Scientific Papers. Series B, Horticulture. Vol. LXVII, No. 1, 2023
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

# ONION STEMPHILIOSIS IN SOUTHERN UKRAINE

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***Abstract***

*Stemphiliosis of onion is a relatively new disease in Ukraine. The disease appears in the form of oblong spots on the leaves and causes premature leaf death. The aim of the work is to propose means of protection for control of onion stemphyliosis based on the study of the pathocenosis species composition, biophenology of the disease agent of onion stemphyliosis and determination of the fungicides efficiency. Field studies were carried out on plantings of onion variety Banco in the farm AF "Petrodolynske" Odessa district, Odessa region of Ukraine in 2019-2021. We identified the causative agents of onion stemphyliosis - Stemphylium vesicarium (Wallr.) Simm, Stemphylium botryosum Wallr, Stemphylium herbarum Simm. which had different frequency of occurrence in the samples. The preparations with the following active substances were found to control the disease most effectively under field conditions: fluopyram (200 g/l) + tebuconazole (200 g/l) and fluoxastrobin (100 g/l) + prothioconazole (100 g/l).*

***Key words**: onion, phytosanitary monitoring, Stemphylium vesicarium, fungicides.*

## INTRODUCTION

Onion (*Allium cepa*) are one of the most important vegetable crops in the world, being the second most valuable after the tomato. Onion yields can be significantly reduced due to many factors, including disease. In recent years, there has been a widespread and increasing harmfulness of onion stemphyliosis or black-gray spot disease. S.M. Stricker et al. (2020) indicate that stemphiliosis, by causing premature leaf dieback, shortening the period of weight gain and ripening, can result in 28-38% loss of onion yield and 74% loss during epiphytotics. M. Hausbeck and B. Werling (2018) note that the disease has been sporadically reported on onion leaves for the past 30 years and was considered a secondary pathogen, but in recent years *Stemphylium vesicarium* has become the dominant pathogen, which appears to have displaced *Alternaria porri*. F. Hay (2018) attributes this to the development of resistance to commonly used fungicides.

The causative agent of Stemphyliosis is the imperfect fungus *Stemphylium vesicarium* (Wallr.) E.G. Simmons, 1969 (teleomorph: *Pleospora alii*). M.E. Miller and H.F. Schwartz (2008) reported that onion stemphiliosis occurs in many regions of the world where onions and garlic are cultivated, but it causes the most problems in warm regions. The main source of the disease is infected plant debris as well as bulbs of infected plants on which the fungus can persist for a long time (Basallote-Ureba et al., 1999). Stemphiliosis reinfestation during onion growing season is carried out by conidia. Conidia can enter onion leaves through stomata (Tayviah, 2017), damage caused by other diseases, insect pests (tobacco thrips, onion flies), herbicides, and wind and hail (Nischwitz, 2016; Du Toit L., 2017). Du Toit L. (2017) stated that onion plants that have been exposed to heat stress become more susceptible to stemphyliosis.

The first signs of the disease on the leaves and leaf sheaths appear as small watery spots that vary in color from light yellow to brown. Increasing in size, the affected areas merge, causing extensive leaf spotting. The central area of the affected areas turns brown to yellowish-brown, then dark olive-brown, and finally black when the fungus spores. Sometimes the fruiting bodies of the fungus, called pseudothecia, may form in the affected tissue as small, black, pinhead-like, raised bodies. The infection usually affects only the leaves and does not spread to the bulbs. The development of stemphiliosis is favored by temperatures between 15 and 25°C, humid conditions, and prolonged periods of leaf wetness lasting 8 hours or more (Suheri and Price, 2000). M.E. Miller and H.F. Schwartz (2008) found that under favorable conditions: temperatures above 18°C, high concentration of conidia in the air, and 24 hours of continuous wet weather, the number of conidia on the leaf surface can exceed 200 pieces per square centimeter. Sporulation usually occurs at the site of initial lesions and is observed 6-14 days after lesion development (Basallote-Ureba et al., 1999). *Stemphylium vesicarium* is generally considered a secondary pathogen that attacks previously damaged tissue; however, severe damage to healthy leaves may be observed during warm weather when leaves are wet for more than 24 hours (Miller and Schwartz, 2008).

Control of stemphyliosis can only be achieved by providing an integrated approach that includes agronomic techniques, biological, chemical and other methods. Agrotechnical methods of controlling stemphyliosis are aimed at reducing the burden of the pathogen on seeds and creating conditions less favorable for infection. Deep tillage after onion harvest promotes decomposition of plant residues and prevents airborne release of ascospores from pseudothecia (Basallote-Ureba et al., 1999; Paibomesai and Celetti, 2012).

Crop rotation with a rotation of 3-4 years reduces the amount of infection in the soil, thereby reducing the incidence of onion stemphiliosis. Cautious use of nitrogen fertilizers is recommended because excessive nitrogen application increases disease severity (Nischwitz, 2016). Du Toit (2017) stated that drip irrigation reduces the risk of stemphiliosis infection by reducing the wetting period of the crop. Other ways to reduce the duration of leaf wetting also include: reducing plant stand density, aligning crop rows with the direction of prevailing winds, and irrigating in the late morning or early afternoon (Paibomesai, Celetti, 2012, Hausbeck 2010). Treatment of plants with fungicides can significantly reduce the degree of disease development, however, in studies C.S. Tayviah (2017) reports that *S. vesicarium* is at risk of developing resistance to fungicides because it has a short asexual reproductive cycle, produces several generations per season, and produces many spores by sexual and asexual reproduction, in addition, multiple applications of fungicides are required during each growing season.

To date, onion stemphiliosis is an understudied disease in Ukraine. In this regard, it is relevant to study the species composition of onion pathocenosis, the isolation and determination of the species identity of the causative agents - stemphiliosis, to determine the effectiveness of modern fungicides used on onions.

## MATERIALS AND METHODS

Field experiments were conducted in 2019-2021 in the farm AF "Petrodolynske", Odessa district, Odessa region of Ukraine, on the plantings of onions of the variety Banko. The culture was sown in the second decade of March. Soil - southern loamy chernozem, humus content 3.67%, pH 6.7. Seeding rate was 11000 seeds/ha. Seeds germinated 20-22 days after sowing. Weed control was performed with Totril 225 EU herbicide according to the recommendations: the first spraying - in the phase of 1-2 leaves, 1.0-1.5 l/ha; the second spraying as weeds grow, 1.0-1.5 l/ha. In the second half of the growing season we conducted two manual weeding operations.

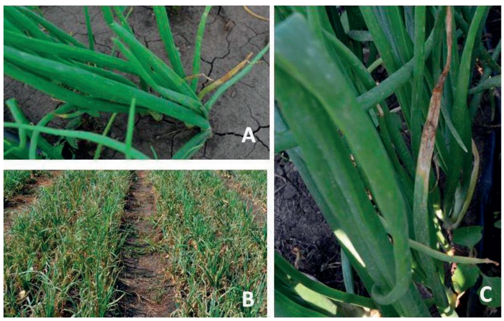


Figure 1. Symptoms of stemphiliosis on onion plants: A - necrotic tips (beginning of the disease); B - general view of affected areas of onion plantings; C - necrotic lesions spreading upwards through the leaves (authors' photos)

To study the species composition of onion mycoflora, we used methods of mycological inoculations on potato-dextrose agar (PDA). Isolates were isolated from leaves of 20-30 bulbs. Small leaf fragments with visible lesions were placed on potato agar and incubated at 22°C for two weeks in a thermostat. After the formation of mycelium, identification of pathogens by morphological features was performed. Identification of phytopathogens was performed according to the methods of V.I. Bilai (1988), N.M. Pidoplychko (1977), N. Shishkoff and J.W. Lorbeer (1989).

Observations of the spread and development of stemphyliosis were carried out dynamically during the growing season, starting from the appearance of the first signs of the diseases. Three leaves on 20 randomly chosen plants from the cutting plot were examined on a scale of 5 points: 0 - no lesions; 1 - up to 10% of surface area affected; 2 - 11 to 25% of surface area affected; 3 - 26 to 50% of surface area affected; 4 - more than 51% of surface area affected.

The disease severity index in all years where the leaves were grouped into classes was calculated as:

$$DSI = \frac{\sum[(class.no)(no.of\ leaves\ in\ each\ class)] \times 100}{(total\ no.leaves\ assessed)(no.classes - 1)}$$

Table 1 shows fungicides and their application rates, which were applied to onions against stemphiliosis. The preparations were applied using a mounted knapsack sprayer with 6 flat jet nozzles, the distance between the nozzles was 50 cm, calibrated for application of 400 l/ha. Experimental plot size is 24 $m^2$, four-quarter repetition. Leaf lesion counts were started with the appearance of the first signs of the disease, followed before each spraying, and 10-15 days after the last treatment. The experimental data were processed using the method of analysis of variance (Little and Hills, 1991).

Table 1. Fungicides, active ingredients, and consumption rates taken to evaluate efficacy in field trials against Stemphiliosis on onions, 2019-2021

| Active ingredient | Trade name | Consumption rates |
|---|---|---|
| Diphenoconazole (250 g/l) | Skor, KE | 0.5 l/ha |
| Azoxystrobin (250 g/l) | Quadris, SC | 1.0 l/ha |
| Praclostrobin (67 g/kg) + boscalid (267 g/kg) | Cygnum, VDG | 1.5 kg/ha |
| Ciprodinil (375 g/l) + fludioxonil (250 g/l) | Switch, VDG | 1.0 kg/ha |
| Fluoxastrobin (100 g/l) + prothioconazole (100 g/l) | Fandango 200 EU, KE | 1.25 l/ha |
| Fluopyram (200 g/l) + tebuconazole (200 g/l) | Luna Expirience 400 SC, KS | 0.75 l/ha |

## RESULTS AND DISCUSSIONS

Phytosanitary monitoring has shown that stemphiliosis in the production conditions of southern Ukraine is very common and harmful disease of onion. The development of onion stemphyliosis is observed in onion fields from about mid-June (BBCH 14-15) until harvesting (Figure 1).

At the first stage of work, we studied the species composition of onion pathogenic mycoflora. For this purpose, we selected isolates and determined their species affiliation. For pathogen diagnosis, we used methods of mycological inoculations on potato dextrose agar (PDA). Isolates were isolated in pure culture according to the conventional methods. After germination of fungi, microscopic preparations were made and analyzed under a light microscope at different magnifications. Fungi were identified by morphological features (morphology of spores, spore carriers, etc.) using phytopathological identifiers.

On the green parts isolated from onion plantations, 8 species of fungi were found, among them various species of *Stemphylium* spp, *Stemphylium herbarum* Simm.), which are considered causative agents of onion stemphyliosis and causative agents of other diseases: *Fusarium oxysporum*, onion head mold (*Aspergillus niger, Penicillium*), false powdery mildew or peronosporosis (*Peronospora destructor*), *Alternaria* spp. The data on the morphological features of fungal mycoflora of onions are summarized and presented in Table 2.

Table 2. Cultural characteristics of fungal mycoflora of onions
(STOV "AF Petrodolynskoe, Odessa district, Odessa region, Ukraine, variety Banko)

| Exciter | Cultural features of the colony | Peculiarities of morphology |
|---|---|---|
| *Stemphylium* spp. | dirty white, light gray color of colonies, later changing to light brown to dark brown with white and brown stripes. | conidia are olive-brown, oval to ovoid, oblong on conidiophores. The conidia range in length from 14.6 µm to 30.6 µm. The conidia vary in width from 4.7 µm to 15.7 µm. |
| *Fusarium oxysporum* | colonies from white to pale lilac, aerial mycelium from almost absent to abundant and fluffy. Abundant macroconidia often form in the central part of the colonies, sometimes producing brownish, blue or purple sclerotia. It often secretes dark purple to dark crimson pigment. | macroconidia spindle-shaped, somewhat curved to almost straight, with 3-5 septa (mainly with 3 septa). Upper cell short, sometimes somewhat hooked, lower cell with stalk or papilla. |
| *Aspergillus niger* Thieg. | The fungus forms a loose mycelium. The conidiose zone is black in color | sterigmas, 20×7 microns, 7×3 microns. Conidia globular, 3 microns assembled in chains. |
| *Peronospora destructor* | The colonies are white to pale gray, the aerial mycelium is abundant and fluffy. | asexual reproduction occurs with the formation of zoosporangia that grow into a conidia-type growth tube. Dichotomously branching conidiophores are formed. Conidia always sprout hyphae. |
| *Alternaria* spp. | gray-purple or pencil-colored velvety plaque of conidial sporulation of the fungus | conidiophores singular 150×3.5 6.5 microns pear-shaped multicellular spores with septa, pencil color, 150×3.5-6.5 microns. |
| *Penicillium* Link. | mycelium yellow-green with yellow-white edging, with fine colorless mottling on the surface | conidiophores smooth, 3-4 microns thick, bearing bilobate tassels. Conidia ellipsoidal, slightly narrowed at the end, 3-4 × 2.5-3.5 microns thick, finely bearded |

Frequency of occurrence in samples (intensity of spread, %) of isolated pathogens on average was: *Stemphylium* spp. - 13.9%, *Fusarium oxysporum* - 20.7%, *Aspergillus niger* - 18.6%, *Penicillium* - 19,4%, *Peronospora destructor* - 9.5%, *Alternaria spp.* - 17.9% (Figure 2).

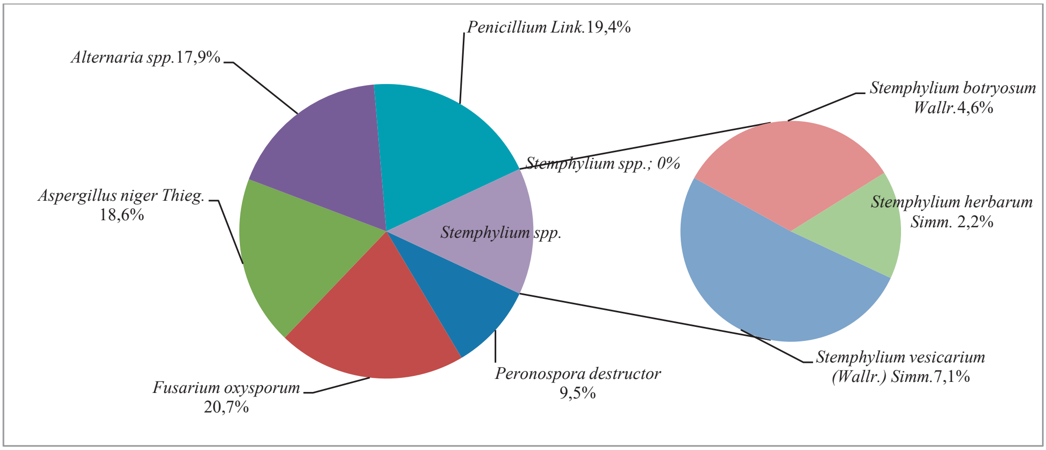


Figure 2. Species composition of fungal mycoflora of onions (% of the number of samples)

Among the eight species of fungi isolated by us, only *Alternaria* and *Stemphylium* are representatives of closely related genera, so they must be identified on the basis of colony culture and conidia morphology (Figure 3).

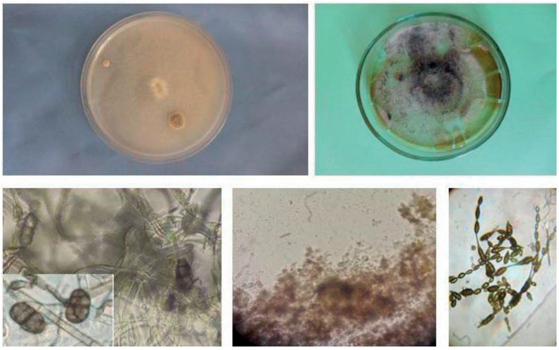

Figure 3. Mycelial colonies on potato dextrose agar (PDA): (A) *Stemphylium* spp.; (B) *Alternaria* spp.; *condyloid* colonies with conidia (C); (D) *Stemphylium* spp.; (E) *Alternaria* spp.

At the second stage, from the spectrum of isolated fungi, we identified the causative agents of onion stemphyliosis. Identification of representatives of the genus *Stemphylium* based on colony culture features and conidia morphology showed the presence of its three representatives in the samples: *Stemphylium vesicarium* (Wallr.) Simm., *Stemphylium botryosum* Wallr., *Stemphylium herbarum* Simm. which are similar in conidia shape, but other characteristics such as size, make them different. Conidia of *S. vesicarium* are oblong or oval with 1-5 transverse septa, sometimes narrowed in the middle, one or three most central of them, and with a complete or nearly complete row of longitudinal septa. The conidia vary in size from 25 to 42 μm in length, 12 to 22 μm in width. *S. botryosum* has spores of subspherical to oblong shape, strongly constricted at the middle septum, 33-35 μm long and N 24-26 μm wide (Shishkoff, and Lorbeer, 1989; Arpita Das et al., 2019). The *S. herbarum* culture was characterized by rapid growth on medium compared to the species described above. The colonies were round in shape, fluffy in texture, and the color of the mycelium ranged from brownish orange to dark brown. The isolated *S. herbarum* produced immature fruiting bodies and many conidia. Spore size range: length 20-30 μm; width 10-15 μm (Lincoln, 2005). The above micromycetes also differed in frequency of occurrence in samples - *Stemphylium vesicarium* (Wallr.) Simm. - 7.1%, *Stemphylium botryosum* Wallr. - 4.6%, *Stemphylium herbarum* Simm. - 2.2%. It is known from the literature that the conditions of the year primarily affect the pathogen, and the degree of this influence depends on its biological characteristics. The maximum lesion of onion with stemphyliosis was noted in 2021 with warm and wet spring and summer periods (Figure 4). However, weather conditions during the growing seasons of 2019 and 2020 were also favorable for disease development (Figure 4). During the 2019 growing season, precipitation was 182.8 mm or 85.4% of the climatic norm for that period (214 mm). The greatest amount of precipitation was observed in the second decade of April (33 mm), the first decade of June (29 mm) and the first decade of August (63 mm). Mean air temperature during the period of tests was 19.6°C, which is 2.4°C above the long-term average (17.2°C). Relative humidity averaged 65%, which was 4% below the multi-year data for this period (69%). Precipitation was 152.0 mm during the growing season in 2020, 29% below the climatic norm for this period. The greatest amount of precipitation was observed in the third decade of May (58.0 mm) and the second decade of June (24.0 mm) in the form of showers. Average air temperature for the period of tests was 18.7°C, which is 1.5°C higher than average multiyear values. Relative humidity averaged 61.0%, which was 8% below the multiyear data for this period. In 2021, precipitation for the growing season was 344.0 mm, 74.0% above the climatic norm for this period. The greatest amount of precipitation was observed in the II decade of May (29.0 mm), I and II decade of June (45.0 and 52 mm) and in the I and III decade of July (39.0 and 60 mm) in the form of showers. Average air temperature during the period of tests was 18.4°C, which is 1.2°C higher than the long-term average. Relative humidity averaged 71.0%, which was 2.0% higher than multiyear data for this period.

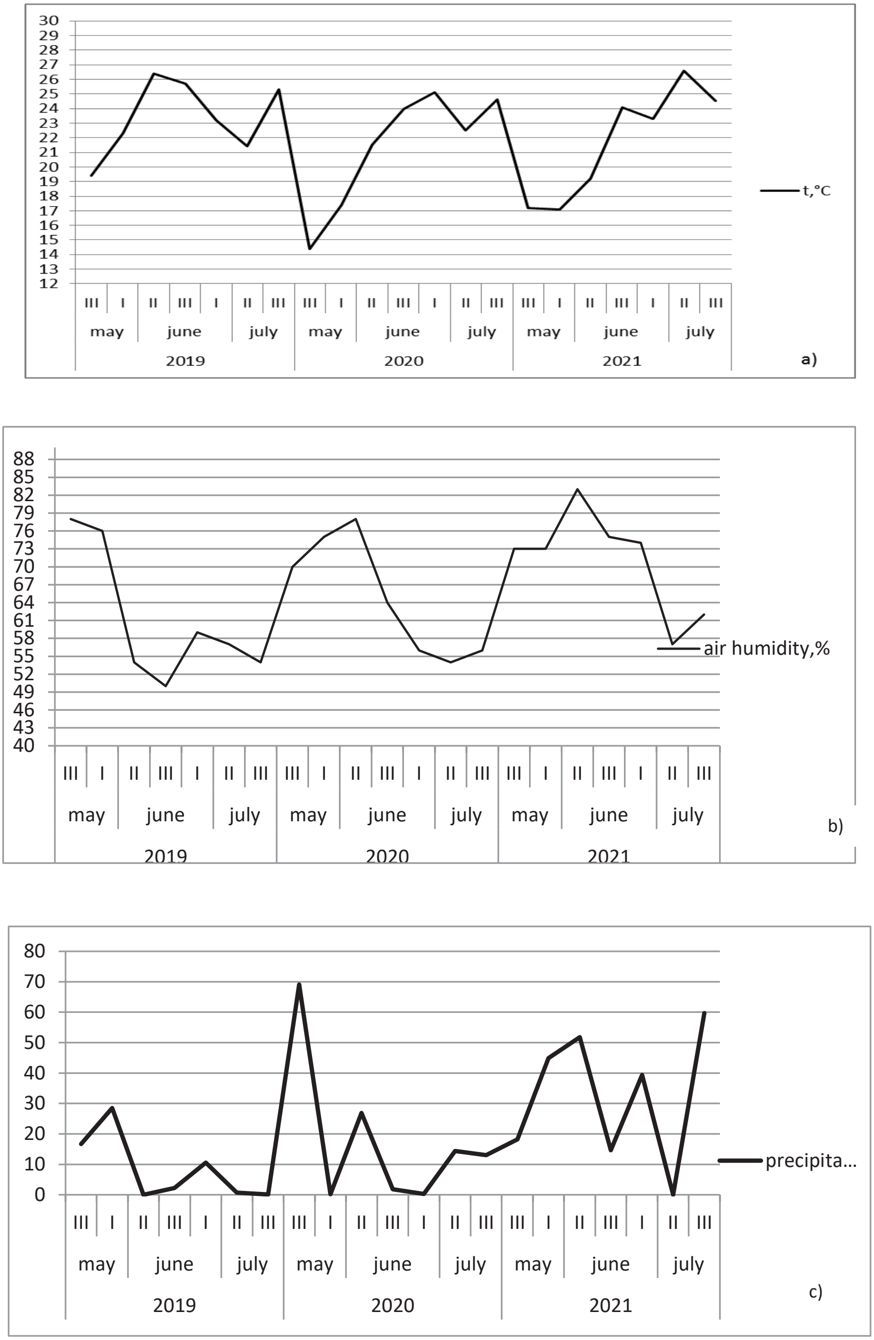


Figure 4. Weather conditions for the growing season of onions, 2019-2021

In onion crops, the disease develops in the following pattern: a low incidence in June (<10%) followed by a rapid increase in July. By late July and early August (during the onion phase of BVSH 43-45), disease incidence can reach 50 to 80% (Figure 5).

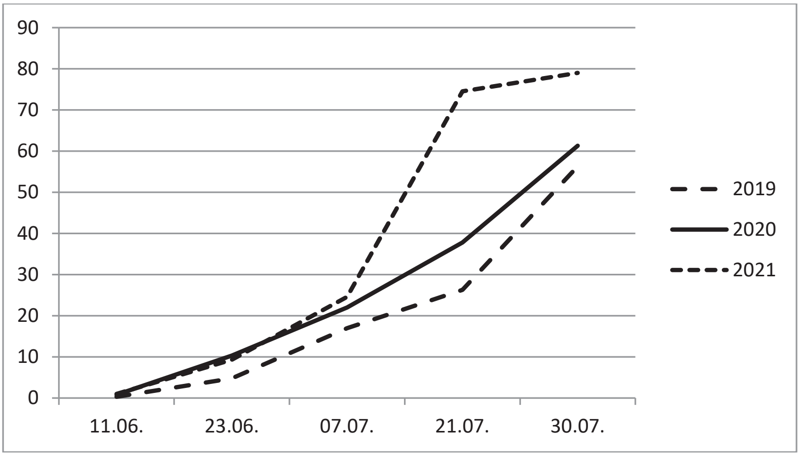


Figure 5. Development of stemphyliosis in onion crops

F. Hay et al. (2021) note that at this time there is a rapid increase in vegetative mass of plants, which contributes to reduction of air movement between plants, increase in its humidity and further development of stemphiliosis. This is usually accompanied by necrosis of the tips and die-off of the outer leaves.

Field studies were conducted in 2019-2021 to determine the most effective fungicides. Five fungicide treatments were conducted throughout the growing season: diphenoconazole (250 g/l), azoxystrobin (250 g/l), pyraclostrobin (67 g/kg) + boscalid (267 g/kg), ciprodinil (375 g/l) + fludioxonil (250 g/l), fluopyram (200 g/l) + tebuconazole (200 g/l) and fluoxastrobin (100 g/l) + protioconazole (100 g/l). The first treatment was given at the detection of the first symptoms which were stated in the second decade of June in the onion BBCH 13-14 phase in the form of single spots, the subsequent treatments were given at an interval of 10-14 days, and the last treatment was given at the WWSN 47 stage (the beginning of feather drop: up to 10% of plants were lodged). Diphenoconazole (250 g/l), azoxystrobin (250 g/l), pyraclostrobin (67 g/kg) + boscalid (267 g/kg), ciprodinil (375 g/l) + fludioxonil (250 g/l) were not effective enough. The fungicides fluopyram (200 g/l) + tebuconazole (200 g/l) and fluoxastrobin (100 g/l) + prothioconazole (100 g/l) controlled the disease most effectively. Their application during the growing season reduced the lesion of onion plants by 67.1-77.1% and 66.8-79.1%, respectively.

The efficacy of fungicides in the third year (2021) of application decreased compared to 2019 and 2020, which may indicate the emergence of resistance to the tested preparations. Therefore, to develop recommendations for control of onion stemphiliosis, it is necessary to continue studies to evaluate the effectiveness of new fungicides in the field in order to warn producers against using ineffective products.

## CONCLUSIONS

Phytosanitary monitoring showed that hemophiliosis in production conditions in the south of Ukraine is a very common and harmful disease of onion. On the control variants the spread of the disease reached at the end of vegetation up to 100.0%, and the intensity of the lesion up to 79.0%.

For the first time in Ukraine, it was found that 8 species of micromycetes belonging to different systematic groups develop on onion. The most frequent are *Peronospora destructor* - 9.5%, *Stemphylium* spp. - 7.1%. The following species dominate by intensity of development: *Fusarium oxysporum* 20.7%, *Aspergillus niger* 18.6%, *Penicillium* 19.4%, *Alternaria* spp. - 17.9%.

From a spectrum of isolated fungi of genus *Stemphylium* spp. we identified species - *Stemphylium vesicarium* (Wallr.) Simm, *Stemphylium botryosum* Wallr, *Stemphylium herbarum* Simm. which had different frequency of occurrence in samples.

It was found that under field conditions, the preparations with the active substances fluopyram (200 g/l) + tebuconazole (200 g/l) and fluoxastrobin (100 g/l) + prothioconazole (100 g/l) controlled the disease most effectively.

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