

Development and reproduction of spider mites *Tetranychus turkestanii* (Acari: Tetranychidae) under water deficit condition in soybeans

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SUMMARY

One of the major pests of soybeans in Bulgaria is the spider mite *Tetranychus turkestanii* Ug et Nik (Acari: Tetranychidae) and different results have been reported about the impact of water stress on its development and reproduction. Soybean plants exposed to natural infestation by spider mites, water deficit and treatment with imidacloprid were examined under greenhouse conditions at the Institute of Forage Crops, Pleven, Bulgaria, over the period 2011-2012. The development of mites and their eggs was faster when plants were exposed to water deficit, which created favorable conditions for high density and reproduction of *T. turkestanii*. There was a vertical distribution of protonymphs, deutonymphs and adults as their numbers and egg counts were significantly higher under conditions of water deficit on the upper and middle plant parts, compared with their bottom parts, and imidacloprid treatment had a stronger and more prolonged activity against mites, compared to its influence on well-watered plants. Spider mites on water-stressed plants caused a 24.8% reduction in the contents of plastid pigments, and carotenoids, and 21.5% decrease on well-watered plants.

Keywords: Spider mites; Imidacloprid; Soybeans; Water; Reproduction

INTRODUCTION

Climate change and global warming affect entomofauna in different countries. Particularly important is their impact on economically important pests. Spider mites (Acari: Tetranychidae) are serious pests of soybean crops in Bulgaria, Serbia, Romania, Italy, France, Australia, the USA (California) and other

countries. There have been different hypotheses about the effects of water stress on mite development and reproduction. According to some authors, such stress leads to an increase in plant damage and mite numbers, as well as their nutritional activity (Chandler et al., 1979; DeAngelis et al., 1982; Hollingsworth & Berry, 1982; DeAngelis et al., 1983; Youngman & Barnes, 1986). However, Mellors et al. (1984) and Oloumi-Sadeghi

et al. (1988) found water stress to reduce the density of mites and especially to affect the density of females and eggs. The results of tests carried out by Ferree and Hall (1980) showed that low soil moisture did not affect the intensity of mite reproduction. Similar results were later reported by Gillman et al. (1999) and Sadras et al. (1998), according to whom water-stressed plants could not affect the density of spider mites. Visual symptoms of damage were more pronounced on well-watered plants, where females preferred to feed and lay eggs (Sadras et al., 1998). In comparison with unstressed host plants, the abundance of spider mites on water-stressed hosts may increase, decrease or remain unchanged (English-Loeb, 1989).

The impact of imidacloprid on the vitality and reproduction of spider mites is questionable. The use of imidacloprid has been found to increase (James & Price, 2002) or significantly reduce the density of females and their eggs (Ako et al., 2006).

The aim of this study was to determine the influence of water deficit and treatment of soybean plants with imidacloprid on spider mite development and reproduction.

MATERIAL AND METHODS

The study was conducted on the soybean variety "Richie" in a greenhouse of the Institute of Forage Crops, Pleven, Bulgaria, over the period 2011-2012. „Wagner" containers were used with 4 plants grown in each. Population density and the number of eggs of *Tetranychus turkestanii* Ug et Nik (Acari: Tetranychidae) were monitored under the following factors: factor A - irrigation mode (water deficit and irrigation), and factor B - imidacloprid (with and without imidacloprid treatment). All plants received equal amounts of water to maintain optimum soil moisture until the end of stage R5/early stage R6 (Fehr & Caviness, 1977), when some plants were exposed to a 10-day water deficit, i.e. the uptake of only 1/2 irrigation rate. After the 10-day water dearth, the initial irrigation regime was recovered and the neonicotinoid insecticide Confidor 70 WG – 150 g/ha (700 g/kg imidacloprid) was applied to some of the experimental variants. Each variant had 11 replications. Leaf area damage was visually observed and assessed as a percentage of leaf area visibly injured by mites (discoloration) using the following scale: 0 – no damage, 1 – 1-10%, 2 – 11-20%, 3 – 21-30%, 4 – 31-40%, 5 – 41-50%, 6 – 51-60%, 7 – 61-70%, 8 – 71-80%, 9 – 81-90%, and 10 – 91-100% damaged leaf

area (Sclar et al., 1998; Shrewsbury & Hardin, 2003). Leaf damage was scored on 20 leaves per variant during the water-stress period and after it.

The content of leaf plastid pigments was determined using the method of Zelenskiy and Mogilev (1980) at the end of stage R5/early stage R6. Biochemical changes resulting from the damage caused by spider mites to soybean leaves, i.e. the content of plastid pigments, were measured and compared to control plants uninfested by spider mites and grown under corresponding conditions of the studied factors: A - irrigation mode and B - imidacloprid.

The statistical processing of experimental data was conducted using the Statgraphics Plus for Windows Ver. 2.1. software program and one-way ANOVA for statistical analysis.

Variants:

+WD +M +I treatment: water deficit + mites + imidacloprid treatment

+WD +M -I treatment: water deficit + mites - without imidacloprid treatment

-WD +M +I treatment: well-watered plants + mites + imidacloprid treatment

-WD +M -I treatment: well-watered plants + mites - without imidacloprid treatment

RESULTS

The mode of irrigation had a significant influence on the development and dynamic of spider mite population density (Table 1).

Cultivation of plants under conditions of water deficit was associated with a significantly higher number of mites. This trend was observed as early as on the first day after plant exposure to water deficit, when their density (13.8 mobile forms/leaf) was 34.0% higher on the average than the density under irrigation (10.3 mobile forms/leaf), and the differences were statistically significant. Differences in the density of mites were found between the two modes of irrigation, and a statistically significant increase from 55.3 to 130.1% occurred in the following days of drought. It was noteworthy that the value of this indicator increased twice, on the 7th and 10th day under drought condition, reaching 97.1 mobile forms/leaf, compared with the well-irrigated plants (42.2 mobile forms). The intensity of increase in the number of mites on plants exposed to water deficit was significantly more pronounced throughout the period, and it reached a twofold increase

Table 1. The number of spider mites and degree of leaf damage after 10 days of water deficit or irrigation of soybean plants, average (2011-2012)

Duration of water deficit/irrigation	+WD		-WD		LSD _{0.05%}
	Number of mobile forms/leaf	Leaf area damage, %/rank	Number of mobile forms/leaf	Leaf area damage, %/rank	
1 st day	13.8 a	16.7 / 2	10.3 b	12.5 / 2	1.309
3 rd day	23.6 a	28.5 / 3	15.2 b	21.4 / 3	5.104
5 th day	36.8 a	35.4 / 4	23.1 b	27.0 / 3	6.142
7 th day	74.3 a	46.3 / 5	35.6 b	35.5 / 4	11.913
10 th day	97.1 a	52.4 / 6	42.2 b	42.2 / 5	10.163
Average	49.1		25.3		

+WD – water deficit; -WD – no water deficit (irrigation)

Means in a row marked by the same letter are not significantly different ($\alpha = 0.05$, LSD)

Table 2. Fecundity of spider mites on soybean plants exposed to 10-day water deficit, average (2011-2012)

Duration of water deficit/irrigation	Number of eggs/leaf		LSD _{0.05%}
	+WD	-WD	
1 st day	14.2 a	12.2 b	1.579
3 rd day	26.5 a	16.3 b	8.256
5 th day	58.85 a	31.15 b	5.817
7 th day	81.55 a	46.65 b	7.222
10 th day	106.9 a	57.1 b	9.416
Average	57.6	32.7	

+WD – water deficit; -WD – no water deficit (irrigation)

Means in a row marked by the same letter are not significantly different ($\alpha = 0.05$, LSD)

(on 7th day), while such an increase was not detected under irrigation. Mite fecundity increased in proportion from 14.2 to 106.9 under drought conditions, and from 12.2 to 57.1 mobile forms/leaf under irrigation. The number of hatched eggs was 16.4-87.2% higher under water stress (Table 2). The development of eggs, protonymphs, deutonymphs and adults proceeded for a short time on the stressed plants. After 10 days of water stress mite numbers and fecundity accelerated by 94.4 and 76.3% on the average.

The result of mite feeding was the yellowing and drying of damaged sections. The proportion of leaf area damaged by mites was greater in water-stressed plants. Visual symptoms of damage were more pronounced on water-stressed plants than on well-irrigated plants. The ranking of this indicator showed that water-stressed plants had a considerably higher rank (Table 1). It was due to the higher density and fecundity of spider mites, as well as their active nutritional activity under drought conditions.

The increasing *T. turkestanii* numbers in the variant + WD + M continued after irrigation of plants was recovered. The effects of water deficit on mite development were also recorded after its discontinuation. The tendency of higher numbers of mites and their fecundity on water-stressed plants than on well-irrigated plants persisted and was much more pronounced (Table 3 and 4). On the first and third day after discontinuation of water stress, mite numbers varied from 100.0 to 177.0 mobile forms/leaf, with an average density increase of 99.8-102.0% in the water deficit variants, regardless of treatment with imidacloprid. Five days after restoring irrigation, the density of mites on water-stressed plants (average 287.8) was significantly higher statistically than it was in variants with well-irrigated plants (average 89.3), i.e. three times higher (222.4%). Factor A - water deficit had a significant impact on the development of mites one week after stress surcease and resulted in an average of 118.2% increase in their numbers. Similar impact was not found after that period.

Table 3. Number of spider mites and degree of leaf damage on soybean plants after discontinuation of water deficit, average (2011-2012)

Day after discontinuation of water deficit	+WD +I		+WD -I		-WD +I		-WD -I		LSD _{0.05%}
	Mobile forms/leaf	Leaf area damage, %/rank							
1 st	100.3 c	53.1 / 6	165.4 d	62.5 / 7	55.2 a	44.9 / 5	77.8 b	47.6 / 5	15.367
3 rd	114.0 c	58.1 / 6	177.0 d	67.3 / 7	62.8 a	49.2 / 5	81.3 b	53.0 / 6	12.318
5 th	227.9 b	76.0 / 8	347.6 c	78.5 / 8	81.2 a	56.5 / 6	97.3 a	58.5 / 6	53.113
7 th	313.8 b	83.3 / 9	384.3 c	87.6 / 9	238.9 a	65.3 / 7	230.8 a	63.8 / 7	68.329
12 th	244.7 a	87.5 / 9	220.3 a	88.1 / 9	305.5 a	76.3 / 8	295.6 a	78.1 / 8	85.404
14 th	150.8 a	90.3 / 10	143.4 a	90.0 / 10	102.4 a	82.9 / 9	92.8 a	80.0 / 9	59.523
20 th	67.4 a	94.4 / 10	78.1 b	91.6 / 10	49.2 a	82.7 / 9	47.6 a	83.2 / 9	21.823
Average	174.1		216.6		110.7		131.9		

+WD +I – water deficit and treatment with imidacloprid; +WD -I – water deficit and no treatment with imidacloprid; -WD +I – no water deficit (irrigation) and treatment with imidacloprid; -WD -I – no water deficit (irrigation) and no treatment with imidacloprid. Means in a column followed by the same letter are not significantly different ($\alpha=0.05$, LSD)

Table 4. Fecundity of spider mites after discontinuation of water deficit in soybean plants, average (2011-2012)

Day after discontinuation of water deficit	Number of eggs/leaf				LSD _{0.05%}
	+WD +I	+WD -I	-WD +I	-WD -I	
1 st	104.7 b	155.5 b	53.3 a	72.3 a	51.092
3 rd	116.5 c	184.2 d	69.8 a	103.1 b	12.621
5 th	255.6 b	319.8 b	117.0 a	143.9 a	65.934
7 th	793.1 b	759.1 b	702.4 a	664.3 a	54.067
12 th	300.8 a	349.0 a	392.8 a	348.0 a	96.94
14 th	178.2 a	179.9 a	132.1 a	148.8 a	71.969
20 th	25.6 a	28.6 a	36.8 ab	55.7 b	24.686
Average	253.5	282.3	214.9	219.4	

+WD +I – water deficit and treatment with imidacloprid; +WD -I – water deficit and no treatment with imidacloprid; -WD +I – no water deficit (irrigation) and treatment with imidacloprid; -WD -I – no water deficit (irrigation) and no treatment with imidacloprid. Means in a row followed by the same letter are not significantly different ($\alpha=0.05$, LSD)

With the advent of seed maturing stage (R7) the number of mites was significantly reduced, regardless of the mode of irrigation. It should be noted that the action of the water deficit factor resulted in a shortening of the reproductive period and earlier seed maturing which was associated with an earlier decrease in mite density. Reduced values of that indicator on water-stressed plants were observed significantly earlier - seven days after discontinuation of stress, and 12 days on well-

irrigated plants. Earlier reaching of the stage R7, its shorter duration, as well as earlier coarsening and loss of leaves, and reduced possibility of foliar penetration worsened the nutritional conditions for mite development on water-stressed plants and resulted in an early decrease in pest density. At seed maturation, the water deficit factor did not influence mite development and their numbers under both modes of irrigation were similar without significant differences.

Table 5. Vertical distribution of population density and eggs of spider mites on soybean leaves, average (2011-2012)

Periods	Vertical distribution of leaves	+WD		-WD	
		Number of mobile forms/leaf	Number of eggs/leaf	Number of mobile forms/leaf	Number of eggs/leaf
10-day water deficit	upper and middle	76 b	79 b	35 b	44 a
	bottom	48 a	56 a	30 a	38 a
LSD _{0,05%}		21.513	17.510	4.303	6.085
20 days after discontinuation of WD	upper and middle	235 b	256 b	138 b	169 b
	bottom	166 a	172 a	125 a	148 a
LSD _{0,05%}		50.177	38.484	6.085	13.606

+WD – water deficit; -WD – no water deficit (irrigation)

Means in a column marked by the same letter are not significantly different ($\alpha = 0.05$, LSD)

A similar dependence was observed regarding egg quantity (Table 4). At first, a linearity was observed in the increase of egg numbers up to the 7th day, as mite reproduction was greater on water-stressed plants. The amount of hatched eggs varied from 104.7 to 793.1 mobile forms/leaf under water-stress conditions and from 53.3 to 702.4 on well-watered plants. The average increase in eggs was 78.8% over the period, and it was followed by a decrease in the proportion of eggs without significant differences between the two modes of irrigation.

Over the 20-day period after restoring irrigation, the mites on water-stressed plants averaged 195.4 mobile forms/leaf, or 50.4% more than mites on well-irrigated plants.

The degree of leaf damage was significantly higher on water-stressed plants and ranged from 53.1 (rank 6) to 94.4% (rank 10), reaching rank 9 as early as 7 days after discontinuation of stress. On well-irrigated plants, leaf area damage was from 44.9 (rank 5) to 83.2% (rank 9), reaching rank 9 no sooner than the 14th day (Table 3). It should be emphasized that leaf area with clearly marked symptoms of damage by mites was significantly greater in water-stressed plants (Tables 1 and 3).

Treatment of plants with imidacloprid affected the spider mite population density at both levels of factor A (mode of irrigation), but to varying degrees. Imidacloprid had acaricidal activity against spider mites from the 1st to 7th day after treatment on water-stressed plants, and significantly reduced mite numbers by 64.9% (from 165.4 to 100.3 mobile forms) and 22.5% (from 384.3 to 313.8), respectively. The toxic action of imidacloprid had a shorter duration on well-irrigated plants, it was observed from the first up to fifth day after treatment, reducing mite numbers by 41.1% (from 77.8 to 55.2) and 19.9% (from 97.3 to 81.2), compared to untreated

plants. Differences on the fifth day were insignificant compared to plants not treated with imidacloprid.

The insecticide exhibited markedly strong and lasting effect against mites on plants with preimposed water deficit by reducing their density at an average of 48.8% one week after its application, while an average 30.2% reduction was recorded after five-day action under conditions of irrigation. No impact of imidacloprid on egg productivity was observed (Table 4).

A vertical distribution of density and fecundity of spider mites on soybean leaves was detected in the study (Table 5). Mite numbers were significantly higher on the upper and middle part of the plants under the conditions of water deficit (average 155 mobile forms in the WD period and after discontinuation) and irrigation (average 87 mobile forms over the whole period). The average increase, in comparison with the bottom leaf level, was from 31.5% for mites to 32.4% for eggs, regardless of the water deficit factor. It should be noted that vertical distribution of mites was considerably more pronounced on plants pre-exposed to water deficit. Over the 10 day period of water stress, the number of mites was 58.3% higher on the average on the upper and middle leaves (76 mobile forms, compared to 48 on bottom leaves), while mite density was only 18.6% higher under irrigation (35 mobile forms on upper and middle leaves, compared to 30 on bottom leaves). Similar results were observed after discontinuation of water deficit, when their density was higher from 42.0 (after ten-day stress) to 10.8% (without ten-day stress), compared to the lower leaves. Water stress depressed plant growth and the number of mites and eggs on newly formed leaves, compared to those on bottom leaves, were considerably higher, i.e. by 45.3 and 46.5%, respectively, while the corresponding values on well-irrigated plants were only 12.3 and 14.5%.

Table 6. Content of plastid pigments in soybean leaves, mg/L (2011-2012)

Variant	Chlorophyll a	Chlorophyll b	Carotenoids	Total	Chlorophyll a/ chlorophyll b	Chlorophyll a+b/ carotenoids
+WD +M +I	6.413	2.862	1.801	11.076	2.241	5.150
+WD -M +I	8.303	3.389	2.313	14.005	2.450	5.055
+WD +M -I	6.338	2.520	1.452	10.310	2.515	6.101
+WD -M -I	7.805	3.080	2.001	12.886	2.534	5.440
-WD +M +I	6.707	2.607	1.537	10.851	2.573	6.060
-WD -M +I	9.284	2.849	2.131	14.264	3.259	5.694
-WD +M -I	5.679	3.210	1.876	10.765	1.769	4.738
-WD -M -I	7.405	3.972	2.209	13.586	1.864	5.150

The results of an analysis of plastid pigments in leaves of soybean (Table 6) showed that spider mites reduced the total pigment content based on chlorophyll a, b and carotenoids. The presence of mites, water deficit and treatment with imidacloprid reduced pigment content by 22.1%, compared with the variant without mites but with water deficit and imidacloprid treatment. Under water deficit and presence of mites but without insecticide treatment, plastid pigments were reduced to a greater extent by 27.4%, compared to plants grown under the same conditions but uninfested by mites. A similar trend was observed in the variants without water deficit (with irrigation) and the presence of mites, regardless of imidacloprid treatment. The reduction of total pigment content ranged from 15.1 to 27.9%, compared to the variants devoid of the impact of spider mites. It should be noted that mites generally caused a reduction in leaf pigment of 24.8% on water stress plants, and 21.5% on well-irrigated plants. The chlorophyll a/chlorophyll b ratio, as well as the ratio of green pigments (chlorophyll a + chlorophyll b) and carotenoids, which are indicators of the physiological state of green plants (Petkova & Poryazov, 2007), varied from 1.769 to 3.259 and from 5.055 to 6.101 respectively.

DISCUSSION

The sensitivity of plants to impact by mites under drought conditions has been discussed by many authors (Mattson & Haack, 1987; Oi et al., 1989; Louda & Collingey, 1992; Huberty & Denno, 2004). According to them, water deficit causes physiological changes in plants, including a reduced synthesis of secondary metabolites or protective compounds, which has a negative effect on feeding mites, increased content of soluble nitrogen (amino acids, amides) and free sugars, which improves

the overall nutritional potential of plant tissues, and raised temperature of leaf surface, which stimulates the development of mites and contributes to a shorter duration of their individual stages, i.e. eggs, larvae, protonymphs, deutonymphs and adults. Other authors (House, 1974; Haack & Slansky, 1987) have also observed that water-stressed plants may be a more suitable background for the development and fecundity of insects as many plant nutrients are more concentrated or better balanced in them, compared to well-irrigated plants. Increasing the content of those nutrients and improving their balance should logically encourage pest reproduction. The degree of imposed water stress was also of substantial importance as mite development was strongly suppressed under heavy drought (Mattson & Haack, 1987).

In comparison with unstressed host plants, the abundance of spider mites on water-stressed hosts can increase, decrease or remain unchanged (English-Loeb, 1989). Actual mite responses seem to depend on the intensity of water deficit experienced by the host plant (English-Loeb, 1989, 1990). In our experiment, soybean water status affected the abundance of adult mites. Further analysis of the effects of water-stressed soybean on mites requires details on the life history, population dynamics and behaviour of mites (Smitley & Kennedy, 1985).

In a number of studies covering a wide water stress intensity range it has been shown that: 1. mite fecundity is affected by plant water status, and 2. mites develop faster on water-stressed hosts due to their increased foliar temperature (Youngman et al., 1988; English-Loeb, 1989; Oi et al., 1989).

Leaf damage per mite was slighter on well-watered than on water-stressed plants and, importantly, the area of severely damaged leaf was far smaller on well-watered plants. The low ratio of intense damage to overall leaf damage on the well-watered plants contrasts with that observed on

water-stressed plants, where mites tend to feed preferentially in protected sites on the leaf surface, spreading only as the damaged area becomes unsuitable (Wilson, 1994). Other changes induced by water deficit that may influence leaf suitability for mites include: first - more, and qualitatively different, epicuticular waxes (Bondada et al., 1996) that are known to influence herbivory (Eigenbrode & Espelie, 1995); and second - changes in concentration of nutrients and of secondary metabolites in plant tissues, often invoked to explain differences in herbivore responses to host plant water status (Jones & Coleman, 1991).

The distribution of density and fecundity of spider mites on soybean leaves was found in this study to be vertical as their numbers were higher on the upper and middle leaves of both water-stressed and well-irrigated plants. Vertical migration was associated with a more intensive movement of plant sap in the upper and middle leaves, compared to the aging leaves below. Related information has been reported by Atanasov (1970, 1991), Rajković (1982), Carter & Rypstra (1995). Gillman et al. (1999) found that plants subjected to water stress had suppressed their growth, and the number of mites and eggs on newly formed leaves was significantly higher.

CONCLUSIONS

The development of mite eggs, protonymphs, deutonymphs and adults required less time on plants water-stressed for 10 days as it created favorable conditions for mites and accelerated an increase in their numbers as well as eggs by the respective 94.4 and 76.3%.

The water deficit factor had a significant impact on the development of spider mites one week after discontinuation of water stress. During the stress period the number of mites and their eggs were found to increase by 118.2 and 78.8%, respectively. At the stage of seed maturation (R7), the influence of water deficit on mite development was suspended.

Damaged leaf area was significantly larger on water-stressed plants than on well-watered plants and reached as far as rank 10 on the damage scale.

A vertical distribution of spider mites was detected. The number of mobile forms and eggs on newly-grown leaves in the top and middle sections, compared to the bottom section, were significantly higher under water deficit conditions, by 45.3 and 46.5%, while only 12.3 and 14.5% under irrigation.

Treatment with imidacloprid had a markedly stronger and more extended activity against mites on plants with pre-imposed water deficit, reducing their density by an average of 48.8% a week after application. Imidacloprid

reduced mite numbers on well-irrigated plants by an average 30.2% over five days. No influence on egg productivity was detected.

Spider mites on water-stressed plants caused a reduction in the content of plastid pigments based on chlorophyll **a**, **b** and carotenoids by 24.8%, while the reduction was 21.5% on well-watered plants.

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Razvoj i reprodukcija grinja paučinara *Tetranychus turkestanii* (Acari: Tetranychidae) na biljkama soje u uslovima vodnog deficita

REZIME

Grinje paučinari *Tetranychus turkestanii* Ug et Nik (Acari: Tetranychidae) predstavljaju jednu od najznačajnijih štetočina u usevima soje u Bugarskoj, a rezultati istraživanja uticaja vodnog stresa na njihov razvoj i reprodukciju su do sada bili različiti. Ispitivane su biljke soje izložene prirodnoj infestaciji grinjama paučinarima, vodni deficit i tretmani imidaklopridom u uslovima staklenika u Institutu za krmno bilje u Plevenu, Bugarska, u periodu 2011-2012. Razvoj grinja i njihovih jaja bio je brži kada su biljke izlagane vodnom deficitu, što je stvorilo povoljne uslove za visoku gustinu populacije i reprodukciju *T. turkestanii*. Rasprostranjenost grinja je bila vertikalna u uslovima vodnog deficita, jer je broj protonimfi, deutonimfi i adulta, kao i jaja bio značajno viši na gornjim i srednjim delovima biljaka sa novoformiranim listovima, u poređenju sa donjim delovima, dok je tretman imidaklopridom imao jače i produženo delovanje na grinje, u poređenju sa uticajem na dobro navodnjenim biljkama. Grinje su prouzrokovale smanjenje od 24.8% u sadržaju hlorofila *a* i *b*, kao i karotenoida u biljkama izloženim vodnom stresu, a 21.5% u dobro navodnjenim biljkama.

Ključne reči: Grinje paučinari; imidakloprid; soja; voda; razmnožavanje