., Saksirirat, W. and Patanothai, A. 2010. Pathogenicity test of *Sclerotium rolfsii*, a causal agent of jrusalem artichoke (*Helianthus tuberosus* L.) stem rot. *Asian Journal of Plant Science,* 9(5): 281-284.

Toribio, J.A. and Rodriguez-Kabana, R. 1992. The effects of inoculum density and physical factors on the assessment of disease caused by *Sclerotium rolfsii. Plant and Soil,* 142: 107-112.

Yaqub, F. and Shahzad, S. 2005. Pathogencity of *Sclerotium rolfsii* on different crops and effect of inoculum density on colonization of mungbean and sunflower roots. *Pakistan Journal of Botany,* 37(1): 175-180.

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Αξιολόγηση της μολυσματικότητας απομονώσεων του *Sclerotium rolfsii* στην αραχίδα (*Arachis hypogaea*) και διερεύνηση της ανθεκτικότητας γονοτύπων αραχίδας σε συνθήκες θερμοκηπίου

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Ο μύκητας *Sclerotium rolfsii* είναι εδαφογενές παθογόνο που προκαλεί σήψη ριζών και στελεχών σε ένα μεγάλο εύρος καλλιεργειών. Η παρούσα μελέτη πραγματοποιήθηκε με σκοπό τον προσδιορισμό της μολυσματικότητας διάφορων απομονώσεων του *S. rolfsii* σε ευπαθές τοπικό γενετικό υλικό αραχίδας, τον καθορισμό του βαθμού ανθεκτικότητας 20 γονοτύπων αραχίδας στην πιο μολυσματική απομόνωση του παθογόνου καθώς και τη διερεύνηση της σχέσης μεταξύ μολυσματικότητας και ομάδων μυκηλιακής συμβατότητας (Mycelial Compatibility Groups, MCGs). Χρησιμοποιήθηκαν 78 απομονώσεις του μύκητα που προέρχονταν από δέκα διαφορετικά φυτά-ξενιστές και ανήκαν σε έξι γνωστές MCGs. Το πείραμα πραγματοποιήθηκε σε συνθήκες θερμοκηπίου (25 ± 5°C) εφαρμόζοντας σχέδιο τυχαιοποιημένων πλήρων ομάδων με τρεις επαναλήψεις. Γλάστρες που περιείχαν αποστειρωμένο έδαφος (pH=6,7) εμβολιάστηκαν με σπόρους κριθής εποικισμένους με καθεμία από τις απομονώσεις του παθογόνου ξεχωριστά πριν από τη σπορά του τοπικού γενετικού υλικού αραχίδας. Η εκτίμηση της έντασης της ασθένειας έγινε βαθμολογώντας τη μάρανση, χλώρωση ή νέκρωση των φυτών, την ανάπτυξη μυκηλίου ή το σχηματισμό σκληρωτίων στην επιφάνεια του εδάφους ή στο στέλεχος των φυτών, το ποσοστό (%) της προσβεβλημένης επιφάνειας του στελέχους και το μήκος της κηλίδας στη βάση του στελέχους στο στάδιο της ωρίμανσης των φυτών. Επίσης έγιναν μετρήσεις του νωπού βάρους του στελέχους και του ύψους των φυτών. Με βάση τα αποτελέσματα των δοκιμών παθογένειας, όλες οι απομονώσεις ήταν μο-

λυσματικές στο τοπικό γενετικό υλικό αραχίδας και υπήρχαν στατιστικά σημαντικές διαφορές (P≤0,01) μεταξύ των απομονώσεων ως προς το βαθμό μολυσματικότητας. Δε διαπιστώθηκε συσχέτιση μεταξύ των πέντε ομάδων μολυσματικών απομονώσεων του μύκητα που προσδιορίστηκαν στην παρούσα μελέτη και των MCGs. Ο γονότυπος αραχίδας 140, ο οποίος ήταν ο καλύτερος σε σχέση με τους υπόλοιπους με βάση το μέγεθος του σπόρου, το ύψος των φυτών, και το μέγεθος της κόμης ήταν και ο πιο ανθεκτικός στις μολύνσεις του παθογόνου.

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SHORT COMMUNICATION

Record of *Phenacoccus peruvianus* Granara de Willing and *Phenacoccus madeirensis* (Hemiptera: Pseudococcidae) on new host ornamental plants in Greece

G.J. Stathas*, E.D. Kartsonas and A.I. Darras

Summary Two invasive mealybug species, *Phenacoccus peruvianus* Granara de Willink and *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), have been recorded on new species of ornamental plants in different regions of Greece. *Phenacoccus peruvianus* was recorded in Athens on *Cestrum nocturnum* L. (Solanaceae) in September 2013. *Phenacoccus madeirensis* was found in Kalamata (Peloponnese) on *Aloysia citriodora* Paláu (Verbenaceae) in May 2014 and on *Osteospermum jucundum* (Phillips) (Asteraceae) in July 2014. This is the first record of *O. jucundum* as host plant of *P. madeirensis*.

During the recent years, mealybugs (Hemiptera: Pseudococcidae) have been spreading in many countries, causing severe damage mainly on ornamental plants. Scale insects represent the second largest group of alien insects in Europe after aphids (DAISIE, 2008; Beltrà *et al*., 2010). The mealybug scale insects are typical invasive species (Miller *et al.,* 2005) due to their small size and cryptic behaviour (Beltrà *et al*., 2010). Their entrance to an area without natural enemies, facilitates their easy establishment, and increases the possibility of causing economically significant damage.

The literature review provided the occurrence of 6 species from the genus *Phenacoccus* in Greece, namely: *Phenacoccus bicerarius* Borchsenius (Kozár *et al.,* 1991), *P. hordei* (Lindeman) (Milonas and Kozár, 2008), *P. peruvianus* Granara de Willink, (Gkounti and Milonas, 2013), *P. interruptus* Green (Kozár, 1985), *P. madeirensis* Green (Jansen *et al.,* 2010; Papadopoulou and Chryssohoides, 2012) and *P. yerushalmi* Ben-Dov (Ben-Dov *et al.,* 2006).

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In this study, the presence of *P. peruvianus* and *P. madeirensis* have been recorded in new locations on new host plant species, only a short time after their first appearance in Greece. The mealybugs were found during phytosanitary inspections on ornamental plants in public and private gardens in the towns of Kalamata (Peloponnese) and Athens.

The bougainvillea mealybug *Phenacoccus peruvianus* is native to Neotropic region (Peru, Argentina). In Europe, it was first reported in the region of Almeria, Spain (1999) on *Bougainvillea glabra* Choisy (Nyctaginaceae). Later it was found in Sicily, Italy (2002), south East England and Corsica (2005), Portugal (2006), Monaco (2008) and France (2008) on *Bougainvillea* sp., while in 2009 was first recorded on *Justicia suberecta* (Acanthaceae) and *Solanum vespertilio* Alion (Solanaceae) in the island of Majorca (Spain) (Beltrà *et al*., 2010). Its fast spread throughout the Mediterranean basin is believed to be facilitated by the increase of world trade of ornamental plants (Beltrà *et al*., 2010; Gkounti and Milonas, 2013). *Phenacoccus peruvianus* was first recorded in Greece (in the island of Paros) on *Bougainvillea* sp. in autumn of 2012 (Gkounti and Milonas, 2013).

During the present study, *P. peruvianus*

was recorded in the suburb of Athens, Papagou (37°59′35.2″N, 23°48′31.8″E), where the mealybug was found on *Cestrum nocturnum* (Solanaceae) in September 2013 (Fig. 1). The identification of *P. peruvianus* was made by Dr M. Bora Kaydan (Çukurova University of Adana, Turkey).

Figure 1. *Phenacoccus peruvianus* on *Cestrum nocturnum* (Photo by G.J. Stathas).

The Madeira mealybug, *P. madeirensis*, is considered to have a New World origin. It was described by Green (1923) and its name was derived from Madeira Islands. In Europe, its first reports were from Western Mediterranean region, Sicily and later from France (Jansen *et al.,* 2010). Jansen *et al.* (2010) recorded Madeira mealybug in Crete on *Hibiscus rosa-sinensis* (Solanaceae) in 2010 and indicated that this mealybug has been spreading to wider territories in the Mediterranean region.

In this study, *P. madeirensis* was recorded in the town of Kalamata (37°02′17.0″N, 22°04′41.2″E) on *Aloysia citriodora* in May 2014 and at the same area on *Osteospermum jucundum* (Phillips) (Asteraceae) in July 2014 (Fig. 2, Fig. 3). The identification of the species was made by Professor Giuseppina Pellizzari (University of Padua, Italy). According to ScaleNet Database (Ben-Dov *et al.,* 2013), *O. jucundum* is a new host plant of *P. madeirensis*.

Although the above mentioned invasive mealybug species have already been recorded in Greece, it is wise to encourage the study of their distribution in other areas of the country. Invasive species can sometimes have a great impact on biodiversity, modify the habitat and cause extensive environmental and economic destruction. In recent years, several species of economically important mealybug pests (Pseudococcidae) have been introduced into different countries in the Mediterranean Region and other areas of the Palaearctic Region (Kaydan *et al.,* 2012).

Figure 2. *Phenacoccus madeirensis* on *Aloysia citriodora* (Photo by G.J. Stathas).

Figure 3. *Phenacoccus madeirensis* on *Osteospermum jucundum* (Photo by G.J. Stathas).

The authors would like to express their gratitude to Dr M. Bora Kaydan, Çukurova University, Adana, Turkey, for the identification of Phenacoccus peruvianus *and to Professor Giuseppina Pellizzari, University of Padova, for the identification of* Phenacoccus madeirensis.

Literature cited

Beltrà, A., Soto, A., Germain, J.F., Matile-Ferrero, D., Mazzeo, G., Pellizzari, G., Russo, A., Franco, J.C. and Williams, D.J. 2010. The Bougainvillea mealybug *Phenacoccus peruvianus*, a rapid invader from South America to Europe. (Summary in Greek). *Entomologia Hellenica*, 19: 137-143.

Ben-Dov, Y., Gounari, S., Kaydan, M.B. and Hadina, F. 2006. *Phenacoccus yerushalmi* Ben-Dov newly recorded from Greece and Turkey (Hem., Coccoidea, Pseudococcidae). *Bulletin de la Societe Entomologique de France*, 111(1): 42.

Ben-Dov, Y., Miller, D.R. and Gibson, G.A.P. 2013. ScaleNet: a database of the scale insects of the World. Available in: http://www.sel.barc.usda.gov/scalenet/query.htm (accessed 12 September 2014).

DAISIE (Delivering Alien Invasive Species Inventories for Europe) 2008. European Invasive Alien Species Gateway. http://www.europe-aliens.org/ (last accessed on 12 September 2014).

Gkounti V. and Milonas, P. 2013. First record of the bougainvillea mealybug *Phenacoccus peruvianus* in Greece. *Entomologia Hellenica*, 22: 16-18.

Jansen, M.G.M., Ben-Dov, Y. and Kaydan, M.B. 2010. New records of scale insects from Crete Island, Greece (Hem., Coccoidea). *Bulletin de la Société Entomologique de France*, 115 (4): 483-484.

Kaydan, M.B., Erciliç, L. and Ülgentürk, S. 2012. An invasive mealybug species *Phenacoccus madeirensis* Green (Hemiptera: Coccoidea, Pseudococcidae) introduced recently into Turkey. *Türkiye Entomoloji Bülteni*, 2 (2): 67-74.

Kozár, F. 1985. New data to the knowledge of scale insects of Bulgaria, Greece and Rumania (Homoptera: Coccoidea). *Acta Phytopathologica Academiae Scientiarum Hungaricae*, 20: 201-205.

Kozár, F., Paloukis, S. and Papadopoulos, N. 1991. New scale insects (Homoptera: Coccoidea) in the Greek entomofauna. *Entomologia Hellenica*, 9: 63-68.

Miller, D.R., G.L. Miller, Hodges, G.S. and Davidson, J.A. 2005. Introduced scale insects (Hemiptera: Coccoidea) of the United States and their impact on US agriculture. *Proceedings of the Entomological Society of Washington*, 107: 123-158.

Milonas, P.G. and Kozár, F. 2008. Check list of mealybugs (Homoptera: Pseudococcidae) in Greece: three new records. *Hellenic Plant Protection Journal*, 1: 35-38.

Papadopoulou, S. and Chryssohoides, C. 2012. *Phenacoccus madeirensis* Green, 1923 (Homoptera: Pseudococcidae) on *Ocimum basilicum*: a new geographical record for Greece. *EPPO Bulletin*, 42(1):146–147.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Καταγραφή των *Phenacoccus peruvianus* Granara de Willing και *Phenacoccus madeirensis* (Hemiptera: Pseudococcidae) σε νέα καλλωπιστικά φυτά-ξενιστές στην Ελλάδα

Γ.Ι. Σταθάς, Ε.Δ. Κάρτσωνας και Α.Ι. Δάρρας

Περίληψη Καταγράφεται η παρουσία των επεκτατικών αλλόχθονων κοκκοειδών εντόμων *Phenacoccus peruvianus* Granara de Willink και *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae) επί νέων ειδών καλλωπιστικών φυτών, σε νέες περιοχές της Ελλάδος, λίγα μόλις έτη μετά από την πρώτη καταγραφή τους στη χώρα μας. Το είδος *Phenacoccus peruvianus* βρέθηκε στην Αθήνα επί του καλλωπιστικού φυτού *Cestrum nocturnum* L. (Solanaceae) το Σεπτέμβριο του 2013. Το είδος *Phenacoccus madeirensis* βρέθηκε στην Καλαμάτα (Πελοπόννησος) επί του καλλωπιστικού φυτού *Aloysia citriodora* Paláu (Verbenaceae) το Μάιο του 2014 και επί του *Osteospermum jucundum* (Phillips) (Asteraceae) τον Ιούλιο του 2014. Το καλλωπιστικό φυτό *O. jucundum*, αναφέρεται για πρώτη φορά ως ξενιστής του *P. madeirensis*.

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SHORT COMMUNICATION

Common burdock (*Arctium minus*): a common weed of non-arable land in Orestiada, Greece

C.A. Damalas*, C. Alexoudis and S.D. Koutroubas

Summary Common burdock (*Arctium minus*) is a common biennial weed of non-arable land in typical rural settings of Orestiada, Greece. The aim of this study was to describe the basic morphological traits of this species throughout the main phenological stages of its life cycle and to obtain some insight into its growth and productivity in Orestiada. Based on our observations, the plants occurred most commonly in moist and fertile soils, usually as isolated individuals or in small patches near the parent plants. The species is characterized by its large basal 'elephant-ear' leaves during the vegetative stage, appearing in alternate arrangement, with irregularly wavy and non-toothed edges, as well as with long hollow stalks forming a noticeable furrow on the top. By monitoring individual plants, it was found that flowering (in the second year of growth) mostly occurred from late June up to early August. The flowers were purple, occurring in bristly heads at the top of the stem. The bristly heads formed a fruit, containing small black seeds. The average number of capitula per plant, from randomly selected populations in Orestiada, was found to be 69.7 and 57.7 respectively, whereas the mean seed number per capitulum reached 30.3 and 33.3 seeds, respectively.

Additional keywords: biology, growth, identification, life cycle, morphology, seed productivity

Common burdock (*Arctium minus*) is a common biennial weed of non-arable land in typical rural settings (abandoned fields, roadsides, pastures, meadows, grazing plains, stream banks and woodland edges) of Orestiada, regional unit of Evros, in northern Greece. The species has been recorded by the authors also in the margins of corn fields in the rural area of Eordea, regional unit of Kozani, in western Greece. In fact, it is a common herb of the Greek flora and occurs throughout the Greek territory.

The genus name *Arctium* was derived from the Greek word for 'bear' and most likely refers to the scruffy and brown appearance of the plants' bristly heads (burs) at maturity. Common names of this weed species in Greek include: 'kollitsida', 'arkoudovotano' and 'platanomantilida' (Anonymous, 2013). Common burdock is originated in Europe and was likely brought to North America by early French and English colonists (Gross *et al*., 1980). Actually, common burdock is a successful global invader, present in Europe, North and South America, Australia and New Zealand (Nawrocki, 2010). Common burdock can grow in a wide range of soils from sandy clay to moist loam, most preferably in nitrogen-rich soils (Gross *et al*., 1980; Van Vleet, 2009). Despite its wide distribution, limited data exist in the formal literature about the biology and the agronomic value of this weed; the available information is scattered in the so-called grey literature. The aim of this study was to describe the basic morphological traits of this species throughout the main phenological stages of its life cycle and to obtain some insight into its growth and productivity in Orestiada.

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In the spring of 2014, randomly selected populations of *Arctium minus* were studied either from abandoned fields or along roadsides near the Farm of Democritus University of Thrace in the rural area of Orestiada (41°30′N latitude, 26°32′E, 22 m asl). Two populations were sampled and certain morphological data (i.e. leaf shape, arrangement, structure and color, flower shape and color as well as fruit shape and color) were collected from three individuals per population. All capitula per sampled individual were collected in brown paper bags and brought to the laboratory. Three randomly selected capitula per individual were opened and the number of seeds was recorded. Information on seed productivity is important because common burdock is reproduced only by seeds (Wax *et al.*, 1999). The average number of capitula per plant, measured from randomly selected populations in Orestiada, was found to be 69.7 ± 12.50 and 57.7 ± 12.22, respectively, whereas the average seed number per capitulum reached 30.3 ± 6.03 and 33.3 ± 2.08, respectively. According to the literature, there is evidence of great variability in the seed productivity of plants (Reed and Stephenson, 1972, 1973; Gross *et al.*, 1980; Straw, 1985), which may be attributed to various factors, such as the genetic background of populations, the growth conditions and the level of insect predation of the seeds as proposed by other authors (Hawthorn and Hayne, 1978; Straw, 1985; Kambo and Kotanen, 2014). Therefore, more research on seed productivity is needed.

From our observations in the field, it was found that the cotyledons are large, spoon-shaped, with a waxy surface. The first true leaves are stalked and ovate with entire and slightly wavy margins (Gross *et al.*, 1980) (Figure 1). In the first year of growth, the plant grows as a typical low-growing rosette of leaves (Figure 2) and then in the second year it produces a tall and erect flowering stem (Gross *et al.*, 1980). Rosette leaves are distinctive due to their large size (elephant ear), heart-shaped base, wooly undersurface and hollow leaf stalks (petioles) (Figures 3 and 4). Careful examination of the plants revealed that the upper leaf surface is distinct green and coarse, whereas the underside is pale green to gray and wooly. Subsequent leaves are alternate, oval-shaped (elliptical), with short hairs, wrinkled between the veins and bitter tasting. Leaves gradually become smaller than the basal leaves, less heart-shaped and attenuated at both ends as their location progresses up towards the head of the stem. Additionally, their petioles become shorter and solid rather than hollow. Stem leaves are similar in shape to the rosette leaves but smaller than them.

Based on our observations in the field, the stem stays flattened and close to the soil surface during the rosette stage of growth. When flowering sets up, the stem elongates producing an erect flower stem that is much-branched, rough-hairy, hollow and angular (Figure 5). Flower heads are located at the ends of the branches or at leaf axils on the flower stem and are comprised of a bur with hooked bristles appearing beneath a closely packed cluster of tubular purplish flowers (Figure 6). Each head has purple disk flowers with involucral (covering) bracts modified into narrow hooked bristles. Through this structure a bur is formed that aids in dispersal of common burdock seeds by animals and humans. Indeed, the weed is best known for the hooked bristles on its burs that stick to fur and clothing (Figure 7). The seeds within the bur are oblong, smooth and mottled. In each bur, there are

Figure 1. Young seedling of *Arctium minus* soon after emergence (original photo by Theodore Webster, USA).

Figure 2. Young seedling of *Arctium minus* in a rosette form.

Figure 3. Characteristics of basal leaves (size, shape, arrangement and color) of *Arctium minus*.

Figure 4. Grown plant of *Arctium minus* at the vegetative stage.

Figure 5. Plant of *Arctium minus* at the flowering stage.

Figure 6. Flower details (flowering stage) of *Arctium minus*.

Figure 7. Fruit (achene) details (maturity stage) of *Arctium minus* (original photo by Bob Osborn, UK).

many single-seeded, brown, oblong, angular fruits having a short, stiff bristle at one end. Flowering stems emerge in June and flowers are formed from late June up to October in Orestiada. Flowering usually takes place during the second year, but occasionally flowers are not formed until the third or fourth year of growth (Gross and Werner, 1983). When burs dry, their hooked bristles attach to fur or clothing and the bur separates from the plant, thereby dispersing the seeds. Dispersal of burs and seeds begins in September and continues throughout winter and into the following spring.

Common burdock can be often confused with other species in various growth stages. To avoid confusion with other species, the following information should be taken into account. At the cotyledon stage, common burdock may show some similarity with giant ragweed (*Ambrosia trifida*) at the same growth stage, but the cotyledons of giant ragweed are obviously smaller (Alex, 1992). At the seedling stage, common burdock can be easily confused with broadleaf dock (*Rumex obtusifolius*) and curly dock (*Rumex crispus*), but these two species do not have hairs on the underside of the leaves (Alex, 1992). At the rosette stage, common burdock resembles a popular garden vegetable, i.e. the cultivated rhubarb (*Rheum rhabarbarum*), but the leaves of the latter do not have wooly undersides and its petioles are solid and tinged red (Alex, 1992). Common burdock flowers are similar to those of bull thistle (*Cirsium vulgare*), but the stems and leaves of the latter have spines and its leaves are deeply lobed (Alex, 1992). Common burdock is similar in appearance to great burdock (*Arctium lappa*), except that the latter grows taller, has larger flower heads arranged in clusters with flattened upper surfaces and the petioles of basal leaves are not hollow (Alex, 1992).

Common burdock is not considered to be a serious weed in cropland, because it can be easily controlled by cultivation, particularly in the first year of growth. Gross *et al.* (1980) and Van Vleet (2009) reported that the plant does not tolerate frequent cultivation (Gross *et al*., 1980; Van Vleet, 2009). However, as more farmers adopt no-till farming practices, this weed can become important, even causing yield losses, if not controlled. Other areas of economic damage by common burdock reported include the reduction of wool value, when the dry heads of the plant cling to the fur of sheep and the bitter taste of milk, when the cows eat large quantities of the plant (Gross *et al*., 1980). In addition, certain microorganisms can grow on common burdock, with two of them having major economic importance: i) *Erysiphe cichoracearum* (powdery mildew) that usually affects squashes and cucumbers as well as many species of Asteraceae, such as *Dahlia*, *Helianthus* and *Chrysanthemum*, and ii) *Phymatotrichum omnivorum* (root rot) that attacks mainly cotton and secondarily numerous other crops. Root-knot nematodes of the genus *Meloidogyne*, which attack many cultivated plants and especially legumes, have been reported from *A. minus* (Gross *et al*., 1980).

Based on reports from the so-called grey literature, common burdock can be controlled by the application of several types of herbicides, including atrazine, 2,4-D, 2,4,5-T, 2,4,5-TP and MCPA with treatments of plants preferably in the first year of growth (Nawrocki, 2010). Glyphosate-based herbicides are effective as well as other herbicides, such as clopyralid, clopyralid plus triclopyr, aminopyralid, picloram and dicamba when applied preferably between the rosette stage and the flowering stage (Klingman *et al*., 1983; Van Vleet, 2009). Also, mowing can assist in eliminating seed production, when repeated multiple times per season. Defoliated plants have been found to produce fewer seeds per head, fewer heads per plant and thus fewer seeds per plant (Reed and Stephenson, 1973). Infestations can be controlled by digging to remove the plant and as much of the taproot as possible. Plants will re-grow, unless the taproot is removed (Van Vleet, 2009).

In conclusion, this preliminary study could be a useful guide to determine common burdock occurrence in Orestiada,

where the species is abundant. In addition, given that information about this species is limited in the formal literature, certain attributes reported herein (such as seed production) could be first clues for better understanding of the species importance and also for the adoption of sustainable management strategies to control it. Obviously, more detailed information will be needed to design such strategies.

Literature cited

Alex, J.F. 1992. Ontario Weeds: Descriptions, illustrations and keys to their identification. Consumer Information Centre, Ontario Ministry of Agriculture and Food, Guelph, Ontario, Canada.

Anonymous, 2013. Common Burdock: One of the most powerful herbs of the world (in Greek). Available at: http://www.back-to-nature.gr/2013/05/k-10.html

Gross, R.S. and Werner, P.A. 1983. Probabilities of survival and reproduction relative to rosette size in the common burdock (*Arctium minus*: Compositae). *American Midland Naturalist*, 109: 184-193.

Gross, R.S., Werner, P.A. and Hawthorn, W.R. 1980. The biology of Canadian weeds. 38. *Arctium minus* (Hill) Bernh. and *A. lappa* L. *Canadian Journal of Plant Science*, 60: 621-634.

Hawthorn, W.R. and Hayne, P.D. 1978. Seed production and predispersal seed predation in the biennial composite species, *Arctium minus* (Hill) Bernh. and *A. lappa* L. *Oecologia*, 34: 283-295.

Kambo, D. and Kotanen, P.M. 2014. Latitudinal trends in herbivory and performance of an invasive species, common burdock (*Arctium minus*). *Biological Invasions*, 16: 101-112.

Klingman, D.L., Bovey, R.W., Knake, E.L., Lange, A.H., Meade, J.A., Skroach, W.A., Stewart, R.E. and Wyse, D.L. 1983. USDA Weed Control Compendium: Systemic herbicides for weed control. USDA Bulletin AD-BU-2281. USDA, Extension Service, Washington, DC, USA.

Nawrocki, T. 2010. Common burdock [*Arctium minus* (Hill) Bernh.]. Alaska Natural Heritage Program, University of Alaska, Anchorage, Alaska, USA.

Reed, F.C. and Stephenson, S.N. 1972. The effects of simulated herbivory on *Ambrosia artemisiifolia* and *Arctium minus* Schk. *Michigan Academician*, 4: 359-364.

Reed, F.C. and Stephenson, S.N. 1973. Factors affecting seed number and size in burdock, *Arctium minus* Schk. *Michigan Academician*, 5: 449-455.

Straw, N.A. 1985. Seed loss caused by flower head inhabiting insects of lesser burdock. In: Delfosse, E.S. (ed.) Proceedings of the VI International Symposium on Biological Control of Weeds, 19-25 August 1984, Vancouver, Canada, pp. 479-484.

Wax, L.M., Fawcett, R.S. and Isely D. 1999. Weeds of the North Central States. Diane Publishing Co, Darby, PA, USA.

Van Vleet, S. 2009. Steve's weed of the month: common burdock. Whitman County Extension, Washington State University, Pullman, WA, USA.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Κολλητσίδα (*Arctium minus*): ένα κοινό ζιζάνιο των μη καλλιεργούμενων εκτάσεων στην Ορεστιάδα

Χ.Α. Δαμαλάς, Χ. Αλεξούδης και Σ.Δ. Κουτρούμπας

Περίληψη Η κολλητσίδα (*Arctium minus*) είναι κοινό διετές ζιζάνιο των μη καλλιεργήσιμων εκτάσεων στον αγροτικό χώρο της Ορεστιάδας. Ο σκοπός αυτής της μελέτης ήταν η περιγραφή βασικών μορφολογικών γνωρισμάτων αυτού του είδους σε όλα τα φαινολογικά στάδια του κύκλου ζωής του και η απόκτηση στοιχείων σχετικά με την ανάπτυξη και την παραγωγικότητά του στην Ορεστιάδα. Με βάση τις παρατηρήσεις μας, τα φυτά εμφανίζονται πιο συχνά σε υγρά και γόνιμα εδάφη, συνήθως ως μεμονωμένα άτομα ή σε μικρές κηλίδες κοντά στα μητρικά φυτά. Το ζιζάνιο χαρακτηρίζεται από τα μεγάλου με-

γέθους φύλλα της βάσης (γνωστά ως 'αυτί του ελέφαντα') κατά το βλαστικό στάδιο, τα οποία εμφανίζονται κατ' εναλλαγή, φέροντα ακανόνιστα κυματιστή και μη-οδοντωτή περιφέρεια, καθώς επίσης και χαρακτηριστικά μακρύ και κοίλο μίσχο που σχηματίζει ευδιάκριτο αυλάκι στην κορυφή. Από την παρακολούθηση ατομικών φυτών βρέθηκε ότι η άνθηση (κατά το δεύτερο έτος της ανάπτυξης) λαμβάνει χώρα κυρίως από τα τέλη Ιουνίου έως και τις αρχές Αυγούστου. Τα άνθη είναι μοβ και εμφανίζονται σε αγκαθωτές κεφαλές στο πάνω μέρος του βλαστού. Οι κεφαλές σχηματίζουν τον καρπό, ο οποίος περιέχει μικρούς μαύρους σπόρους. Ο μέσος αριθμός κεφαλών ανά φυτό από δύο τυχαία επιλεγμένους πληθυσμούς στην Ορεστιάδα βρέθηκε να είναι 69,7 και 57,7 αντίστοιχα, ενώ ο μέσος αριθμός σπόρων ανά κεφαλή έφτασε τους 30,3 και 33,3 σπόρους, αντίστοιχα.

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Evaluation of Aquatain™ monomolecular surface film against mosquito larvae of *Culex pipiens* in a full-grown rice field in Greece

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Summary The impact of the monomolecular surface film Aquatain™ against mosquito larvae was tested in a rice field in Central Greece. Aquatain™ was poured in a 1.2 hectare rice paddy along the one side of the field. Laboratory reared mosquito larvae of *Culex pipiens* were introduced into cages placed in three different transects along the short side of the rice paddy. Larval mortality was counted 3, 6, 15 and 25 days after application. In the line located closer to the site of Aquatain™ application, larval mortality ranged from 100% to 70%, 25 days after application. Mortality ranged from 88% to 25% in the middle transect and from 42%, to 10% in the more distant line. The results indicated that Aquatain™ provides sufficient larval control in wide mosquito larval habitats.

Introduction

Mosquitoes are the most important insects of public health due to the pathogens they transmit. Mosquito larvae require water to breed. Larval habitats vary among extensive surfaces with fresh or brackish water to small containers (used tyres). In agricultural areas, rice fields are often the most important extensive mosquito larval breeding sites for both culicines and anophelines, vectors of serious human diseases (Lacey and Lacey, 1990).

Rice cultivation in Greece covers some 30.000 hectares every year, grown under constant flooding (FAOSTAT, 2012). In areas with rice cultivation, mosquito bites impede human activities, especially at dusk. Moreover, in rice paddies many Culicinae and Anophelinae mosquito species proliferate including the main malaria vector in Greece, *Anopheles sacharovi* (Voyadjoglou-Samanidou *et al*., 1989). Mosquito control in rice paddies is currently performed by aerial spraying of larvicides after a specific local authorization and under the supervision of the authorities, as an exception to the prohibition of aerial application of pesticides in EU on the grounds of the threat posed on public health by these breeding sites of mosquitoes. The effectiveness of the applied larvicides is highly impeded by vegetation, especially when rice plants are full-grown.

A method to overcome such problems can be the use of alternative to biocides larvicidal agents which spreads efficiently covering the aquatic surface without accumulating around debris or vegetation. Such a solution can be the use of monomolecular surface films that can be effective on killing mosquito larvae and pupae physically and furthermore non-toxic and safe to the environment (Reiter, 1978; Das *et al*., 1986; Batra *et al*., 2006; Bashir *et al*., 2008). Aquatain™ is a silicone-based monomolecular surface film and its larvicidal and pupicidal properties have been well documented in the laboratory (Bukhari and Knols, 2009; Webb and Russell, 2009). Mosquito control potential of Aquatain™ has also been investigated in

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field trials in backyard habitats in Australia where it successfully managed to keep mosquito larval habitats free of mosquito eggs, larvae and pupae up to 5 weeks post-application (Webb and Russell, 2012). Because of its non-biochemical mode of action no resistance is anticipated. In the present study the effectiveness of Aquatain™ as a mosquito control agent was tested in rice paddies under the common cultivation practices followed in Greece. For this purpose, a field experiment was set up in rice fields close to Anthile, Prefecture of Phthiotida, Greece (38°49′08.36″N- 22°29′51.89″E) in a difficult for mosquito control period when rice plants had completed tillering and flowering, from August to September 2012.

Materials and methods

Cylindrical cages were specially constructed by plastic bottles (30cm high, 8cm diameter) with an opening (3.5x15 cm) at 3.5cm height above the bottom. The opening was covered with gauze of an appropriate mesh capable to prevent mosquito larvae from escaping but allowing the free movement of water in the cages (Figure 1). Nine sampling cages, along three different lines (three cages per line), were selected and marked in a 1.2 ha (120x100m) experimental paddy (Figure 2). The first line (line 1) of replicates was set close to the short edge of the paddy where Aquatain™ was applied, the second one in the middle of the paddy (line 2) and the third one at the far distant edge of the release site (line 3). The sampling cages were 25m apart in the same line and 30m apart from the corresponding point in the adjacent line and from the short edge of the paddy (Figure 2). An adjacent rice paddy of the same dimensions which received the same cultivation and plant protection management as the experimental paddy but was not treated with Aquatain™, served as a control paddy. Sampling cages were set in the same pattern in the control paddy. An irrigation ditch provided water constantly to both paddies (experimental and control), whereas excess water was exiting to the drainage channel (Figure 2, blue arrows). Plastic cages were tightened on supporting poles and placed in the rice paddy prior to Aquatain™ application. All supporting poles were submerged in such a way that the opening of the cages was half submerged (10cm from the base of the cage, Figure 2). Adjustment of the level of the cages was performed on the assessment days to match water fluctuation. Twenty fourth-instar larvae (L_4) of *Culex pipiens* from a laboratory colony (reared at Benaki Phytopathological Institute, T=25 ± 2°C and 75 ± 5%RH) were placed into each cage just before Aquatain™ application.

Aquatain™ was applied into the experimental field by pouring from five different spots along the short edge (A) of the paddy (Figure 2) at the labelled rate of 1 ml/m² (12L in total). A single application was performed on 27th August 2012. Efficacy of Aquatain™ was assessed by recording larval mortality 3, 6, 15 and 25 days after application. To record larval mortality, the contents of the cages were poured into a white pan in order to count the remained larvae (dead or alive). After each assessment another batch

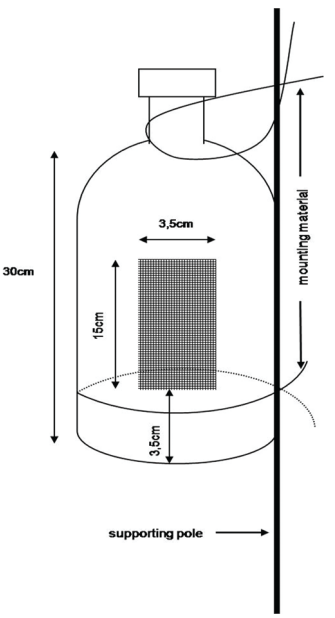

Figure 1. Plastic cage used in the study.

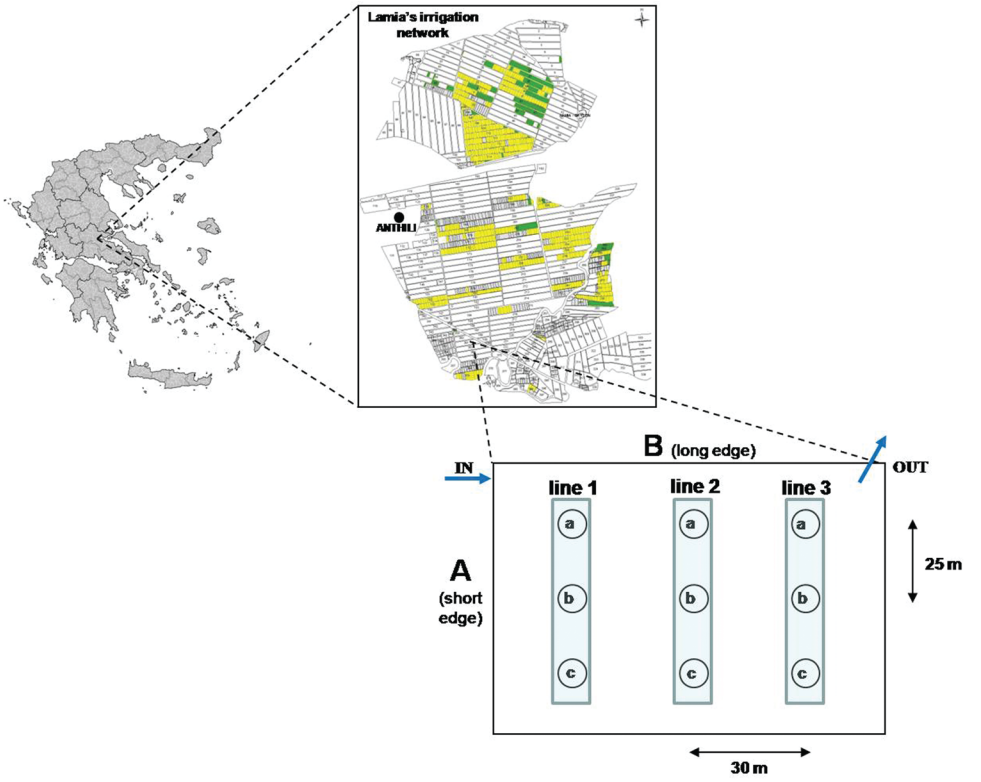

Figure 2. Map presenting the location of the experimental rice in Phthiotida, Lamia, the irrigation system of the rice fields, and a drawing showing the positions of the cages in the experimental paddy.

of twenty fourth *Cx. pipiens* larvae were introduced into the cages (each batch of larvae remained for three days in the cages before mortality evaluation) and adjustment of the level of the cages was performed to match water fluctuation.

Statistical analysis

To identify differences in percent larval mortality between lines in the rice paddy, indicating distances from Aquatain™ application site, the data of each assessment were analyzed using Kruskal-Wallis H-test. When significant differences were detected, Mann – Whitney U-test were carried out for pairwise comparisons. All analyses were conducted using the statistical package SPSS 14.0.

Results and Discussion

The results indicated that Aquatain™ managed successfully to cover the entire water surface of the 1.2 ha rice paddy within a time period of 6 days even though it was applied along the one side only (A, short edge, Figure 2).

Larval mortality reached 100% in the proximate line to the application site (line 1) three days after application. Mortality remained at 100% six days after application and ranged from 95% to 73%, 15 and 25 days after application respectively. Mortality in line 1 was significantly higher than mortality in the other lines, in all assessments, indicating that the distance from the release site affected the larval mortality (Table 1). In the middle of the paddy (line 2), larval mortality was quite low (28%) three days after applica-

tion but it increased significantly during the following days (88% and 83%, 6 and 15 days after Aquatain™ application, respectively) and was significantly higher than mortality in line 3. Larval mortality in line 3 increased and reached its highest level (42%) six days after application.

Increase of larval mortality over time in lines 2 and 3 can be attributed to the time needed for the spread of the product. Furthermore, three days after the application of Aquatain™ (first assessment) all cages in the paddy were found above the water surface as the amount of irrigation water could not maintain the water level. Thus, there was adequate water in the traps to keep mosquito larvae alive but Aquatain™ could not enter into the cages except for those in line 1. Adjustment of the supporting poles after the first assessment, allowing the water to cover half of the cage opening, is also associated with time period of the mortality levels increase in lines 2 and 3.

The complete coverage of the experimental paddy occurred at slower mode than that reported in other studies (Bukhari *et al.,* 2011). This could possibly be due to higher density of the rice plants as in our case Aquatain™ was applied relatively late in the season, after tillering had been completed.

Aquatain™ remained highly effective in lines1 and 2 for at least 15 days (larval mortality rates ranged up to 95% and 83%, respectively). Environmental factors (rainfall, strong winds) as well as cultivation techniques may possibly influence the efficacy of Aquatain™ over extensive and verdant mosquito breeding sites. For this reason Bukhari *et al.* (2011) recommended a reapplication of 10 – 14 days in rice paddies in Kenya in order to effectively control mosquito larvae. However, in container mosquito habitats Aquatain™ provided effective control of mosquito larvae for at least 4 weeks at the labelled dose-rate (1ml/m^2) and thus the recommended reapplication frequency is every 4 weeks even though a longer larval control may be achieved with a single application (Webb and Russell, 2012).

In conclusion, our study showed that Aquatain™ is an effective control agent against mosquitoes not only in small or closed water ecosystems (Webb and Russell, 2012), but also in extensive water surfaces such as rice paddies. It causes death of the existing mosquito larvae and pupae of a breeding site (Webb and Russell, 2009) and it can offer sufficient protection from the development of new mosquito generations for at least 3 weeks.

Table 1. Mean larval mortality (±SE) and percentage mortality (%) of L_4 mosquito larvae of *Culex pipiens* in cages over a period of 25 days after application of the product Aquatain™ in a rice paddy in the Prefecture of Phthiotida, Greece.

	Experimental paddy			Control paddy*
Days after application of Aquatain™	line 1	line 2	line 3	lines 1,2,3 (pooled data)
3	20.0 ± 0.0 (100%) a§	5.67 ± 5.13 (28%) b	0.0 ± 0.0 (0%) b	1.0 ± 1.0 (1.67%) b
6	20.0 ± 0.0 (100%) a	17.67 ± 3.21 (88.3%) a	8.33 ± 2.08 (41.7%) b	1.33 ± 0.56 (2.22%) c
15	19.0 ± 1.0 (95%) a	16.67 ± 0.58 (83.3%) b	5.33 ± 2.08 (26.7%) c	0.33 ± 0.57 (0.56%) d
25	14.0 ± 1.0 (70%) a	5.0 ± 1.0 (25%) b	2.0 ± 1.0 (10%) c	1.33 ± 1.15 (2.21%) c

* Without application of Aquatain™
§ Different letters within rows indicate statistically significant differences.

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Literature cited

Bashir, A., Hassan A., Salmah C. and Rahman W. 2008. Efficacy of Agnique (MMF) monomolecular surface film against immature stages of *Anopheles arabiensis* Patton and*Culex* spp (Diptera: Culicidae) in Khartoum, Sudan. *Southeast Asian Journal of Tropical Medicine & Public Health*, 39: 222-228.

Batra, C.P., Mittal P.K., Adak T. and Subbarao S.K. 2006. Efficacy of Agnique MMF monomolecular surface film against *Anopheles stephensi* breeding in urban habitats in India. *Journal of the American Mosquito Control Association,* 22: 426-432.

Bukhari, T. and Knols B.G.J. 2009. Efficacy of Aquatain™, a monomolecular surface film, against the malaria vectors *Anopheles stephensi* and *An. gambiae* s.s. in the laboratory. *American Journal of Tropical Medicine and Hygiene*, 80: 758-763.

Bukhari, T., Takken W., Githeko A. and Koenraadt J.M.C. 2011. Efficacy of Aquatain, a monomolecular film, for the control of malaria vectors in rice paddies. *PloS ONE*. 6:e21713. doi:21710.21371/journl.pone0021713.

Das, P.K., Tyagi B.K., Somachari N. and Venkatesan V. 1986. Efficacy of arosurf- a monomolecular surface film, in controlling *Culex quinquefasciatus* Say, *Anopheles stephensi* Liston and*Aedes aegypti* (L). *Indian Journal of Medical Research,* 83: 271-276.

Food and Agriculture Organization of the United Nations. Statistics division. 2012. (http://faostat3.fao.org/download/G1/GR/E).

Lacey, L.A. and Lacey C.M. 1990. The medical importance of riceland mosquitoes and their control using alternatives to chemical insecticides. *Journal of the American Mosquito Control Association,* 6: 1-93.

Reiter, P. 1978. The action of lecithin monolayers on mosquitoes. II. Action on the respiratory structures. *Annals of Tropical Medicine and Parasitology*, 72: 169-176.

SPSS Inc. 2004. *SPSS 14 for Windows user's guide*. SPSS Inc.,Chicago, IL.

Voyadjoglou-Samanidou, A., Papadimitriou, E. and Marcelou O. 1989. Geographical distribution and breeding sites of common and anopheline mosquitoes in Phthiotis district, Greece. In-*Proceedingsof the 3rd Entomological Conference*, Thessaloniki, Greece 1989, p. 78-83.

Webb, E.C. and Russell R.C. 2009. A laboratory investigation of the mosquito control potential of the monomolecurlar film Aquatain mosquito formula against immature stages of *Aedes aegypti* and *Culex quinquefasciatus*. *Journal of the American Mosquito Control Association*, 25: 106-109.

Webb, E.C. and Russell R.C. 2012. Does the monomolecular film Aquatain® mosquito formula provide effective control of container-breeding mosquitoes in Australia? *Journal of the American Mosquito Control Association*, 28: 53-58.

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Μελέτη της αποτελεσματικότητας του σκευάσματος μονομοριακής μεμβράνης Aquatain™ εναντίον προνυμφών κουνουπιών *Culex pipiens* σε ορυζώνες

Η. Κιούλος και Γ. Κολιόπουλος

Περίληψη Η δράση του προϊόντος μονομοριακής μεμβράνης Aquatain™ εναντίον προνυμφών κουνουπιών εξετάστηκε σε ορυζώνα της κεντρικής Ελλάδας (Ανθήλη, Φθιώτιδα). Το Aquatain™ εφαρμόστηκε με απλή έγχυση μόνο από τη μία πλευρά ορυζώνα έκτασης 12 στρεμμάτων. Προνύμφες κουνουπιών 4ης ηλικίας (*Culex pipiens* από εργαστηριακή εκτροφή) τοποθετήθηκαν σε ειδικά κατασκευασμένους κλωβούς. Οι κλωβοί εγκαταστάθηκαν σε τρεις παράλληλες γραμμές που βρίσκονταν σε διαφορετικές αποστάσεις από την πλευρά εφαρμογής του Aquatain™. Η θνησιμότητα των προνυμφών καταγράφηκε 3, 6, 15 και 25 μέρες μετά την εφαρμογή. Στην πλησιέστερη γραμμή στην πλευρά εφαρμογής του Aquatain™ η θνησιμότητα κυμάνθηκε από 100% έως 70% καθ' όλη τη διάρκεια του πειράμα-

τος. Στη δεύτερη γραμμή στο μέσο του πειραματικού αγρού, η θνησιμότητα κυμάνθηκε από 88% έως 25% ενώ στην πιο απομακρυσμένη γραμμή η θνησιμότητα ήταν 42% έως 10%. Σύμφωνα με τα αποτελέσματα το Aquatain™ αποδεικνύεται ικανό να καταπολεμήσει τις υπάρχουσες προνύμφες των κουνουπιών και να εμποδίσει την ανάπτυξη νέων για διάστημα τουλάχιστον τριών εβδομάδων σε μεγάλα υδρόβια συστήματα όπως οι ορυζώνες.

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SHORT COMMUNICATION

Wild poinsettia (*Euphorbia heterophylla*): an emerging weed in cotton and processing tomato in Greece

D. Chachalis

Summary *Euphorbia heterophylla* (wild poinsettia) is reported as an emerging weed in cotton and processing tomato in Kopaida plain, region of Viotia, in central Greece. This is the first record of *E. heterophylla* in tomato crop in Greece. In a field experiment, mature plants grown under weed-free conditions produced on average 19 heads, 64 capsules, and 192 seeds per individual plant. Mature seeds exhibited no dormancy and the maximum germination (82 to 90%) occurred at temperatures from 25 to 35°C, with a drastic decline (<38%) at 15 and 40°C. Light had no significant effect on seed germination in the whole range of temperatures tested. Fully mature plants were taller than cotton, exerting strong competition. Having no light dependence for germination, seeds might have the potential to germinate and emerge from greater soil depths. This short communication summarizes information for the identification, seed germination and growth of this weed species that would support a proper weed management.

Additional keywords: germination, growth, invasive, noxious, weeds

Euphorbia heterophylla (wild poinsettia) is native to Central and South America (Wilson, 1981). Today it is widely distributed throughout the tropics, subtropics and the Mediterranean region. Moreover, it is widely spread as an important weed in at least 28 tropical countries and it is present in another 37 countries (Wilson, 1981). In countries where the species is present, research has been directed mainly towards suitable methods for its control in crops such as peanuts and soybeans (Moore *et al.* 1990; Willard and Griffin, 1993; Brecke and Tobola, 1996). First occurrence of *E. heterophylla* in Greece, was recorded in cotton crop in 2008 (Chachalis and Travlos, 2009). Since then, the species has become a major weed problem in the area Anthochori, Kopaida plain, Viotia, infesting mainly cotton and processing tomato fields. This is the first record of *E. heterophylla* in tomato crop in Greece. This study investigated the seed germination and growth of this species contributing with useful results for the effective management of the weed.

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Morphological Description

Euphorbia heterophylla is a monoecious annual plant, sparsely branched, up to 140 cm tall (Figure 1). Stem is often tinged red towards the apex; leaves are arranged spirally, crowded at stem apex, simple having stipules modified into purplish glands (Mosango, 2008). Inflorescence is a compact axillary or terminal cyme consisting of clusters of flowers (cyanthia), each with basal bracts similar to the leaves, but paler green, with involucres containing one female flower surrounded by many male flowers (Mosango, 2008). Fruit is a deeply 3-lobed capsule; seeds are ovoid, 2.5 mm in diameter, warty, blackish brown, and the embryo is located towards the apex of the seed (Figure 2).

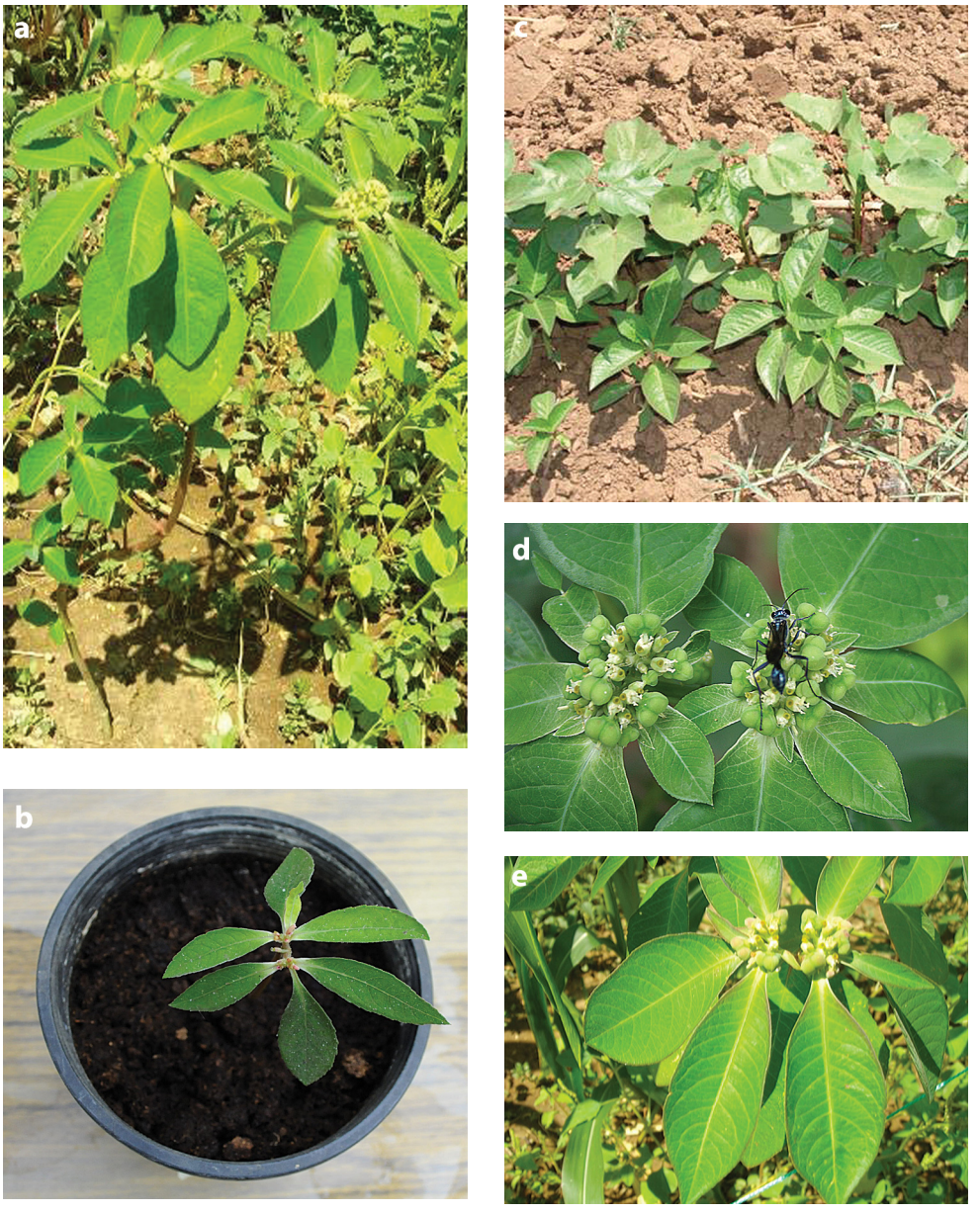

Figure 1. Plant and plant parts of *Euphorbia heterophylla* at different growth stages: a. mature plant; b. young plant; c. newly emerged plants in a cotton field; d. inflorescence; e. leaves

Seed germination and emergence

Seeds of *E. heterophylla* were collected in 2009 from cotton fields at Anthohori, Kopaida region, and stored at approximately 5°C. Mature plants grown under weed-free conditions in field experiments produced on average 19 heads, 64 capsules, and 192 seeds per individual plant. Seed germination was evaluated by evenly placing 25 seeds on a 9 cm-diameter Petri dish containing two layers of filter paper Whatman No. 1, moistened

with 4 ml of distilled water. Petri dishes were sealed with Parafilm. Seed germination was determined in growth chambers under constant temperatures of 10, 15, 20, 25, 30, 35, and 40°C with (95μmol m^{-2} s^{-1}) or without light. Germination percentage was recorded 1 week after incubation (visible radicle protrusion 1 week after incubation). Each mean germination test was replicated four times.

Maximum germination (82 to 90%) occurred at temperatures from 25 to 35°C with a drastic decline (<38%) at 15 and 40°C (Figure 3). These results are in line with previous studies (Bannon *et al*., 1978). Light had no significant effect on seed germination in the whole range of temperatures tested (Figure 3). However, the effect of light on seed germination of this species has been contradictory, with some reports recording no effect on seed germination (Brecke, 1995) as opposed to others showing significant positive effect (Suda and Giorgini, 2000). Apparently, the growth conditions of the mother plants and both the time and the conditions of seed storage prior to germination tests might explain to some extent such discrepancies. In our study, no light dependence for germination indicates that seeds could ger-

Figure 2. Fruit and seeds of *Euphorbia heterophylla*: a. Capsule; b. Seeds; c. Longtudinal section of the seed.

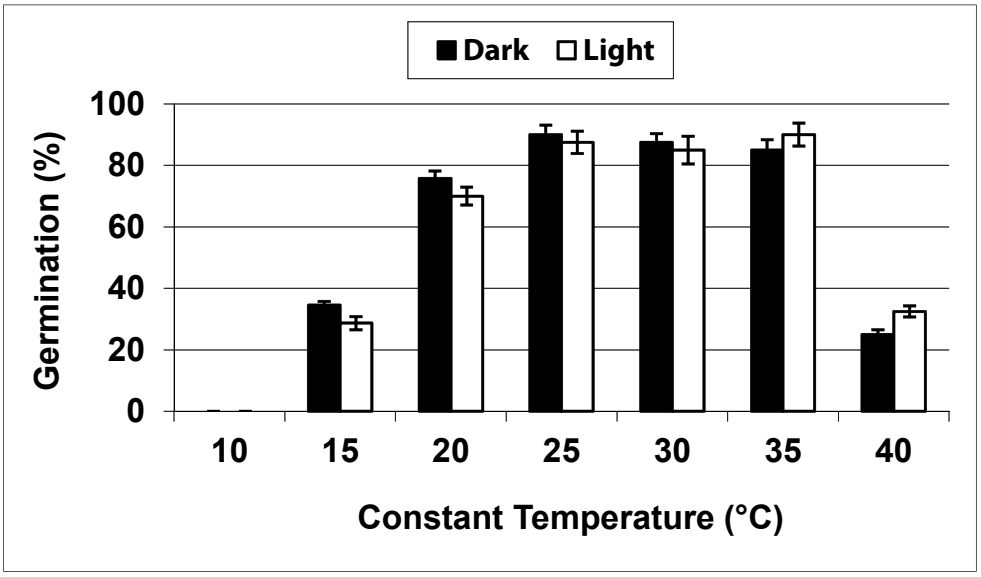

Figure 3. Effect of constant temperature on germination of *Euphorbia heterophylla* seeds incubated in the dark or under a 12-h photoperiod for 1 week. Error bars represent standard error of the means.

minate and emerge from deeper soil, given that light is poorly transmitted to the soil at a depth more than 4 mm (Benvenuti, 1995). The optimum temperatures for *E. heterophylla* seed germination seems to coincide with periods from late spring up to early autumn in the Kopaida region as well as in the whole country and therefore this species could become a significant weed problem for many spring crops in many lowland regions in the country, including major crops such as cotton, processing tomato and maize.

Growth measurements

Cotton (cv. Celia) was seeded on 26 April 2009 in Anthohori region; the seedbed was prepared using a cultivator and later disked for a proper seedbed. Basic pre-plant fertilization (400 kg ha^{-1} of NPK 11-15-15) and urea (N 46-0-0, 80 kg ha^{-1} at 5WAP) was applied, according to standard agronomic practices. Total irrigation was 6,360 mm ha^{-1} based on farmers' empirical estimation. Cotton seeds were sown (25 seeds per meter) and final cotton population was approximately 180,000 plants ha^{-1}. Emergence of *E. heterophylla* was approximately 2 weeks behind that of cotton, however soon afterwards there was a flush of newly emerged seedlings that formed a very dense weed community (Figure 1c). Initial growth rate of the weed was less than that of cotton until the emergence of squares (65 DAP) (Figure 4). At 1st bloom (70 DAP), cotton and weed plants had similar height (~58 cm), whereas after that stage the weed plants were always taller than cotton, reaching a maximum difference at the late ball stage (125 DAP), when weed plants were approximately 40% taller than cotton (Figure 4).

The above data indicate that fully grown weed plants could exert very strong competitive pressure on cotton. In addition, given the lower initial weed growth, it would be important to study competition in cotton cultivation systems with narrow or ultra-narrow rows. In Greece, there has been a renounced interest in such cropping systems mainly due to the significant positive effect on irrigation water and competition on weeds (Darawsheh *et al*., 2009).

Conclusion

In this study, the presence of *E. heterophylla* is recorded in tomato crop for the first time in Greece. In addition, *E. heterophylla* is reported as an emerging weed problem in cotton and processed tomato crops in Kopaida region, Southern Greece. A basic description of *E. heterophylla* along with several images of the species were provided for accurate identification in future recordings in new areas. *Euphorbia heterophylla* was shown to have a relatively short growth cy-

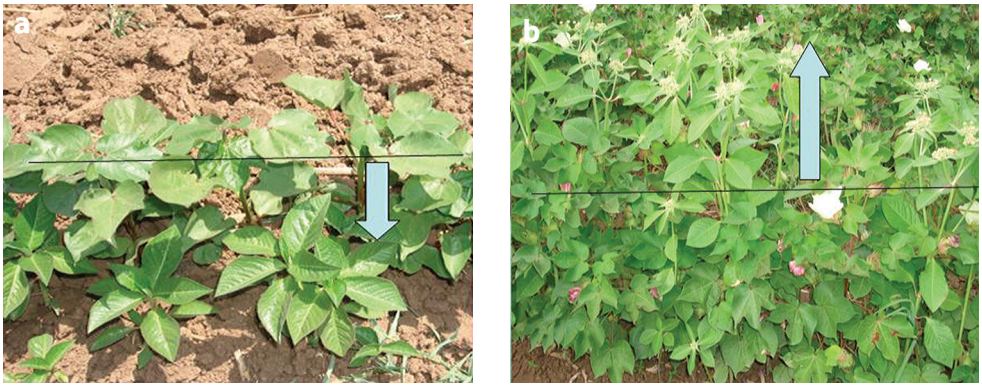

Figure 4. Images illustrating the relative growth of cotton *vs Euphorbia heterophylla* in field: a. At the stage of 4th cotton node; b. At the stage of first balls. Arrows indicate the relative canopy height difference between cotton and weed plants.

cle. Seeds were capable of germination within a wide range of temperatures, without light requirement. Fully mature plants were taller than cotton, exerting strong competition. Special attention should be given to this species to prevent its dispersal to new areas, given its ability to infest many spring crops in the country.

Mrs Sofia Liberopoulou is acknowledged for providing technical support for the species identification.

Literature cited

Bannon, J.S., Baker J.B., and Rogers, R.L. 1978. Germination of wild poinsettia (*Euphorbia heterophylla*). *Weed Science,* 26: 221-225.

Benvenuti, S. 1995. Soil light penetration and dormancy of jimsonweed (*Datura stramonium*) seeds. *Weed Science,* 43: 389-393.

Brecke, B.J. 1995. Wild poinsettia (*Euphorbia heterophylla*) germination and emergence. *Weed Science,* 43: 103-106.

Brecke, B.J. and Tobola, P. 1996. Growth and development of wild poinsettia (*Euphorbia heterophylla*) selections in peanut (*Arachis hypogaea*). *Weed Science,* 44: 575-578.

Chachalis, D. and Travlos, I.S. 2009. Studies on *Euphorbia* spp. and *Sida* spp. as new emerging weed problems in cotton in Greece. In Rubin, B. and Economou, G. (Eds) Proceedings of the 2nd International Conference "Novel and sustainable weed management in arid and semi-arid systems", 7-10 September 2009, Santorini, Greece, p. 45.

Darawsheh, M.K., Chachalis, D., Aivalakis, G. and Khah, E. M. 2009. Cotton row spacing and plant density cropping systems II. Effects of seed cotton yield, ball components, and lint quality. *Journal of Food, Agriculture and Environment,* 7: 262-265.

Moore, J.D., Banks, P.A. and Pinnel-Alison, C.L. 1990. Wild poinsettia (*Euphorbia heterophylla*) control in peanut (*Arachis hypogaea*). *Weed Science,* 38: 536-540.

Mosango, D.M., 2008. *Euphorbia heterophylla* L. In: Schmelzer, G.H. & Gurib-Fakim, A. (Editors). Prota 11(1): Medicinal plants/Plantes médicinales 1. [CD-Rom]. PROTA, Wageningen, Netherlands.

Suda, C.N.K. and Giorgini, J.F. 2000. Seed reserve composition and mobilization during germination and initial seedling development of *Euphorbia heterophylla*. *Revista Brasileira de Fisiologia Vegetal,* 12: 226-245.

Willard, T.S. and Griffin, J.L. 1993. Soybean (*Glycine max*) yield and quality responses associated with wild poinsettia (*Euphorbia heterophylla*) control programs. *Weed Technology,* 7: 118-122.

Wilson, A.K. 1981 *Euphorbia heterophylla:* a review of distribution, importance and control. *Tropical Pest Management,* 27: 32-38.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Η άγρια ποϊνσέτια (*Euphorbia heterophylla*): ένα νέο ζιζάνιο στο βαμβάκι και στη βιομηχανική τομάτα στην Ελλάδα

Δ. Χάχαλης

Περίληψη Η άγρια ποϊνσέτια *(Euphorbia heterophylla*) παρουσιάζεται ως νέο ζιζάνιο στο βαμβάκι και τη βιομηχανική τομάτα στην περιοχή της Κωπαΐδας Βοιωτίας. Αυτή είναι η πρώτη αναφορά του ζιζανίου στην καλλιέργεια της τομάτας στην Ελλάδα. Σε πείραμα αγρού ώριμα φυτά του ζιζανίου, που αναπτύχθηκαν σε συνθήκες έλλειψης ανταγωνισμού, παρήγαγαν κατά μέσο όρο 19 κεφαλές με 64 κάψουλες και 192 σπόρους ανά φυτό. Οι ώριμοι σπόροι δεν εμφάνισαν λήθαργο και η μέγιστη βλαστικότητα (από 82 έως 90%) μετρήθηκε σε θερμοκρασίες από 25-35°C, με μια δραστική πτώση της βλαστικότητας (<38%) σε θερμοκρασίες 15 και 40°C. Το φως δεν είχε σημαντική επίδραση στη βλάστηση των σπόρων σε όλο το θερμοκρασιακό εύρος (από 15 έως 40°C), με αποτέλεσμα οι σπόροι πιθανώς να έχουν τη δυνατότητα να βλαστήσουν και σε μεγαλύτερα βάθη από την επιφάνεια του εδάφους. Τα ώριμα φυτά

του ζιζανίου ήταν υψηλότερα από το βαμβάκι και έτσι ασκούν ισχυρό ανταγωνισμό σε αυτά. Αυτή η σύντομη ανακοίνωση συγκεντρώνει τις σχετικές πληροφορίες για την επιτυχή αναγνώριση του είδους, όπως επίσης και δεδομένα για τη βλάστηση και ανάπτυξή του, στοιχεία απαραίτητα για τη διαχείριση του ζιζανίου.

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