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# THE EFFECT OF MONO- AND MIXED PHYTOPLASMA AND BACTERIAL INFECTIONS ON *GALEGA ORIENTALIS* L. PLANTS

*In recent years, in regions of intensive agriculture, due to unfavorable agro-ecological conditions, mixed infections have become a serious threat to the production of high-quality crop products, in particular, Galega orientalis L.* ***Objective.*** *To investigate the effect of mono- and mixed phytoplasma and bacterial infection on Galega orientalis L. plants.* ***Methods.*** *Plants of Galega orientalis L. were inoculated with phytoplasma strains: A. laidlawii var. granulum 118 — the causative agent of pale green dwarfism of wheat; A. laidlawii 101 and A. laidlawii 178 — pathogens of tomato trunk, and Pseudomonas syringae pv. atrofaciens D13 — the causative agent of wheat basal bacteriosis. Different schemes of inoculation of Galega orientalis L. plants were used in the experiments, which were applied both in mono- and mixed phytoplasma and phytoplasma-bacterial infection. Symptoms were recorded by visual examination starting from the 14$^{th}$ day. Uninfected plants of this crop served as a control.* ***Results.*** *Under both mono- and mixed phytoplasma-infection, both typical (anthocyanin coloration of stems and formation of additional shoots from the base of internodes («witch's brooms») and atypical (small leaves and shortened internode spacing, which are characteristic of pale green dwarfism of wheat (PGD) symptoms of phytoplasmosis, were established on plants of Galega orientalis L. Under the conditions of phytoplasma-bacterial infection in plants, a noticeable thickening of the stem and stunted growth were observed, i.e., the symptoms that are characteristic of the development of phytoplasmosis, and were recorded, but their manifestation was atypical for legumes. Thus, in the case of phytoplasma-bacterial infection, the vast majority of symptoms characteristic of phytoplasmosis (namely, pale green dwarfism of wheat) were recorded such as dwarfism, small leaves, shortened distances between internodes, which are atypical for legumes.* ***Conclusions.*** *Typical and atypical symptoms of phytoplasmosis and bacteriosis for Galega orientalis L. under mono- and mixed infection have been established. Mono- and especially mixed infections with phytoplasmas and phytopathogenic bacteria significantly affect the development of Galega orientalis L. plants. The increase in the infectious load on Galega orientalis L. plants, as well as the priority of the pathogen's penetration into plant tissues, leads to a significant inhibition of the development of infected plants of this crop. The obtained data can be useful for diagnostics of Galega orientalis L. crops affected by phytoplasmosis or bacteriosis.*

***Keywords:*** *Galega orientalis L., Acholeplasma laidlawii var. granulum 118, Pseudomonas syringae pv. atrofaciens D13, mono- and mixed infection, typical and atypical symptoms.*

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Today, to avoid protein deficiency and create a sustainable fodder base, along with the cultivation of conventional legumes, *Galega orientalis* L., or Eastern galega, deserves special attention.

*Galega orientalis* L. is a species of flowering plant in the legume family. It is known commonly as fodder galega and eastern galega. It is a valuable high-protein crop capable of perennial growth, with a high content of protein, vitamins, nutrients, and amino acids and is resistant to changing climatic conditions (Żarczyński et al., 2021; Darmohray et al., 2021).

This crop is extremely promising in modern ecologically oriented agriculture. *Galega orientalis* L. plays an important role in strengthening the livestock feed base and restoring soil fertility.

In terms of its fodder qualities, *Galega orientalis* L. is not inferior to the well-known clover and alfalfa, significantly exceeding them in terms of spring regrowth rate, green mass yield, and productive longevity. The fodder qualities of *Galega orientalis* L. and the possibility of accumulation of nitrogen (humus, phosphorus) in the soil, a long period of productive growth in one place (more than 10 years of cultivation in one field) put it in a number of promising fodder crops in the fodder production (Kuzmenko & Zhukov, 2016; Savenko, 2000; Voronetska & Yurchuk, 2023; Ignaczak et al., 2023).

However, the most important property of *Galega orientalis* L. is its nitrogen supply through symbiotic fixation from the atmosphere, and, as a result, enrichment of the soil with biological nitrogen and improvement of the ecological state of the agrocenosis. Thanks to symbiosis with nodule bacteria, this crop is able to accumulate up to 300 kg/ha of biological nitrogen in the soil during the growing season. Therefore, *Galega orientalis* L. is a good predecessor for other crops, especially cereals (Abramov, 1996; Zabolotna et al., 2004; Vorobey & Kovalevskaya, 2008; Didovych et al., 2008).

However, recently, due to unfavorable agro-environmental conditions, there has been an increase in the intensity of the spread of diseases in cultivated plants. As a result, crop yields are decreasing and the quality of the harvest is deteriorating.

However, in nature, it is usually rare for plants to be affected by a single pathogen (mono-infection), and plants usually come into contact with several phytopathogens at the same time. The severity and nature of the disease symptoms largely depend on how many pathogens the plant is infected with. Likely, infection with several pathogens (in the case of mixed infection) leads to more severe disease symptoms compared to mono-infection, and the diagnosis becomes much more difficult (Bianco et al., 1993; Alma et al., 1996; Bertaccini et al., 2014).

Climate change can also significantly affect the course of the phytopathological process of *Galega orientalis* L. (Anderson et al., 2004; Mohamed Mahgoub Azooz, Parvaiz Ahmad, 2016; Ignaczak et al., 2023; Paige Van de Vuurst & Luis E. Escobar, 2023).

Thus, mixed infections pose a serious threat to the production of high-quality crop products, in particular, *Galega orientalis* L., as pathogens of phytoplasmosis and bacteriosis can cause significant harmful effects on *Galega orientalis* L. crops, affecting the ground parts of plants and causing premature leaf fall. This worsens the quality of green mass and reduces the yield of this crop by 32—42%.

However, the range of phytopathogens of *Galega orientalis* L. is not sufficiently studied in Ukraine and the world, and there is practically no information on the defeat of this crop by phytoplasmosis, there are only a few reports

(Bogoutdinov & Zudilin, 2000; Bogoutdinov, 2013; Bertaccini et al., 2014; Patyka et al., 2016).

Therefore, given the rapid spread of phytoplasmosis and bacteriosis to much larger areas in recent years and the increasing aggressiveness of the pathogens of these diseases, consideration of issues related to mixed infection with *Galega orientalis* L. is relevant and timely today.

When studying the mixed infection of *Galega orientalis* L., it is important to establish the degree of damage to this crop depending on the infectious

load on plants, in particular, to investigate how the degree of damage to *Galega orientalis* plants inoculated with different phytopathogens will change.

Knowledge of the peculiarities of the development of *Galega orientalis* L. under the influence of mono- and mixed infection with phytopathogenic microorganisms is necessary to understand the degree of development of the pathological process in plants of this crop to assess possible yield losses. This knowledge will allow us to establish criteria for the preliminary identification of a pathogen at the early stages of damage during the examination of *Galega orientalis* crops, which will prevent the spread of diseases and help minimize yield losses from the harmful effects of phytopathogenic microorganisms.

Visual inspection of crops is a necessary element of phytosanitary monitoring. Thus, to prevent massive disease damage, a visual inspection of crops is carried out, which is necessary when analyzing the condition of crops and determining the percentage of affected plants in crops.

Since, unfortunately, the analysis of the phytosanitary condition of legume crops in recent years has shown its deterioration, it is important to conduct timely inspections of crops to identify pathogens of cultivated plants to preserve their yield. When conducting mass inspections, the visual method of disease diagnosis should be widely used, as it makes it possible to understand the nature of the disease.

Thus, monitoring of *Galega orientalis* L. diseases and their diagnosis is of great practical importance.

**Aim** of this study was to investigate the effect of mono- and mixed phytoplasmal and bacterial infection on *Galega orientalis* L. plants.

**Materials and Methods.** For inoculation of *Galega orientalis* L. plants with different strains of phytoplasmas and phytopathogenic bacteria, plants of *Galega orientalis* L. of the 2nd year of cultivation were used. Plants were grown in the field at the experimental site of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

Plants were inoculated with phytopathogenic microorganisms — phytoplasmas (class *Mollicutes*) and phytopathogenic bacteria of different taxonomic groups.

All strains of mollicutes and phytopathogenic bacteria used in the study were obtained from the Ukrainian Collection of Microorganisms (UCM) of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

For inoculation of *Galega orientalis* L. plants with phytoplasmas, strains isolated from different sources were used: *Acholeplasma laidlawii* var. *granulum* 118, the causative agent of pale green dwarfism of wheat (PGD), isolated from wheat (UCM BM-34); *Acholeplasma laidlawii* 101 (UCM BM-45) and *Acholeplasma laidlawii* 178 (UCM BM-37) — pathogens of tomato trunk, as well as phytopathogenic bacterium *Pseudomonas syringae* pv. *atrofaciens* D13 — the causative agent of basal bacteriosis, isolated from wheat.

To obtain an inoculum, phytoplasma strains were cultivated in selective liquid culture medium SM IMV-72 for 72 hours at a temperature of 37 °C (Skripal' & Malinovskaya, 1984). To determine the concentration of the inoculum for each phytoplasma strain, a turbidity standard was used. The concentration of the inoculum for each phytoplasma strain was $1.5 \times 10^7$ CFU/mL.

*P. syringae* pv. *atrofaciens* D13 was cultivated on potato agar in a thermostat at 26—28 °C. For artificial infection, a bacterial suspension according to the turbidity standard was prepared. The concentration of a bacterial suspension of *P. syringae* pv. *atrofaciens* D13 was $1 \times 10^9$ CFU/mL.

*Galega orientalis* plants were inoculated with phytopathogenic strains of microorganisms — phytoplasmas and/or phytopathogenic bacterium by subepidermal injection into the stem, at the level of the 2nd internode, using the Klement method (Klement, 1963).

During the experiments, *Galega orientalis* plants were infected on the 21st day after sowing. Symptoms were recorded by visual examination

starting from the 14th day. Uninfected plants of this crop served as a control.

To study the symptoms of one (monoinfection) or several (mixed infection) diseases — phytoplasmosis and/or bacteriosis, *Galega orientalis* L. plants were infected by introducing a culture suspension of one (in the case of monoinfection) or several strains (in the case of mixed infection) of phytopathogenic microorganisms.

During the studies on reproduction of symptoms of phytoplasmosis and/or bacteriosis on *Galega orientalis* L., depending on the type of infection (mono- or mixed), different schemes of inoculation of experimental plants of *Galega orientalis* L. were used — simultaneous, staged, or both simultaneous and staged introduction of cell suspensions of the studied strains of phytopathogenic microorganisms.

Thus, to reproduce the symptoms of one of the diseases — phytoplasmosis or bacteriosis on *Galega orientalis* L. plants, in the case of monoinfection, plants of this culture were artificially infected by introducing a suspension of cells of one of the studied strains of a phytopathogenic microorganism — phytoplasma or phytopathogenic bacterium.

In mixed phytoplasma infection, both staged inoculation and a combination of simultaneous and staged infection with phytopathogenic mollicutes were used.

In the case of using staged inoculation of *Galega orientalis* L. plants with the studied phytopathogens in mixed infection, inoculation with the second phytopathogen was carried out 7 days after inoculation with the first pathogen. Such an interval of 7 days between inoculation with the first phytopathogen and inoculation with the second phytopathogen was selected experimentally taking into account the incubation period of phytopathogen, the time sufficient for its penetration, growth, and reproduction in the tissues of experimental plants.

Thus, in the case of staged infection with mixed phytoplasma-infection, *Galega orientalis* L. plants were first inoculated with phytoplasma of *A. laidlawii* var. *granulum* 118, after 7 days — infection of experimental plants was carried out by simultaneous injection of suspensions of phytoplasma strains *A. laidlawii* 101 and *A. laidlawii* 178.

In the case of simultaneous and staged infection with phytopathogenic mollicutes in mixed phytoplasma infection, *Galega orientalis* L. plants were first infected by simultaneously inoculating cell suspensions of two strains of phytoplasmas — *A. laidlawii* var. *granulum* 118 and *A. laidlawii* 101, after 7 days the experimental plants were re-inoculated with *A. laidlawii* var. *granulum* 118.

Both staged and simultaneous inoculation with two phytopathogens was also used in the case of mixed phytoplasma-bacterial infection. Thus, when applying the scheme with staged inoculation of *Galega orientalis* L., plants of this culture were first infected with phytoplasma of *A. laidlawii* var. *granulum* 118, and after 7 days — with phytopathogenic bacterium *Pseudomonas syringae* pv. *atrofaciens* D13.

In studying the effect of mixed phytoplasma-bacterial infection on the damage of *Galega orientalis* L. plants, simultaneous inoculation of suspensions of *A. laidlawii* var. *granulum* 118 and *Pseudomonas syringae* pv. *atrofaciens* D13 cells was used.

During the visual inspection of the experimental plants, their condition and signs of phytoplasmosis and/or bacteriosis were recorded.

**Results.** As a result of the research, the effect of mono- and mixed infections on the symptoms of *Galega orientalis* L. plants artificially affected by phytoplasmosis and bacteriosis was studied, and both typical and atypical signs of phytoplasmosis were identified.

Thus, during the examination of *Galega orientalis* L. plants infected with *A. laidlawii* var. *granulum* 118, in the case of monoinfection, anthocyanin coloration of stems was detected. This sign is typical in the development of legume phytoplasmosis (Fig. 1).

When *Galega orientalis* L. plants were infected with another strain, *A. laidlawii* 178, the following symptoms typical for legumes were observed in the

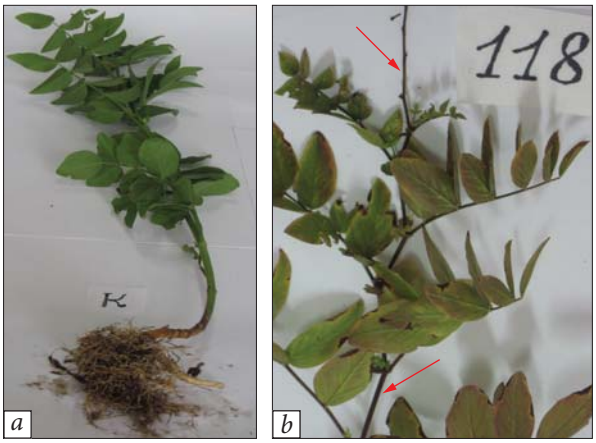


***Fig. 1.*** *Galega orientalis* L. infected with *A. laidlawii* var. *granulum* 118: anthocyanin coloration of stems (monoinfection). On the left — uninfected, control plant (K)

studied plants, such as the formation of additional shoots from the base of the internode (Fig. 2).

When using a mixed phytoplasma infection during the staged inoculation of *Galega orientalis* L. plants with phytoplasmas (first *A. laidlawii* var. *granulum* 118, after 7 days — *A. laidlawii* 101 and *A. laidlawii* 178), a much wider range of symptoms characteristic for phytoplasmosis was recorded. Thus, among the affected plants of *Galega orientalis*, plants with signs of «witch's brooms» prevailed — with the formation of many (from 3 to 7) additional shoots located in «bunches» at the base of each internode. This main symptom of witches' broomstick phytoplasmosis is typical for legumes affected by phytoplasmosis (Fig. 3).

It is important to note that as a result of mixed phytoplasma infection *Galega orientalis* L. plants, with a staged infection (*A. laidlawii* var. *granulum* 118 and *A. laidlawii* 101, and again — *A. laidlawii* var. *granulum* 118), the studied plants were most affected and had more pronounced signs of phytoplasmosis compared to plants infected by other schemes. For example, such plants had leaves of a rich green color (Fig. 4, left), and we also observed the presence of very branched tops with anthocyanin-coloured stems and additional shoots of the same color (Fig. 4).

***Fig. 2.*** Formation of additional shoots under artificial infection of *Galega orientalis* L. plants with *A. laidlawii* 178 (monoinfection)

***Fig. 3.*** *Galega orientalis* L. infected with *A. laidlawii* var. *granulum* 118, *Acholeplasma laidlawii* 101, and *Acholeplasma laidlawii* 178 (mixed phytoplasma-infection, staged introduction): plants with witch's broom signs and many shoots arranged in «bunches» at the base of each internode; on the right — uninfected, control plant

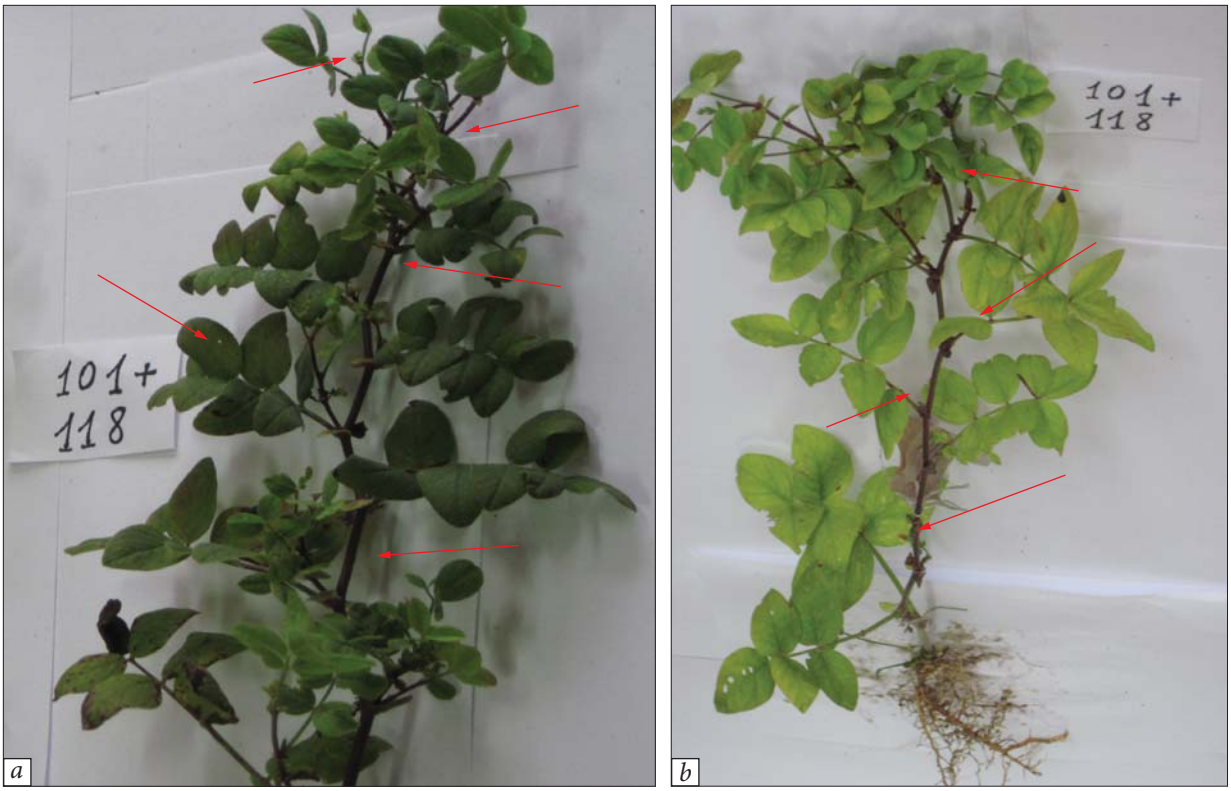


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***Fig. 4.*** *Galega orientalis* L. after mixed phytoplasma infection with *A. laidlawii* var. *granulum* 118 and*A. laidlawii* 101 and repeatedly with *A. laidlawii* var. *granulum* 118: branched tops with elongated anthocyanin-colored stems and adventitious shoots of the same color are present

***Fig. 5.*** Manifestation of lesion symptoms on leaves of *Galega orientalis* L. inoculated with the phytopathogenic bacterium *P. syringae* pv. *atrophaciens* D13: dry light cork necrotic spots of irregular shape with a chlorotic border in the central part and along the perimeter of the leaf and the burn of the leaf tip (monoinfection)


***Fig. 6.*** *Galega orientalis* L. under staged mixed phytoplasma-bacterial infection with *A. laidlawii* var. *granulum* 118 and *Pseudomonas syringae* pv. *atrofaciens* D13: most leaves are of brown or dark anthocyanin color, with very modified tops of light green color; numerous additional shoots formed from internode bases, with small leaves; anthocyanin color of stems

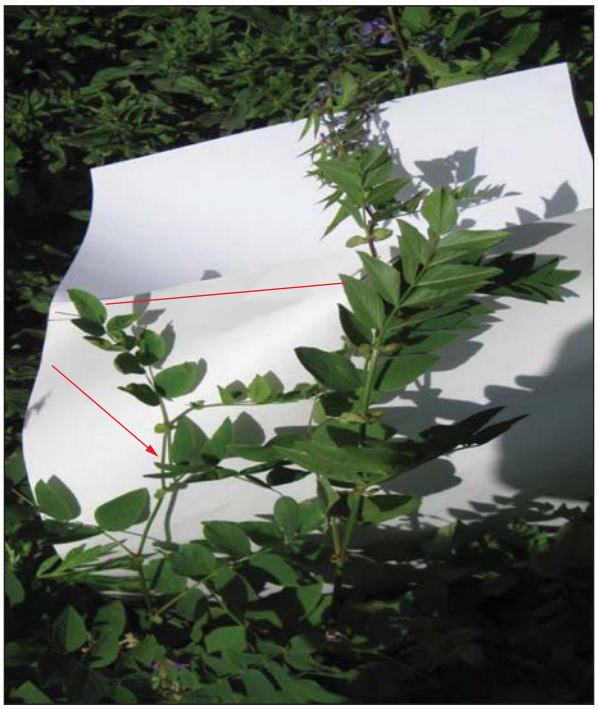

***Fig. 7.*** Symptoms of phytoplasmosis and bacteriosis of *Galega orientalis* L. in mixed infection — simultaneous inoculation with phytoplasma *A. laidlawii* var. *granulum* 118 and phytopathogenic bacterium *Pseudomonas syringae* pv. *atrofaciens* D13: left — affected plant with signs of stunting and thickened stems; right — uninfected, control plant

It should be noted that such an intense green color of the leaves that we recorded is a characteristic symptoms of tomato stem blight caused, in particular, by *A. laidlawii* 101, the inoculum of which infected the experimental plants in this variant, but this feature is atypical for legumes (Fig. 4, left).

During artificial infection of *Galega orientalis* L. plants with phytopathogenic bacteria *P. syringae* pv. *atrofaciens* D13 (monoinfection), the following symptoms of bacteriosis damage to this crop were recorded: dry light corky necrotic spots of irregular shape with a chlorotic border in the central part and along the perimeter of the leaf and the burn of the leaf tip (Fig. 5 ). Such symptoms are typical for this legume crop when it is affected by bacteriosis (Savenko, 2000).

As a result of the examination of *Galega orientalis* L. plants inoculated with two different pathogens — phytoplasma *A. laidlawii* var. *granulum* 118 and phytopathogenic bacterium *Pseudomonas syringae* pv. *atrofaciens* D 13 during their gradual introduction (mixed phytoplasma-bacterial infection), it was found that *Galega orientalis* plants severely damaged by such infection had brown leaves and highly modified tops, and the formation of additional shoots was also observed (Fig. 6).

As a result of the examination of *Galega orientalis* L., infected simultaneously with two phytopathogens — phytoplasma *A. laidlawii* var. *granulum* 118 and phytopathogenic bacterium *Pseudomonas syringae* pv. *atrofaciens* D 13 (mixed phytoplasma-bacterial infection, simultaneous inoculation), signs characteristic of plant damage by phytoplasmosis were revealed — significant slowdown in growth and thickened stems, which are atypical symptoms for legumes (Fig. 7).

**Discussion.** In recent years, in regions of intensive farming, there has been a tendency for phytopathogens to spread more and more due to the changes in climatic conditions and disruption of the symbiotic balance of ecosystems (Garcia-Salazar et al., 1991; Chakraborty et al., 2000; Anderson et al., 2004; Chakraborty et al., 2008; Stephen Cohen & Jan Leach, 2020; Amna M. Al Ruheili et al., 2021; Clarke et al., 2022).

As known, varieties of phytoplasmosis pathogens infect a very wide range of host plants. It should be borne in mind that the same type of phytopathogenic mollicute can infect many plant species (Foissac & Wilson, 2010; Hogenhout et al., 2008; Maramorosh & Raychaudhuri, 2013; Bertaccini et al., 2014; Bertaccini, 2022).

However, in nature, it is rare for plants to be affected by one pathogen, and we often deal with mixed phytoplasma infection, which increases the harmfulness of the corresponding diseases (Bianco et al., 1993; Banttari & Zeyen, 1979; Alma et al., 1996; Rao et al., 2018; Moreno & López-Moya, 2019). It should be noted that the symptoms of diseases caused by phytopathogenic mollicutes vary greatly, especially in the case of mixed infection (Alma et al., 1996; Banttari & Zeyen, 1979; Bianco et al., 1993; Bertaccini & Duduk, 2009). In addition, the condition of such plants deteriorates significantly when they are affected by other pathogens, such as fungi, viruses, etc. (Banttari & Zeyen, 1979; Alma et al., 1996; Moreno & López-Moya, 2019; Xu et al., 2022).

Therefore, mixed infections pose a serious threat to the production of high-quality crop products, in particular, *Galega orientalis* L., as pathogens of phytoplasmosis and bacteriosis can cause significant damage to *Galega orientalis* L. crops.

When studying the effect of mono- and mixed phytoplasma and bacterial infection on *Galega orientalis* L. plants, using different schemes of artificial infection of plants of this legume, we recorded the manifestation of both typical and atypical signs of phytoplasmosis and bacteriosis on artificially infected plants of *Galega orientalis* L. (Table 1).

As presented in Table 1, when *Galega orientalis* L. plants were inoculated with one phytopathogen, in the case of monoinfection, they manifested typical symptoms characteristic of phytoplasmosis or bacteriosis of legumes. At the same time, there were no symptoms of phytoplasmosis or bacteriosis atypical for legumes.

Let's take a closer look at the symptoms detected on the affected plants of *Galega orientalis* L. after inoculation with one of the above phytopathogens (monoinfection).

Thus, when *Galega orientalis* L. plants were affected by *A. laidlawii* var. *granulum* 118 phytoplasma, we recorded the manifestation of such symptoms of phytoplasmosis as anthocyanin coloration of stems and formation of additional shoots from the base of internodes (Table 1, Fig. 1).

Such a typical symptom as anthocyanin coloration of stems in phytoplasma affected *Galega orientalis* L. plants is most often found during the inspection of soybean crops affected by phytoplasmosis (Lee et al., 2011; Mollov et al., 2014).

A typical feature of legumes affected by phytoplasmosis is the formation of additional shoots from the base of internodes, which we observed after inoculation of experimental plants of *Galega orientalis* L. with *A. laidlawii* 178 phytoplasma, which causes tomato trunk (monoinfection) (Table 1, Fig. 2). Such type of lesions as the formation of additional shoots («witch's brooms»), in the case of phytoplasmosis infection, is most often observed in alfalfa and eastern galega crops (Bogoutdinov & Zudilin, 2000; Bogoutdinov, 2013; Hosseini et al., 2013; Donkersley et al., 2018). Around the same time, in 2011, Lava Kumar et al. first reported the detection of plants with symptoms of «witch's brooms» on soybeans in Malawi and Mozambique (Lava Kumar et al., 2011).

*Table 1.* **Manifestation of typical and atypical symptoms of phytoplasmosis and bacteriosis on artificially infected plants of *Galega orientalis* L. under different infection schemes**

| Type of infection | Causative agents | Symptoms of legume damage | |
|---|---|---|---|
| | | Typical | Atypical |
| Monoinfection | *A. laidlawii* var. *granulum* 118 (Fig. 1) | Anthocyanin coloration of stems | Not detected |
| Monoinfection | *A. laidlawii* 178 (Fig. 2) | Formation of additional shoots from the base of internodes | Not detected |
| Mixed phytoplasma-infection (staged inoculation) | *A. laidlawii* var. *granulum* 118, later — *A. laidlawii* 101 and 178 (Fig. 3) | Signs of «witch's brooms» — the formation of many additional shoots from the base of internodes | Shortened distances between internodes, Small leavedness |
| Mixed phytoplasma-infection (simultaneous and staged inoculation) | *A. laidlawii* var. *granulum* 118 and *A. laidlawii* 101, later — *A. laidlawii* var. *granulum* 118 (Fig. 4) | Very branched tops with anthocyanin-colored stems and the same colour of the adventitious shoots | Intense green colour of leaves |
| Monoinfection | *Pseudomonas syringae* pv. *atrofaciens* D 13 (Fig. 5) | Dry light cinnamon necrotic spots of irregular shape with chlorotic border in the central part and along the perimeter of the leaf, burn of the leaf tip | Not detected |
| Mixed phytoplasma-bacterial infection (staged inoculation) | *A. laidlawii* var. *granulum* 118, later — *Pseudomonas syringae* pv. *atrofaciens* D 13 (Fig. 6) | Most of the leaves are of dark anthocyanin color, with highly modified light green apices; formation of numerous additional shoots from the bases of internodes; anthocyanin color of the stems | Shortened internode spacing and small-leavedness |
| Mixed phytoplasma-bacterial infection (simultaneous inoculation with two pathogens) | *A. laidlawii* var. *granulum* 118 and *Pseudomonas syringae* pv. *atrofaciens* D 13 simultaneously (Fig. 7) | No signs typical for legumes were found | Significant stunted growth, thickened stems |

During the examination of *Galega orientalis* L. plants inoculated with the phytopathogenic bacterium *P. syringae* pv. *atrofaciens* D13 (monoinfection), we found the following symptoms: dry light corky necrotic spots of irregular shape with a chlorotic border in the central part and along the perimeter of the leaf and burns of the leaf tips (Table 1, Fig. 5). Such symptoms are typical for this legume affected by bacteriosis (Savenko, 2000).

Thus, in the case of monoinfection, as a result of artificial infection of *Galega orientalis* L. plants with a phytopathogenic mollicute or phytopathogenic bacterium, the manifestations of disease signs characteristic of plant damage by phytoplasmosis or bacteriosis, respectively, were recorded. It should be noted that even in the case of nonspecific infection of *Galega orientalis* L. plants with phytoplasma, symptoms typical of legumes affected by phytoplasma were reproduced.

Significantly more symptoms were recorded during the examination of affected plants of *Galega orientalis* L. in the case of mixed infection with the studied phytopathogens.

Thus, in the case of mixed phytoplasma infection, with the use of staged inoculation (first — *A. laidlawii* var. *granulum* 118, later — *A. laidlawii* 101 and *A. laidlawii* 178), both typical and atypical symptoms for this crop were found during the examination of infected plants of *Galega orientalis* L. (Table 1, Fig. 3). In contrast to monoinfection, in the case of mixed phytoplasma infection with inoculation, *Galega orientalis* L. plants were additionally inoculated with two more strains of phytoplasmas — tomato trunk pathogens *A. laidlawii* 101 and *A. laidlawii* 178 (in addition to *A. laidlawii* var. *granulum* 118) (Table 1, Fig. 3). As a result of such staged inoculation with two phytopathogenic strains of mollicutes — pathogens of the tomato trunk *A. laidlawii* 101 and *A. laidlawii* 178, the infection load on experimental plants of this crop increased accordingly, which led to the appearance of a wider range of symptoms of this disease. Thus, in addition to the symptoms typical for legumes (plants with signs of «witch's brooms» (the formation of many additional shoots from the base of nternodes), atypical symptoms such as shortening of the distance between internodes and small leaves were also recorded.

As follows, in the case of mixed phytoplasma infection, using staged inoculation with two phytopathogenic strains of mollicutes — pathogens of tomato trunk diseases (*A. laidlawii* 101 and *A. laidlawii* 178), the effect of additional infection load on experimental plants was shown. In addition to the symptoms typical for legumes with phytoplasmosis, such as «witch's brooms», a wide range of atypical symptoms was observed on *Galega orientalis* L. plants, namely, shortening of the distance between internodes and small leaves.

A significantly higher degree of damage to *Galega orientalis* L. plants was observed in mixed phytoplasma infection with simultaneous and staged inoculation (first — *A. laidlawii* var. *granulum* 118 and *A. laidlawii* 101, later — *A. laidlawii* var. *granulum* 118) (Table 1, Fig. 4). Manifestations of both typical (very strongly branched tops with anthocyanin-colored stems and additional shoots of the same color) and atypical (leaves of intense green color) symptoms were recorded. The intense green color of the leaves has not been previously described for legumes affected by these phytopathogens. The presence of such a symptom as the intense green color of leaves may indicate a disruption of the photosynthetic process in infected plants.

It should be noted that as a result of the use of this particular infection scheme (mixed phytoplasma infection, simultaneous and staged inoculation), the degree of damage to the experimental plants of *Galega orientalis* L. was the most significant (Table 1, Fig. 4).

The highest degree of damage by phytoplasmas and phytopathogenic bacteria was observed in *Galega orientalis* L. plants in the case of mixed phytoplasma-bacterial infection using staged inoculation of the studied plants: first — *A. laidlawii* var. *granulum* 118, and later — *Pseudomonas syringae* pv. *atrofaciens* D 13 (Table 1,

Fig. 6). When using the above scheme, the largest number of typical symptoms was recorded: brown or dark anthocyanin color of leaves and stems, highly modified light green tops, and the formation of numerous additional shoots from internode bases. A shortened distance between internodes and small leaves were noted among the atypical symptoms (Table 1, Fig. 6).

Interestingly, such an atypical symptom as the shortened distance between the internodes was detected on the affected experimental plants of *Galega orientalis* L. using staged inoculation both in the case of mixed phytoplasma-infection (first — *A. laidlawii* var. *granulum* 118, later — *A. laidlawii* 101 and *A. laidlawii* 178) (Table 1, Fig. 3 ), and in the case of mixed phytoplasma-bacterial infection (initially — *A. laidlawii* var. *granulum* 118, later — *Pseudomonas syringae* pv. *atrofaciens* D 13) (Table 1, Fig. 6). In both cases, at the firsta stage, *Galega orientalis* L. plants were inoculated with phytoplasma of *A. laidlawii* var. *granulum* 118, which is the causative agent of wheat pale green dwarfism (PGD).

It is important to note that such a symptom as the shortened distance between the internodes is atypical for affected legumes and, in particular, for *Galega orientalis* L., but is typical for wheat plants affected by PGD.

In addition, *Galega orientalis* L. plants inoculated with phytoplasma strains isolated from different sources clearly show symptoms of phytoplasmosis, such as small leaves, dwarfism, chlorosis, and witch's brooms (Figs. 1—4, 6, 7; Table 1). Some of the above symptoms, such as witch's broom, are typical for legumes affected by phytoplasma (Figs. 1—4, 6, 7; Table 1). However, symptoms such as dwarfism, small leaves, and chlorosis are all signs of PGD. The appearance of these symptoms may be caused by the inoculation of *Galega orientalis* L. plants with the phytopathogenic phytoplasma of *A. laidlawii* var. *granulum* 118, which is the causative agent of PGD. Although this is a nonspecific infection, all symptoms characteristic of cereals affected by phytoplasmosis (in particular, PGD) were recorded on plants of *G. oryentalis* L. artificially infected with *A. laidlawii* var. *granulum* 118. Thus, all symptoms, both typical and atypical, were reproduced on plants of this crop infected with phytoplasmas during nonspecific infection, which confirms their ability to infect many plant species. This ability of phytoplasma strains from different sources (tomatoes, wheat) to infect *Galega oryentalis* L. with symptoms characteristic of phytoplasmosis indicates a wide phylogenetic specialization of these pathogens.

Thus, under conditions of mixed phytoplasma infection, the symptoms of phytoplasmosis were more intense, and a much wider range of symptoms characteristic of phytoplasmosis was recorded.

We have found that mono- and especially mixed infection with phytoplasmas and phytopathogenic bacteria significantly affects the development of *Galega orientalis* L. plants. At the same time, the types of symptoms are combined to varying degrees, which depends on which pathogen of a particular disease penetrates the plant first. Thus, the most significant damage to *Galega orientalis* L. plants was observed when applying the scheme of mixed phytoplasma-bacterial infection using a staged inoculation, by which experimental plants of this culture were inoculated with the phytopathogenic bacterium *Pseudomonas syringae* pv. *atrofaciens* D13 7 days after inoculation of *Galega orientalis* L. plants with phytoplasma *A. laidlawii* var. *granulum* 118. Due to using such an inoculation scheme, a much wider range of symptoms typical of legumes affected by phytoplasma was recorded compared to the use of the other infection schemes.

It should be noted that in the case of mixed phytoplasma-bacterial infection of *Galega orientalis* L. plants, with the simultaneous introduction of two pathogens ( simultaneous inoculation with two pathogens) — phytoplasma and phytopathogenic bacteria — no typical symptoms characteristic of legumes were found. Among the atypical symptoms, a significant

delay in plant growth and stem thickening were noted (Table 1, Fig. 7).

Thus, it is likely that in the development of the pathological process when two phytopathogens enter the plant, both the aggressiveness of each of them and the duration of their incubation period are important. The pathogen that entered the plant earlier and began to develop actively can displace another pathogen in the future. So, in such cases, the phytopathogen that enters the plant earlier and has a shorter incubation period has an advantage.

Having analyzed the data obtained from the study of the effect of mono- and mixed phytoplasma and bacterial infection on *Galega orientalis* L., it can be concluded that the aggressiveness of the studied strains of phytoplasmas and phytopathogenic bacteria — phytoplasma of *A. laidlawii* var. *granulum* 118 was more aggressive compared to the phytopathogenic bacterium *P. syringae* pv. *atrofaciens* D13 under conditions of artificial infection of *Galega orientalis* L. plants.

Thus, as a result of the studies, it was found that an increase in the infection load on *Galega orientalis* L. plants, as well as the priority of the phytopathogen penetration into plant tissues, leads to a significant inhibition of the development of infected plants of this crop.

It is also important to note that the manifestations of such symptoms as stunted growth and thickened stems of *Galega orientalis* L. plants are atypical not only for legumes affected by phytoplasmosis but also for plants of these crops affected by bacteriosis. The data obtained as a result of these studies using plants of the legume *Galega orientalis* L. infected with two pathogens at the same time — phytoplasma and phytopathogenic bacterium showed quite interesting and unexpected results that require additional research in the future.

**Conclusions.** As a result of the conducted research, both typical and atypical for legumes symptoms of phytoplasmosis and bacteriosis were established on artificially infected *Galega orientalis* L. plants, both in mono- and mixed infection. All symptoms, both typical and atypical, were reproduced on plants of this crop infected with phytoplasmas under nonspecific infection, which confirms their ability to affect many plant species and indicates a wide phylogenetic specialization of these phytopathogens. It has been shown that mono- and especially mixed infections with phytoplasmas and phytopathogenic bacteria significantly affect the development of *Galega orientalis* L. plants.

Thus, as a result of the studies, it was found that an increase in the infectious load on *Galega orientalis* L. plants, as well as the priority of the phytopathogen penetration into plant tissues, leads to significant inhibition of the development of infected plants of this crop.

**Conflicts of Interest.** The authors declare no conflict of interest.

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## ВПЛИВ МОНО- ТА ЗМІШАНОЇ ФІТОПЛАЗМОВОЇ І БАКТЕРІАЛЬНОЇ ІНФЕКЦІЇ НА РОСЛИНИ *GALEGA ORIENTALIS* L.

Останніми роками в регіонах інтенсивного землеробства внаслідок несприятливих агроекологічних умов змішані інфекції стають серйозною загрозою для отримання високоякісної продукції рослинництва, зокрема — *Galega orientalis* L. **Мета.** Дослідити вплив моно- та змішаної фітоплазмової та бактеріальної інфекції на рослини *Galega orientalis* L. **Методи.** Рослини *Galega orientalis* L. інокулювали штамами фітоплазм: *A. laidlawii* var. *granulum* 118 — збудник блідо-зеленої карликовості пшениці; *A. laidlawii* 101 та *A. laidlawii* 178 — збудники стовбуру томатів та фітопатогенна бактерія *Pseudomonas syringae* pv. *atrofaciens* D13 — збудник базального бактеріозу пшениці. У дослідах використовували різні схеми інокуляції рослин *Galega orientalis* L., які застосовували як за моно-, так і за змішаної фітоплазмової та фітоплазмо-бактеріальної інфекції. Симптоми фіксували шляхом візуального огляду, починаючи з 14-ї доби. Контролем слугували неінфіковані рослини тієї ж культури. **Результати.** За моно- та змішаної фітоплазмової інфекції на рослинах *Galega orientalis* L. виявлено як типові (антоціанове забарвлення стебел та утворення додаткових пагонів від основи міжвузля («відьмині мітли»), так і нетипові (дрібне листя та вкорочені міжвузля, характерні для блідо-зеленої карликовості пшениці) ознаки фітоплазмозу. За умов фітоплазмо-бактеріальної інфекції в рослин спостерігали помітне потовщення стебла та затримку росту, тобто були зафіксовані симптоми, які є характерними при розвитку фітоплазмозу, але прояв їх є нетиповим для бобових культур. Отже, за умов фітоплазмо-бактеріальної інфекції було зафіксовано переважну більшість симптомів, характерних для фітоплазмозу блідо-зеленої карликовості пшениці — карликовість, дрібне листя, вкорочені відстані між міжвузлями, які є нетиповими для бобових культур. **Висновки.** Встановлено типові та атипові симптоми фітоплазмозу та бактеріозу для *Galega orientalis* L., за моно- та змішаної інфекції. Моно- і особливо змішана інфекція суттєво впливають на розвиток рослин *Galega orientalis* L., уражених фітоплазмами та фітопатогенними бактеріями. Збільшення інфекційного навантаження на рослини *Galega orientalis* L., а також пріоритетність проникнення патогена в рослинні тканини призводить до значного пригнічення розвитку заражених рослин цієї культури. Отримані дані можуть бути корисними в діагностиці посівів *Galega orientalis* L., уражених фітоплазмозом та бактеріозом.

***Ключові слова:*** *Galega orientalis L., Acholeplasma laidlawii var. granulum 118, Pseudomonas syringae pv. atrofaciens D13, моно- та змішана інфекції, типові та нетипові симптоми.*