

(*Leucojum aestivum* L.)

1*, 2, 3, 1, 1, 8,
1164, 2, 1, 8,
1164, a, 7, 1080, 281,
5600, *E-mail: lilka81@abv.bg

Influence of genotype on the development of the disease process in Bulgarian populations of summer snowflake (*Leucojum aestivum* L.)

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SUMMARY

Summer snowflake is a valuable, endangered species, from which the Bulgarian drug Nivalin[®] is produced. The artificial infections were carried out in sterile conditions on *in vitro* bulbs from six natural populations of Bulgarian summer snowflake with 4 bacterial isolates from bulbs with symptoms of decay. The symptoms were traced on the 3rd, 6th and 9th day after infection. The percentage of infected bulbs from each isolate was calculated. The results showed that the expression of virulence of the pathogen is dependent on the genotype of the plants,

L.)
 Amarillydace.
 (Sener *et al*, 1998;
 Szlávik *et al.*, 2004).
 ®,
 (Stefanov, 1990).
 (Astadjov, 1980).
 (Georgieva *et al*,
 2007; Berkov *et al.*, 2013).
Leucojum aestivum L.
 (Russeva, 1984; Mirkova and
 Karadzkhova, 1993).

even at population levels.

Key words: summer snowflake,
 bacterial decay, artificial infection, plant-
 pathogen interactions

INTRODUCTION

Summer snowflake (*Leucojum aestivum* L.) is a medical bulbous plant which belongs to the Amarillydaceae family. Its extracts have proven antibacterial, antiviral, and antifungal effects (Sener *et al.*, 1998; Szlávik *et al.*, 2004). The high content of galantamine makes *L. aestivum* a valuable resource for the world-known Bulgarian medicine Nivalin® (galantamine hydrobromide), which is used for the treatment of severe neurodegenerative diseases like Alzheimer's disease. The natural habitats of summer snowflake in Bulgaria are valuable because of their high galantamine content compared to populations in other Balkan countries (Stefanov, 1990). The Bulgarian summer snowflake is spread in low, wet forests, wetlands, periodically swamped meadows and riverside terraces along the rivers Tundzha, Maritsa, Diyavolska, Fakiyska, Kamchia and the the Danube (Astadjov, 1980). The Danube populations are most rich in the alkaloid homolycorine, those around Kamchia - in lycorine, while those in southern Bulgaria synthesize mostly galantamine in varying amounts (Georgieva *et al.*, 2007; Berkov *et al.*, 2013).

The available natural reserve of *Leucojum aestivum* L. in Bulgaria determines long-term multidisciplinary studies related to the presevation, maintenance and effective use of this unique plant species.

There are almost no reports of pathogens in *Leucojum sp.*, except for a few ones of fungal diseases in plantations more than 10 years ago (Ruseva, 1984; Mirkova and Karadzkhova, 1993). There is no information are there more pathogens

and how they affect the galantamine contents in plants. However, different bacterial phytopathogens have been described on bulbous plants from other families, many of which are new species or species with new unique properties of antagonists and bioremediation agents (Bradbury, 1986; Schaad, 2001).

(Bradbury, 1986; Schaad, 2001).
 et al., 2012), (Stoyanova et al., 2012), symptoms of bacterial decay in plants from the region of Tutrakan (near the Danube River, Silistra region) it was found. A total of 10 bacterial isolates were separated from their bulbs. Their pathogenicity was confirmed by artificial inoculation on scales of the summer snowflake and other hosts (onion, hyacinth, tulip, and narcissus). Some of the isolates were identified by sequence analysis of the 16S rRNA gene. Sequencing results showed matches for *Serratia plymuthica* 97-98% (three isolates), *Rahnella aquatilis* 95% (one isolate), *Bacillus subtilis* 96% (1 isolate), *Stenotrophomonas maltophilia* 98% (1 isolate).

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The purpose of this study was to determine the pathogenicity of the new isolates on bulbs of *Leucojum aestivum* from different Bulgarian populations. Because of protected status of summer snowflake (Gussev et al., 2007) the artificial inoculations were performed on bulbils propagated *in vitro* which reduces the risk that the resulting symptoms were due to a secondary infection.

(Gussev et al., 2007)
in vitro,
 Georgieva et al. (2010).
 (Georgieva et al., 2007).

MATERIAL AND METHODS

Plant material: *In vitro* bulbils (first leaf phase), cut in halves were used for artificial inoculation. They were propagated and maintained according to Georgieva et al., (2010). Six clones were selected, originating from the territories of Prasad (Burgas region), Svilengrad (Haskovo reg.), Vinica (Plovdiv reg.), Kochovo (Shumen reg.), Palauzovo and Yambol (Yambol region) (Georgieva et al., 2007).

LB (Bertani, 1951) (King's medium B) (King, 1954)
Stoyanova *et al.* (2012).

4
16S :
1 - *Serratia plymuthica* (Genbank No. JF896806),
3 - *Serratia plymuthica* (JF896808),
5 - *Rahnella aquatilis* (JN400360),
6 - *Bacillus subtilis* (JN400359).

12

S
(Murashige and Skoog, 1962).
10-20 µl

in vitro

9 (Georgieva *et al.* 2010).

3 (3, 6 9-
).

Microsoft Excel 2013.

K

Bacterial strains: Bacterial isolates were maintained on both media - LB (Bertani, 1951) and KB (King's medium B) (King, 1954) and were described in details by Stoyanova *et al.* (2012).

Four bacterial isolates were selected and identified by sequence analysis of the 16S RNA gene: Isolate 1 - *Serratia plymuthica* (Genbank No. JF896806), Isolate 3 - *Serratia plymuthica* (JF896808), Isolate 5 - *Rahnella aquatilis* (JN400360), Isolate 6 - *Bacillus subtilis* (JN400359).

The applied method of inoculation has been suited and adapted to the purpose of the study. We have not used separate scales but bulbils that were cut longitudinally in halves in order for us to be able to monitor the infectivity of bacteria on any part of the bulb at any point of inoculation. At the same time explants possess relative integrity (with all parts of the bulbs (internal, external scales, basal steam, leaf).

For each variant 12 halves of the bulbs (longitudinal cut) were used. The halves were placed in Petri dishes with sterile filter paper soaked in MS macroelements (Murashige and Skoog, 1962). Bacterial suspension was added to each half at an amount 10-20 µl. Sterile water instead of bacterial suspension was added to the control bulbs. For every inoculated clone *in vitro* bulb controls of the same clone were used.

Cultivation of the infected explants: The variants of the artificial infection (plant-bacterium) were maintained in a cultivation room 9 days (Georgieva *et al.*, 2010). The development of the infectious process was recorded every 3 days (on the 3rd, 6th and 9th day after inoculation).

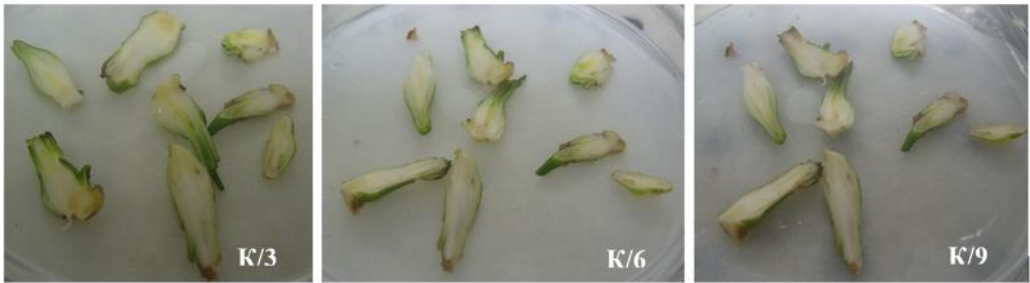
Statistical analyses: The results were analyzed with the commercial spreadsheet application Microsoft Excel 2013.

RESULTS AND DISCUSSION

The control bulbs kept their appearance for the entire period of the study. Because of the very similar

(1).

appearance of controls, only the one of Vinica origin is shown as an example (Figure 1).



. 1. - *in vitro*

3- , 6- 9-

Fig. 1. Controls - *in vitro* bulbs originating from Vinica on days 3rd, 6th and 9th (from left to right)

plymuthica

1 - *Serratia*

Inoculation with Isolate 1 - *Serratia plymuthica*

1 *in vitro*

The inoculation with the bacterial Isolate 1 of *in vitro* bulbs, obtained from different populations caused creamy color and a watery consistency of the affected bulb tissue. Their color gradually changes to dark brown and they subsequently wither. The inner layers were affected primarily. The first symptoms were appeared after the 3rd day of infection, but not with all explants. By the 9th day of inoculation symptoms were observed on bulbs from four origins - Prasad, Vinica, Svilengrad and Kochovo, whereas the bulbs from Palauzovo and Yambol had no symptoms (Figure 2). This isolate affected approximately 1/5 of all explants.

(2).
1/5



. 2.

1 9-

Fig. 2. Symptoms of bulbs inoculated with Isolate 1 on the 9th day after the infestation by Prasad, Vinica, Kochovo (from left to right)



6- 9-
Fig. 3. Symptoms of bulbs inoculated with Isolate 3 on days 3rd, 6th and 9th

Legend: — Prasad, — Svilengrad, — Vinica, — Palauzovo, — Yambol

plymuthica 3 - *Serratia*

3
 9-
 3 (*Serratia* 75%)
 (50 %),
 100%
 (,)
 - 25% (3, 6).

Inoculation with Isolate 3 - *Serratia plymuthica*

Inoculation with isolate 3 was caused strongly expressed symptoms on plant tissues that appeared on the second day after inoculation. Initially, the pathogen affects the pith of the bulb, gradually the infection affects the peripheral scales, and by the 9th day, almost all the explants died. The color of the affected tissues was dark-gray. They emit a strong, unpleasant smell. Isolate 3 (*Serratia plymuthica*) affects on average more than 75% of inoculated bulbs. However, there were differences in symptomatology in different genotypes. The weakest is the reaction of bulbs from Yambol and Kochovo, where the tissues look dark and dry (about 50%), Explants from Prasad and Svilengrad were affected 100% and they show wet decay. The remaining three genotypes (Vinica, Palauzovo and Prasad) survived less than 25% (Figures 3, 6).

aquatilis 5- *Rahnella*

5-6-
 (4, 6). 14 %

Inoculation with Isolate 5- *Rahnella aquatilis*

5-6 days after inoculation with Isolate 5, symptoms was occurred. It affected mainly the inner and some peripheral scales, and the spots were dark and dry. Such a reaction was observed in bulbs from Prasad, Svilengrad, Vinica and Kochovo, but in those from Palauzovo and Yambol no symptoms were observed (Figures 4, 6). An average of 14% of the explants from each clone were affected.



4. (5 9-)
Fig. 4. Symptoms of bulbs inoculated with Isolate 5 on the 9th day after infection from Svilengrad, Vinica and Palauzovo (from left to right)

subtilis

6 - *Bacillus*

Inoculation with Isolate 6 - *Bacillus subtilis*

Isolate 6 also affects partially infected bulbs. Many explants were unaffected, and in others damage was mainly peripheral and with very dark color. Isolate 6 was one of the least pathogenic isolates included in our study. In explants from Prasad, Palauzovo and Yambol no symptoms were observed after the artificial inoculation. A very weak reaction (less than 10%) occurring on the 9th day of inoculation (darkening of the inner part) was recorded in the bulbs originating in Vinica, Kochovo and Svilengrad (Figures 5, 6).

6

6

(10%)

9-

(5, 6).



. 5.

6 9-

Fig. 5. Symptoms of bulbs inoculated with Isolate 6 on the 9th day after the infection from Svilengrad, Vinica and Kochovo (from left to right)

3

(. 6).

6).

9-

80% (7).

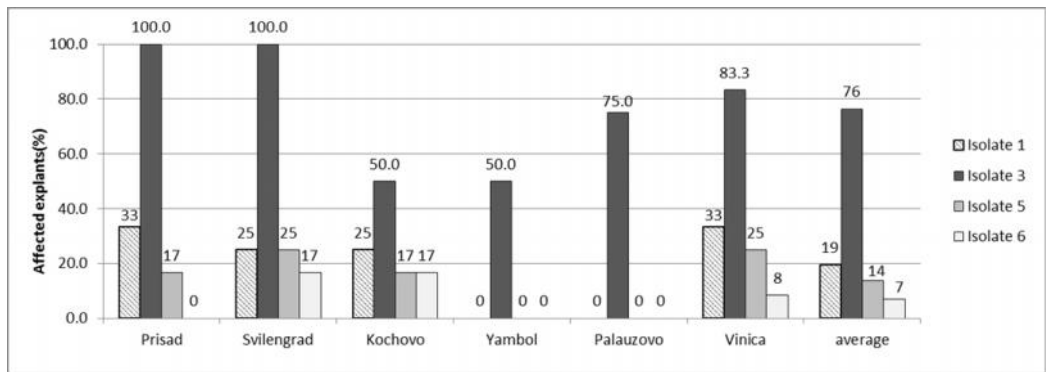
6,

5% (

6, 7).

When comparing the number of infected explants, Isolate 3 stands out significantly against the background of the other isolates tested (Figure 6). This isolate affects all artificially infected explants from Prasad and Svilengrad, most of the explants from Palauzovo and Vinica and half of Kochovo and Yambol. At the same time this was the only isolate that was struggled the bulbs from Yambol and Palauzovo (Figure 6). The total percentage of affected tissues on the 9th day is over 80% (Figure 7).

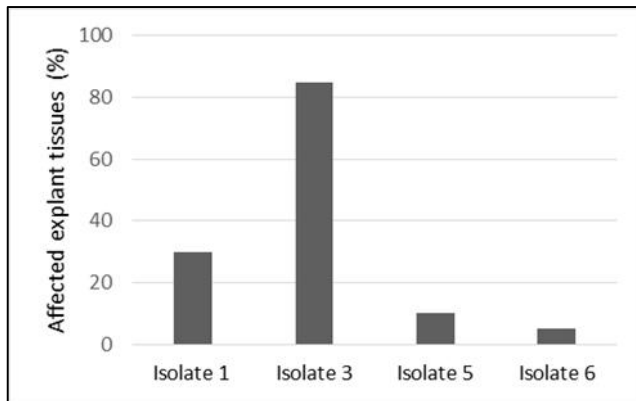
The least pathogenic is Isolate 6, which infects a relatively small percentage of explants from three origins and the percentage of affected tissues is only 5% on average (Figures 6, 7).



. 6.

(%)

Fig. 6. Summer snowflake explants symptoms of bacteriosis (in% of the total number of inoculated explants from each clone)



. 7.

(%)

Fig. 7. Affected explant tissues (% of total inoculated explants)

1 5 -
 (6), -
 (30%
 10%) (7).
 : 3 >>>
 1 > 5 > 6, -
 1 , 6 - - .
 1 3
 (*Serratia plymutica*), -

Isolates 1 and 5 relate to a relatively close number of bulbs, except those of Prasad (Figure 6), but the degree of invasion of individual explants was different (30% and 10%, respectively) (Figure 7).

About their virulence and aggression, the isolates were arranged in the order: Isolate 3 >>> Isolate 1 > Isolate 5 > Isolate 6, In which Isolate 1 exhibits these properties to the highest degree, and Isolate 6 at the lowest.

According to sequencing analysis, Isolate 1 and Isolate 3 were of the same species (*Serratia plymutica*), but they had different virulence and host attack rate.

in vitro

4

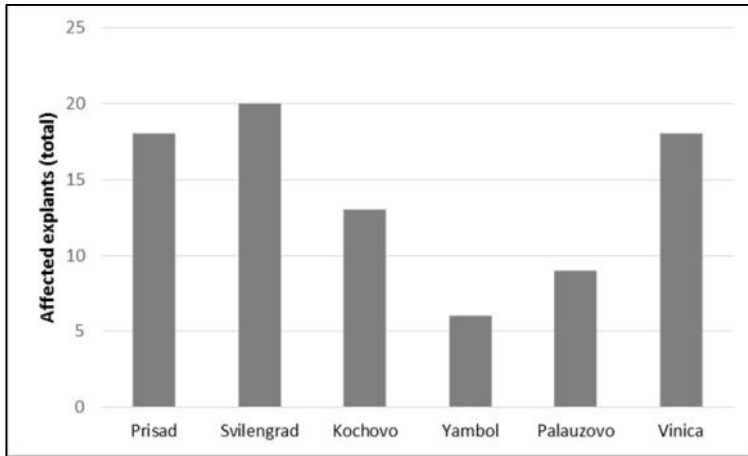
6).

3

8),

6).

The artificial inoculation of *in vitro* bulbs of different origins gives information on the stability of each genotype to the pathogens tested. The genotypes from Yambol and Palauzovo proved to be resistant to 3 of the 4 strains of pathogens and the genotype of Yambol was characterized by the lowest percentage of affected bulbs from the most virulent bacterial strain (Figure 6). The largest number of explants with symptoms of bacteriosis were observed in the clones originating from Svilengrad, Prasad and Vinnitsa (Figure 8), although Prasad origin explants did not infect the least virulent strain (Figure 6).



8. Fig. 8. Affected explants from summer snowflake (total for each genotype)

50%.

17%.

Explants of Kochovo were a special case compared to other genotypes. Like the Yambol clone, the number of infected bulbs with the most virulent strain was the lowest - 50%. At the same time, this genotype was susceptible to all bacterial strains tested and the percentage infected with the least virulent bulb strain was highest - 17%.

CONCLUSIONS

in vitro

This study of the *in vitro* response of *in vitro* bulbs of summer snowflake of different origins to four bacterial isolates gives us reason to assert that the virulence of the pathogen is dependent on the

genotype of the plants. According to the results obtained, the clones originating in Svilengrad and Vinica were the most sensitive to the tested bacterial isolates, while the Yambol branch stands out as the most sustainable. Prison Branch was also among the sensitive genotypes, but three of the four tested bacterial strains.

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HF-333

, 4004 , .” ”, 12,
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Effect of some soil herbicides on growth of the vegetative pear rootstock HF-333

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SUMMARY

2014-2015 .
- .
33 , 4 ,
50 ,
OHF-333
3
180 -
180-
(cm).

Studies were carried out in the period 2014-2015 at the Fruit-Growing Institute – Plovdiv. The effect of the herbicides Devrinol 4F, Stomp 33 EC, Goal 4 F, Metofen, Pledge 50 WP and Bonalin on the growth habits of *in vitro* propagated and rooted plants of the vegetative pear rootstock OHF-333 was studied under the conditions of a pot experiment. The herbicide rate was recalculated according to the surface area of the cultivation container. The height of the source plants in the separate variants was measured before treatment. The experiment was set by standard methods in 3 replications. After treatment with herbicides, the plants were grown in a glass-house for 180 days. Visual observations for incidence of external symptoms of phytotoxicity were made. The biometric characteristic stem height increment (cm) was reported on 180th day.
The results showed that treatment with the soil herbicides, included in the study, did not cause the incidence of external symptoms of phytotoxicity in *in vitro* propagated and rooted plants of

OHF-333.

- OHF-333. The plants of the variants treated with napropamide, pendimethalin, oxyfluorfen, flumioxazine and benfluralin showed a larger stem height increment compared to the untreated control. This gives reason to assume that these active substances in the administered doses do not exert a suppressive effect on plant growth. The largest growth was recorded in plants treated with pendimethalin. The lowest values on growth were reported after treatment with Metofen, which shows the inhibitory effect of the herbicide on plant growth.

: OHF-333,

Key words: OHF–333, herbicides, phytotoxicity, stem height increment.

INTRODUCTION

- Control of weed vegetation is a major agrotechnical problem in fruit tree nurseries, determining to a great degree the production of good quality planting material. Nowadays weed control in fruit nurseries is realized by applying mainly herbicides of the group of the selective herbicides having an effect against Johnson grass species, which exert an efficient control on annual and perennial grass species, such as loose silky-bent, blackgrass, annual meadow grass, Johnson grass, Bermuda grass, coach grass, etc. Selective soil herbicides, which have different effects on plant development, should be applied for the control of broad-leaf weed species. Fruit species rootstocks show different responses to herbicide treatment, expressed in incidence of visual symptoms of phytotoxicity caused by the herbicides, as well as possible growth suppression. In literature there are data about different effects of herbicides on growth habits of the fruit species, used as rootstocks (Rankova, 2007; Hanson and Schneider, 2008; Rankova, 2011; Rankova et al., 2012; Thakur et al., 2012; Rankova and Tityanov, 2013; Rankova and Zhivondov, 2013; Abit and Hanson, 2013; Rankova and Ivanova, 2016).

(Rankova, 2007; Hanson and Schneider, 2008; Rankova, 2011; Rankova et al., 2012; Thakur et al., 2012; Rankova and Tityanov, 2013; Rankova and Zhivondov, 2013; Abit and Hanson, 2013; Rankova and Ivanova, 2016).

(Old home x Farmingdale) OHF 333

(*Erwinia amylovora*),
Phytophthora Cactorum.

in vitro. (Kornova and Popov, 2014).

OHF 333
(Urbina et al., 2003).

HF-333.

2014-2015

(4), (33
) , (4),
+ (50)
()

OHF-333

333

1. ()
2. - 4 - 400 ml/da
3. - 33 - 400 ml/da
4. - 4 - 250 ml/da

There is a demand for the pear rootstock OHF 333 ('Old Home' x 'Farmingdale') in the production of pear planting material because of the many advantages. It is resistant to Fire blight (*Erwinia amylovora*), nematodes and *Phytophthora Cactorum*. It is suitable to be grown in dry and calcareous soils; it shows an excellent compatibility with the pear cultivars for grafting. The best way for mass production of that rootstock is offered by *in vitro* propagation (Kornova and Popov, 2014).

The information about the effect of herbicide applications on the vegetative pear rootstock OHF 333 is limited in literature (Urbina et al., 2003).

The aim of the present study was to follow out the effect of some soil and leaf herbicides on the growth habits of *in vitro* propagated and rooted plants of the vegetative rootstock HF-333 under the conditions of a pot experiment.

MATERIAL AND METHODS

Studies were carried out in the period 2014-2015 at the Fruit-Growing Institute – Plovdiv. The effect of the herbicides napropamide (Devrinol 4F), pendimethalin (Stomp 33 EC), oxyfluorfen (Goal 4 F), metolachlor + oxyfluorfen (Metofen), flumioxazine (Pledge 50 WP) and benfluralin (Bonalin) on the growth habits of *in vitro* propagated and rooted plants of the vegetative pear rootstock OHF-333 was studied under the conditions of a pot experiment. Micropropagated and rooted plants of the vegetative pear rootstock HF-333 were planted in a peat-and-perlite mixture in pots of 2 L volume. Treatment with herbicides was applied immediately after planting.

The following variants were set:

1. Control (untreated)
2. Napropamide – Devrinol 4 F – 400 ml/da
3. Pendimethalin – Stomp 33 EC – 400 ml/da

- 5. +
- 160 ml/da
- 6. - 50 - 8
- g/da
- 7. - 300 ml/da

- 4. Oxyfluorfen – Goal 4 F - 250 ml/da
- 5. Oxyfluorfen + metolachlor – Metofen – 160 ml/da
- 6. Flumioxazine – Pledge 50 WP – 8 g/da
- 7. Benfluralin – Bonalin – 300 ml/da

180-
e
(cm).

The herbicide rate was recalculated according to the surface area of the cultivation container. The height of the source plants in the separate variants was measured before treatment. The trial was set by standard methods, in three replications. After treatment the plants were grown in a glass-house for 180 days. During that period visual observations for eventual incidence of external symptoms of phytotoxicity, caused by the herbicides, had been made. The biometric characteristic stem height increment (cm) was reported on 180th day. The results obtained were processed by standard dispersion analysis methods.

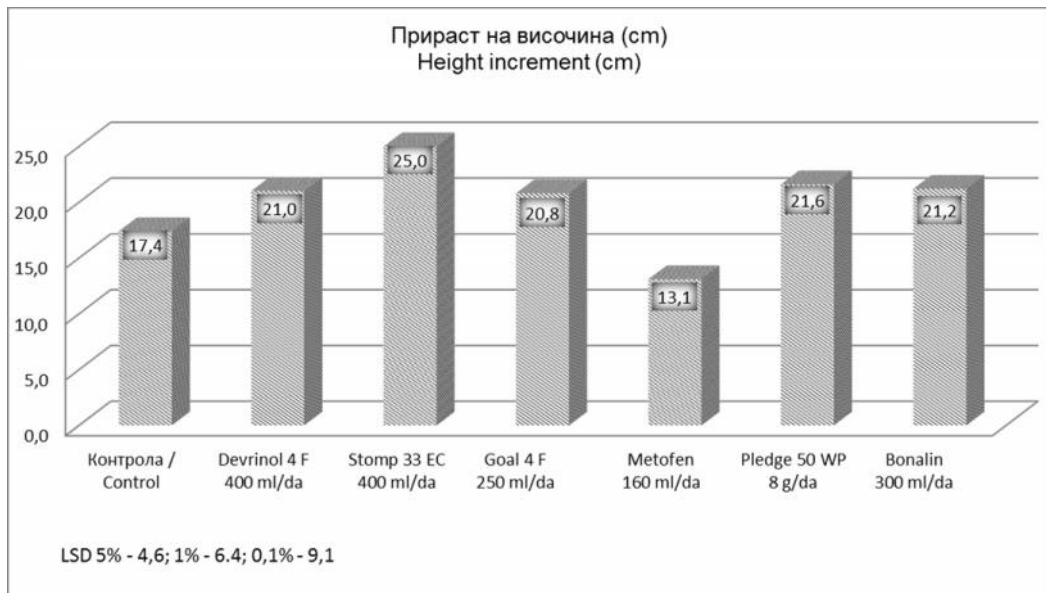
RESULTS AND DISCUSSION

In all the variants treated with herbicides, external symptoms of phytotoxicity (chlorosis, necrosis, plant growth suppression) were not observed. The plants treated with herbicides did not differ in the external characteristics from the plants of the untreated control. The vegetative tips of all the plants were fresh and actively growing. That gave the grounds to assume that the soil herbicides included in the present study and applied at the rates mentioned, do not cause external symptoms of phytotoxicity in micropropagated and rooted plants of OHF-333.

OHF-333.

The results of the biometric analysis are presented in Figure 1.

1.



.1

OHF-333

Fig. 1. Effect of soil herbicides on plant height of *in vitro* propagated and rooted plants of OHF-333

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 ,
 -
 .
 ,
 -
 (. 3).
 -
 (. 5),
 (2).

The plants of the variants treated with napropamide, pendimethalin, oxyfluorfen, flumioxazine and benfluralin showed a larger stem height increment compared to the untreated control. That gave the grounds to assume that those active substances applied at the rates mentioned, do not have a detrimental effect on plant growth. The largest height increment was reported in the plants treated with pendimethalin (Var. 3). The lowest values of that characteristic were reported after treatment with Metofen (Var. 5), which shows an inhibitory effect of the herbicide on plant growth.



. 2.

OHF-333

(5)

Fig. 2. Plants of pear rootstock OHF-333 with suppressed growth after treatment with Metofen (Variant 5)

CONCLUSIONS

Treatment with the soil herbicides included in the study did not cause any external symptoms of toxicity to *in vitro* propagated and rooted plants by OHF-333.

The plants of the variants treated with pendimethalin, showed the largest stem height increment. The lowest values on growth were reported after treatment with Metofen, which shows the inhibitory effect of the herbicide on plant growth.

OHF-333.

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(*Cacopsylla pyri* L.) (Hemiptera: Psyllidae)

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Improved strategy for control of pear psylla (*Cacopsylla pyri* L.) (Hemiptera: Psyllidae)

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SUMMARY

(*Cacopsylla pyri* L.)
(Hemiptera: Psyllidae)
2015 2016.
(Envidor® 240 SC)
(Movento® 100 SC),
e
(Akarzin),
10
(Vertimec®)
(Akarzin).
(Vertimec®)

Pear psylla (*Cacopsylla pyri* L.) is one of the key insect pests in pear orchards of Bulgaria. Field trials to evaluate the efficacy of two new active ingredients spirodiclofen and spirotetramat against *Cacopsylla pyri* L. (Hemiptera: Psyllidae) were carried out in a production pear orchard in the surroundings of Plovdiv (Bulgaria) in 2015 and 2016. The aim of the study was to assess and compare the efficacy of spirodiclofen (Envidor®) and spirotetramat (Movento®), when applied once or twice in combination with mineral oil (Akarzin) or in strategy with other products, which involves treatment with one of two products in combination with mineral oil, followed by treatment after 10 days with abamectin (Vertimec®) in combination with mineral oil (Akarzin).

The efficacy of the two products was compared with that of abamectin, which is considered the best standard now.

Spirodiclofen and spirotetramat, when implemented in a strategy with abamectin (Vertimec®) in combination with mineral oil, show better efficacy than the same

(Vertimec®)

C. pyri

: *Cacopsylla pyri*,

(*Cacopsylla pyri* L.)

“*Candidatus Phytoplasma pyri*”,
“ (Pear Decline) (Seemüller and Schneider, 2004). ”

(, , .)

(*Anthocoris nemoralis*).

(*Anthocoris*

C. pyri,

active substances used once in combination with mineral oil or twice-applied of abamectin (Vertimec®) in combination with mineral oil. The importance of these results for improve chemical control of pear psyllid in Bulgaria is discussed.

Key words: *Cacopsylla pyri*, pear, spiroticlofen, spirotetramat, efficacy, Bulgaria

INTRODUCTION

Pear psylla (*Cacopsylla pyri* L.) is one of the most important pests of pear trees in Bulgaria, which can cause direct damage to the production of pears and indirect ones such as the vector of ‘*Candidatus Phytoplasma pyri*’, causal agent of the disease ‘Pear Decline’, (Seemüller and Schneider, 2004).

It is considered a "key pest" in pear trees, but its populations can often be regulated by natural a factor, which does not always require chemical treatments. In fact, however, the improvement of agro-technical conditions (fertilization, irrigation, pruning, etc.) and especially the side effect of specific insecticides directed against other pests and their non-selectivity favor the development of pear psylla and often require the application of measures to limit its populations and damage.

In recent years new environmental friendly chemical products have been introduced to control of the codling moth, tortricids and leaf miners, with a pronounced protective effect on the populations of the main predator of pear psylla, *Anthocoris nemoralis* Fabr.

The use of selective chemicals for control against *C. pyri* allows better preservation of natural predators of psyllids and maintenance of ‘key pest’ populations below the threshold of economic harm.

At present, control of this pest focuses on the use of insecticides with specific activity such as abamectin, some neonicotinoid insecticides and acaricides with insecticidal activity to which *C. pyri* has not yet developed resistance.

Recently, two new active substances, spirotetramat (Movento®) and spirotetramat (Movento®), a new class of products – ketoenols derived from spirocyclic tetrone acid produced by Bayer CropScience, appeared on the market. These products are characterized by a new mode of action, which is expressed in blocking lipid synthesis in the body of a large number of insect species, including *C. pyri*, allowing them to successfully controlling the resistant populations of this and other similar pests (De Maeyer et al., 2002, Mar i et al., 2007, Pasqualini and Civolani, 2010; Pasqualini et al., 2012; Civolani et al., 2015).

The aim of this study is to investigate and compare the efficacy of Spirodiclofen (Envidor®) and Spirotetramat (Movento®) when applied in combination with mineral oil (Acarzine) or in a strategy with other products involving treatment with either Tested insecticide in combination with mineral oil followed by 10 days of treatment with abamectin in combination with mineral oil or treatment with the same insecticide combinations but in reverse order.

MATERIAL AND METHODS

Field tests to evaluate the efficacy of spirotetramat (Movento®) and spirotetramat (Movento®) in combination with mineral oil applied singly or a strategy involving their alternation with other products for control of *C. pyri* were carried out in a commercial pear tree garden located in the village of Tsarimir near Plovdiv in 2015 and 2016. The products used in the tests and their basic characteristics are presented in Table 1.

Table 1. Products used in tests

Active substance	Product	Commercial formulation	Dose . f. ml/hl
Spirodiclofen	Envidor®	240 g/L SC	60
Spirotetramat	Movento®	1000 g/L SC	150
Abamectin	Vertimec®	18 g/L EC	150
Mineral oil + mulsifier	Akarzin	85% + 15% EC	250

SC) (Envidor® 240 (Movento® 100 SC) 60 150 ml/hl, (Akarzin – 250 ml/hl) (1) (Akarzin – 250 ml/hl), (2) 10 (Vertimec® 18 EC – 150 ml/hl) (Akarzin – 250 ml/hl), (Vertimec® – 150 ml/hl) + (Akarzin – 250 ml/hl), (T 2). (1) (2) (10 3 (” “ ” “ 1- 28- (L) (N) 7- 14- , 21- 28- (T+7, 14, 21 28).

Spirodiclofen (Envidor® 240 SC) and spirotetramat (Movento® 100 SC) were applied once at a dose of 60 or 150 ml/hl in combination with mineral oil (Akarzin 250 ml/hl) or in a strategy with other products including initial treatment (T1) with one of the two tested insecticides (at the same doses) in combination with mineral oil (Akarzin – 250 ml/hl) followed after 10 days by treatment (T2) with abamectin (Vertimec® 18 EC – 150 ml/in combination with mineral oil (Akarzin – 250 ml / hl), or treatments with the same insecticide combinations applied at the same doses but in the reverse order.

The efficacy of both products in combination with mineral oil was compared to that of abamectin (Vertimec® – 150 ml/hl) + mineral oil (Akarzin – 250 ml/hl) considered to be the best standard at present (Table 2). The first treatment (T1) was conducted in the presence of predominantly "yellow eggs" or first hatched larvae and the second treatment (T2) – 10 days later (for the experimental plots where such treatment was provided). The experiments are set on a block random scheme in 3 replicates for each treatment, on 6-year pear trees with spindle-shaped form, from the 'Beurré Bosc' cultivar. Efficacy estimates were made on marked shoots without sampling, from 1 to 28 days after treatment.

The results are presented as mean number of larvae (L) and nymph (N) on shoot on the 7th, 14th, 21st and 28th days after treatment (T + 7, 14, 21 and 28). The efficacy of the tested chemicals

Abbott (1925).
 (ANOVA),
 Tukey HDS (p 0,05).

- was evaluated according to the formula of
 - Abbott (1925). All values were analyzed
 (ANOVA), and mean differences were
 compared with the Tukey HDS test at
 (p 0.05).

2.

Table 2. Experimental conditions

Treatment	Insecticide combinations	Active substance	/Dose ml / hl	Time of application	Date treatment	
					2015	2016
1.	Envidor 240 SC + karzin	spirodiclofen + mineral oil	60+250	1 " " 1 - "yellow egg" and the first hatched larvae	30.04	25.04
2.	Envidor 240 SC + karzin	spirodiclofen + mineral oil	60+250	1	30.04	25.04
	Vertimec 18 EC + karzin	bamectin + mineral oil	150+250	2 – 10 (1) T2 – treatment 10 days after first application (T1)	10.05	5.05
3.	Vertimec 18 EC + karzin	bamectin + mineral oil	150+250	1	30.04	25.04
	Envidor 240 SC + karzin	spirodiclofen + mineral oil	60+250	2	10.05	5.05
4.	Movento 100 SC + karzin	spirotetramat + mineral oil	150+250	1	30.04	25.04
5.	Movento 100 SC + karzin	spirotetramat + mineral oil	150+250	1	30.04	25.04
	Vertimec 18 EC + karzin	bamectin + mineral oil	150+250	2	10.05	5.05
6.	Vertimec 18 EC + karzin	bamectin + mineral oil	150+250	1	30.04	25.04
	Movento 100 SC + karzin	spirotetramat + mineral oil	150+250	2	10.05	5.05
7.	Vertimec 18 EC + karzin	bamectin + mineral oil	150+250	1	30.04	25.04
8.	Vertimec 18 EC + karzin	bamectin + mineral oil	150+250	1	30.04	25.04
	Vertimec 18 EC + karzin	bamectin + mineral oil	150+250	2	10.05	5.05
9.	Untreated Kontrola					

RESULTS AND DISCUSSION

The results, expressed as average number of larvae/nymphs (L/N) per shoot, presented in Table 3-4 for each year separately. The reduction of the total number of larvae/ nymphs expressed as % is shown on Figure 1 and 2.

, / (L/N)
 3-4
 .
 / -
 %
 1 2.

3. () *C. pyri* 7- , 14- , 21- 28-

Table 3. Average number of *C. pyri* larvae (nymphs) of at the 7th, 14th, 21st and 28th days after the first treatment, Plovdiv 2015

	Products	Dose ml/hl	Date f treatment	Average number of <i>C. pyri</i> larvae (nymphs) per shoot			
				T1+ 7	T1+ 14	T1+ 21	T1+ 28
				MF*	MF	MF	MF
1.	Envidor + karzin	60+250	30.04	3,1 b	2,5 b	1,6 b	0,20 b
2.	Envidor + karzin	60+250	30.04	3,0 b	2,0 b	1,4 b	0,05 b
	Vertimec + karzin	150+250	10.05				
3.	Vertimec+ karzin	150+250	30.04	3,2 b	1,5 b	0,2 b	0,00 b
	Envidor + karzin	60+250	10.05				
4.	Movento+ karzin	150+250	30.04	2,7 b	1,1 b	0,9 b	0,25 b
5.	Movento+ karzin	150+250	30.04	2,7 b	1,2 b	0,4 b	0,00 b
	Vertimec+ karzin	150+250	10.05				
6.	Vertimec+ karzin	150+250	30.04	3,3 b	0,8 b	0,2 b	0,00 b
	Movento+ karzin	150+250	10.05				
7.	Vertimec+ karzin	150+250	30.04	3,2 b	1,0 b	2,4 b	2,06 b
8.	Vertimec+ karzin	150+250	30.04	3,3 b	0,6 b	1,1 b	0,85 b
	Vertimec+ karzin	150+250	10.05				
9.	Untreated Kontrola	-		31,2 a	73,8 a	91,2 a	52,8 a

*MF – (/); mobile forms (larvae / nymphs per shoot)

4. () *C. pyri* 7- , 14- , 21- 28-

Table 4. Average number of *C. pyri* larvae (nymphs) of at the 7th, 14th, 21st and 28th days after the first treatment, Plovdiv 2016

	Products	Dose ml/hl	Date f treatment	Average number of <i>C. pyri</i> larvae (nymphs) per shoot			
				T1+ 7	T1+ 14	T1+ 21	T1+ 28
				MF*	MF	MF	MF
1.	Envidor + karzin	60+250	25.04	1,5 b	1,2 b	0,7 b	0,15 b
2.	Envidor + karzin	60+250	25.04	1,4 b	1,0 b	0,5 b	0,05 b
	Vertimec+ karzin	150+250	5.05				
3.	Vertimec+ karzin	150+250	25.04	1,6 b	1,1 b	0,3 b	0,04 b
	Envidor + karzin	60+250	5.05				
4.	Movento + karzin	150+250	25.04	1,2 b	0,6 b	0,4 b	0,08 b
5.	Movento+ karzin	150+250	25.04	1,3 b	0,5 b	0,1 b	0,00 b
	Vertimec+ karzin	150+250	5.05				
6.	Vertimec+ karzin	150+250	25.04	1,6 b	0,5 b	0,2 b	0,00 b
	Movento+ karzin	150+250	5.05				
7.	Vertimec + karzin	150+250	25.04	1,6 b	0,4 b	0,8 b	1,27 b
8.	Vertimec+ karzin	150+250	25.04	1,4 b	0,3 b	0,2 b	0,74 b
	Vertimec+ karzin	150+250	5.05				
9.	Untreated Kontrola	-		23,4 a	51,5 a	67,6 a	49,2 a

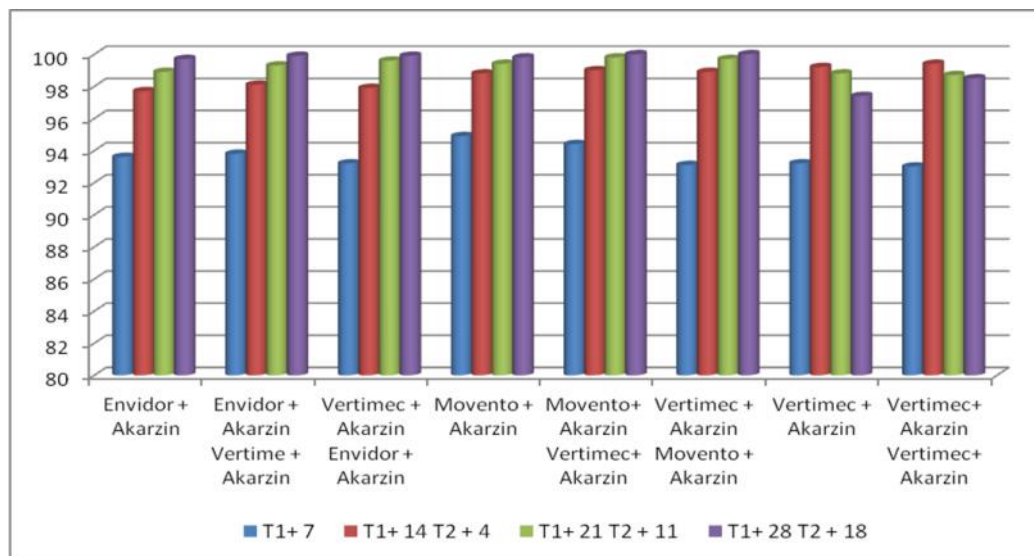
*MF – (/); mobile forms (larvae / nymphs per shoot)

() *C. pyri*

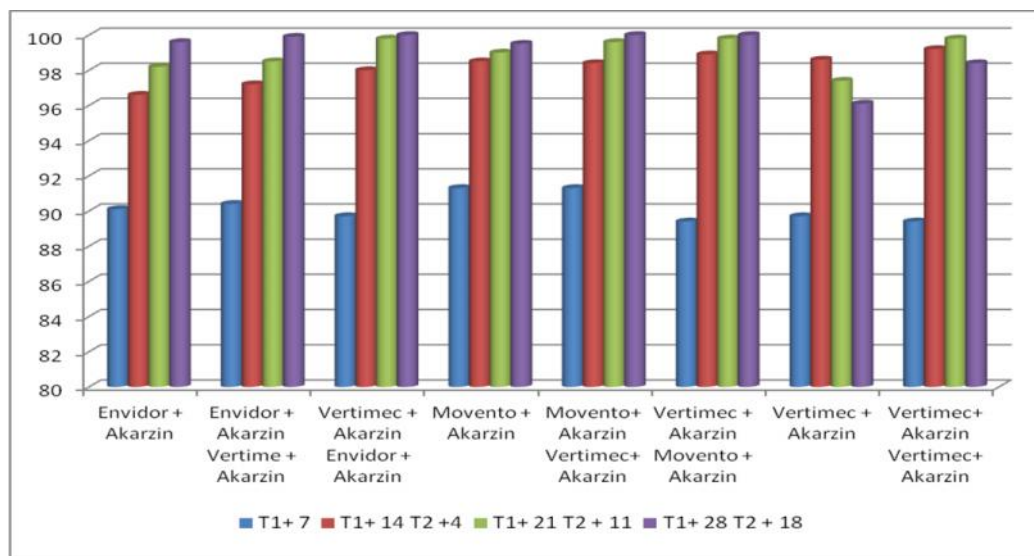
- | Significant differences in population density (number of eggs and larvae per shoot) of *C. pyri* in the different experimental plots prior to treatment were not established during both years of the

study. In contrast to the population density, the degree of hatching of the eggs at the time of treatment was not identical in both years. At the first counts (T1 + 7 days), live larvae were found in all experimental plots during both years of study (Table 3-4).

(1 + 7)
(3-4).



1. Efficacy of insecticides against *C. pyri* (in %), Plovdiv, 2015



2. Efficacy of insecticides against *C. pyri* (in %), Plovdiv, 2016

(3-4).

2016,

(3-4).

(Envidor® 240 SC)
(Movento® 100 SC)
(Akarzin)

e
C. pyri,

(Vertimec® 1.8 EC) +
(Akarzin).

240 SC Movento® 100 SC
99,5-99,8%

(Vertimec® 1.8
EC + Akarzin) – 97,4-96,1%,
(1 2).

(Envidor® 240 SC)
(Movento® 100 SC)

(Vertimec® 1.8
EC),

, Envidor® 240 SC
Movento®

(Envidor® 240 SC)
(Movento®)

However, significant differences in the numbers of living larvae were observed between pesticide-treated trees and untreated trees as a result of the chemical treatments during both years of study (Table 3-4).

The results of two-year tests are identical and outline similar trends despite the apparently lower efficacy of applied insecticides at the end of the first week after treatment in 2016 due to the higher percentage of hatched larvae at the time of treatment (Table 3-4). This gives us reason to make a joint analysis of the results and to discuss them together.

Spirodiclofen (Envidor® 240 SC) and spirotetramat (Movento® 100 SC) in combination with mineral oil (Akarzin) applied singly show a high efficacy in control of *C. pyri*, which is insignificantly better than that of the reference combination abamectin (Vertimec® 1.8 EC) + mineral oil (Akarzin). Once applied in combination with mineral oil, Envidor® 240 SC and Movento® 100 SC effectively reduce the pest population, reaching 99.5-99.8% efficacy at 28 days post-treatment, which is slightly better than that of (Vertimec® 1.8 EC + Akarzin) – 97.4-96.1%, with one application (Figure 1 and 2). Significant differences in the overall efficacy of spirodiclofen (Envidor® 240 SC) and spirotetramat (Movento® 100 SC) were not found, although spirodiclofen demonstrated a slightly slower effect over the first two weeks after treatment. Unlike abamectin (Vertimec® 1.8 EC), which is characterized by a low initial toxicity and a relatively short-lasting effect, Envidor® 240 SC and Movento® show a poor initial effect that gradually improves over time.

The use of spirodiclofen (Envidor® 240 SC) and spirotetramat (Movento®) in strategy with other products involving initial treatment with one of the two

insecticides in combination with mineral oil after 10 days followed by abamectin treatment in combination with mineral oil or the same Insecticides, but applied in reverse order, contributes to significantly improve the control of *C. pyri*. Incorporation of these products into one of the two strategies leading to a significant increase in the efficacy (up to 100%), resulting in complete reduction of pest populations up to four weeks after the initial treatment.

Spirodiclofen and spirotetramat when used in a strategy with other products (abamectin + mineral oil) that preceded or followed the main treatment showed better efficacy than spirodiclofen (Envidor 240 SC), spirotetramat (Movento®) and abamectin (Vertimec®) applied once in combination with mineral oil or abamectin (Vertimec®) in combination with mineral oil but in two consecutive treatments.

insecticides in combination with mineral oil after 10 days followed by abamectin treatment in combination with mineral oil or the same Insecticides, but applied in reverse order, contributes to significantly improve the control of *C. pyri*. Incorporation of these products into one of the two strategies leading to a significant increase in the efficacy (up to 100%), resulting in complete reduction of pest populations up to four weeks after the initial treatment.

Spirodiclofen and spirotetramat when used in a strategy with other products (abamectin + mineral oil) that preceded or followed the main treatment showed better efficacy than spirodiclofen (Envidor 240 SC), spirotetramat (Movento®) and abamectin (Vertimec®) applied once in combination with mineral oil or abamectin (Vertimec®) in combination with mineral oil but in two consecutive treatments.

Movento® 100 SC + Envidor® 240 SC
 (Arnaudov, 2016a; Arnaudov, 2016b).
C. pyri

Movento® 100 SC + Envidor® 240 SC + Vertimec® +
 Movento® 100 SC + Envidor® 240 SC + Vertimec® +
 Movento® 100 SC + Envidor® 240 SC

CONCLUSIONS

Experiments confirm the good efficacy of Envidor® and Movento® in control of *C. pyri*, which is also reported in previous studies (Arnaudov, 2016a, Arnaudov, 2016b). The addition of mineral oil to them improves their efficacy and makes them even more reliable in limiting the key pest populations. The control of *C. pyri* populations is as better and longer as the right time for treatment is better defined. Early application of these products, as well as their later application, in the presence of already hatched larvae, can significantly reduce their efficacy.

The activity of Envidor® 240 SC + mineral oil and Movento® 100 SC + mineral oil, once applied, seems to be superior to this Vertimec® + mineral oil under the specific garden conditions.

The use of Envidor® 240 SC or Movento® 100 SC in combination with mineral oil in a strategy with prior or subsequent treatment with Vertimec® 18

Vertimec® 18 EC +	-	EC + mineral oil gives similar or negligibly improved results than single treatment with Envidor 240 SC, Movento® 100 SC Or Vertimec® 18 EC + mineral oil, or twice-treatment with Vertimec® 18 EC + mineral oil. This strategy, with two consecutive treatments with different active substances (spirodiclofen or spirotetramat, followed by abamectin or vice versa in combination with mineral oil), can be a significantly better solution as well as a possible alternative to once-and-twice treatment with abamectin + mineral oil, in cases of severe and prolonged attack by <i>C. pyri</i> .
Envidor® 240 SC, Movento® 100 SC Vertimec® 18 EC + EC+	-	
Vertimec® 18	-	
SC	-	
Envidor® 240 SC Movento® 100	-	
SC	-	
Envidor® 240 SC Movento® 100	-	
<i>C. pyri</i> ,	-	
<i>C. pyri</i> .	-	In the case of a lesser attack from this pest, single treatment with Envidor® 240 SC or Movento® 100 SC in combination with mineral oil may be enough to keep the pest population at an economically safe level. The inclusion of Envidor® 240 SC and Movento® 100 SC in the strategy for controlling the populations of <i>C. pyri</i> by single application or in a scheme with other products will make the latter more flexible and more reliable in controlling this key pest.

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(*Pyrus communis* L.)

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1 , , .” “ 12, 4004 ,
2 , .” “ 12, 4000 ,
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Optimisation of acclimatization of micropropagated pear plants (*Pyrus communis* L.) by new plant biostimulators of natural origin

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SUMMARY

A - Acclimatization is one of the key
- steps in the micropropagation process.
- The aim of the present investigation is to
- study the possibility to improve
- acclimatization of micropropagated plants
- by new generation of plant biostimulators
- of natural origin – Regoplant and Stimpo
(Agrobiotech, Ukraina).
- These biostimulators contain metabolism
- products of *in vitro* cultivation of
- endophyte fungus, isolated from ginseng
- roots. For the purpose of this study
- micropropagated and rooted plantlets
- from pear rootstock OHF 333 (*Pyrus
communis* L.) are treated with Regoplant
or Stimpo in concentrations 50 µl l⁻¹ or
100 µl l⁻¹.
- Plantlets treated with distilled water
(without plant growth regulators) serve as
a control sample. Multi-cell bedding plant
trays filled with peat: perlite (1:1) are used

(1:1)
 16 (150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD)
 30-

50 $\mu\text{l l}^{-1}$

in vitro

ex vitro *in vitro* *in vitro*

2.

in vitro

vitro

for acclimatization. Plants were kept in a growth chamber under 16 h photoperiod (150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) and high humidity. Data on fresh and dry matter, mean number of roots per plant, stem and root length, photosynthetic pigments, antioxidant activity and chlorophyll fluorescence are collected 30 days after treatment. The best result is obtained with plants treated with 50 $\mu\text{l l}^{-1}$ Regoplant.

Keywords: micropropagation, acclimatization, pear, biostimulators, Regoplant, Stimpo

INTRODUCTION

In vitro propagation is an efficient method for large scale production of valuable species. In many species, especially in woody plants, micropropagation is limited due to rooting difficulties and the low survival rate of the plantlets during the *ex vitro* acclimatization. During *in vitro* conditions, plantlets grow under specific climatic conditions – in small tightly closed vessels with high air humidity, low gas exchange and thus a CO₂-shortage during almost the whole photoperiod, ethylene production and relatively low light intensity, in a culture medium with a large concentration of sugar.

These special conditions result in the formation of plants with abnormal morphology, anatomy and physiology.

After the transfer from *in vitro* to *ex vitro* conditions, plants have to correct the abnormalities and to acclimatize to the new environments in the greenhouse or in the field. Acclimatization is one of the key steps in the micropropagation process.

Some approaches are often used to increase plant survival rate after transplanting, such as natural light shading, antitranspirant application for reducing plant transpiration etc.

Plant growth stimulators Regoplant and Stimpo are composite natural plant biostimulators. Their action is based on synergic effect of products of biotechnological cultivation of fungi-micromycetes from root system of ginseng and aversectine – biological product with antiparasitic action (Agrobiotech, Ukraina, <http://www.agrobiotech.com.ua>).

The pear rootstock OHF 333 (*Pyrus ommunis* L. 'Old Home' x 'Farmingdale') (Van der Zwet and Beer, 1995; Wertheim, 2002).

(Jones and Webster, 1989).

OHF 333 (Agrobiotech, Ukraina).

OHF 333 (*Pyrus ommunis* L. 'Old Home' x 'Farmingdale').

In vitro

22±2° 16/8 (40
 µmol m⁻² s⁻¹ PPF, OSRAM, 40 W).
 30 mm

81,35 GE/day (Microbox, Belgium). (600 ml) 100 ml 10

14-

10

Plant growth stimulators Regoplant and Stimpo are composite natural plant biostimulators. Their action is based on synergic effect of products of biotechnological cultivation of fungi-micromycetes from root system of ginseng and aversectine – biological product with antiparasitic action (Agrobiotech, Ukraina, <http://www.agrobiotech.com.ua>).

The pear rootstock OHF 333 (*Pyrus cuommunis* L. 'Old Home' x 'Farmingdale') has moderate growth, good compatibility with most pear varieties and is slightly susceptible to fire blight (Van der Zwet and Beer, 1995; Wertheim, 2002). It is relatively difficult to propagate through cuttings or micropropagation, especially rooting (Jones and Webster, 1989).

The aim of the present investigation is to study the possibility of improving acclimatization of micropropagated plants by new generation of plant growth stimulators of natural origin – Regoplant and Stimpo (Agrobiotech, Ukraina).

MATERIAL AND METHODS

The experiments are carried out with plants of pear rootstock OHF 333 (*Pyrus communis* L. 'Old Home' x 'Farmingdale'). *In vitro* plantlets are cultivated in a growth chamber at 22±2° and 16 h photoperiod (40 µmol m⁻² s⁻¹ PPF, fluorescent tubes OSRAM, 40 W). Shoot tips (30 mm) are set for rooting in polypropylene vessels with green antibacterial filter (gas exchange rate 81,35 GE/day, Microbox, Belgium). In each vessel (600 ml) onto 100 ml nutrient medium 10 shoots are inoculated. On the 14th day, the plants are very well rooted and, after being removed from the agar, are treated by dipping in a solution of Regoplant or Stimpo for 10 minutes. As a control sample served microshoots dipped in water.

1. - ;
 2. - 50 $\mu\text{l l}^{-1}$ (R1);
 3. - 100 $\mu\text{l l}^{-1}$ (R2);
 4. - 50 $\mu\text{l l}^{-1}$ (S1);
 5. - 100 $\mu\text{l l}^{-1}$ (S2).

(105)
 (2:1)

22±2°

16/8 (150
 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD,
 OSRAM, 40 W)

30-

(FW) -
 (DW)

80 ° 48 h

(Beadle, 1993).

85% (v/v)
 a 3.

663 nm, 644 nm 440 nm

4500 g 10 min.

Lichtenthaler Wellburn (1983)
 mg g^{-1}

MINI-PAM
 (Heinz Walz, Germany)

30-

(F₀) (F_m),

2 - Y = (F_m - F₀)/F_m (Genty
 et al., 1989).

Variants:

1. Control – distilled water;
2. 50 $\mu\text{l l}^{-1}$ Regoplant (R1);
3. 100 $\mu\text{l l}^{-1}$ Regoplant (R2);
4. 50 $\mu\text{l l}^{-1}$ Stimpo (S1);
5. 100 $\mu\text{l l}^{-1}$ Stimpo (S2).

Multi-cell bedding plant trays filled with peat: perlite (1:1) are used for acclimatization. Plants are kept in growth chamber at 22±2° under 16 h photoperiod (150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, fluorescent tubes OSRAM, 40 W) and high humidity conditions for 2 weeks and then humidity was gradually decreased.

After 30 days in *ex vitro* conditions growth parameters, physiological and biochemical analysis are performed.

Fresh weight of whole plants (FW) is determined immediately after removing the plants from the soil. The dry weight of plants (DW) is measured after drying the material at 80 ° for 48 h (Beadle, 1993).

The photosynthetic pigments are extracted in the dark, with cooled to 4 ° C 85% (v/v) acetone, in the presence of CaCO₃. The optical density of the obtained extracts is determined spectrophotometrically at 663 nm, 644 nm and 440 nm after centrifugation of the extracts at 4500 g for 10 min. The content of photosynthetic pigments is calculated according to Lichtenthaler and Wellburn (1983) and expressed in mg g^{-1} fresh weight of sample.

The chlorophyll fluorescence is measured by MINI-PAM, Heinz Walz, Germany on the first (top-down) fully formed unattached leaves. After 30 min in the dark initial (F₀) and, maximal fluorescence (F_m) are measured and overall quantum yield of photochemical energy conversion (Yield) Y = (F_m - F₀)/F_m (Genty et al., 1989) is calculated.

Rapid light curves are generated with the MINI-PAM and photosynthetic

2 (ETR = Y*0.5. *0.84)
 (Handbook of operation
 with MINI-PAM, 1996).

Yen and Chen
 Rossi et al.
 (1995)
 (2003).

30
 (One-way ANOVA) Duncan
 95%.

electron transport rates (ETR) are
 calculated from chlorophyll fluorescence
 (ETR = YxPARx0.5x0.84) (Handbook of
 operation with MINI-PAM, 1996).

Antioxidant activity is determined
 according to the method of Yen and Chen
 (1995) and the percentage of DPPH radical
 scavenging capacity was calculated
 according to Rossi et al. (2003).

The experiment is conducted twice
 with 30 explants for variant. Statistical
 analyses were carried out by One-Way
 ANOVA using Duncan's test to validate
 the different significance at p = 95%.

RESULTS AND DISCUSSION

As early as the 14th day after
 treatment with Regoplant and Stimpo,
 apparent differences are found between
 the variants, with the best status
 distinguishing the plants treated with 50 µl
 l⁻¹ Regoplant (Figure 1).

14-
 -
 50
 µl l⁻¹ (1).



1. Regoplant
 Stimpo 14 *ex vitro*

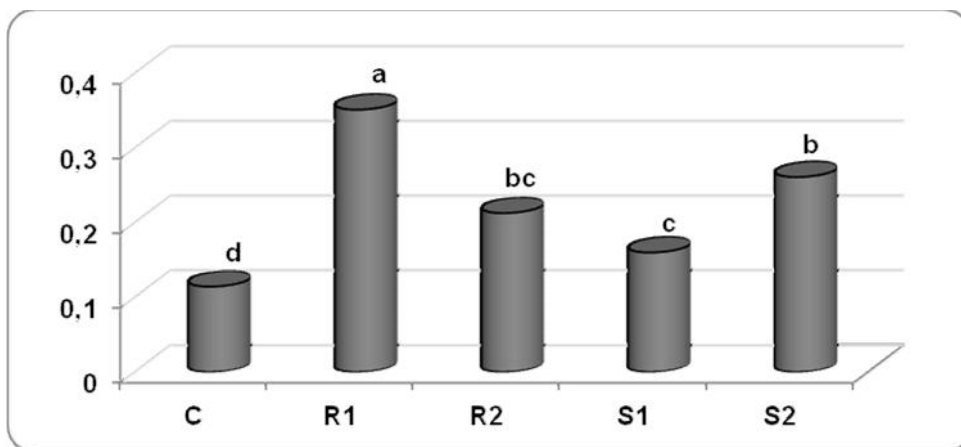
Fig. 1. General view of the pear plants, treated with 50 µl l⁻¹ Regoplant or 50 µl l⁻¹ Stimpo 14 days after transplanting to *ex vitro* conditions

Control - distilled water; R1 - 50 µl l⁻¹ Regoplant; R2 - 100 µl l⁻¹ Regoplant; S1 - 50 µl l⁻¹ Stimpo; S2 - 100 µl l⁻¹ Stimpo

30-
 -
 (FW) (DW)
 (2).
 50 µl l⁻¹

The results at the end of the
 acclimatization (30th day) confirm this
 observation. In all treated variants, higher
 fresh (FW) and dry (DW) weight
 accumulation is observed compared to
 the control sample (Figure 2). The
 highest values are recorded for plants
 treated with 50 µl l⁻¹ Regoplant (R1)

(R1) (1, 2), (Table 1, Figure 2), followed by those of the variant treated with 100 $\mu\text{l l}^{-1}$ Stimpo (S2). (S2).



. 2. (g) 30 *ex vitro*

Fig. 2. Fresh weight of one pear plant (g), treated with Regoplant or Stimpo 30 days after transplanting to *ex vitro* conditions

C - control; R1 - 50 $\mu\text{l l}^{-1}$ Regoplant; R2 - 100 $\mu\text{l l}^{-1}$ Regoplant; S1 - 50 $\mu\text{l l}^{-1}$ Stimpo; S2 - 100 $\mu\text{l l}^{-1}$ Stimpo. (P<0.05)

Different letters within each column indicates significant difference (P<0.05) by DMRT

1. 30 *ex vitro*

Table 1. Growth and biochemical parameters of plants, treated with Regoplant and Stimpo 30 days after treatment and transplanting to *ex vitro* conditions

Variants	DW g	DW/FW %	DPPH %	Yield Y=Fv/Fm
Control (C)	0,0515 ± 0,001 ^d	45,01 ± 1,48 ^a	21,44 ± 1,76 ^a	0.768
50 $\mu\text{l l}^{-1}$ Regoplant (R1)	0,1190 ± 0,022 ^a	33,64 ± 1,50 ^b	20,82 ± 2,20 ^a	0.803
100 $\mu\text{l l}^{-1}$ Regoplant (R2)	0,0809 ± 0,001 ^c	38,11 ± 2,10 ^{ab}	25,94 ± 0,63 ^a	0.779
50 $\mu\text{l l}^{-1}$ Stimpo (S1)	0,06 ± 0,004 ^{cd}	35,93 ± 0,45 ^b	21,31 ± 0,50 ^a	0.776
100 $\mu\text{l l}^{-1}$ Stimpo (S2)	0,0972 ± 0.005 ^b	37,19 ± 1,66 ^b	26,37 ± 4,99 ^a	0.783

* (P<0.05)

Different letters within each column indicates significant difference (P<0.05) by DMRT

(FW) 1 (DW), DPPH – ;
() II – Yield (Y= Fv/Fm)

Fresh weight (FW) and dry weight (DW) were based on one plant. DPPH (%) - radical scavenging capacity; Maximum quantum yield of photosystem II - Yield – (Y = Fv/ Fm)

FW
50 $\mu\text{l l}^{-1}$ (R1),
, DW
(1, 2). Fresh weight (FW) of plant, treated with 50 $\mu\text{l l}^{-1}$ Regoplant (R1), is three times greater and dry weight (DW) is two times greater than the control variant (Table 1, Figure 2). The ratio of DW to

(DW/FW) , (1).
 -
 -
 100 $\mu\text{l l}^{-1}$,
 100
 $\mu\text{l l}^{-1}$. R1 (50 $\mu\text{l l}^{-1}$)
)
 -
 ,
 ,
 ,
 FW.
 (Fujiwara et al. 1992; Pospisilova 1996).
 ,
 . / . b
 (2).

FW does not differ significantly in the plants treated with Regoplant or Stimplo but is significantly higher in the control (Table 1).

The highest values of photosynthetic pigments are recorded in the leaves of the plants treated by Stympo at a dose of 100 $\mu\text{l l}^{-1}$, followed by the control and the variants with 100 $\mu\text{l l}^{-1}$ Regoplant.

The total chlorophyll content in variant R1 (50 $\mu\text{l l}^{-1}$ Regoplant) is the lowest. This is most likely due to the fact that the values obtained are determined on fresh weight basis, namely that this variant has a maximum value of FW. This is well documented by other authors (Fujiwara et al. 1992; Pospisilova 1996).

A similar trend is observed in carotenoids, with no statistically proven difference between the variants. The ratio of chl.a/chl.b. and total chlorophyll/carotenoids are within the normal range for all tested variants (Table 2).

2.

(mg g⁻¹ FW)
 30

ex vitro

Table 2. The content of photosynthetic pigments (mg g⁻¹ FW) in the leaves of pear plants treated by Regoplant or Stimplo 30 days after treatment and transplanting to *ex vitro* conditions

Variants	Chl a mg g ⁻¹ FW	Chl b mg g ⁻¹ FW	Chl (a+b) mg g ⁻¹ FW	Carotenoides mg g ⁻¹ FW	Chl a/Chl b	Chl (a+b)/Car
Control (C)	3,09±0,14 ^{ab}	1,03±0,03 ^b	4,44±0,10 ^{ab}	1,24±0,09 ^a	2,99±0,22 ^{ab}	3,58±0,19 ^a
50 $\mu\text{l l}^{-1}$ Regoplant (R1)	2,31±0,40 ^b	0,78±0,04 ^b	3,33±0,46 ^b	0,94±0,18 ^a	2,92±0,35 ^{ab}	3,58±0,21 ^a
100 $\mu\text{l l}^{-1}$ Regoplant (R2)	3,09±0,01 ^{ab}	1,01±0,08 ^b	4,39±0,08 ^{ab}	1,29±0,02 ^a	3,07±0,25 ^a	3,39±0,13 ^a
50 $\mu\text{l l}^{-1}$ Stimplo (S1)	2,66±0,53 ^b	0,90±0,17 ^b	3,81±0,76 ^b	1,10±0,22 ^a	2,95±0,03 ^{ab}	3,45±0,02 ^a
100 $\mu\text{l l}^{-1}$ Stimplo (S2)	3,62±0,19 ^a	1,53±0,26 ^a	5,54±0,52 ^a	1,33±0,04 ^a	2,41±0,29 ^b	4,15±0,51 ^a

* (P<0.05)
 Different letters within each column indicates significant difference (P<0.05) by DMRT

(DPPH)
 (Y) 1).
 2
 ,

Total antioxidant activity (DPPH) does not differ significantly in the tested treatments (Table 1).

Quantum yield of photochemical energy conversion (Y) is an indicator of photochemical activity of Photosystem II in dark-adapted plants. In our experiment, the results show that the photosynthetic function in the leaves of the plants is negligibly improved in the treated plants.

Nordenkampf and Oquist, 1993).
 1
 2.
 (ETR)
 (White
 ETR
 50 $\mu\text{l l}^{-1}$
 (S1)
 and Critchley, 1999).
 (R1) 50 $\mu\text{l l}^{-1}$
 (3).

The difference between the variants is not statistically proven. Values ranging from 0.75 to 0.83 are characteristic of healthy leaves (Bolhar-Nordenkampf and Oquist, 1993). The results presented in Table 1 show that not significant alterations are found in the photochemical potential of Photosystem II in the test plants (treated and control).

The rapid light curves provide information about the electronic transport rate (ETR) through Photosystem II and give information about the plasticity of the photosynthetic apparatus to the increasing intensity of the light (White and Critchley, 1999). The values of ETR in plants treated with $\mu\text{l l}^{-1}$ Regoplant (R1) and $50 \mu\text{l l}^{-1}$ Stimpo (S1) are higher than the controls (Figure 3).

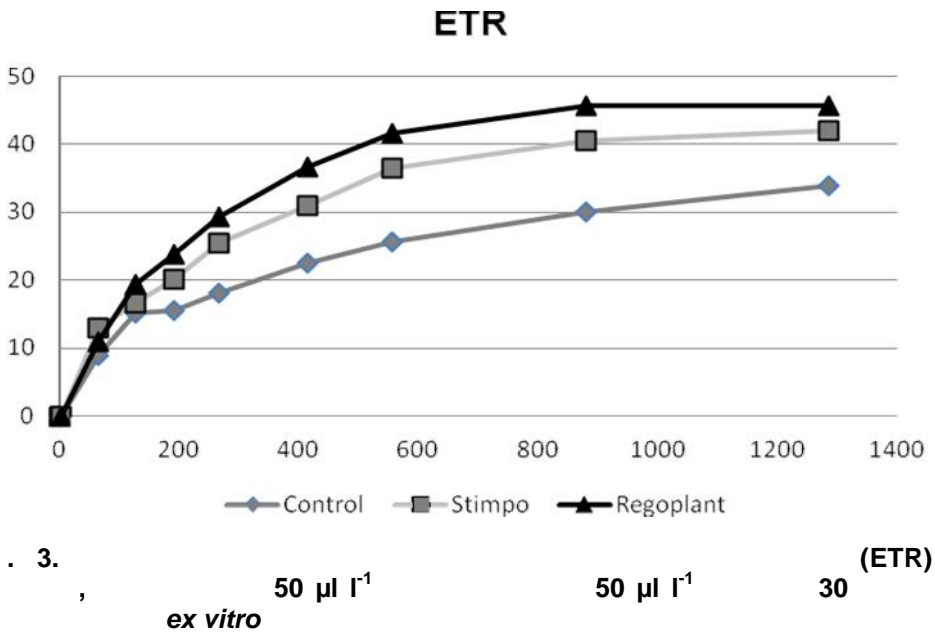


Fig. 3. Apparent electron transport rate (ETR) of plants, treated with $50 \mu\text{l l}^{-1}$ Regoplant or $50 \mu\text{l l}^{-1}$ Stimpo 30 days after transplanting to *ex vitro* conditions

(Y) ETR
 50 $\mu\text{l l}^{-1}$
 (R1),

The significantly higher FW and DW, larger quantum yield (Y) and ETR in plants treated with $50 \mu\text{l l}^{-1}$ Ragoplant (R1) give us a reason to suppose that these plants have more intensive

in vitro ex vitro

in vitro

50 µl l⁻¹

OHF 333 (*Pyrus communis* L.).

photosynthesis, accumulate more effective biomass and more easily overcome stress from the transfer from *in vitro* to *ex vitro* conditions. Therefore, we could recommend the use of Regoplant in the acclimatization of *in vitro* cultivated plants for easier acclimatization to new *ex vitro* conditions.

CONCLUSIONS

The application of biostimulator of natural origin Regoplant (50 µl l⁻¹) could significantly stimulate the growth and improve the acclimatization of micropropagated pear rootstock OHF 333 plants (*Pyrus communis* L.).

ACKNOWLEDGEMENTS

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HTC/ /01/9

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