THE USE OF ARTHROPODS AS TEST OBJECTS FOR THE DEVELOPMENT OF NEW BIOTECHNOLOGIES

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Authors:
K. Holoborodko, O. Seliutina, I. Ivanko, S. Sytnyk, V. Lovynska,
O. Pakhomov, L. Faly, O. Boyko, V. Komlyk, O. Tykhanova, T. Kolombar

Reviewer:
Prof. **Olga Khunah** (Oles Honchar Dnipro National University)
Prof. **Yurii Kulbaschko** (Oles Honchar Dnipro National University)

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The monograph studies the matters of the use of arthropods and mites as objects for the development of new biological technologies. The application of biosensor technologies in assessing the influence of miner caterpillars (Lepidoptera: Gracillariidae) on the process of photosynthesis in woody plants was demonstrated. The results of using insects as test objects (Diptera, Chironomidae) for studying the impact of feed additives on the environment were presented. The results of the development of an environmentally safe and cost-effective alternative to agricultural products fumigation with pesticides were shown.

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THE USE OF BIOSENSOR TECHNOLOGIES TO DETERMINE THE EFFECT OF INVASIVE LEAF-MINING MOTHS ON PHOTOSYNTHESIS OF TREES

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I. Ivanko, S. Sytnyk,
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The use of biosensor technologies to determine the effect of invasive leaf-mining moths on photosynthesis of trees

K. K. Holoborodko [1], O. V. Seliutina [1],
I. A. Ivanko [1], S. A. Sytnyk [2],
V. M. Lovynska [2], O. Y. Pakhomov [1]

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[1] Oles Honchar Dnipro National University, Dnipro, Ukraine
Oles Honchar Dnipro National University, Gagarin av., 72, Dnipro, 49010, Ukraine.
E-mail: goloborodko@ua.fm

[2] Dnipro State Agrarian and Economic University, Dnipro, Ukraine
Dnipro State Agrarian and Economic University, Sergiy Efremov st., 25, Dnipro, 49000, Ukraine.
E-mail: sytnyk.s.a@dsau.dp.ua

1.1. Effect of feeding *Cameraria ohridella* on photosynthesis of *Aesculus hippocastanum* during different generations

According to estimates of the Invasive Species Specialist Group (ISSG) of the World Conservation Union (IUCN) and the European Plant Protection Organization (EPPO), the number of species entered into the novel untypical environment under the influence of direct or indirect human action annually increases (Lopez-Vaamonde et al., 2010). Having adopted, some of these live organisms begin to compete with native species, interfering with the established ecological functions of various ecosystems. Such invasions can often bring irreparable environmental consequences that cause significant biological disturbances in the function of entire ecosystems resulting in significant economic losses to various economic sectors (Kirichenko et al., 2019). Currently, a list of 435 species was made for quarantine organisms (Holoborodko et al., 2016) having different hazard statuses, both environmental and economic, because their life activities annually cause direct economic losses.

The range of potential invasive species that can enter the territory of Ukraine is currently estimated at 1.500 (Holoborodko et al., 2016). Disturbances in the natural functioning of ecosystems caused by the effect of invasive species can cause both direct and indirect risks directly to human health (Inghilesi et al., 2013; Marenkov et al., 2017; Voronkova et al., 2018).

The horse-chestnut leaf miner (*Cameraria ohridella* Deschka & Dimič, 1986) is an invasive phytophage from the family of leaf blotch miner moths (Gracillariidae Stainton, 1854) completely settled Ukraine territory at the beginning of the XXI century (Akimov et al., 2003). *C. ohridella* is trophically associated with horse chestnut (*Aesculus hippocastanum* (Linnaeus, 1753)). By its vital activity, this invasive species threatens the existence of *A. hippocastanum*, inhabiting its trees sometimes by 95 %. Taking into account that horse chestnut is one of the main tree species in populated areas of Ukraine, invasion by this insect species is of increased interest.

Results obtained (Seliutina et al., 2020) demonstrate activation of the enzymatic antioxidant defense system of *A. hippocastanum* in response to the damaging effect of the horse-chestnut leaf miner, which allows the plant to survive and complete the ontogenesis program in unfavorable conditions. The most significant factor in protecting cells from caterpillar activity involves an increase in the activity of guaiacol peroxidase, which indicates an increase in the cell barrier properties (Shupranova et al., 2019).

On the other hand, the effect of *C. ohridella* on the processes of *A. hippocastanum* photosynthesis remains unclear. Photosynthesis is one of the most vulnerable processes to stress factors, so significant information about the state of the photosynthetic apparatus in a plant under the influence of various abiotic and biotic factors can be obtained using fluorescence analysis (Huang et al., 2012; Kargar et al., 2019; Pérez-Bueno et al., 2019).

Chlorophyll fluorescence is an indicator that allows us to study the passage of photochemical reactions in living objects associated with the operation of photosystem II (FSII), the most sensitive to environmental factors. The results of studies on chlorophyll fluorescence intensity contribute to a deeper understanding of the regulatory mechanisms that ensure efficient energy conversion in the primary and subsequent stages of photosynthesis (Kalaji et al., 2017; Chen et al., 2019; da Silva et al., 2020). Our study aimed to establish the features of the influence of vital activity *C. ohridella* on the process of *A. hippocastanum* photosynthesis using the chlorophyll fluorescence induction estimation method.

The study was conducted during the 2019 growing season in Dnipro city (Ukrainian North Steppe subzone). The city is located in the zone of temperate latitudes with a fairly active atmospheric circulation (the predominant movement of air masses from East to West). The territory has temperate-continental climate. Significant fluctuations in weather conditions from year to year is one of the features of the climate in the territory. Moderately wet years alternate with sharply dry ones, and hot dry winds are not uncommon. In general, the climate is characterized by rather cool winters and hot summers.

In 1931, the Botanical Garden at Oles Honchar Dnipro National University (48.430957, 35.040264, 127 m above sea level) was founded on the territory of Dnipro city. A test site was laid on its territory, with 4 trees of the horse chestnut (*Aesculus hippocastanum* Linnaeus, 1753) having similar morphological and tax features (trunk diameter 132–151 cm; height 17–21 m). In general, the edaphotope of the selected test site is represented by degraded ordinary low-humus chernozem (1.0–4.0 %).

To study the effect of caterpillars of *C. ohridella* feeding on photosynthesis processes in *A. hippocastanum* selected leaves of medium formation, we sampled five leaves in illuminated and shaded sides from annual vegetative growth in the lower third of the crown of different exposures in dry weather. Each examined leaf was marked separately. The study was conducted from 17.05.2019 to 13.06.2019, which corresponded to the full development cycle (5-age caterpillars) of the second-generation *C. ohridella.*

Thus, the study elucidated the influence of each age of the second-generation caterpillar. The age of the caterpillar was determined by the visual parameters of the mine. The damage degree of the leaf blades of the horse chestnut by *C. ohridella* was evaluated visually with a scale previously developed by us (Shupranova et al., 2019).

Light intensity measurements were made with RCE-174 luxmeter (PCE Instruments, Germany, 2018). Temperature and relative humidity measurements were made with HE-173 thermohygrometer (HUATO ELECTRONIC CO.LTD, China, 2018).

Portable fluorometer “Floratest” was used for the diagnosis of photosynthetic disorders of native chlorophyll in living *A. hippocastanum* leaf. Portable fluorometer “Floratest” comprises a base unit with a graphic liquid crystal display, control buttons, a remote optoelectronic sensor, connecting cable to the USB port of a personal computer, and a network adapter (Fig. 1.1, see p. 10).

a *b*

Fig. 1.1. Experimental *Aesculus hippocastanum* (Linnaeus, 1753) trees (*a*) and portable fluorometer "Floratest" (*b*)

The remote optoelectronic sensor includes an LED that has a maximum radiation intensity of λ = (470 ± 20) nm. Irradiation indicators in the sensor were the following: irradiation wavelength 470 ± 15 nm; irradiated spot area not less than 15 mm^2; illumination within the spot at least 2.4 W/m^2. Signal reception indicators in an optoelectronic sensor: the spectral range of fluorescence intensity measurement was 670–800 nm; receiving window area 9 mm^2; photodetector sensitivity at λ = 650 Nm was 0.45 A/W.

A light filter at the input of the photodiode amplifier was used to study the intensity of chlorophyll fluorescence that occurs in the red region of the spectrum. In the optoelectronic head (clip), LEDs are mounted, the light from which is directed to one point of the leaf under study, and one photodetector. It is provided that the radiation intensity of light diodes and the sensitivity of the photodetector of the sensor head can be changed during the measurement process.

The internal memory capacity of the device provides long-term storage of information about 40 chlorophyll fluorescence induction curves.

Observations were made on live *A. hippocastanum* leaves. After the start of light exposure, the intensity of chlorophyll fluorescence (fluorescence induction or fluorescence induced (induced) by light) begins to change significantly over time. The time dependence of the chlorophyll fluorescence intensity has the characteristic form of a curve with one or more maximum and is called the chlorophyll fluorescence induction curve (Kautsky curve) (Fig. 1.2).

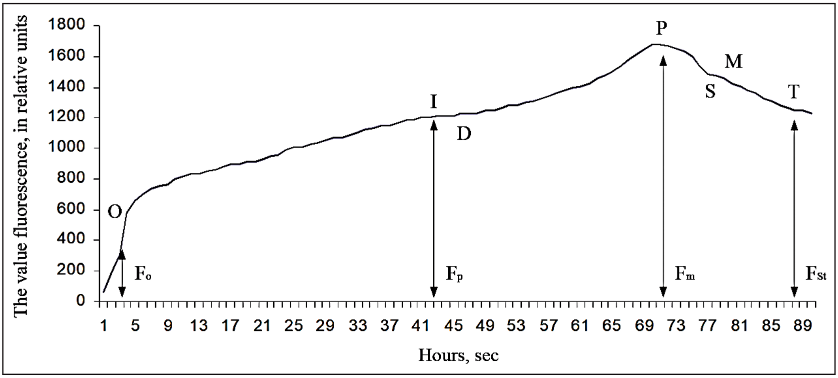

Fig. 1.2. Typical chlorophyll fluorescence induction curve (Kautsky & Hirsch, 1931):

F_0 is the initial value of fluorescence induction after irradiation is turned on; F_p is the "plateau" fluorescence induction value; Fm is the maximum value of fluorescence induction; F_{St} is the stationary value of fluorescence induction after light adaptation of a plant leaf

The shape of this curve is quite sensitive to changes that occur in the photosynthetic apparatus in plants when adapting to different environmental conditions, which has become the basis for the widespread use of the Kautsky effect in the study of photosynthesis.

At the initial time point, all channels of photosynthetic electron transfer are open and the maximum energy of excited electrons goes to the photosynthetic process. During this period, chlorophyll fluorescence is minimal and its intensity on the Kautsky curve is denoted by F_0. The transition from F_0 to F_p is caused by the transfer of electrons from the reaction centers of FS II via pheotine to the primary acceptors (chions). The transition from F_0 to Fp is observed, for example, with a short period of dark adaptation. The entire segment from F_0 to F_m is called the fast fluorescence phase or variable fluorescence. The slow phase of chlorophyll fluorescence induction represents all induction transitions after reaching the peak value by F_m (Gorbunov & Falkowski, 2021). It is known that certain segments of the chlorophyll fluorescence induction curve are indicators of the corresponding physiological processes in the photosynthetic chain (Table 1.1).

Table 1.1

Characteristic segments of the chlorophyll fluorescence induction curve and their diagnostic significance

The CFI segment	Type (attributes) of the segment	Possible time interval, s	Stages of the photosynthetic process that this segment provides information about
Point O	initial segment	0–5	Efficiency of chlorophyll II light collection and reaction centers
O – I – D – P	exit to the main maximum	0.1–10.0 (0.1–1.0)	The electron transport link ((from H_2O to PD (ferrodoxin) and NADP)) is the so-called “Light Stage” of photosynthesis
P – S – M	descending and moving to the second maximum	3.0–50.0 (0.5–10.0)	Activation (via PD) of the Calvin cycle enzyme proteins, establishment of the pH gradient in membranes, reduction of competing acceptors (O_2, NO_2, etc)
M – T	descending and entering stationary mode	10.0–2000.0 (10.0–300.0)	Adjustment of reactions in the Calvin cycle and flows of substances through membranes and through leaf vessels

Therefore, violations of individual links of photosynthesis caused by exo- and endogenous factors are manifested in characteristic changes in the corresponding segments of the chlorophyll fluorescence induction curve.

Features of chlorophyll fluorescence induction depend on the state of the entire photosynthetic system and reflect the kinetics of the flow of all links in the biochemical chain of photosynthesis (Govindjee, 2004). Changes in any part of photosynthesis cause a change in the appearance of the chlorophyll fluorescence induction curve. Therefore, based on the appearance of this curve, it is possible to diagnose the current state of the photosynthetic apparatus in the plant, evaluate changes in the photosynthesis efficiency with changes in the light regime, temperature, humidity, and other factors.

The microclimatic parameters in which the experimental tree is located affect the vital activity of all ages of *C. ohridella* caterpillars (Fig. 1.3, see p, 14). The temperature regime and humidity regime significantly affect the features of induction changes in chlorophyll fluorescence that occur under the influence of *C. ohridella* caterpillar feeding. The difference in such impact is significant both for different generations of *C. ohridella* and for different positions of experimental leaves in the crown (shaded-illuminated parts). There was no significant effect of leaf lighting conditions on caterpillar feeding (Fig. 1.3, *b*).

During the caterpillar development (5 generations), there is a gradual decrease in the values of all the main indicators (F_o, F_m, F_p, and F_{St}) of the chlorophyll fluorescence induction curve (Kautsky curve) (Fig. 1.4, see p, 15) of damaged *A. hippocastanum* leaves. A significant difference in the influence of different caterpillar ages and leaf crown position was established for all the main indicators of the chlorophyll fluorescence induction curve. The only exception is the initial value of fluorescence induction (F_o), for which no such dependence has been established (Fig. 1.4, *a*).

The calculated parameters as variable chlorophyll fluorescence ($F_v = F_m - F_0$) and the maximum efficiency of primary photosynthesis processes ($E_f = F_v/F_m$) reliably depend on the leaf position on which the mine is located (Fig. 1.5, see p, 16). We can see a significant difference between the shadow part and the illuminated part

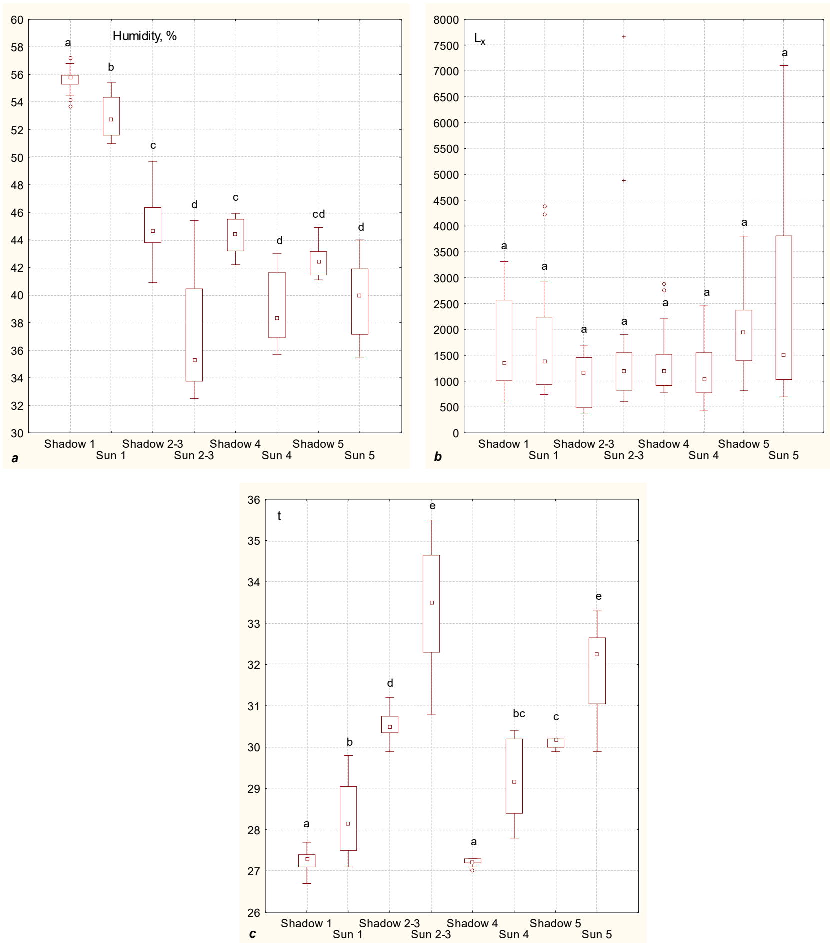

Fig. 1.3. Influence of *Cameraria ohridella* Deschka & Dimič, 1986 caterpillars on the process of *Aesculus hippocastanum* (Linnaeus, 1753) photosynthesis depending on microclimatic factors: *a* – humidity; *b* – light intensity; *c* – temperature

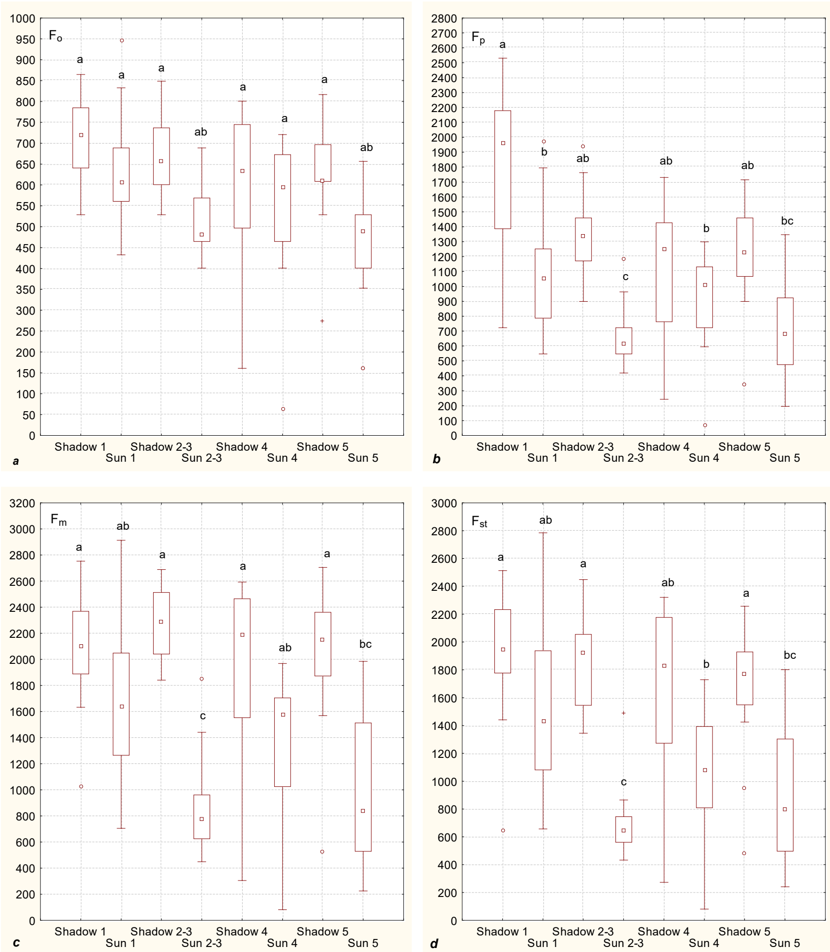

Fig. 1.4. Variability of indicators of the chlorophyll fluorescence induction curve (Kautsky curve) in *Aesculus hippocastanum* (Linnaeus, 1753) leaves damaged by *Cameraria ohridella* Deschka & Dimič, 1986:

F_0 – the initial value of fluorescence induction after switching on irradiation; F_p – the value of fluorescence induction “plateau”; F_m – the maximum value of fluorescence induction; F_{St} – the steady value of fluorescence induction after light adaptation of the leaf of the plant

(Fig. 1.5, *b*, *c*). The effect of different caterpillar generations on the efficiency of photochemical energy conversion in FS II $(F_m - F_{St})/F_{St}$) has not been established (Fig. 1.5, *d*). We see only the difference for the I and V generations in the position of the leaf in the crown.

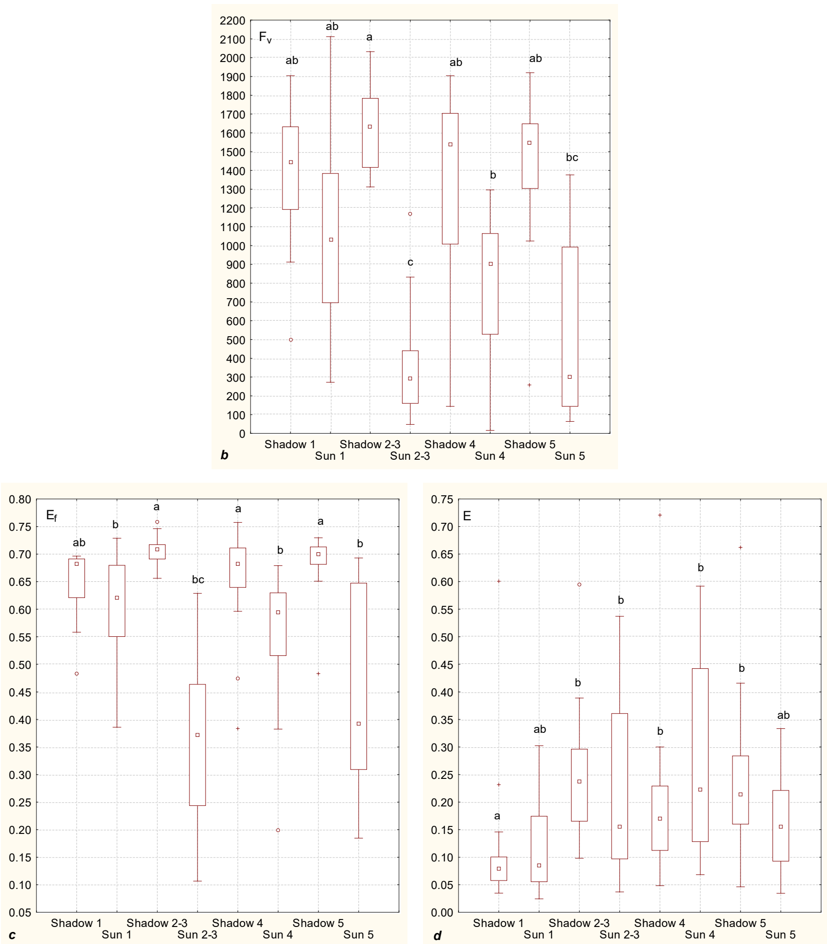

Fig. 1.5. Critical parameters of chlorophyll fluorescence induction in *Aesculus hippocastanum* leaves (Linnaeus, 1753) damaged by *Cameraria ohridella* Deschka & Dimič, 1986

Under the conditions of caterpillar feeding, the time dependence of the chlorophyll fluorescence intensity had the characteristic form of a curve with one maximum, graphically reflecting the Kautsky effect. The shape of the Kautsky curves shows that background fluorescence under the influence of caterpillar feeding increased in the leaves of the shaded part of the crown compared to the illuminated part. This indicates an increase in the amount of chlorophyll not involved in photosynthetic energy transfer to reaction centers in damaged leaves.

Changes in the fluorescence intensity of chlorophyll FSI reflect oxidative processes in the reaction center of this photosystem. The absorbed energy of light quanta can be transferred in three ways: photochemical reactions, heat dissipation, and light emission as chlorophyll fluorescence. These processes compete with each other in such a way that the efficiency increasing of one results in the suppression of the other two. Therefore, the fluorescence intensity is sensitive to changes in the intensity of photochemical processes and thermal dissipation (Essemine et al., 2012; Rühle et al., 2018). An increase in temperature leads to the appearance of thermoinduction of chlorophyll at certain temperature values. The method for determining the parameters of the chlorophyll photosynthesis phenomenon in plant leaves (Kautsky curve) ensures the independence of measurement results from the temperature of the plant leaf, which leads to the appearance of thermoinduction of chlorophyll at certain temperature values and to an increase in accuracy (Starodub et al, 2015; Kalaji et al., 2016; Chen et al., 2018).

Under normal conditions, the level of fluorescence is insignificant (1–2 % of the total absorbed light), which indicates the active use of absorbed light energy by cells. After lightening the leaves of plants adapted to the dark, first for a few seconds, there is a sharp increase in the intensity of chlorophyll fluorescence as a fast phase. Then within a few minutes, there is a gradual decrease through certain stages from the slow phase to the stationary level of F_{St} (Martinazzo et al., 2012; Ruban, 2016).

The chart of changes in fluorescence from the beginning of illumination to reaching a stationary level consists of information about the state of the photosynthetic apparatus of the plant leaf. Changes O–I–D–P are called the first wave or rapid induction of fluorescence. It occurs in 1–3 seconds, depending on the illumination intensity and other factors, and is observed both in living objects and isolated chloroplasts. Slower changes in P–S–M–T are known as second-wave or slow induction of fluorescence. These changes occur over a period of several tens of seconds to several minutes, depending on the object and conditions of the experiment.

At the initial point in time, all channels of photosynthetic electron transfer are open, and the maximum energy of electron excitation goes to the photosynthetic process. During this period, chlorophyll fluorescence is minimal, and its intensity on the Kautsky curve is indicated by the letter F with the “0” index, *i. e.* F_0. Background fluorescence index (F_0) characterizes the inactive chlorophyll amount that does not have a functional connection with the reaction centers; that is, it acts as the initial level of induction of chlorophyll fluorescence. It depends on the loss of excitation energy during migration along with the pigment matrix.

Thus, during active photosynthesis, when all the reaction centers are in an open working process, almost all the absorbed sunlight energy is used, and only a small amount of it (about 3 %) is converted into light energy in the form of background fluorescence (F_0). As a rule, the F_0 index under normal conditions is low due to the active use of absorbed light energy by cells. The influence of *C. ohridella* feeding or other unfavorable conditions disrupts the state of photosynthetic membranes, and the reaction centers become inactive, stopping the flow of electrons. At the same time, the absorbed light energy can no longer be used in photosynthesis, and therefore chlorophyll fluorescence increases (Fig. 1.4).

F_o–F_p transition is associated with a decrease in electron transport. It characterizes the thermal adaptation period of the leaf. If the adaptation period is short, the transition is steeper, but it is slower if the adaptation period is large. The F_m parameter indicates the highest level of fluorescence, which is recorded on the induction curve as the maximum. The entire F_o–F_m area is called the fast fluorescence phase. The slow phase of chlorophyll fluorescence induction represents all induction transitions after reaching the maximum value (peak) of P. The steady-state level of fluorescence (F_{St}) is characterized by a dynamic equilibrium between the processes that cause an increase in fluorescence and the processes that lead to its decrease. As shown in Figure 1.4, there is a significant difference in the value of all key parameters of chlorophyll fluorescence induction. We have shown that with the caterpillar development (during all 5 of its generations), a gradual decrease in the values of all the main indicators (F_o, F_m, F_p та F_{St}) is observed, which affects the general physiological state of the leaf on which *C. ohridella* mine is located.

To characterize the photosynthetic apparatus under stress at the leaf level during quick stage, the ratio of variable fluorescence F_v to the maximum F_m level is often used, which is considered an indicator of potential photosynthetic activity in the leaf (Starychenko et al., 2016; Tseng, Chu, 2017; Scognamiglio et al., 2019). Fluorescence variability (F_v) is calculated as the difference between F_m and F_o values and it is a physiological indicator that reflects the influence of environmental and experimental factors on the plant (Alonso et al., 2017; Chitband et al., 2017; Huliaieva et al., 2018).

However, this parameter has some limitations since it applies exclusively to primary photochemical processes in PSII and only during the first 100–500 ms. The effect of any adverse factor (in our case, this is possible damage caused by *C. ohridella*) reduces the attragative ability of the Calvin cycle, which stops the flow of electrons, and the reaction centers go into an inactive (closed) state. At the same time, photosynthesis can no longer use the absorbed light energy, and therefore chlorophyll fluorescence increases over the entire time range of recording its induction changes (Duarte et al., 2017; Rühle et al., 2018).

The F_v/F_m index depends on the efficiency of photochemical reactions of FSII. In dark-adapted plants, it reflects the potential quantum efficiency of FSII. This indicator is used as an indicator of photosynthetic productivity. Its optimal value for most plant species under conditions of saturating intensity of exciting light does not exceed 0.83 (Martinazzo et al., 2012).

It was found that an increase in the ambient temperature relative to the optimal one for this plant species leads to a decrease in the difference $F_v = (F_m - F_0)$. The reason is a decrease in the electron transport chain activity or the light stage of photosynthesis. When the temperature rises to the level of destruction (45…50 °C), the level of F_0 intensity increases markedly. In the breeding process, plant varieties resistant to high temperatures were quickly selected based on these indicators (Essemine et al., 2012, Martinazzo et al., 2012). Reducing the ambient temperature to the optimal level for this plant species also leads to a decrease in the value of $F_v = (F_m - F_0)$, which is caused by inhibition of the photochemical activity of FSII.

The ratio $(F_m - F_0)/F_v$ increases. Based on these characteristics, cold-resistant plants can be selected. Water deficiency leads to a decrease in the $F_m - F_0$ difference proportionally to the decrease in the water potential of the leaf. This indicator can be used to select drought-tolerant plant specimens (Chen et al., 2019). To estimate the fluorescence induction of chlorophyll-bearing tissues, the calculated parameter is used as the chlorophyll fluorescence variable (F_v), which is expressed as the difference between the highest level of fluorescence and background fluorescence $F_v = F_m - F_0$ giving information about the amplitude of changes in the Kautsky curve. In Fig. 1.5, *b*, we see a significant difference in the influence of the leaf position in the crown.

The maximum efficiency of primary photosynthesis processes resulting from the physiological state of the plant is characterized by the parameter $E_f = F_v/F_m$. The efficiency of photosynthesis, as well as a mentioned parameter, depends on the intensity of exposure to abiotic factors. The highest integrated indicator that characterizes the effective structure of the organization of the FS II pigment system is the F_v/F_m coefficient.

The F_m parameter characterizes the highest level of chlorophyll fluorescence, which is recorded as the maximum on the induction curve. At this point, photosynthesis corresponds to the minimum level, and its value depends on the dynamic balance between the processes of fluorescence, photochemistry, and thermal dissipation. The obtained data demonstrate a decrease in the quantum efficiency of FS II (photosynthetic activity inhibition) in the leaves of the illuminated part of the crown, which indicates a destructive effect of *C. ohridella* caterpillar feeding on the photosynthetic apparatus of *A. hippocastanum* (Fig. 1.5, *c*).

The efficiency of photochemical energy conversion in FS II is calculated by the Formula $E=(F_m-F_{St})/F_{St}$, which characterizes the rate of linear electron transport and is an integrated indicator of the photosynthetic process. The ratio $((F_m-F_{St})/F_{St}$ is called the plant viability Index, where F_m is the maximum fluorescence level, and F_{St} is the steady level. Study of the feeding effect of *C. ohridella* caterpillars on the efficiency of the main enzyme of the Calvin cycle, which closely correlates with the fluorescence induction coefficient, which characterizes the efficiency of dark photosynthetic processes, demonstrated a significant decrease in its activity in the leaves of both the illuminated and shaded parts of the crown (Fig. 1.5, *d*) for I and V generations.

One of the problems of comparing the parameters of the Kautsky curve for plants of both the same and different species is that the above time intervals can differ significantly for different plant species and also for different leaves of the same plant that have different ages or adapted to different lighting conditions (for example, grow at the roots or on the crown, on old branches or on young shoots, well-illuminated or always darkened).

As the stress factor increases, indicators of the fluorescence phenomenon, such as the “viability Index”, as well as the intensity of photosynthesis, usually decrease. The possibility of estimating the state of plants by changing the shape of the Kautsky curve has been confirmed experimentally (Elahifard et al., 2013; Duarte et al., 2017; Kyrychenko et al., 2019). The current limitations are because today there are no samples of the Kautsky curve for different plant species under normal conditions of their existence and for different ages. This means no "measure" by which it is possible to compare Kautsky curves under other conditions of plant existence.

Feeding of *C. ohridella* caterpillars causes a decrease in the photosynthetic apparatus activity of *A. hippocastanum* regardless of the spatial arrangement of leaves in the tree's crown. In *A. hippocastanum* leaves under the influence of *C. ohridella* feeding, the amount of chlorophyll not involved in photosynthetic energy transfer to reaction centers increases.

Conversely, during the development of only one generation of *C. ohridella* observed a constant decrease in the quantum efficiency of FS II (inhibition of photosynthetic activity). Already at the beginning of the development of the mine (the first-age *C. ohridella* caterpillars) on the leaves in the illuminated and shaded part of the crown, a significant decrease in activity and an increase in the plateau coefficient were observed, which in turn indicates inhibition of photophysical and photochemical processes of photosynthesis and a reduction in the pool of electron acceptors in the electron transport chain.

Such pathological changes were caused by a decrease in the content of active chlorophyll (a component of pigment-protein complexes FS II) and its destruction. The values of the key parameters of chlorophyll fluorescence induction indicate a significant inhibition of photosynthesis processes and a violation of the coherence of Calvin cycle reactions. The consequence of the photosynthetic apparatus damage in plants is a decrease in the content of photosynthetic pigments since these metabolic transformations are determined by local changes in the structure and functions of chloroplasts.

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1.2. The Impact of *Cameraria ohridella* on the State of *Aesculus hippocastanum* Photosynthetic Apparatus in the Urban Environment (Dnipro city)

The environment of an industrial city encompasses a large number of stressors for plants that inhibit their development and vital activity (Blum, 2017; Kosova et al., 2018; Zandalinas et al., 2018). Among the dominant stress factors that affect living organisms in urban conditions, effects of climate changes associated with rising air temperatures and drought occurrence, pesticide contamination from surrounding agrocenoses, motor transport and industrial enterprises emissions are of particular interest (Langraf et al., 2018; Langraf et al., 2019). Harmful effects of pollutants can manifest themselves in various functional changes in trees, including their interactions with lepidopteran phytophages and predators that feed on these phytophages.

In an urban environment, disruption of biochemical reactions, physiological functions, morphostructure, and reduction of resistance to pests and diseases are observed in woody plants depending on concentration of the toxic substances and duration of their exposure (Shupranova et al., 2019; Seliutina et al., 2020); it worsens the living state of plants and sometimes causes their premature death (Sett, 2017; Dimitrijevic et al., 2019). Diagnostics of the influence of environmental factors for the purpose of rapid assessment of plant functional state requires the use of express and informative techniques that would allow conducting analysis both in the laboratory and in the field conditions with minimal violation of the studied object integrity.

Such techniques include the method of chlorophyll fluorescence induction widely applied in modern studies of photosynthetic processes (Martínez-Ferri et al., 2016; Urban et al., 2017; Wan et al., 2020; Berner et al., 2021; Holoborodko et al., 2021).

Photosynthesis is one of the processes most vulnerable to stressors, so valuable information on the state of the photosynthetic apparatus in a plant under the impact of phytophage feeding can be obtained by fluorescence analysis (Baghbani et al., 2019; Koski et al., 2017). The influence of many different urbanized factors on the functional state of woody plant leaves resulted in adaptive changes of plants accompanied by certain morphological changes in the assimilation apparatus, as well as a shift in seasonal developmental rhythms (Uhrin et al., 2018; Cheng et al., 2019).

It is known that certain sections of the chlorophyll fluorescence induction curve may be used as indicators of the corresponding physiological processes in the photosynthetic chain. Violations of its particular components caused by exo- and endogenous factors show themselves in specific changes in the corresponding sections of the curve. The photosynthetic apparatus in plants was characterized using the method in many woody plants growing in an urban environment due to its close relationship with chlorophyll fluorescence intensity (Li et al., 2017; Lin et al., 2019; Castillo-Campohermoso et al., 2020). White oak (*Quercus alba* L.) compared to red maple (*Acer rubrum* L.) shows more significant differences in chlorophyll fluorescence parameters under megalopolis conditions compared to these species growing in native forest conditions (Sonti et al., 2020). The results of Uhrin & Supuka (2016) confirmed that the F_v/F_m (maximum efficiency of primary photosynthesis processes) parameter proved to be an effective tool for measuring the growth response of roadside sycamore maple (*Acer pseudoplatanus* L.) in the transformed urban environment. Analysis of the F_v/F_m, Frd (fluorescence reduction coefficient, which characterizes the quantum efficiency of photosynthesis or the viability index), and PCII (stability of light-harvesting complexes photosystem II to the influence of different strains of pathogens) parameters allowed assessing the adaptation potential of wild pear (*Pyrus pyraster* L.) and European mountain ash (*Sorbus domestica* L.) trees to water deficiency (Šajbidorová et al., 2015).

Results obtained demonstrate the possibility to use changes in certain sections of the chlorophyll fluorescence induction curve to detect deterioration in the life state of Moreton Bay fig (*Ficus macrophylla* Pers.), london plane (*Platanus × acerifolia* (Aiton)

Willd.), Chinese elm (*Ulmus parvifolia* Jacq.) in drought conditions (Sepúlveda, Johnstone, 2019), and to assess the resistance of horse chestnut (*Aesculus hippocastanum* L.), small-leaved lime (*Tilia cordata* Mill.), and European white birch (*Betula pendula* Roth.) trees to soil salinization due to the use of salt as deicing agent (Swoczyna, Latocha, 2020).

It was found that the chlorophyll fluorescence F_v/F_m parameter of eucalyptus (*Eucalyptus saligna* Sm.) leaves have statistically significant association with wood density and the amount of wood decomposition in summer period (Johnstone et al., 2014). The effect of heavy trimming of roadside small-leaved lime trees (*Tilia cordata* Mill.) on the photosynthesis process was investigated compared to neighboring non-trimmed trees (Suchocka et al., 2021).

Changes in chlorophyll fluorescence parameters may indicate the effect of phytophagous insects on the plant photosynthetic apparatus (Carvalho de Almeida et al., 2018; Ullah et al., 2020; Moustaka et al., 2021). For example, it has been demonstrated a close relationship between the level of damage of cork oak (*Quercus suber* L.) and holm oak (*Quercus ilex* L.) trees by the great capricorn beetle and chlorophyll content in leaves depending on the age of the phytophage (Cárdenas, Gallardo, 2016). The effect of different residential densities of *Coccus hesperidum* L. (Hemiptera, Coccidae) per leaf on the plant pigments concentration (chlorophyll a, chlorophyll b, and carotenoids) and chlorophyll fluorescence parameters (maximum quantum yield of photosystem II F_v/F_m, the effectiveness of "open" reaction enters (RC) in the light Fv/Fm, and coefficient of non-photochemical of chlorophyll QN and coefficient of photochemical quenching of chlorophyll QP) was studied in lemon plants (*Citrus limon var. ponderosa* L.) and ferns (*Nephrolepis biserrata* (Swartz) Schott.). The effect of the degree of infestation with *C. hesperidum* on the pigments loss in plants and changes in the photosynthetic productivity of host plants was characterized (Golan et al., 2015).

The objective of our research was to establish the peculiarities of the effect of *Cameraria ohridella* Deschka & Dimić, 1986

(Lepidoptera, Gracillariidae) vital activity on the photosynthesis process of Aesculus hippocastanum in various urban environments.

The research was conducted during the 2019 growing season in Dnipro city (Ukrainian North Steppe subzone). The city is situated in temperate zone with a relatively active atmospheric circulation (the atmospheric circulation is predominantly from east to west). The climate is temperate continental (Seliutina et al., 2020). One of the climate features in the territory is the wide fluctuations in weather conditions from year to year. Moderately wet years alternate with sharply dry ones, and hot dry winds occurrs fairly common. In general, the climate is characterized by rather cool winters and hot summers.

Within Dnipro city territory, we selected eight park ecosystems (Fig. 1.6 (see p. 31), Table 1.2 (see p. 32)) that have different conditions of horse chestnut growth (Table 1.3, see p. 33–34). Four trees of *Ae. hippocastanum* were selected on the territory of each park area with similar morphological and taxational characteristics (trunk diameter 132–151 cm; height 17–21 m).

The research was conducted on June 14, 2019, which corresponded to the development of the 5th-age caterpillars of *C. ohridella* second generation, which begins to give an irruption in this generation in the conditions of Dnipro city. The age of the caterpillars was determined with the visual parameters of the mines made by their. The damage degree of the horse chestnut leaf blades by *C. ohridella* was assessed visually with a previously self-developed scale. Light intensity measurements were conducted with RCE-174 luxometer (PCE Instruments, Germany, 2018). Temperature and relative humidity measurements were conducted with HE-173 thermohygrometer (Huato Electronic Co.LTD, China, 2018).

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blades by *C. ohridella* was assessed visually with a previously self-developed scale. Light intensity measurements were conducted with RCE-174 luxometer (PCE Instruments, Germany, 2018). Temperature and relative humidity measurements were conducted with HE-173 thermohygrometer (Huato Electronic Co.LTD, China, 2018).

Fig. 1.6. Map of the sampling units within the territory of Dnipro city (Ukraine) To study the effect of *C. ohridella* caterpillars feeding on the photosynthesis processes in *Ae. hippocastanum* plants, leaves of medium formation were selected at 5 pcs. from the illuminated crown exposition (which was mostly infected by a miner)

Portable fluorometer “Floratest” was used for the diagnosis of native chlorophyll disorders in fresh *Ae. hippocastanum* leaves. Portable fluorometer “Floratest” comprises a base unit with a graphic liquid crystal display, control buttons, a remote optoelectronic sensor, connecting cable to the USB port of a personal computer, and a network adapter.

Table 1.2

Characteristics of research areas

No.	Name	Park coordinates	Altitude above sea level, m
1	Botanical Garden of DNU	48°26'14" N, 35°02'35" E	127
2	Novokodatskyi Park	48°29'08" N, 34°56'42" E	82
3	Taras G. Shevchenko Park	48°27'48" N, 35°04'23" E	83
4	Pridneprovsky Park	48°23'59" N, 35°07'59" E	75
5	Metallurgists Square	48°28'26" N, 34°59'31" E	65
6	Lazaria Hloby Park	48°28'11" N, 35°01'48" E	56
7	Druzhby narodiv Forest Park	48°32'02" N, 35°05'42" E	65
8	Park Sahaydak	48°29'13" N, 35°03'41" E	50

The remote optoelectronic sensor includes an LED that has a maximum radiation intensity of $\lambda = 470 \pm 20$ nm. Irradiation indicators in the sensor were the following: irradiation wavelength 470 ± 15 nm; irradiated spot area not less than 15 mm^2; light intensity within the spot at least 2.4 W/m^2. Signal reception indicators in an optoelectronic sensor: the spectral range of fluorescence intensity measurement was 670–800 nm; receiving window area 9 mm^2; photodetector sensitivity at $\lambda = 650$ nm was 0.45 A/W.

Observations were made using fresh *Ae. hippocastanum* leaves. After the start of light exposure, the intensity of chlorophyll fluorescence (fluorescence induction or fluorescence induced (caused) by light) begins to change significantly over time. The time dependence of the chlorophyll fluorescence intensity has the characteristic form of a curve having one or more maximum, and it is called the chlorophyll fluorescence induction curve (the Kautsky curve).

The shape of this curve is quite sensitive to changes that occurred in the photosynthetic apparatus in plants when adapting to different environmental conditions, which has become the basis for the widespread use of the Kautsky effect in the study of photosynthesis. To interpretation the Kautsky curve (Kautsky, Hirsch, 1931), we used its known critical parameters: F_0 means the initial value of fluorescence induction after irradiation is turned on; F_p means the

Table 1.3

Characteristics of the location of parks within the gradient of landscape and soil-climatic conditions

No.	Name	Relief part (floodplain, sandy terrace, third terrace, ravine, watershed)	Mechanical composition of soil (sand, sandy loam, loam, clay)	Soil humus content, %	Slope exposure and steepness (for example, 3 % northwest-facing slope)	Park area, ha	Degree and predominant type of anthropogenic impact (for example, moderate recreation, heavy aerogenic pollution)	Share of a target tree of all the trees in the park, %
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	*Botanical Garden of DNU*	Watershed, upper third of ravine	loam	2.3–5.7	7° northeast-facing slope	46	moderate recreation, moderate aerogenic motor vehicle pollution	<1
2	Novokodatskyi Park	floodplane	loam	3.1–3.8	various-exposured slopes, 2–7°	35	moderate recreation, moderate aerogenic motor vehicle pollution, heavy aerogenic industrial pollution	1.4
3	*Taras G. Shevchenko Park*	upland with access to watershed	loam	3.2–4.8	15° northwest-facing	57	moderate recreation, moderate aerogenic industrial pollution	16
4	Pridneprovsky Park	sandy terrace	sandy loam	0.9–1.4	–	7	moderate recreation, heavy aerogenic industrial pollution	12

End of Table 1.3

1	2	3	4	5	6	7	8	9
5	Metallurgists Square	watershed	loam	2.8–3.4	–	3.8	moderate recreation, moderate aerogenic motor vehicle pollution, moderate aerogenic industrial pollution	8
6	Lazaria Hloby Park	floodplane	loam	3.3–4.7	–	26	moderate recreation, moderate aerogenic motor vehicle pollution, mild aerogenic industrial pollution	<1
7	Druzhby narodiv Forest Park	third terrace	loam	2.2–4.6	–	90	moderate recreation, moderate aerogenic motor vehicle pollution	<1
8	Park Sahaydak	floodplane	Sandy loam (filling artificial soils)	0.7–1.6	–	34	moderate recreation, moderate aerogenic motor vehicle pollution, moderate aerogenic industrial pollution	<1

value of "plateau" fluorescence induction; Fm means the maximum value of fluorescence induction; F_{St} means the stationary value of fluorescence induction after light adaptation of a plant leaf In addition to the critical parameters of the Kautsky curve, we used calculated parameters as variable chlorophyll fluorescence ($F_v = F_m - F_0$); maximum efficiency of primary photosynthesis processes ($E_f = F_v / F_m$), and coefficient of photochemical processes efficiency ($E = (F_m - F_{St}) / F_{St}$).

The data were analyzed using Statistica 8.0 program (StatSoft Inc., USA). The Table 1.4 demonstrate the results as $x \pm SD$ (mean ± standard deviation). Differences between the values of the experimental groups were determined using the Tukey test, where the differences were considered significant at $P < 0.05$. In the Figures small square is median, the upper and lower line of the rectangle is 75 % and 25 % quartiles, the upper line is minimum and maximum values, circles and asterisks is outliers.

Table 1.4

Average damage level of *Ae. hippocastanum* leaf blades by *C. ohridella* miner ($x = SD$, $n = 40$)

No.	Name	Average damage level
1	Botanical Garden of DNU	0.36 ± 0.008
2	Novokodatskyi Park	0.06 ±0.003
3	Taras G. Shevchenko Park	0.16 ± 0.007
4	Pridneprovsky Park	0.36 ± 0.006
5	Metallurgists Square	0.12 ± 0.004
6	Lazaria Hloby Park	0.47 ± 0.017
7	Druzhby narodiv Forest Park	0.35 ± 0.011
8	Park Sahaydak	0.08 ± 0.009

As a result of the phytosanitary monitoring of park areas in Dnipro city, it was found that the trees of *Ae. hippocastanum* were not equally infected with *C. ohridella* (Table 1.4). The analysis of chlorophyll fluorescence induction variability showed specific patterns of changes in a number of physiological parameters of photosynthesis. Significant changes in the critical parameters of the Kautsky curve depending on the degree of leaf blade damage by *C. ohridella* caterpillars were not detected (Fig. 1.7, see p. 38).

The use of the fluorescence analysis method allowed the determination the effect of growing site conditions on individual indicators of chlorophyll fluorescence induction in the leaves of the studied *Ae. hippocastanum* trees (Fig. 1.7). High plasticity was established in the structure of chloroplasts in horse chestnut leaves which was characterized with F_0, F_v, F_p, and F_{St} parameters.

We found significant differences in the above parameters in *Ae. hippocastanum* leaves sampled from Botanical Garden of DNU compared to the leaves of the studied plant species sampled from different parks in Dnipro city. It was noted that the efficiency coefficients of photochemical processes (E) in *Ae. hippocastanum* trees in the parks located at low terrain levels were grouped separately (Fig. 1.8, *c*, see p. 39). The lowest E values were recorded in horse chestnut trees in Botanical Garden of DNU, Novokodatskyi Park, Taras G. Shevchenko Park, and Metallurgists Square; it may be associated with the reaction of Calvin cycle enzymes.

The analysis of chlorophyll fluorescence induction variability allowed determining specific patterns of changes in series of photosynthesis physiological parameters. The background fluorescence level (F_0) depends on the loss of excitation energy during migration along pigment matrix, as well as on the content of chlorophyll molecules that do not functionally associated with reaction centers (Antal et al., 2018; Zhang et al., 2020).

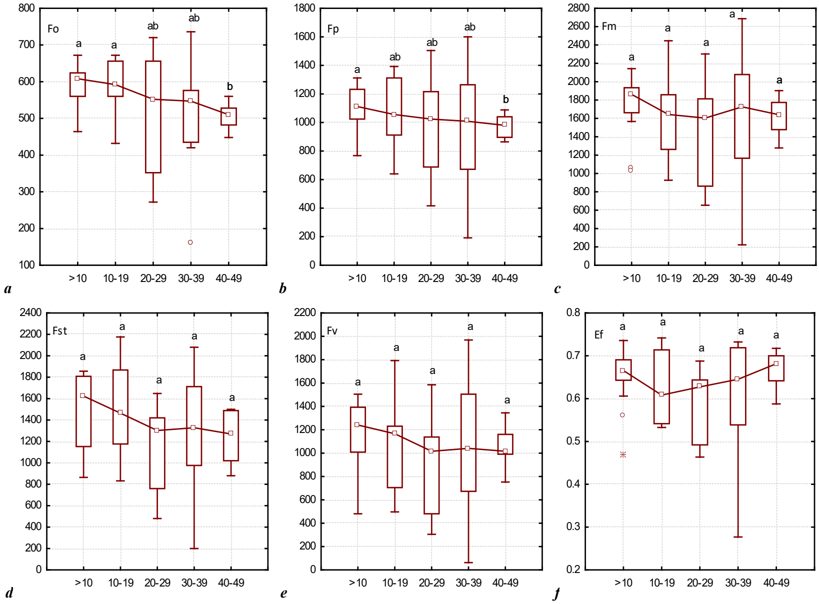
The maximum values of F_0 parameter were recorded in the *Ae. hippocastanum* leaves sampled from Park Sahaydak and Novokodatskyi Park, and the lowest values were observed in leaves sampled from Lazaria Hloby Park. This is due to the fact that a structural change in the pigment complex associated with the loss of green leaf tissues under the phytophage influence. As the number of antenna chlorophylls decreases, the initial level of fluorescence decreases, and vice versa.

The F_p parameter characterizes the highest level of fluorescence, i. e. it means the maximum value on the induction curve. It has the most variable pattern characterized by adaptive changes (Zhao et al., 2018). In the structure of the pigment complex in the studied *Ae. hippocastanum* plants, the lowest F_p values were found in trees having the highest degree of leaf damage by *C. ohridella* larvae (Botanical Garden of DNU, Lazaria Hloby Park, and Pridneprovsky Park).

It was caused by a decrease in the number of both light-harvesting and antenna chlorophylls. The obtained data are supported by the tendency to decrease the variable fluorescence of chlorophyll (F_v) with an increase in the damage degree of horse chestnut leaves by mining moth larvae. The calculated F_v parameter is expressed as the difference between the highest level of fluorescence and background fluorescence indicating the amplitude value of changes in the Kautsky curve (Alonso et al., 2017; Ayyaz et al., 2020).

The value of the steady-state fluorescence level (F_{St}) also decreased with an increasing degree of damage of *Ae. hippocastanum* leaves. This parameter is characterized by a dynamic equilibrium between the processes that cause an increase in fluorescence and the processes that lead to its decrease (Ruban, 2016). As shown in Figure 1.8, there is a significant difference in the values of all key parameters of chlorophyll fluorescence induction. We have shown that an increase in the number of *C. ohridella* mines reduces the values of all major indicators (F_0, F_v, F_p and F_{St}), which affects the overall physiological state of *Ae. hippocastanum* leaves. In general, plant defense responses to insect attacks are very often associated with a decrease in the rate of photosynthesis (Balabanova et al., 2016). Genotypes that are capable to maintain the rate of photosynthesis under these conditions probably show greater resistance, i. e. the plant's ability to grow, develop, and bear fruit satisfactorily even at a certain level of herbivorous insect infestation (Blacutt et al., 2018).

Another indicator important for assessing the functional state of leaves is the efficiency coefficient of dark photochemical reaction, E. This parameter reflects the activity of the main enzyme in the



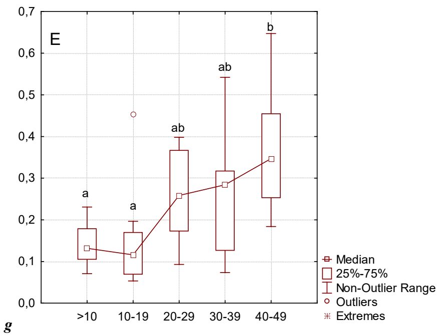


Fig. 1.7. Measurements of physiological parameters of photosynthesis depending on the leaf damage degree:

a – F_0 – the initial value of fluorescence induction after switching on irradiation, *b* – F_p – the value of fluorescence induction “plateau”, *c* – F_m – the maximum value of fluorescence induction, *d* – F_{St} – the steady value of fluorescence induction after light adaptation of the leaf of the plant, *e* – F_V – variable chlorophyll fluorescence, *f* – Ef – the maximum efficiency of primary photosynthesis processes, *g* – E – efficiency coefficients of photochemical processes; small square – median, the upper and lower line of the rectangle – 75 % and 25 % quartiles, the upper line – minimum and maximum values, circles and asterisks – outliers; different letters within each figure indicate significant differences between the groups ($P < 0.05$) according to the results of Tukey test, $n = 7$

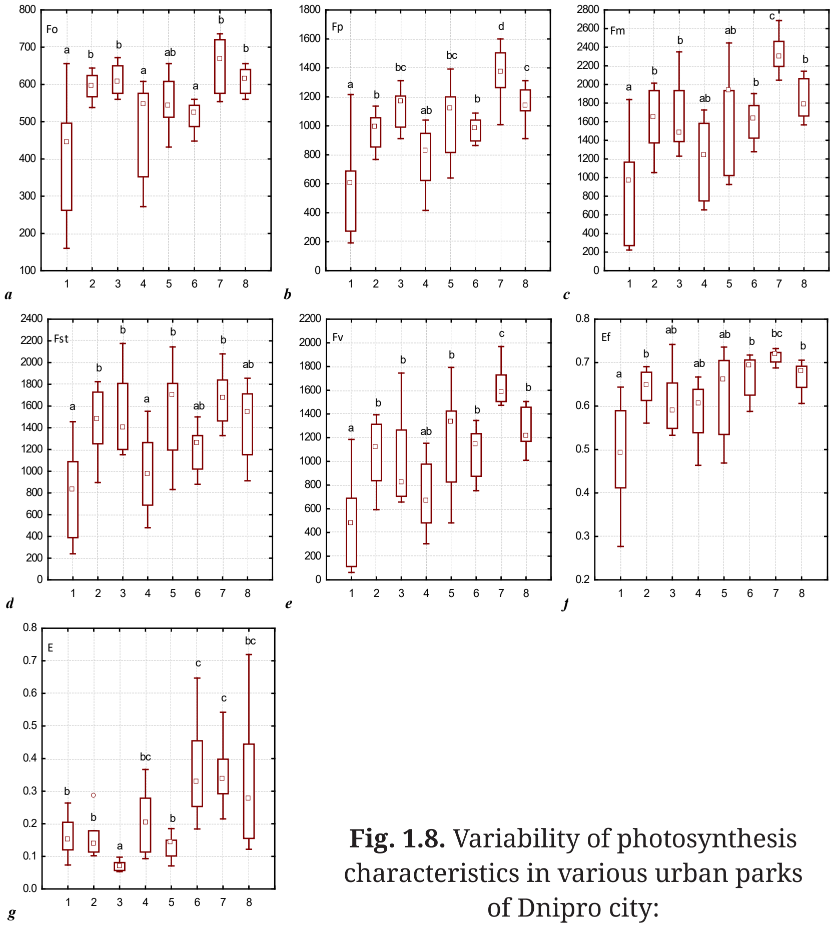


Fig. 1.8. Variability of photosynthesis characteristics in various urban parks of Dnipro city:

a – F_0 – the initial value of fluorescence induction after switching on irradiation, *b* – F_p – the value of fluorescence induction “plateau”, *c* – F_m – the maximum value of fluorescence induction, *d* – F_{St} – the steady value of fluorescence induction after light adaptation of the leaf of the plant, *e* – F_V – variable chlorophyll fluorescence, *f* – Ef – the maximum efficiency of primary photosynthesis processes, *g* – E – efficiency coefficients of photochemical processes; at the x-axis: 1 – Botanical Garden of DNU, 2 – Novokodatskyi Park, 3 – Taras G. Shevchenko Park, 4 – Pridneprovsky Park, 5 – Metallurgists Square, 6 – Lazaria Hloby Park, 7 – Druzhby narodiv Forest Park, 8 – Park Sahaydak; at the y-axis: the values of parameters indicated; please see Fig. 1.7 for an explanation of statistical processing

Calvin cycle, ribulose bisphosphate carboxylase. It is known that ribulose bisphosphate carboxylase has not only carboxylase, but also oxygenase activity, which means that the increase in the activity of this enzyme can be caused by increase in the competitive process for photosynthesis, photorespiration, which can reach up to 50 % of the enzyme activity. Photorespiration is also known to have a protective effect on the photosynthetic apparatus in plants and increases markedly under the influence of stress factors (Chen et al., 2019; Scognamiglio et al., 2019).

Effect of *C. ohridella* caterpillars on the photosynthetic apparatus in *Ae. hippocastanum* allowed determining specific patterns of changes in critical parameters of chlorophyll fluorescence induction. Significant changes in the critical parameters of the Kautsky curve depending on the degree of leaf blade damage by *C. ohridella* caterpillars were not identified. The use of the fluorescence analysis method allowed determining the effect of growing site conditions on individual indicators of chlorophyll fluorescence induction in the leaves of the studied *Ae. hippocastanum* trees. High plasticity was established in the structure of chloroplasts in horse chestnut leaves which was characterized with F_o, F_v, F_p, and F_{St} parameters. We found significant differences in the above parameters in *Ae. hippocastanum* leaves sampled from Botanical Garden of DNU compared to the leaves of the studied plant species sampled from different parks in Dnipro city. It was noted that the efficiency coefficients of photochemical processes (E) in *Ae. hippocastanum* trees in parks located at low terrain levels were differed markedly. The lowest efficiency coefficients of dark photochemical reactions were recorded in horse chestnut trees from the Botanical Garden of DNU, Novokodatskyi Park, Taras G. Shevchenko Park, and Metallurgists Square, which can probably be associated with the urban environment characteristics.

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1.3. The Impact of *Parectopa robiniella* on the State of *Robinia pseudoacacia* Photosynthetic Apparatus in the Urban Environment (Dnipro city)

North American species *Robinia pseudoacacia* L. (black locust) is adventive tree species currently the most common in Europe, and the total square of its secondary area in the world is estimated at approximately 2.3×10^6 ha (Vítková et al., 2020; Nicolescu et al., 2020). It is a drought-resistant, fast-growing tree species that has a wide ecological amplitude in relation to soil conditions (Vitkova et al., 2017); plantings from this species play an important role in the economics of many countries and perform a large number of necessary ecosystem resources: carbon sequestration, soil enrichment with nitrogen, soil protection from erosion, field-protective functions, microclimate optimization, bioenergy and wood production (Zverkovsky et al., 2018; Gritsan et al., 2019; Nicolescu et al., 2020).

However, despite its ecological, social and economic significance, Robinia pseudoacacia is recognized as an invasive species due to its active population strategy and introduction into various types of ecosystems in many countries of the world (Rumlerová et al., 2016; Wagner et al., 2017; Burda & Koniakin, 2019; Nicolescu et al., 2020; Vítková et al., 2020), and is included in the current list of the worst invasive alien species in Europe that cause the greatest negative ecological and socio-economic impact (Nentwig et al. 2018). The effect of Robinia pseudoacacia plantings on the reduction of species richness, unification and homogenization of forest vegetation has already been confirmed in Central Europe (Šibíková et al., 2019; Montecchiari et al., 2020). It is considered that Robinia has a high acclimatization potential in secondary area. In the context of climate change, it is predicted that its potential climate niches in Europe will be spread easterly, with their reduction in southern Europe and expansion in Central and North-Eastern Europe (Klisz et al., 2021; Puchałka et al., 2021), where an expansion of its invasive activity is possible in the future.

The successful Robinia naturalization in the secondary area and high potential for its invasiveness were largely determined by successful reproduction strategies of this species, among which the formation of root growth is a high-priority (Vítková et al., 2020). The rate and intensity of colonization of nearby biotopes by overgrowth largely depend on the type of their vegetation, the presence or absence of soil and vegetation disturbances, and the specifics of agricultural activities. Vegetative activity with the formation of a greater number of ramets increases in conditions of high light intensity in open areas and decreases in shading (Carl et al., 2019), which reduces the potential threat of colonization of natural broad-leaved forests with undisturbed canopy (Vitkova et al., 2017), but contributes to the active Robinia expansion in illuminated areas of meadows, steppes, roadsides, etc. adjacent to parent Robinia plantings. It is known that root growth colonizes most often and intensively the abandoned agricultural lands (Carl et al., 2019; Sitzia et al., 2018, Vitkova et al., 2020).

Previously, it was believed that the absence of natural enemies outside the native area could be one of the main reasons for the long period of spontaneous Robinia populations' existence in the secondary area (Cierjacks et al., 2013). But at the beginning of the 21st century, as with other massively introduced trees (Shupranova et al., 2019), the phytophages mainly specialized for *R. pseudoacacia* began to be recorded on the territory of Ukraine; their invasions began to cause a phytosanitary threat to the normal existence of this plant species. The studies (Holoborodko et al., 2021) showed that primarily 2 species in the family of leaf blotch miners (Lepidoptera: Grasillariidae Stainton, 1854) currently associated with the main phytosanitary risk of *R. pseudoacacia* existence in the Ukrainian area. Among the complex of phytophages-invaders of *R. pseudoacacia*, *Parectopa robiniella* is characterized by the largest scale of invasion in Ukraine (Clemens, 1863) (Holoborodko et al., 2021; Shvydenko et al., 2021). Since its introduction in Europe, *P. robiniella* is subjected to numerous environmental and biological studies (Guo et al., 2018; Kirichenko et al., 2018; Wilkaniec et al., 2021). The absence of natural enemies and diseases within the novel area and an almost unlimited food resource contribute to their rapid and wide spread.

The objective of our research was to determine the impact of invasive *P. robiniella* on critical parameters of the Kautsky curve for *R. pseudoacacia* trees in the main types of forest-growing conditions in the Steppe zone of Ukraine.

The research was conducted in September 2021 in Dnipro city and its surroundings: the Majorca village in the Dnipro district (northern subzone of the steppe zone of Ukraine) (Table 1.5, see p. 47–48). The locations were situated in the zone of temperate latitudes with a relatively active atmospheric circulation (the predominant movement of air masses from East to West).

Table 1.5

Description of the study location

Feature	**(L1) Taras G. Shevchenko Park Monastyrsky island (Dnipro city)**	**(L2) The landscape reserve of local importance "Levoberezhny" (Dnipro city)**	**(L3) The forest planting surrounding the Majorca village (Dnipro district)**
1	**2**	**3**	**4**
GPS latitude, longitude (height above sea level)	48º27'34.02'' N 35º04'58.01'' E (height above sea level is 54 m)	48º30'36.50'' N 34º58'54.89'' E (height above sea level is 54 m)	48º15'49.97'' N 35º09'47.90'' E (height above sea level is 104 m)
Landscape type	valley-terrace landscape. The Dnieper River valley (artificially washed sandbar)	valley-terrace landscape The Dnieper River valley	the watershed-gully landscape. Watershed
Soil, texture Granulometric composition of soils	Arenosol	Fluvic Arenosol, sandy loam	Calcic Chernozem, loamy
Groundwater depth (m)* *	0.9–1.2	2–2.5	20–25

End of Table 1.5

1	2	3	4
Dominant tree species	*Robinia pseudoacacia* L.	*Robinia pseudoacacia* L.	*Robinia pseudoacacia* L.
Other tree species	*Populus alba* L.	*Elaeagnus angustifolia* L., *Populus alba* L., *Ulmus pumila* L.	*Fraxinus pennsylvanica* Marshall., *Ulmus pumila* L., *Ulmus minor* Mill., *Morus alba* L.
Canopy density	0.61 ± 0.04	0.74 ± 0.03	0.96 ± 0.07
Relevant vegetation	*Carex ligerica* J. Gay. (dominant), *Calamagrostis epigeios* (L.) Roth, *Anisantha tectorum* (L.) Nevski, *Secale sylvestre* Host, *Taraxacum officinale* Wigg., *Polygonum arenarium* Waldst. et Kit.	*Hordeum murinum* L. (dominant), *Calamagrostis epigeios* (L.) Roth, *Elytrigia repens* (L.) Nevski, *Coniza canadensis* (L.) Cronq, *Chenopodium album* L., *Ambrosia artemisifolia* L., *Berteroa incana* DC.	*Poa angustifolia* L. (dominant), *Gallium aparine* L., *Elytrigia repens* (L.) Nevski, *Ballota nigra* L., *Convolvulus arvensis* L.
The percentage grass cover	56.3 ± 0.11	84.7 ± 0.07	66.7 ± 0.09

* Notes: soil classification was presented in accordance with the International Classification System IUSS Working Group WRB 2015 [IUSs Working Group WR. (2015). World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome. URL: http://www.fao.org/3/i3794en/I3794en.pdf].

The climate of the territory is temperate-continental. Significant fluctuations in weather conditions from one year to another are one of the features of the climate in the territory. Moderately wet years alternate with sharply dry ones, and hot dry winds occur quite often. In general, the climate regime is characterized by rather cool winters and hot summers.

Black locust (*Robinia pseudoacacia* L.) was chosen as the research object because it is one of the tree species most commonly introduced within the research region. Trees of 10–14 years of age having similar morphological and taxational characteristics were studied. The studies were conducted on model *Robinia pseudoacacia* L. trees in local spontaneous plant communities dominated by black locust which were spontaneously formed near the parent artificial Robinia stands in different urban and suburban habitats characterized by different soil and hydrological conditions. In the studied plant communities, young trees of Robinia pseudoacacia were mainly of vegetative origin (root growth).

Location 1 (L1). Spontaneous planting in the Taras G. Shevchenko Park (Monastyrsky island) was formed in a recreational area within an artificially washed sandbar on a site with a shallow groundwater depth. In addition to the dominant black locust, the spontaneously formed community fragmentally includes the white poplar (*Populus alba* L.) undergrowth which occupies up to 10 % of the plantation composition. The grass cover under the canopy of the plant community was native and consisted mainly of mesophilic oligotrophic and eurytopic light-demanding species with complete dominance of *Carex ligerica* J. Gay.

Location 2 (L2). In the landscape reserve of local importance "Levoberezhny", a local spontaneous Robinia community was formed in a recreational zone within the valley-terraced landscape of the Dnieper River on natural soils characterized by sandy loam granulometric composition and fairly shallow groundwater. In addition to the dominant Robinia, the spontaneous ruderal community fragmentally includes young trees and undergrowth of *Elaeagnus angustifolia* L., *Populus alba* L. and *Ulmus pumila* L. The share of these species' participation reaches up to 30 % of the plantation composition. Naturally formed vegetation cover within this plantation was mainly represented by eurytopic ruderal species with a predominance of adventive *Hordeum murinum* L.

Location 3 (L3). Spontaneous Robinia pseudoacacia planting in the vicinity of the Majorca village was located on agricultural land abandoned more than 20 years ago near the parent artificial field-protective Robinia planting on the watershed area within the watershed-gully landscape. The habitat area is characterized by deep groundwater and the loamy granulometric composition of the soil. The young ruderal community was completely dominated by Robinia pseudoacacia, but there was also a few undergrowth of *Fraxinus pennsylvanica* Marshall., *Ulmus pumila* L., *Ulmus minor* Mill. and *Morus alba* L. (no more than 10 % of the plant composition), which was also present in the parent planting. Under the canopy of the spontaneous plant community, the vegetation is mainly represented by mesotrophic long-rhizome-forming cereals (with dominance of *Poa angustifolia* L.) and perennial dicotyledons (for example, *Ballota nigra* L.), *Gallium aparine* L. completely dominates in the spring period drying up in the summer.

Leaves of the middle formation were examined on annual vegetative growth simultaneously sampled from the lower third of the crown in the illuminated (5 complex leaves) and shaded part (5 complex leaves) in dry clear weather in mid-September 2021 on five Robinia trees from each experimental site. The period of accounting for damaged leaves corresponds to the period of development of the most massive second generation of *Parectopa robiniella* Clemens, 1863. The degree of damage to Robinia leaf blades caused by *P. robiniella* was evaluated visually. Light intensity was measured using a Lux Meter RCE-174 (PCE Instruments, Germany, 2018). Temperature and relative humidity measurements were made using HE-173 thermohygrometer (HUATO ELECTRONIC CO.LTD, China, 2018).

We used portable fluorometer "Floratest" for the diagnostics of photosynthetic disorders of native chlorophyll in living *R. pseudoacacia* leaves. The system of portable fluorometer "Floratest" comprises a base unit with a graphic liquid crystal display, control buttons, a remote optoelectronic sensor, connecting cable to the USB port of a personal computer, and a network adapter (Fig. 1.9, see p. 51). The remote optoelectronic sensor includes an LED that has a maximum radiation intensity at $\lambda = (470 + 20)$ nm.

Irradiation indicators in the sensor were the following: irradiation wavelength 470 + 15 nm; spectral range of fluorescence intensity measurement 670–800 nm; receiving window area 9 mm^2; photodetector sensitivity at λ = 650 nm 0.45 A/W.

Fig. 1.9. Instrumental support for the field experiment: portable fluorometer “Floratest”

The observations were made using fresh *R. pseudoacacia* leaves. After the start of light exposure, the intensity of chlorophyll fluorescence (fluorescence induction or fluorescence induced (caused) by light) begins to change significantly over time. A curve of the time dependence of the chlorophyll fluorescence intensity has the characteristic form with one or more maximum and is called the chlorophyll fluorescence induction curve (the Kautsky curve) (Kautsky & Hirsch, 1931). Changes in any link of the photosynthetic chain cause a change in the appearance of the chlorophyll fluorescence induction curve. Therefore, based on the appearance of this curve, it was possible to diagnose the current state of the plant photosynthetic apparatus and to evaluate changes in the photosynthesis efficiency at changes in the light regime, temperature, humidity, and other factors (Holoborodko et al., 2022).

To interpret the Kautsky curve, we used its known critical parameters: F_0 is the initial value of fluorescence induction after irradiation is turned on; F_p is the value of "plateau" fluorescence induction; F_m is the maximum value of fluorescence induction; F_{St} is the stationary value of fluorescence induction after light adaptation of a plant leaf. In addition to the critical parameters of the Kautsky curve, we used calculated parameters such as variable chlorophyll fluorescence ($F_v = F_m - F_0$); maximum efficiency of primary photosynthesis processes ($E_f = F_v / F_m$), and coefficient of photochemical processes efficiency ($E = (F_m - F_{St}) / F_{St}$). The chlorophyll concentration was determined in the undamaged parts of the leaves in acetone extraction using SF-46 spectrophotometer at wavelengths of 662 nm and 641 nm. Then the obtained results were recalculated to mg/g of raw mass of plant tissues with the use of Wettstein's formula.

The data were processed using variational statistics and presented as mean and root-mean-square deviation ($x \pm SD$). Comparison of samples in tables was carried out using ANOVA, and the differences between individual samples were considered reliable at $P < 0.05$ according to the results of the Tukey Test with Bonferroni adjustment. The influence of environmental factors on the parameters of the Kautsky curve and the chlorophyll content was assessed using three-way variance and regression analysis (Table 1.6, see p. 53–54).

The values of the initial induction of chlorophyll fluorescence (F_0) were within the range of 196–284 RFU (Table 1.6). The minimum value was recorded in Robinia plantings at the L3 location in the damaged leaf under shading conditions, while the maximum background fluorescence was observed in the undamaged leaf, but also under shading conditions.

A decrease in this indicator in damaged leaves in relation to undamaged ones was indicated in the planting of a local spontaneous Robinia community in the landscape reserve of local importance "Levoberezhny" (L2). An increase in the background concentration on the illuminated side of the leaf in relation to shading conditions was noted in the planting of the L3 location, and this trend was not significantly affected by caterpillar feeding.

Table 1.6

Influence of biotic and abiotic factors on photosynthesis indicators of *Robinia pseudoacacia* ($x \pm$ SD, $n = 120$)

Ecosystem	Side of the plant leaf and its damage by phytophage	Indicators of fluorescence, in relative fluorescence units (RFU)			
		F_0	F_p	F_m	F_{st}
1	2	3	4	5	6
L1	The light side of a leaf undamaged by phytophage	261 ± 12 ^{cd}	863 ± 46 ^{d}	1345 ± 75 ^{d}	1194 ± 64 ^{c}
	The shadow side of a leaf undamaged by phytophage	284 ± 16 ^{d}	908 ± 64 ^{d}	1704 ± 86 ^{e}	1451 ± 86 ^{d}
	The light side of a leaf damaged by phytophage	266 ± 17 ^{cd}	760 ± 47 ^{c}	1225 ± 66 ^{cd}	1110 ± 61 ^{bc}
	The shadow side of a leaf damaged by phytophage	254 ± 7 ^{c}	637 ± 75 ^{ab}	1138 ± 141 ^{c}	930 ± 131 ^{b}
L2	The light side of a leaf undamaged by phytophage	223 ± 15 ^{b}	510 ± 5^{9} a	656 ± 81 ^{a}	584 ± 75 ^{a}
	The shadow side of a leaf undamaged by phytophage	269 ± 8 ^{c}	1142 ± 4^{2} e	1680 ± 8^{6} e	1534 ± 92 ^{d}
	The light side of a leaf damaged by phytophage	214 ± 10 ^{ab}	549 ± 60 ^{a}	712 ± 9^{1} a	649 ± 90 ^{a}
	The shadow side of a leaf damaged by phytophage	214 ± 11 ^{ab}	639 ± 40 ^{b}	926 ± 95 ^{b}	839 ± 82 ^{b}
L3	The light side of a leaf undamaged by phytophage	259 ± 16 ^{c}	1149 ± 2^{9} e	1399 ± 49 ^{d}	1194 ± 58 ^{c}

End of Table 1.6

1	2	3	4	5	6
L3	The shadow side of a leaf undamaged by phytophage	211 ± 11 [ab]	855 ± 51 [d]	1033 ± 68 [bc]	948 ± 62 [b]
	The light side of a leaf damaged by phytophage	266 ± 19 [c]	849 ± 38 [d]	1012 ± 74 [bc]	875 ± 32 [b]
	The shadow side of a leaf damaged by phytophage	196 ± 6 [a]	637 ± 3[5] b	776 ± 51 [a]	704 ± 46 [ab]

Notes: different letters within the column indicate datasets reliably ($P < 0.05$) differ from each other according to the results of the Tukey Test with Bonferroni adjustment; L1, L2, L3: ecosystems which characteristics were shown in Table 1.5.

In the study conditions, the parameter F_p fluorescence reached a “plateau” at the level of 510–1149 RFU. A significant difference in achieving saturation of reaction centres is defined by the insect influence and the light intensity. The damage by insects, as well as shading, caused a significant decrease in F_p values, both in the conditions of an artificially washed sandbar (639 ± 40) and in the watershed area of the watershed-gully landscape (637 ± 35, while without damage these values were 1142 ± 42 and 1149 ± 29, respectively). The values of Fm as an indicator of photosynthesis under the simultaneous influence of the studied factors were fairly highly variable in spontaneous planting within the valley-terrace landscape. A more than twofold excess of the maximum fluorescence level was recorded compared to the damaged leaves: 656 ± 81 to 1680 ± 86 (Table 1.6). Minimal fluorescence values were observed at all locations when the leaf mesophyll was damaged by insects under both intense lighting and shading conditions. The maximum fluorescence reached the highest values in the absence of insect damage under shading conditions.

Stationary fluorescence level (F_{st}) was provided by chlorophyll molecules that are not involved in energy transfer to the reaction centres of photosystem II. An increase in the values of this indicator evidences an inhibition of the outflow of reduced photoproducts from the reaction centres as a result of various factors. The steady-state fluorescence level was significantly higher in the absence of insect damage, both under shading (1534 ± 92) and lighting (1194 ± 58) conditions.

As can be seen from the data shown in Table 1.7 (see p. 56–57), the initial induction of photochemical processes depends on the action of exogenous factors. Each of them in its separate action did not have a statistically significant effect on the fluorescence index. Further, the statistically significant influence of factors of forest-growing conditions, as well as phytophages, on the F_p indicator was determined (Table 1.7). The complex effect of all three factors of exogenous nature was revealed as statistically significant in comparison with the mono-influence of individual factors.

Indicators of the maximum fluorescence value showed a different trend (Table 1.7). This indicator depends to the greatest extent on the intake of solar radiation and on the degree of damage to the leaf surface by phytophages, which cannot be noted in the case of the edaphic factor exposure on the plants. In the case of intensification of solar energy supply, the F_m indicator is expected to increase. In contrast, leaf surface damage by phytophages resulted in inhibition of the reactions of maximum fluorescence.

Statistical analysis of F_v values showed that, like F_m, the most significant effect on this indicator was achieved in the event of solar radiation effect on plants and in the case of plant damage by phytophages. Statistical processing of data on the effect of environmental factors and phytophages on the E value separately and with their combined effect did not reveal statistically significant results (Table 1.8, see p. 57).

Studies of changes in the level of chlorophyll components (Table 1.9, see p. 58) revealed statistically significant dependences of chlorophylls a and b on the factors of plant localization and the amount of incoming solar radiation, while the effect of phytophages on the studied parameter was insignificant. No statistically significant effect on chlorophyll levels was observed a, as well as amounts of a + b chlorophylls in the case of a combination of environmental factors.

Table 1.7

Results of three-way dispersion and regression analysis of the influence of location, light intensity, and influence of phytophages on the fluorescence in the leaves of *Robinia pseudoacacia* L. model trees (*N* = 120)

Fluorescence parameters	Factor of influence (*n* = 120)	Beta	Standard error	B	Standard error	t_{116}	P	R^2	F	P
1	2	3	4	5	6	7	8	9	10	11
F_0	Location	–0.025	0.091	–1.475	5.348	–0.276	0.783	0.039	1.562	0.20230
	Light intensity	–0.106	0.091	–10.200	8.733	–1.168	0.245			
	Influence of phytophages	–0.164	0.091	–15.733	8.733	–1.802	0.074			
	Intercept	–	–	2904.183	1265.930	2.294	0.024			
F_p	Location	–0.246	0.081	–81.29	26.827	–3.030	0.003	0.237	12.041	<0.00001
	Light intensity	0.043	0.081	23.02	43.808	0.525	0.600			
	Influence of phytophages	–0.419	0.081	–226.22	43.808	–5.164	<0.001			
	Intercept	–	–	21781.88	6350.582	3.429	<0.001			
F_m	Location	–0.059	0.084	–30.82	43.89	–0.702	0.484	0.190	9.046	0.00010
	Light intensity	0.176	0.084	151.27	71.68	2.110	0.037			
	Influence of phytophages	–0.394	0.084	–337.67	71.68	–4.711	<0.001			
	Intercept	–	–	20301.52	10390.80	1.954	0.053			
F_{st}	Location	–0.031	0.084	–14.43	39.316	–0.367	0.714	0.163	8.743	0.00003
	Light intensity	0.174	0.084	133.50	64.203	2.079	0.039			

End of Table 1.7

1	2	3	4	5	6	7	8	9	10	11
F_{st}	Influence of phytophages	–0.391	0.084	–299.57	64.203	–4.665	<0.001	0.163	8.743	0.00003
	Intercept	–	–	18051.70	9307.026	1.939	0.054			
F_v	Location	–0.060	0.082	–29.35	40.361	–0.727	0.468	0.234	10.130	0.00001
	Light intensity	0.202	0.082	161.47	65.909	2.449	0.016			
	Influence of phytophages	–0.404	0.083	–321.93	65.909	–4.884	<0.001			
	Intercept	–	–	17397.33	9554.376	1.820	0.071			

Table 1.8

Results of three-way dispersion and regression analysis of the effect of location, light intensity, and influence of phytophages on the calculated fluorescence parameters in the leaves of *Robinia pseudoacacia* L. model trees ($N=120$)

Fluorescence parameters	Factor of influence ($n=120$)	Beta	Standard error	B	Standard error	t (116)	p-level	R^2	F	p-level
E_f	Location	–0.229	0.082	–0.024	0.008	–2.766	0.006		9.940	0.00001
	Light intensity	0.274	0.082	0.046	0.014	3.303	0.001			
	Influence of phytophages	–0.278	0.082	–0.047	0.014	–3.355	0.001			
	Intercept			0.882	2.044	0.431	0.666	0.205		
E	Location	–0.175	0.091	–0.014	0.007	–1.918	0.057		1.2333	0.30087
	Light intensity	0.009	0.091	0.001	0.012	0.109	0.913			
	Influence of phytophages	0.007	0.091	0.001	0.012	0.079	0.937			
	Intercept			–0.069	1.746	–0.040	0.968	0.031		

Table 1.9

Results of three-way dispersion and regression analysis of the effect of location, light intensity, and influence of phytophages on the content of chlorophyll components in the leaves of *Robinia pseudoacacia* L. model trees ($N=120$)

Chlorophyll components	Factor of influence ($n=120$)	Beta	Standard error	B	Standard error	t (116)	p-level	R^2	F	p-level
Chlorophyll a	Location	–0.186	0.086	–0.144	0.067	–2.150	0.033		5.7632	0.00104
	Light intensity	–0.298	0.086	–0.377	0.109	–3.441	0.000			
	Influence of phytophages	0.078	0.086	0.099	0.109	0.906	0.366			
	Intercept			29.872	15.882	1.880	0.062	0.130		
Chlorophyll b	Location	–0.309	0.084	–0.106	0.029	–3.654	0.000		7.8667	0.00008
	Light intensity	–0.268	0.084	–0.150	0.047	–3.174	0.001			
	Influence of phytophages	0.034	0.084	0.019	0.047	0.411	0.681			
	Intercept			14.139	6.867	2.059	0.041	0.169		
Chlorophyll a+b	Location	–0.301	0.083	–0.141	0.039	–3.615	0.000		9.1193	0.00002
	Light intensity	–0.303	0.083	–0.233	0.064	–3.637	0.000			
	Influence of phytophages	0.085	0.083	0.065	0.064	1.027	0.306			
	Intercept			18.158	9.295	1.953	0.053	0.191		

The results of statistical analysis obtained for the maximum efficiency index of primary photosynthesis processes revealed its highly significant dependence on individual factors of exogenous influence, while the complex effect of these factors did not have a significant effect on E_f. No statistically significant results were found for the efficiency coefficient of dark photochemical reactions.

The process of photosynthesis begins with primary photochemical reactions being the initial link in the light energy conversion chain. In low light intensity under optimal plant growth conditions, primary reactions proceed with high intensity (Zhou et al., 2012; Antal et al., 2018; Guidi et al., 2019). These reactions include several stages: absorption of light energy by pigments, energy migration to the reaction centres of photosystems, and charge separation, after which the process of electron transfer along the electron transport chain is activated (Stirbet et al., 2018). For efficient absorption and migration of light energy, pigment molecules are assembled in antennas and stay in the form of pigment-protein complexes.

As a result of interaction with proteins, chlorophyll changes its optical properties, which makes it possible to obtain a set of spectral forms in the antenna, in which the absorption spectra overlap with each other. This ensures efficient energy migration from antenna chlorophylls to reaction centres. Pigments in the reaction centers are functionally closely related to the electron acceptor and donor, which provides the continuous electron outflow along the electron transport chain and reduction of pigment in the reaction center (Strasser et al., 2004).

Taking into account the research of a number of scientists (Flexas et al., 2002; Petrova et al., 2019; Sáez et al., 2018), it can be assumed that the chlorophyll content serves as a significant short-term indicator of the state of tree species due to its direct role in the photosynthetic reactions. The chlorophyll content is closely related to the plant photosynthetic functioning and this ability varies within the range of soil-environmental factors (Hari & Luukkanen, 2006; He et al., 2018; Lichtenthaler & Babani, 2022). Based on the results obtained by us, taking into account the influence of various factors on the photosynthetic apparatus, the effect on the concentration of chlorophylls under the influence of solar radiation and soil conditions was revealed, which is consistent with the data obtained by many authors (Huang et al., 2017).

At the same time, there was no clear response of plants to the action of phytophages, but according to information available in the literature (Cardenas & Gallardo, 2016), this type of response will vary depending on the type of plant, as well as the type of harmful stress-causing agent.

The output level of induction of background fluorescence corresponds to the minimum fluorescence quantum yield (Zhang et al., 2020). The background fluorescence level is determined by chlorophyll fluorescence under conditions when all reaction centers stay in an open working state and all molecules of the primary quinone acceptor coenzyme are ready to accept an electron from P680. It is likely that of the analyzed factors, insect damage the most affects the activation of reaction centers (Cardenas & Gallardo, 2016).

Fluorescence index at the F_p level is caused by rapid saturation of energy through reaction centers that do not transfer energy to the electron transport circuit. Thus, the reaction centers do not restore the primary coenzyme acceptor and therefore act as reaction centers that do not restore the electron transport chain. The maximum fluorescence value characterizes the potential productivity of plant photosynthesis.

When the acceptor molecules accept coenzyme electrons (i. e., under conditions of the complete reduction occurring at bright light exposure), the reaction centers are closed, since the transfer of electrons from P680 to pheophytin is impossible because of electrostatic repulsion. In this case, the electron excitation energy is realized mainly in the process of fluorescence emission; its absolute value and quantum yield reach the maximum values, corresponding to F_m in the experiment.

With insufficient light intensity, the number of light-absorbing and antenna chlorophylls increases causing to increase in this indicator.

F_v/F_m ratio is widely used as an indicator of the functional state of the photosynthetic system in intact green plant tissues. It was found (Duysens, 1961) that a coenzyme A reduction represents the cause of the increase in fluorescence from the F_0 level up to F_m level. Reducing the values of the F_v/F_m ratio is caused by inhibition of photosystem II (Van Rensburg & Kruger, 1993) and a decrease in the proportion of reaction centers of photosystem II that are unable to reduce coenzyme B (Ouzounidou, 1993).

Sensitivity F_v/F_m to the inhibition of the light stage of photosynthesis makes this indicator an effective means to monitor the stressful effects of the environment on the plant. F_v/F_m value can be easily measured. Due to the reaction's high sensitivity, rate and non-invasiveness, the determination of the F_v/F_m parameter was often preferred in studies of a wide variety of light photosynthesis reactions (Van Rensburg & Kruger, 1993).

Photosystem II makes the main contribution to chlorophyll fluorescence at room temperature (as F_m, so and F_0). F_0 is a component generated in low actinic light or as a rapid response to any actinic light that develops prior to the triggering of primary photochemical reactions (Stirbet et al., 2018). In both cases, the primary electron acceptor plastoquinone cannot be recovered. Actually, F_0 reflects a constant fluorescence component independent of photochemical reactions (Khan et al., 2021).

Background fluorescence F_0 is emitted by chlorophyll molecules that are part of the photosystem II antenna complex (Longoni & Goldschmidt-Clermont, 2021; Pashayeva et al., 2021). Technically, it is measured before the initiation of primary photochemical reactions associated with coenzyme reduction.

The fluorescence yield increases immediately after starting of coenzyme A reduction. Since variable fluorescence F_v is determined by the redox status of coenzyme A, its level serves as an indicator of photochemical redox reactions (Tsai et al., 2019). When electron transport from coenzyme A to the following components of the photosynthetic electron transport chain is blocked or the intensity of actinic illumination exceeds the saturation level, F_v quickly reaches the maximum possible values.

Therefore, any external influences affecting the electron transport process in the electron transport chain of the thylakoid will also affect the F_v value. This circumstance allows the use of F_v as a physiological indicator that reflects the influence of environmental and experimental factors on plants. Chlorophyll fluorescence has been shown to be potentially used in environmental and forestry research due to the relationship demonstrated with a set of leaf economic characteristics indicators of the photosynthetic metabolism (Bucher et al., 2018; Bussotti et al., 2020; Cetner et al., 2020; Guimarães et al., 2022).

Feeding of *P. robiniella* caterpillars causes a decrease in the activity of the photosynthetic apparatus in *R. pseudoacacia*. It is reflected in almost all values of critical chlorophyll fluorescence parameters. The maximum value of the background fluorescence parameter was recorded in undamaged leaves and under shading conditions. Both phytophage exposure and shading factor caused a significant decrease in fluorescence induction values (F_p), which was confirmed by us in all variants of *R. pseudoacacia* habitats.

Under simultaneous exposure of the studied factors, values of the maximum fluorescence index (F_m) of photosynthesis were fairly highly variable. Unlike the F_p parameter, the highest F_m values were achieved in the absence of phytophage damage under conditions of total shading. As revealed by dispersion and regression analyses, the maximum fluorescence index was most dependent on the amount of solar radiation and on the degree of the leaf surface damage by phytophages. Significantly higher values of the steady-state fluorescence induction parameter (F_{st}) were determined in the absence of insect damage in both shading and lighting conditions. A highly significant dependence of the maximum efficiency values of primary photosynthesis processes (E_f) on individual factors of exogenous influence was established, while the complex effect of these factors did not affect this parameter.

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CHAPTER

THE INFLUENCE OF CHEMICALS USED IN FOOD PRODUCTION ON THE COMPONENTS OF BIOCENOSES IN NATURAL ECOSYSTEMS

L. I. Faly, O. O. Boyko, V. O. Komlyk

The influence of chemicals used in food production on the components of biocenoses in natural ecosystems

L. I. Faly[1],
O. O. Boyko[2], V. O. Komlyk[1]

[1] Oles Honchar Dnipro National University, Dnipro, Ukraine
Oles Honchar Dnipro National University, Gagarin av., 72,
Dnipro, 49010, Ukraine.
Tel.: +38-096-64-31-593. E-mail: faly@ua.fm
[2] Dnipro State Agrarian and Economic University, Dnipro, Ukraine
Dnipro State Agrarian and Economic University,
Sergiy Efremov st., 25, Dnipro, 49000, Ukraine.
Tel.: +38-097-296-42-10. E-mail: boikoalexandra1982@gmail.com

Various components of the biocenosis in natural ecosystems are regularly exposed to various chemicals used in food production. The experiment was carried out in vitro in two stages. Both larval stages of insect development (the first stage) and imago (the second stage) were exposed to food additives. The biological models were mosquito larvae (Diptera, Chironomidae, Chironomus sp.) and coleopteran predators of the species Harpalus rufipes (De Geer, 1774), H. smaragdinus (Duftschmid, 1812), Harpalus sp., Calathus fuscipes (Goeze, 1777), Staphylinus caesareus Cederhjelm, 1798 which were fed on mosquito larvae treated with 1 % solution. Mosquito larvae were found to be more susceptible to the negative effects of additives compared with coleopteran predators that feed on them. Methylparaben, raspberry ketone, benzaldehyde, benzyl alcohol, ethyl acetate, benzoic, salicylic, acetic, adipic, propionic acids, sodium metabisulfite, as well as 2-phenylphenol at 1 % concentration have the greatest effect on mosquito larvae: 100 % of the deaths in the first 15 minutes of the experiment. Formic acid, cinnamic aldehyde, and potassium hydroxide have been reported to be less toxic to mosquito larvae. Benzyl acetate, orthophosphoric acid, sodium hydrosulfite, sodium sulfate, sodium nitrite, and calcium formate did not significantly affect the mosquito larvae. More than 50 % of individuals remained viable under influence of the chemicals for 120 minutes. Not one of the studied additives did not cause the death of more than 20 % of insect imagoes. Sodium metabisulfite had the greatest negative impact on the biocenosis components of natural ecosystems under in vitro conditions: 100 % of dead individuals of mosquito larvae in the first 15 minutes of the experiment and 16.7 % of dead imago of coleopteran predators.

There are a great number of food additives in the world that diversely affect living organisms. The study of the toxic effect of these chemicals on individual groups of invertebrates, as well as ways of their entry into the environment, is a promising direction in modern science (Boyko, Brygadyrenko, 2018; 2019b).

The influence of some of these chemicals on certain groups of invertebrates has been previously described in the literature (Table 2.1, see p. 72–78). The decline in insect pest populations is associated not only with the use of targeted insecticides, but also with the increase of the biodiversity of beneficial predators, as well as with changes in the content of nutrients in plants (Blundell et al., 2020). A change in the composition of nutrients is associated with the application of various fertilizers to the soil, as well as with the use of plant growth regulators. A potential approach to insect and disease control is induced host plant resistance (Haghighi et al., 2021). Plant resistance is an effective and environmentally friendly method of pest control. Phenolic acids are a key group of compounds responsible for protecting plants from pests. For example, an increased number of phenolic acids in hazel leaves reduces the number of aphids on them (Gantner et al., 2019).

Table 2.1

Literature data on the effect of food additives on agricultural pests

Chemical name	**The group of invertebrates affected by the chemical**	**Action**	**A source**
1	**2**	**3**	**4**
Benzoic acid		it is used as a component of insecticides	Jiang et al., 2021
Benzofurans, benzoic acid derivatives, diterpenes and pyrrolizidine alkaloids		they have insecticidal and fungicidal properties	Ruiz-Vásquez et al., 2018

Continuation of Table 2.1

1	2	3	4
Phenolic acids: gallic, protocatechuic, p-hydroxybenzoic, salicylic, chlorogenic, feluric, caffeic, α-resolcylic and cinnamic acids	filbert aphids	high concentrations of phenolic acids reduce the number of aphids on hazel leaves	Gantner et al., 2019
Raspberry ketone, zingerone	fruit fly *Bactrocera jarvisi* (Tryon) (Diptera: Tephritidae)	when feeding with these substances, the sexual activity of males increases, and zingerone has a greater effect than raspberry ketone	Wee & Clarke, 2020
Raspberry ketone	melon fly *Zeugodacus cucurbitae* (Coquillett)	raspberry ketone has no effect on *Zeugodacus cucurbitae*	Fezza & Shelly, 2018
Raspberry ketone	fruit fly *Bactrocera tryoni* (Diptera: Tephritidae)	raspberry ketone supplementation promotes early puberty in male fruit flies *Bactrocera tryoni* (Diptera: Tephritidae)	Akter et al., 2017
Methyl eugenol, raspberry ketone, dimethyl dichloro-vinyl phosphate	fruit fly *Bactrocera dorsalis* (Hendel), melon fly *Bactrocera cucurbitae* (Coquillett)	the use of methyl eugenol, raspberry ketone, dichlorovinyl dimethyl phosphate in baits for control of fruit and melon flies	Vargas et al., 2015
Ethyl acetate extract isolated from *Photorhabdus temperata* M1021	*Galleria mellonella* larvae	causes 100 % death of *Galleria mellonella* larvae	Ullah et al., 2015
Benzaldehyde isolated from *Photorhabdus temperata* M1021	*Galleria mellonella*	benzaldehyde has a dose-dependent insecticidal effect against *Galleria mellonella*	Ullah et al., 2015

Continuation of Table 2.1

1	2	3	4
Indolo-pyrosols (derivatives of indole, arylhydrazine and benzaldehyde)	*Anopheles arabiensis* larvae and imago	have larvicidal and insecticidal properties in relation to *Anopheles arabiensis*, cause more than 80 % of the death of larvae, as well as imago	Makhanya et al., 2020
Substituted benzaldehyde derivatives of chloroformates (substituted benzaldehyde-derived chloroformates)		insecticidal properties	Mulvihill et al., 2001
Essential oils of *Cinnamomum aromaticum*	cowpea weevil *Callosobruchus maculatus*	have insecticidal properties against *Callosobruchus maculatus*	Islam et al., 2009
Benzyl acetate	single bees and other insects	traps with benzyl acetate collect single bees and other insects	Ikemoto & Tomoyuki, 2021
Benzyl acetate	*Apis cerana*	*Apis cerana* uses benzyl acetate as an alarm pheromone	Wen et al., 2017
Compound C13-norisoprenoid, phenolic glycoside, alangionoside C, pisumionoside, koaburaside, 3,5-dimethoxy-benzyl alcohol 4-O-β-d-glucopyranoside, 3,4,5-trimethoxy-benzyl-β-d-glucopyranoside, arbutin, salidroside isolated from alcohol extract of *Viburnum fordiae* Hance stems	*Mythimna separata*	C13-norisoprenoid exhibits insecticidal activity against *Mythimna separata*	Shao et al., 2019

Continuation of Table 2.1

1	2	3	4
Benzyl alcohol, benzoic acid, toluene, hydroquinone, phenethyl alcohol, pinene, methylparaben, kojic acid, formic acid, isoamyl alcohol, tartaric acid, glycine, succinic acid, stearic acid, and ethylenediamine-tetraacetic acid	*Tribolium confusum*	do not affect the motor activity of *Tribolium confusum*	Titov & Brygady-renko, 2021
Benzyl alcohol, hydroquinone, phenethyl alcohol, glycine, EDTA, toluene, methylparaben, succinic acid, benzoic acid	*Sitophilus granarius*	attractant effect on *Sitophilus granarius*, reduces its motor activity	Titov & Brygady-renko, 2021
1-octen-3-ol, 6-methyl-5-hepten-2-one, α-pinene, benzyl alcohol, m-cresol, p-cresol and decanal	sandflies	sandflies-attractant property	Hassaballa et al., 2021
Volatile compounds of *Solanum melongena* 2,2'-(ethane-1,2-diylbis(oxy)) bis(ethane-2,1-diyl) dibenzoate, 3,7-dimethylocta-1,6-dien-3-ol, benzyl alcohol	*Leucinodes orbonalis*	volatile compounds have an attractant effect on insect pests *Leucinodes orbonalis*	Nusra et al., 2021
(Z)-3-hexenyl hexanoate, (Z)-jasmone, linalool, ocimene, benzyl alcohol	*Dasychira baibarana* (Matsumura) (Lepidoptera: Lymantriidae)	volatile compounds of tea attract the tea black tussock moth *Dasychira baibarana*	Magsi et al., 2021
Benzyl alcohol, 1,3-diethylbenzene, thymol, 1-hexadecene	*Aphis craccivora*	these substances can be used as baits for *Aphis craccivora*,	Mitra et al., 2021

Continuation of Table 2.1

1	2	3	4
		and their action is similar to the action of *Lathyrus sativus* L. (Fabaceae) natural substances	
Volatile substances of host plants: benzenoids and terpenoids: (Z)-6-nonenal, octanal, (E)-2-octen 1-ol, 1-hexanol, benzyl alcohol, benzaldehyde	*Diaphania indica* (Saunders) (Lepidoptera: Pyralidae)	attract pregnant female pests of cucumbers, pumpkins, melons, watermelon *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae) for egg laying	Gharaei et al., 2020
Benzyl alcohol, 4-oxoisophorone of Fontainea *picrosperma* flowers	various types of insects	attract various insects.	Grant et al., 2021
Lignin	*Sitophilus oryzae*, *Plodia interpunctella*	used to repel *Sitophilus oryzae*, *Plodia interpunctella* insects	Vachon et al., 2020
Betaine-based iodosulfuron-methyl	storage pests	has a weak repellent activity against storage pests	Kaczmarek et al., 2021
Ethyl acetate extract of *Alpinia galanga*	*Spodoptera litura*	exhibits enzymatic-inhibitory activity against *Spodoptera litura*	Datta et al., 2021
Ethyl acetate extract of *Alpinia galanga*	*Spodoptera litura* larvae	exhibits inhibitory activity against *Spodoptera litura* larvae	Datta et al., 2019
Chloroform, ethyl acetate and hexane extracts of *Moringa oleifera* (Lam.)	*Spodoptera litura*	have a negative impact on *Spodoptera litura* development.	Kaur et al., 2021

Continuation of Table 2.1

1	2	3	4
		Most of the larvae fed on a diet with the addition of *M. oleifera* extracts showed incomplete molting and turned into larva-pupa intermediates	
Ethyl acetate, 3-methyl-octan-4-ol	*Rhynchophorus phoenicis* (Coleoptera: Curculionidae)	attract *Rhynchophorus phoenicis* (Coleoptera: Curculionidae	Egonyu et al., 2021
Ethyl acetate extract of *Aspergillus flavus*	*Spodoptera litura*	It has genotoxic and cytotoxic effects on *Spodoptera litura*	Kaur et al., 2019
Ethyl acetate extract of borage (*Borago officinalis* L.)	cat flea *Ctenocephalides felis* and hedgehog flea *Archaeopsylla erinacei*	It has an insecticidal effect after 48 hours of contact	El Haddad et al., 2020
Ethyl acetate extract of *B. bassiana* and *T. harzianum* mushrooms	*A. gossypii*	Have an insecticidal effect	Abdullah, 2019
Ethyl acetate extract of *B. bassiana* mushroom	*S. littoralis*	It has an insecticidal effect	Abdullah, 2019
Salicylic acid	*Agonoscena pistaciae*	Causes an increase in the mortality of the pest Nikhath	Haghighi et al., 2021
Growth regulators: jasmonic and salicylic acids	cotton leafhopper *Amrasca biguttula bigutulla* (Ishida), cotton aphid *Aphis gossypii* (Glover)	Reducing the number of these pests on treatment of plants with these solutions	Nikhat et al., 2019

End of Table 2.1

1	2	3	4
	and cotton thrips Thrips *tabaci* (Lindeman)		
Salicylic acid increases the resistance of tomatoes to pests	Beet Leafhopper *Circulifer tenellus*	The accumulation of salicylic acid in plants reduces the number of pests	Blundell et al., 2020
Silicon, potassium silicate and salicylic acid	Cabbage aphid *Brevicoryne brassicae* (Hemiptera: Aphididae)	Reducing the pest population on application of fertilizers based on these chemicals	Abdollahs et al., 2021

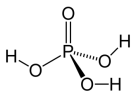
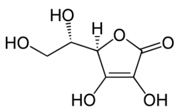
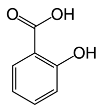
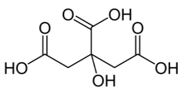
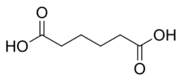
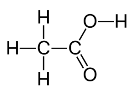
The experiment was divided into two parts in order to study the effect of food additives on the components of biocenoses in steppe ecosystems. The first part of the experiment was carried out using a biological model, larvae of *Chironomus* sp. (Diptera, Chironomidae).

The goal of this work was to study the effect of a group of chemicals on the larvae of *Chironomus* sp., as well as on their natural enemies, predatory litter invertebrates. These were chemicals such as orthophosphoric, ascorbic, salicylic, citric, adipic, acetic, tartaric, formic, propionic, benzoic acids, sodium metabisulfite, methylparaben, sodium nitrite, sodium hydroxide, calcium formate, potasium hydroxide, benzyl acetate, ethyl acetate, benzyl alcohol, cinnamic aldehyde, benzaldehyde, raspberry ketone, potassium nitrite, sodium hydrosulfite, sodium sulfate, 2-phenylphenol (Table 2.2, see p. 79–83).

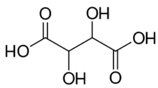
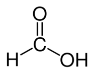
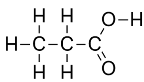
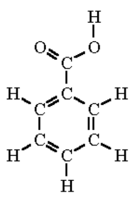
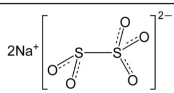
These substances are used not only in the food industry but also in other industries: medicine, pharmacy, cosmetology, chemical industry, etc. (Table 2.3, see p. 83–96).

Table 2.2

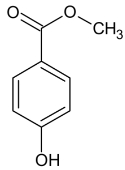
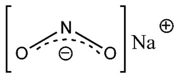
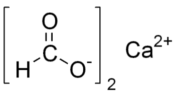
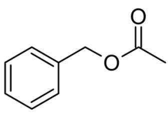
Properties of food additives which were used for establishing the viability level of *Chironomus* sp. larvae

Name of the substance	**Chemical formula**	**Structural formula**	**Properties**	**Content**
1	**2**	**3**	**4**	**5**
Ortho-phos-phoric acid	H_3PO_4		a colorless, odorless phosphorus-containing solid, and inorganic compound	it is produced industrially
ascorbic acid	$C_6H_8O_6$		white crystalline powder with sour taste. It soluble in water and in alcohol easily	find in citrus and other fruits and vegetables (citrus fruits, kiwifruit, guava, broccoli, Brussels sprouts, bell peppers, potatoes, and strawberries)
salicylic acid	$HOC_6H_4CO_2H$		a colorless, bitter-tasting solid	it occurs in plants, numerous fruits and vegetables, sweet potato, nuts, and olive oil
citric acid	$HOC(CO_2H)(CH_2CO_2H)_2$		it is a colorless weak organic acid	it occurs naturally in citrus fruits
adipic acid	$(CH_2)_4(COOH)_2$		it has chemical properties characteristic of carboxylic acids	it rarely occurs in nature
acetic acid	CH_3COOH		it is an acidic, colorless liquid and organic compound	it does not occurs in nature

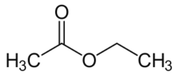
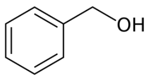
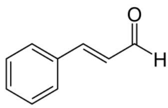
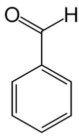
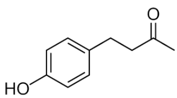
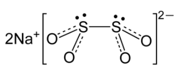
Continuation of Table 2.2

1	2	3	4	5
tartaric acid	$C_4H_6O_6$		is a white, crystalline organic acid	it occurs naturally in many fruits, most notably in grapes, but also in bananas, tamarinds, and citrus
formic acid	CH_2O_2		it is a colorless liquid having a pungent	is found in most ants and in stingless bees of the genus *Oxytrigona*
propio-nic acid	$CH_3CH_2CO_2H$		it is a liquid with a pungent and unpleasant smell	bacteria of the genus *Propionibacte-rium* produce propionic acid, it is the end-product of their anaerobic metabolism, Theese bacteria find in the stomachs of ruminants
benzoic acid	$C_6H_5CO_2H$		it is a white or colorless solid	it occurs naturally in many plant and animal species: in most berries, ripe fruits of several *Vaccinium* species, in apples after infection with the fungus *Nectria galligena*, in omnivorous or phytophageous species
sodium metabi-sulfite	$Na_2S_2O_5$		substance has a strong smell of rotten eggs	it does not occurs in nature

Continuation of Table 2.2

1	2	3	4	5
methyl-paraben	$CH_3(C_6H_4(OH)COO)$		it is a white crystalline powder, has low solubility in water	it occurs naturally in several fruits, particularly in blueberries, was found in the roots of *Tuberous oxalis*
sodium nitrite	$NaNO_2$		it is a white to slightly yellowish crystalline powder that is very soluble in water	it does not occurs in nature
sodium hydro-xide	NaOH	–	it is a white solid ionic compound consisting of sodium cations Na+ and hydroxide anions OH–	it does not occurs in nature
calcium formate	$Ca(HCO_2)_2$		calcium salt of formic acid	it does not occurs in nature
pota-sium hydro-xide	KOH	–	It exhibits high thermal stability	it does not occurs in nature
benzyl acetate	$CH_3C(O)OCH_2C_6H_5$		it is an organic ester, liquid possesses a sweet and pleasant fruit aroma	it is a constituent of jasmin and of the essential oils of ylang-ylang and neroli, in flowers like jasmine (Jasminum), and fruits like pear, apple, etc.

Continuation of Table 2.2

1	2	3	4	5
ethyl acetate	$CH_3CO_2CH_2CH_3$		this colorless liquid has a characteristic sweet smell	present in many organisms, It produce by yeast
benzyl alcohol	$C_6H_5CH_2OH$		it is a colorless liquid with a mild pleasant aromatic odor	it is produced naturally by many plants and is commonly found in fruits and teas
cinnamic aldehyde	$C_6H_5CH = CHCHO$		this pale yellow, viscous liquid	It occurs in the bark of cinnamon trees and other species of the genus *Cinnamomum*
benzaldehyde	C_6H_5CHO		it is a colorless liquid with a characteristic almond-like odor	it is the primary component of bitter almond oil, and other natural sources
raspberry ketone	$C_{10}H_{12}O_2$		it is a white needle or granular crystal with a ripe raspberry odor	it occurs in a variety of fruits, including raspberries, cranberries, and blackberries
potassium nitrite	KNO_2	–	a white or slightly yellow, hygroscopic crystalline powder that is soluble in water	it is present at trace levels in soil, natural waters, plant and animal tissues, and fertilizer
sodium hydrosulfite	$Na_2S_2O_4$		it is a white crystalline powder with a sulfurous odor	it does not occurs in nature

End of Table 2.2

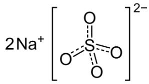
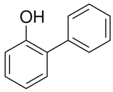
1	2	3	4	5
sodium sulfate	Na_2SO_4		it is white solid that are highly soluble in water	anhydrous sodium sulfate occurs in arid environments as the mineral thenardite
2-phe-nyl-phenol	$C_6H_5C_6H_4OH$		it is a white solid	it does not occurs in nature

Table 2.3

Application of food additives in the food and other industries

Name of the substance	**Food supplement**	**Usage in food industry**	**Usage in other industries**
1	**2**	**3**	**4**
Ortho-phospho-ric acid	E338	It is an acidity regulator used in the production of carbonated beverages	This food additive is used in the aviation industry. In animal husbandry farms, an orthophosphoric acid solution is used to prevent increased gastric acidity, as well as urolithiasis in animals. E338 is also used in hydroponic systems to adjust the acidity level of the nutrient solution. In medicine, this acid is used in dental filling with subsequent washing and drying
ascorbic acid	E300	This antioxidant is used in the food industry in the form of salts (sodium, calcium, potassium) to prevent the oxidation of products.	It is used in various fields of human activity: pharmacology, medicine, cosmetology, photographic development, in analytical chemistry. It is a part of antioxidant preparations used in the treatment of carbon monoxide poisoning, and it promotes the restoration of redox processes.

Continuation of Table 2.3

1	2	3	4
		Its derivative, D-isoascorbic acid (E315), is used as a preservative in the food industry	This acid is also part of pharmacological preparations used for hemorrhagic diathesis, capillary toxicosis, hemorrhagic stroke, nasal, pulmonary, uterine bleeding, slow healing wounds, ulcers, burns, during infectious diseases, idiopathic methemoglobinemia, intoxication, alcoholic and infectious delirium, acute radiation sickness, transfusion-associated complications, Botkin's disease, chronic hepatitis and cirrhosis, gastrointestinal diseases, such as achillea, peptic ulcer, enteritis, colitis, cholecystitis, as well as helminthiasis. Ascorbic acid-containing preparations are used for physical and mental overload, during pregnancy, as well as for vitamin C deficiency. In cosmetology, it is used to heal and restore the skin protective functions in order to moisturize and increase the elasticity of the skin exposed to sunlight. In order to lighten the skin and reduce age spots, this acid is part of creams. In photography, it is used in industrial developers as a developing substance. Photochemistry manufacturers use ascorbic acid as part of photographic developers and photo papers. In analytical chemistry, this acid restores such inorganic substances as iron, mercury, gold, platinum, silver, iodine, and others

Continuation of Table 2.3

1	2	3	4
salicylic acid		It is used as a preservative in food preservation	In medicine, salicylic acid as an antiseptic is used to produce topical ointments and solutions for the treatment of skin diseases. Salicylic acid is a part of some pastes, powders, patches and other medications. Also, among the dosage forms, there is a topical alcohol solution for external use. Derivatives of this acid are also used in medicine. Thus, acetylsalicylic acid (aspirin) is used in medicine as an antipyretic, analgesic, anti-inflammatory, as well as anti-rheumatic agent. Phenylsalicylate is also used as an antiseptic, and para-aminosalicylic acid is used as an antitubercular agent
citric acid	E330	This acid, as well as its derivatives: salts sodium citrate, potassium citrate, calcium citrate, and bismuth tripotassium dicitrate, are used in the food industry as a flavouring additive. Citric acid is also used as an acidity regulator. At the same time, it has the preservative properties. It is used in the production of melted cheeses, as well as dry mixes for the preparation of carbonated drinks	Scientists report that citric acid in small doses is able to activate the Krebs cycle in the body. And this, in turn, contributes to the acceleration of metabolism. Therefore, this acid is used in medicine to improve energy metabolism in the Krebs cycle. It is used as an acidity regulator of cosmetics in cosmetology, and also as a buffer solution in mixtures for the preparation of "effervescent" baths. This acid is used during the drilling of oil and gas wells in order to reduce the acidity of the drilling mud after alkaline baths. In the construction industry, citric acid is added to cement and gypsum solutions as a retarding agent. This acid together with hydrogen peroxide is used to etch away the copper layer on printed circuit boards.

Continuation of Table 2.3

1	2	3	4
			In household chemicals, citric acid is part of the cleaning agent for heating surfaces. So, for example, it is used as a descaling agent to clean kitchen utensils
adipic acid	E355	In the food industry, adipic acid is used to give a sour taste to food products. It is used in the production of non-alcoholic drinks	Like citric acid, in household chemicals it is part of various descaling agents. It is also used on the seams between ceramic tiles to remove residual adhesive material
acetic acid	E260	In the food industry, acetic acid and its solutions are used in the canning of products in household cooking	In household chemicals, it is also used as a descaling agent. In medicine, acetic acid is used to produce medicinal and fragrant substances. For example, it is used as a solvent in the production of acetylcellulose, as well as acetone. Acetic acid is used in printing and dyeing. For the purpose of oxidation of various organic substances, acetic acid is also used as a reaction medium. This can be the oxidation of organic sulfides with hydrogen peroxide in the laboratory, and, on an industrial scale, it can be the oxidation of paraxylene with air oxygen into terephthalic acid. Scientists suggest that having a pungent odour, this acid can be used instead of liquid ammonia for medical purposes to prevent or treat fainting
tartaric acid	E334	Tartaric acid is used as an acidity regulator. In the food industry, this additive is also used as an antioxidant	This acid is used in many fields of human activity, including medicine, analytical chemistry, chemical and pharmacological industries. In medicine, it is used as tartaric acid salts during tissue staining.

Continuation of Table 2.3

1	2	3	4
		in the production of canned food, jams, jellies, as well as various confectionery products	This acid is used in analytical chemistry to detect aldehydes, sugars and other substances
formic acid	E236	It is used in the food industry as a preservative	In agriculture, this acid is used as a preservative and antibacterial agent in forage conservation. It is able to slow down the processes of decay and rotting. It was found that hay and silage treated with formic acid can keep for long time. This acid is also used in wool dyeing. There is a lot of data on the use of formic acid to control the parasites of honey bees. In some chemical reactions, formic acid is used as a solvent. In order to improve the properties of concrete, this acid is used to produce formates. In medicine, formic acid is used to prepare solutions of performic acid as a mixture of hydrogen peroxide and formic acid. It is used as an antiseptic in the surgery. At the same time, formic acid is used in the pharmaceutical industry for the purpose of equipment disinfection
propionic acid	E280	Propionic acid is used as a preservative in the food industry. It is able to delay the development of bacteria and mold on food products	Propionic acid is widely used in agriculture as pesticides. This acid and its derivatives can be comprised in herbicides. In pharmacology, its derivatives are part of medicines, aromatic substances

Continuation of Table 2.3

1	2	3	4
benzoic acid	E210	In the food industry, benzoic acid is used as a preservative, in a concentration up to 0.1 %	This acid is used in the production of caprolactam and viscose. Benzoic acid is also used in the production of salts such as potassium benzoate, sodium benzoate, calcium benzoate, etc. In cosmetology, benzoic acid is used as a preservative, as well as in the food industry. It has anti-corrosion properties. Benzoic acid is used in the production of dyes. In pharmacology, it is used in the manufacture of antimicrobial and fungicidal drugs
sodium metabi-sulfite	E223	This food additive is used in the food industry as a preservative, as well as an antioxidant. It is used as a bleach and raising agent in the production of fruit juices, sweets, as well as beverages such as wine and beer	It is also used in pharmacy, medicine, chemical industry and agriculture. In pharmacy, it is used as a tablet filler, as well as as an excipient in injectable medicines and water filtration systems. Sodium metabisulfite is used in medicine for equipment disinfection. In agriculture, sodium metabisulfite is used to remove tree stumps
methyl-paraben	E218	In the food industry it is used as a preservative	In pharmacy and cosmetology, methylparaben is widely used as a preservative in the manufacture of medicines and cosmetics
sodium nitrite	E250	As a preservative, as an antioxidant, as well as to improve colouring, this additive is used in the food industry. It is used for the preparation of meat and fish products.	Sodium nitrite is used by humans in various fields, including chemical metallurgy, medical, pulp-and-paper and other industries. So, in the chemical industry, sodium nitrite is used in the form of a powder or an aqueous solution as an antifreeze additive to concrete in the production of construction products during

Continuation of Table 2.3

1	2	3	4
		It has antibacterial properties and prevents the growth of *Clostridium botulinum* (the causative agent of botulism), which leads to severe food intoxication caused by botulinum toxin and is characterized by nervous system damage. Sodium nitrite interacts with myoglobin (meat protein). This additive gives meat products a characteristic pinkish color. In the European Union, sodium nitrite is allowed only in a mixture with food salt, with a nitrite content of about 0.6 %, in order to reduce the risk of exceeding daily norms, which can cause fatal poisoning	the construction of monolithic concrete and reinforced concrete structures. Sodium nitrite is also used in the production of diazo dyes, nitro compounds. Sodium nitrite is needed in dyeing by staple and direct (direct printing) methods of textiles made of natural and bleached natural fibers. It is used in metal surface treatment during phosphating treatment and for removing the tin layer, as well as in the production of rubbers. Sodium nitrite is used in the manufacture of alkyl nitrites. It is used in the production of explosives for the preparation of a solution of a gas-generating additive. In photography, it is used as a reagent and an antioxidant (as a corrosion inhibitor in automatic laboratories). In medicine and veterinary, it is used as a vasodilator, bronchodilator. It is able to relieve intestinal spasms and is used as a laxative, as well as an antidote for cyanide poisoning. With sodium nitrite, experiments are conducted on its use in sickle anemia, heart attacks and cardiac ischemia, brain aneurysms and pulmonary hypertension in children
sodium hydroxide	E524	This additive is used to wash and peel fruits and vegetables. Also, sodium hydroxide is used	Sodium hydroxide is used in various industries. So, it is used in the pulp-and-paper industry in the production of paper, cardboard, artificial fibers, and wood-fiber boards.

Continuation of Table 2.3

1	2	3	4
		in the production of chocolate and cocoa, beverages and ice cream. It is used when coloring caramel, as well as to give olives a black color. This additive is also used in bakery production	In the chemical industry, this additive is used in the production of soap, shampoo and other detergents. Products based on sodium hydroxide, heated to +50…+60 °C, are used in the field of industrial washing to clean stainless steel products from grease and other oily substances, as well as mechanical processing residues. In chemical industries, it is used to neutralize acids and acid oxides, as a reagent or a catalyst in chemical reactions. In chemical analysis, it is used for titration, aluminium etching and in the production of pure metals. In the oil refining industry, it is used for the production of oils. Also, sodium hydroxide is used for the manufacture of bio-diesel fuel. This additive is part of the products (gels, dry granules) used for cleaning sewer pipes. Sodium hydroxide is used in civil defense to neutralize toxic substances, as well as to purify exhaled air from carbon dioxide. Sodium hydroxide is also used in the textile industry. The fiber of wool and cotton acquires strength and silky shine. In cosmetology, specialists use sodium hydroxide to remove keratinized skin areas, warts, and papillomas. Among the various fields of use, sodium hydroxide is used in photography. It has an accelerating property in developers for high-speed processing of photographic materials

Continuation of Table 2.3

1	2	3	4
calcium formate	E238	Calcium formate is used as a preservative in the production of non-alcoholic drinks. It is also used in the fermentation of various vegetables (especially cabbage). Calcium formate is used as a salt alternative in all dietary products	In construction, calcium formate is used as a hardening accelerator for building mixes and concrete. It is also used as concrete antifreeze. In cosmetology, this additive is used to protect cosmetic products from spoilage. Calcium formate is also used for tanning leather, as well as dyeing fabric and printing colored wallpaper
potasium hydroxide	E525	In the food industry, it is used as an acidity regulator. It is also used to peel vegetables, root vegetables and fruits	The food additive potassium hydroxide is used in various areas of human activity. It is used to neutralize acids as alkaline elements. This additive is part of detergents, dyes, fertilizers. It is used in gas purification, metallurgical production, oil refining, and paper production. This substance has found wide application in pharmacy, as well as household chemicals. It is also used to produce methane, and absorb acid gases. In cosmetology, it is used in the manufacture of some cosmetic products. It is also widely used as a sewer cleaner. It is used in the form of granules or gels. Potassium hydroxide enhances the movement of waste further down a pipe. It is also used to produce defluorinated zirconium hydroxide. It is used to clean stainless steel items from grease and other oily substances, as well as from mechanical processing residues.

Continuation of Table 2.3

1	2	3	4
			In the manufacture of batteries, potasium hydroxide is used as an electrolyte in alkaline batteries. Solution 5 % is used in medicine to treat warts. In photography, potassium hydroxide is used as a component of developers, toners, and thiosulfate indicators and for removing emulsion from photographic materials
benzyl acetate		In the food industry, benzyl acetate is used in confectionery production	It is a part of fragrant oils, such as jasmine flower oils, gardenia flower oils, and ylang-ylang oil. It is widely used in perfumery
ethyl acetate	E1504	This additive has a pronounced fruity smell. It is used as a component of fruit essences. Addition of ethyl acetate to flavorings	Ethyl acetate is often used as a solvent, as it is low-toxic, and also has an acceptable odor. For example, it is used as a solvent for polyurethane, nitrocellulose, acetyl cellulose, fats, as well as waxes. In the production of artificial leather, it is used to clean printed circuit boards, and mixed with alcohol as a solvent. In entomology, this substance is known as a poison for entomological killing jars for the purpose of killing insects. The body of insects after using ethyl acetate is softer and more suitable for preparation than after killing in chloroform vapors. This substance is often used for extraction, as well as in chromatography (both column and thin layer). It is sometimes used as a solvent for conducting reactions, as well as for obtaining acetoacetic ether

Continuation of Table 2.3

1	2	3	4
benzyl alcohol	E 1519	In the food industry, benzyl alcohol is used in the manufacture of liqueurs, flavored wines, beverages, and cocktails. This additive is part of the flavors	Benzyl alcohol is used in perfumery. In the chemical industry, it is used as a solvent for lacquers. In pharmacology, benzyl alcohol is used for the disinfection of oil solutions of drugs for intramuscular injection
cinnamic aldehyde		Cinnamic aldehyde is a component of food essences, it is used as an aromatic substance	Cinnamic aldehyde is widely used in perfumery to create perfume compositions. This additive is widely used in the production of soap fragrances. In the chemical industry, cinnamic aldehyde is used to produce cinnamon alcohol, cinnamyl cinnamate, and other fragrances. There is scientific evidence of the use of cinnamic aldehyde as a fungicide. It is often used in agriculture as it is low-toxic. It is also used as an insecticide and a means for animal hazing (cats and dogs). In veterinary medicine, good results were obtained when using this substance in vitro against the larvae of nematode parasites of farm animals. Also, cinnamon aldehyde has proven itself as a corrosion inhibitor for steel and iron alloys
benzal-dehyde		Benzaldehyde is used as a food aromatic agent	Benzaldehyde is used for the synthesis of mandelic acid, as well as dyes. In perfumery and cosmetology, this additive is used for the synthesis of fragrant substances in perfume and cosmetic compositions
raspberry ketone		In order to impart a fruity smell raspberry ketone is used in the food industry	Raspberry ketone is used in perfumery as well as cosmetology

Continuation of Table 2.3

1	2	3	4
potassium nitrite	E 249	Potassium nitrite is used as a preservative. It is used similarly to other nitrites and salts, such as sodium chloride, and sodium nitrite. Potassium nitrite prevents the development of botulinum toxin, which can be developed during the vital activity of Clostridium botulinum. In addition to meat, potassium nitrite forms nitrosomyoglobin with a characteristic red color. It can be observed in most food products: sausages, etc. In European Union, potassium nitrite is allowed for food purposes only in the form of a mixture with food salt. At the same time, the nitrite content should be up to 0.6 %	Potassium nitrite is used in the production of azo dyes for diazotization. In analytical chemistry, it is used to identify amines. It also spread in photography as a sensitizer
sodium hydro-sulfite	E222	In the food industry, it is used as a preservative, as well as an antioxidant. It is often used in exported wines	Sodium hydrosulfite is used in various industries. In the textile industry, this substance is used in bleaching and dyeing fabrics as a preservative.

Continuation of Table 2.3

1	2	3	4
		in order to prevent oxidation, as well as preserve the taste. Sodium hydrosulfite is used for fruit canning. This additive is used as antibrowning agent. It is used in germ-fighting. But in very high concentrations, sodium hydrosulfite can cause allergic reactions	In some countries of the world, including the USA, sodium hydrosulfite is prohibited for use on raw vegetables and fruits, as it can cause a number of gastrointestinal diseases
sodium sulfate		Sodium sulfate is an acidity regulator. It is used as a buffer additive that maintains the acidity level	All over the world, sodium sulfate has been widely used in the production of synthetic detergents. But in recent years it has been used less often. Also, this substance is used in large quantities in glass production. It is considered no less common in the production of cellulose by the sulfate method. Sodium sulfate is also used in the textile, leather industries, as well as in non-ferrous metallurgy. It is used in small quantities in chemical laboratories for the dehydration of organic solvents. Sodium sulfate is even less widespread in medicine and veterinary medicine. It is used as a saline laxative and as a component in nasal cleansers. Its aqueous solutions are used in medicine to treat poisoning with soluble barium salts

End of Table 2.3

1	2	3	4
2-phenyl-phenol	E231	2-phenyl-phenol refers to preservatives. In the food industry, it is used for the external processing of fruits	In the textile industry, it is used as an antiseptic in the manufacture of real leather, as well as as an additional reagent when dyeing with dispersed dyes. In photography, the use of this substance was proposed (patent US2252718) as a blue diffusing color-forming component

Note: data are generalized based on OSHA Occupational Chemical Database – Occupational Safety and Health Administration, Nomenclature of Organic Chemistry: IUPAC Recommendations and Preferred Names 2013 (Blue Book), Lide, (2005), Butler et al. (2008), with additions of information from other sources.

Representatives of the family Chironomidae are among the most sensitive insects that are used as bioindicators. Thus, De Bisthoven et al. (2005) conducted an experiment using larvae of the family Chironomidae as bioindicators of acid mine effluents in Portugal. Francis et al. (2018) also used representatives of the family Chironomidae as bioindicators of heavy metal pollution of Lake Shiroro (Niger State, Nigeria). The scientists studied the deformation of oral apparatus in the larvae of these insects under the influence of heavy metals contaminated the water reservoir. At the same time, of the dominant species of Chironomidae (*Chironomus* sp., *Polypedilum* sp. and *Ablabesmyia* sp.), the most pronounced deformities were observed in *Chironomus* sp. The deformation of Chironomidae (*Prodiamesa olivacea* (Meigen, 1818) was also studied by Servia et al. (1998). The authors documented and illustrated morphological deformations of *Prodiamesa olivacea* larvae sampled from a polluted urban area in Santiago de Compostela (northwest Spain).

Similar studies have been conducted in Northeastern Algeria. Youbi et al. (2020) proposed using morphological deformations of the genus *Chironomus* (Diptera: Chironomidae) representatives as a bioindicator of heavy metal contamination. The studies of these scientists have shown the relationship between the concentrations of heavy metals in water, as well as water deposits, and the oral apparatus deformations in larvae of the genus *Chironomus*. Therefore, information on such deformations in *Chironomus* sp. can be used in bioassessment of freshwater ecosystems.

Violations of the oral apparatus structure in larvae of *Chironomus riparius* (Diptera, Chironomidae) were also studied by Tomilina, Grebenyuk (2020). The scientists determined changes in the oral apparatus under the influence of metal-containing nanoparticles. Similar studies were conducted by Veroli et al. (2014). They also studied deformations of Chironomidae larvae as a result of contamination of water reservoirs with heavy metals.

Lee et al. (2009) also evaluated the genotoxicity and ecotoxicity of nanoparticles, cerium dioxide, silicon dioxide and titanium dioxide on *Chironomus riparius* larvae. The scientists have established the genotoxic effect of cerium dioxide on the larvae of this species.

Similar studies on *Chironomus tentans* larvae were conducted by Park and Choi (2007). The scientists used nonylphenol, bisphenol A, bis(2-ethylhexyl)phthalate and paraquat dichloride as model pollutants. According to the results of their studies, nonylphenol and bisphenol A have a pronounced genotoxic effect on larvae.

Lencioni et al. (2012) determined the environmental qualities of mountain springs using Chironomidae bioindicators. The scientists analyzed the influence of environmental factors in 124 springs of Italian Prealps and Alps with varying pollution levels on the reaction of chironomid communities (Diptera: Chironomidae).

Basu et al. (2010) investigated the bioindicator potential of Chironomidae larvae to determine the antibiotic-resistant microbial load of an aquatic ecosystem. According to the results of their research, it can be assumed that midge larvae can be used as a powerful bioindicator to assess the aquatic environment in which they live by assessing the accumulation of microbial load resistant to antibiotics in this environment. At the same time, the larvae of *Chironomus* sp. were a food base for predatory insects (Aditya, Saha, 2006).

At the first stage of the experiment, the effect of 1 % solutions of the following 26 food additives on the viability of *Chironomus* sp. larvae was determined: cinnamic aldehyde, formic acid, benzoic acid, methylparaben, raspberry ketone, benzaldehyde, benzyl acetate, benzyl sprite, ethyl acetate, salicylic acid, tartaric acid, orthophosphoric acid, sodium hydroxide, acetic acid, adipic acid, sodium metabisulfite, citric acid, ascorbic acid, potassium hydroxide, sodium nitrite, sodium hydrosulfite, sodium cyanoxide, sodium nitric acid, calcium formic acid, propionic acid, 2-phenylphenol.

In order to determine the viability of the first-second-stage larvae, *Chironomus* sp. (3–8 specimens) were placed in 1.5 ml test tubes. Then 1 ml of 1 % aqueous solution of each of the used food additives was added in a fivefold repetition. Distilled water was used to prepare an aqueous solution. The reference group of *Chironomus* sp. larvae was kept in distilled water without the addition of food additives. The experiment exposure was 15, 30, 60 and 120 minutes. The temperature values were within the range of 25…28 °C.

A sampling of coleopteran species was carried out on the territory of the green zones of Dnipro city in June-August 2021 (applications). Common herpetobiont species of predatory Coleoptera of the families Carabaidae and Staphylinidae were used as experimental objects (*Harpalus rufipes* (De Geer, 1774), *H. smaragdinus* (Duftschmid, 1812), *Harpalus* sp., *Calathus fuscipes* (Goeze, 1777), *Staphylinus caesareus* Cederhjelm, 1798) (Fig. 2.1, see p. 99).

H. rufipes is the most common of the studied species inhabiting the Tunnel Gully. It is a pest of many agricultural crops. This species causes damage to cereals, legumes, sugar beets, as well as tree nurseries. It can often be found on the territory of anthropogenic landscapes. At the larval stage, *H. rufipes* is a predator, and at the imago stage, it is a myxophytophagus. In the northern part of the range, *H. rufipes* develops for two years. The more rapid development of this beetle species is recorded in its southern part. On the territory of Ukraine, *H. rufipes* is observed from mid-spring to early autumn. This species is considered to be an effective entomophagus. It actively destroys the Colorado potato beetle, weevils, as well as owlet moths.

a

b

c

d

e

Fig. 2.1. Harpalus rufipes (*a*), H. smaragdinus (*b*), H. sp. (*c*), Calathus fuscipes (*d*), Spaphylinus caesareus (*e*)

Beetles were caught in artificial forest plantations of the Tunnel Gully (Fig. 2.2–2.22, see p. 100–120). The characteristics of individual sites were given in the scientific works of the authors (Faly, Brygadyrenko, 2014). Soil traps type Barber (plastic cups with a capacity of 0.2 L without a retainer) were used to collect insects. The sampling of the material was carried out at 2–3-day intervals.

Fig. 2.2. Xeromesophilic coppice Acer negundo plantation in the middle third of the north-facing slope

Fig. 2.3. The edge of xeromesophilic ash-oak plantation with dominance of Elytrigia repens in the middle third of the north-facing slope

Fig. 2.4. Mesophilic oak-maple plantation with ruderal forbs in the thalweg of a ravine

Fig. 2.5. The edge of xeromesophilic Ailanthus altissima and Robinia pseudoacacia plantation with ruderal forbs on the middle third of the south-facing ravine slope

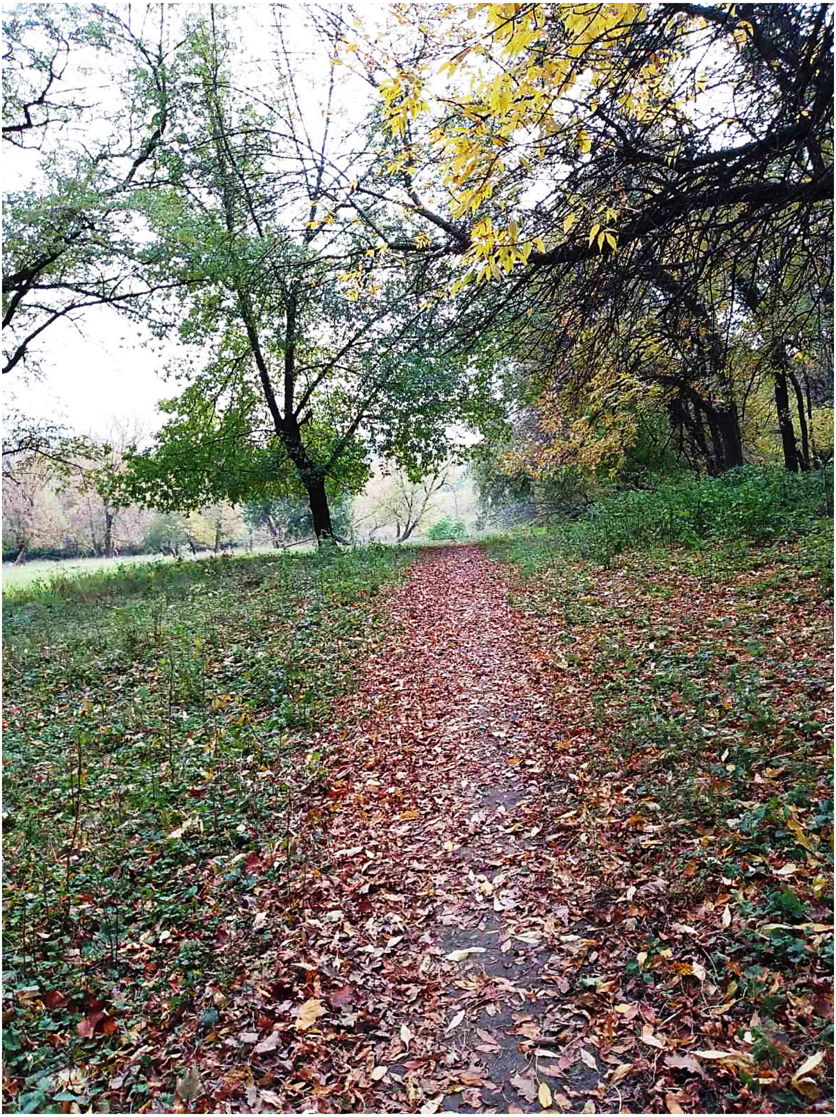


Fig. 2.6. The edge of xeromesophilic maple-ash plantation on the lower third of the north-facing slope of a ravine

Fig. 2.7. Mesohygrophilic bottomland meadow with Phragmites australis in the thalweg of a ravine

Fig. 2.8. Mesophilic willow-elm plantation with Elytrigia repens in the thalweg of a ravine

Fig. 2.9. Mesophilic willow-ash plantations with Elytrigia repens in the thalweg of a ravine

Fig. 2.10. Mesophilic coppice plantations of white mulberry and ash-leaved maple with Elytrigia repens in the lower third of the north-facing slope

Fig. 2.11. The edge of dead-covering mesophilic oak plantation in the lower third of the south-facing slope

Fig. 2.12. Mesophilic ash-oak plantation with broad-leaved grasses in the thalweg of a ravine

Fig. 2.13. Mesohygrophilic meadow community with dominance of Elytrigia repens in the thalweg of a ravine

Fig. 2.14. The edge of mesophilic maple-oak plantation
in the lower third of the north-facing slope

Fig. 2.15. The edge of willow-poplar plantation with mesophilic forbs in the thalweg of a ravine

Fig. 2.16. Mesohygrophilic ash-oak plantation with Viola odorata in the thalweg of a ravine

Fig. 2.17. Xerophilic ruderal forb steppe community with dominance of Bromus inermis in the upper third of the northwest-facing slope

Fig. 2.18. Mesophilic poplar plantation with ruderal forbs in the thalweg of a ravine

Fig. 2.19. Mesophilic oak-maple plantation with Anthriscus sylvestris in the thalweg of a ravine

Fig. 2.20. Coppice growth of an adventive species, Ailanthus altissima, in the lower third of the south-facing slope

Fig. 2.21. Mesophilic meadow community with dominance of Elytrigia repens and Poa pratensis in the thalweg of a ravine

Fig. 2.22. Ruderal mesophilic forbs in the thalweg of a ravine

To conduct experiments on the effect of food additives on the lifespan of litter-dwelling coleopteran species, the chemicals of 1 % concentration most common in the food industry were used (propionic, adipic, acetic acids, sodium metabisulfite, sodium nitric acid, sodium hydroxide, calcium formate, calcium hydroxide, benzyl acetate, ethyl acetate, cinnamic aldehyde, potassium nitrite, sodium hydrosulfite, 2-phenylphenol).

In the laboratory, the captured beetles were kept in insectariums (plastic cages with a volume of 1000 cm^3, tightly covered with a fine mesh) in a thermostat with a constant air temperature of 26 °C. Each species was kept separately, 8–10 individuals per insectarium for a month. Purified fine-grained river sand was used as a substrate. The substrate was periodically moistened to maintain optimal microclimatic conditions. Predatory ground beetles and staphylinids were fed with mosquito larvae (Diptera, Chironomidae, *Chironomus* sp.). The larvae were previously placed in an aqueous solution of the chemicals used in the experiment (at a concentration of 1 %) for 30 minutes. Regardless of whether the mosquito larvae died in a solution of a food additive or not, they were removed from the container and placed in an insectarium containing beetles. Feed was introduced into cages with coleopteran species once a day. The control of the surviving and dead beetles was carried out daily.

According to the results of studies on the effect of food additives on mosquito larvae, 100 % death of these insects was established under the influence of methylparaben, raspberry ketone, benzaldehyde, benzyl alcohol, ethyl acetate, benzoic, salicylic, acetic, adipic, propionic acids, sodium metabisulfite, 2-phenylphenol. The death of insect larvae was also observed under the influence of a 1 % solution of cinnamic aldehyde and formic acid in the exposure for 30 minutes and longer (Table 2.4, see p. 122). Similar results were recorded using potassium hydroxide. Although 100 % death of larvae was observed in the exposure of 120 min, more than 50 % of dead individuals have been identified under the influence of this substance after 60 minutes.

Table 2.4

The effect of 1 % of chemicals used in the food industry on the survival of mosquito larvae (%, ($x \pm$ SE, $n = 5$))

Name of the substance solution	Exposure, min			
	15	**30**	**60**	**120**
cinnamic aldehyde	100.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
formic acid	100.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
benzyl acetate	93.334 ± 6.67	86.668 ± 8.16	80.00 ± 13.33	63.33 ± 13.07
tartaric acid	44.67 ± 4.11	44.67 ± 4.11	44.67 ± 4.11	44.67 ± 4.11
orthophosphoric acid	92.00 ± 8.00	92.00 ± 8.00	92.00 ± 8.00	57.33 ± 14.08
sodium hydroxide	100.00 ± 0.00	53.91 ± 5.26	39.05 ± 10.09	36.19 ± 11.18
citric acid	60.33 ± 16.55	50.33 ± 13.23	45.33 ± 14.18	37.83 ± 13.31
ascorbic acid	52.00 ± 16.79	52.00 ± 16.79	52.00 ± 16.79	44.00 ± 17.71
potasium hydroxide	100.00 ± 0.00	75.24 ± 2.91	36.83 ± 8.52	0.00 ± 0.00
potasium nitrite	100.00 ± 0.00	65.68 ± 5.19	43.44 ± 13.95	26.30 ± 7.88
sodium hydrosulfite	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	71.67 ± 13.33
sodium sulfate	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	93.33 ± 6.67
sodium nitrite	100.00 ± 0.00	100.00 ± 0.00	92.07 ± 1.77	78.05 ± 3.73
calcium formate	100.00 ± 0.00	100.00 ± 0.00	93.33 ± 6.67	66.67 ± 10.54

When treating mosquito larvae with tartaric acid, the death of more than 50 % of individuals was already recorded after 60 minutes. But 100 % insect death during the experiment (15–120 min) was not observed. Less influential were sodium hydroxide, ascorbic and citric acids, as well as potassium nitrite. The death of more than 50 % of individuals occurred only with an exposure of 60 minutes and longer.

The other food additives studied (benzyl acetate, orthophosphoric acid, sodium hydrosulfite, sodium sulfate, sodium nitrite, and calcium formate) did not significantly affect the vital activity of mosquito larvae. With an exposure of 120 min, more than 50 % of the individuals remained viable under the chemicals' influence.

The results of studies on the effects of food additives on the coleopteran species viability show that the chemicals used in the experiment with a concentration of 1 % practically do not affect the lifespan of predatory litter-dwelling coleopteran species. When processing food objects with propionic, adipic, acetic acids, cinnamic aldehyde, calcium hydroxide, and benzyl acetate, 100 % survival of beetles of all species was observed. When used with most other food additives, such as calcium formate, sodium nitrite, potassium nitrite, sodium hydroxide, 2-phenylphenol, sodium hydrosulfite, beetle mortality did not exceed 1.2 %; 1.8 %; 2.0 %; 2.5 %; 2.8 % 3.4 % respectively. Only for ethyl acetate and sodium metabisulfite, the mortality rates of coleopteran species were slightly higher (6.5 and 16.7 %, respectively).

Every day our planet comes under anthropogenic influence. Tens of thousands of various chemical substances enter the environment: household, industrial, radioactive and other types of waste, dioxins, pesticides, medicines, and other substances that humans use in their everyday life. Today, the food industry is developing intensively. The use of a variety of food additives to enhance taste, change color, and for other purposes during storage, processing and preparation of products is a mandatory process in this field of industry. Regularly, food waste, together with preservatives, acidity regulators, dyes and other additives, enter the environment, affecting the components of the biocenosis of various ecosystems in different ways. Some of the food additives are used in agriculture for pest control. Thus, the resistance of plants to sucking pests (aphids, leafhoppers, and psyllas) increases when treating plants and soil with substances containing salicylic acid (Haghighi et al., 2021). A decrease in the number of pests such as *Amrasca biguttula bigutulla* Ishida, 1912, *Aphis gossypii* Glover, 1877, Thrips *tabaci* Lindeman, 1889, *Circulifer tenellus* (Baker, 1896), *Brevicoryne brassicae* (Linnaeus, 1758) was proved in plants treated with salicylic acid (Nikhath et al., 2019; Blundell et al., 2020; Abdollahi et al., 2021). In our in vitro experiment, we also observed a significant effect of salicylic acid on mosquito larvae.

Tartaric, kojic, formic, stearic, and succinic acids are often included in the composition of synthetic aromatic substances. Under experimental conditions, the presence of these acids did not affect the motor activity of *Tribolium confusum* Jacquelin du Val, 1868 (Titov & Brygadyrenko, 2021). We also observed fewer active effects of tartaric and formic acids on mosquito larvae than salicylic acid. During the whole experiment, the larvae were recorded alive under the influence of 1 % tartaric acid solution. But viable individuals were observed in 1 % formic acid solution only for the first 15 minutes of the experiment. At the same time, succinic and benzoic acids have an attractant effect on *Sitophilus granarius* (Linnaeus, 1875), and reduce its motor activity (Titov & Brygadyrenko, 2021). Benzoic acid is also often used in the composition of insecticides (Jiang et al., 2021). Some substances, such as monoterpenes, sesquiterpenes, iridoid monoterpenes, cyanogenic glycosides, benzoic acid derivatives, benzoquinones and naphthoquinones, can be synthesized in both insects and plants. Sometimes these substances are produced by plants to interfere with their functioning in insects (Beran et al., 2019). It has been proved that benzofurans, benzoic acid derivatives, diterpenes and pyrrolizidine alkaloids of *Senecio subcandidus* A. Gray plants, native to Peru, have insecticidal properties (Ruiz-Vasquez et al., 2018). In this experiment, we also recorded 100 % death of mosquito larvae on exposure to 1 % solution of this acid. We have shown such a strong effect of benzoic acid on living organisms in our previous works regarding the effect of acids on the larvae of nematode parasites of farm animals in vitro (Boyko, Brygadyrenko, 2019a).

Benzyl alcohol is a component of essential oils, and it is widely used in perfumery and pharmacology. Benzyl alcohol has attractant properties in relation to different types of parasites (Hassaballa et al., 2021) and pests (Gharaei et al., 2020; Grant et al., 2021; Nusra et al., 2021). This substance can be used as baits (Magsi et al., 2021; Mitra et al., 2021). Benzyl alcohol also has insecticidal properties (Nagarajan et al., 2001; Shao et al., 2019). This was also confirmed by our experiments on mosquito larvae. During the exposure to its

1 % solution, 100 % death of individuals was observed in the first 15 minutes of the experiment.

Benzaldehyde is widely used as a food flavor, and as a fragrant substance in perfumery. This substance can be used for the production of new pesticides (Ullah et al., 2015), since benzaldehyde and its derivatives have broad larvicidal and insecticidal activity (Mulvihill et al., 2001; Makhanya et al., 2020). In our experiment, benzaldehyde and cinnamon aldehyde also had a negative effect on mosquito larvae, although 100 % of predatory coleopteran species that fed on insect larvae treated with a 1 % solution of cinnamon aldehyde survived in our experiment in vitro.

Benzyl acetate is a fruity-smelling natural organic substance. Benzyl acetate is an important component of some essential oils, and it is widely used in perfumery and confectionery production as a flavoring agent. Traps baited with benzyl acetate attract solitary bees and other insects (Ikemoto & Tomoyuki, 2021). The use of benzyl acetate for *Apis cerana* Fabricius, 1793 as an alarm pheromone, which is avoided by other honeybee species, has also been established (Wen et al., 2017). This additive also had no negative effect on mosquito larvae, as well as on predatory coleopteran beetles that feed on these larvae.

Ethyl acetate is widely used as a solvent, it is slightly toxic and has the appropriate odor. Very often it is used in entomological traps for killing insects. It is a frequent component of various fruit essences, and was registered as an E1504 flavor. There are a large number of scientific papers devoted to the research of ethyl acetate and its effects on various groups of invertebrates. Ethyl acetate extract isolated from the bacterium *Photorhabdus temperata* M1021 causes 100 % death of *Galleria mellonella* (Linnaeus, 1758) larvae (Ullah et al., 2015). Ethyl acetate extract of *Beauveria bassiana* (Bals.-Criv.) Vuill. (1912) mushroom has an insecticidal effect on *Aphis gossypii* Glover, 1877 and *Spodoptera littoralis* (Boisduval, 1833), and ethyl acetate extract of *Trichoderma harzianum* Rifai, 1969 has such effect only on *Spodoptera littoralis* (Boisduval, 1833) (Abdullah, 2019). Ethyl acetate extract of *Aspergillus flavus*

Link (1809) mushroom exhibits genotoxic and cytotoxic effects on *Spodoptera litura* (Fabricius, 1775) (Kaur et al., 2019). Ethyl acetate attracts *Rhynchophorus phoenicis* (Fabricius, 1801) (Coleoptera: Curculionidae) (Egonyu et al., 2021). Chloroform, ethyl acetate and hexane extracts of *Moringa oleifera* Lam. have a negative impact on the development of *Spodoptera litura* (Fabricius, 1775). Most of the larvae fed on a diet with the addition of *M. oleifera* extracts showed incomplete molting and turned into larval-pupal intermediates (Kaur et al., 2021). Ethyl acetate extract of *Alpinia galanga* (L.) Willd. also exhibits enzymatic-inhibitory activity against *Spodoptera litura* (Fabricius, 1775) (Datta et al., 2019; 2021). El Haddad and co-authors (2020) evaluated the insecticidal activity of ethyl acetate extract of *Borago officinalis* L. widely distributed in northern Algeria, on fleas *Ctenocephalides felis* (Bouché, 1835) and *Archaeopsylla erinacei* Bouché, 1835 as carriers of serious diseases. The results showed that ethyl acetate extract of Borage significantly reduces the number of flea populations after 48 hours of contact.

There is also information regarding raspberry ketone used as a food flavor, and also widely used in perfumes and cosmetics. This substance can be used to control fruit flies. Raspberry ketone causes physiological and behavioral changes, and changes in sexual activity of male fruit flies *Bactrocera jarvisi* (Tryon, 1927) (Diptera: Tephritidae) (Wee & Clarke, 2020). The addition of raspberry ketone to the diet promotes early puberty in males of *B. tryoni* (Froggatt, 1897) fruit fly (Akter et al., 2017). A combination of insecticide and raspberry ketone (male attractant) can be used in pest traps (Vargas et al., 2015; Fezza & Shelly, 2018). We also recorded 100 % death of mosquito larvae when exposed to its 1 % solution even within 15 minutes.

Thus, the use of food additives in various fields of human activity may not equally affect the components of the biocenosis in natural ecosystems. Mosquito larvae were more susceptible to the negative effects of additives compared with coleopteran predators that feed on them. Thus, 100 % death of mosquito larvae is recorded in the first 15 minutes of the experiment under the influence of methylparaben, raspberry ketone, benzaldehyde, benzyl alcohol, ethyl acetate, benzoic, salicylic, acetic, adipic, propionic acids, sodium metabisulfite, 2-phenylphenol. Formic acid, cinnamic aldehyde, and potassium hydroxide were less toxic in respect to these larvae. But until the end of the experiment (120 minutes), the larvae did not survive in a 1 % solution of these food additives. Benzyl acetate, orthophosphoric acid, sodium hydrosulfite, sodium sulfate, sodium nitrite, and calcium formate did not significantly affect mosquito larvae. More than 50 % of individuals remained viable under influence of the chemicals for 120 minutes. More than 80 % of predatory coleopteran species did not die when feeding on mosquito larvae treated with a 1 % solution of the studied food additives. Sodium metabisulfite had the greatest negative impact on the components of the biocenosis of natural ecosystems in vitro. Under its influence in a concentration of 1 %, 100 % of mosquito larvae died in the first 15 minutes of the experiment, as well as 16.7 % of imago of predatory coleopteran species.

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EFFECT OF AROMATIC SUBSTANCES ON THE MOTOR ACTIVITY OF MITES *TYROPHAGUS PUTRESCENTIAE* (SCHRANK, 1781)

O. Tykhanova, T. Kolombar

Effect of aromatic substances on the motor activity of mites *Tyrophagus putrescentiae* (Schrank, 1781)

Oleksandra Tykhanova [1],
Tetiana Kolombar [2]

[1] *KZO "Scientific Medical Lyceum 'Dnipro' DOR"*
[2] *Oles Honchar Dnipro National University, Gangarin av., 72, Dnipro, 49010, Ukraine. Tel.: +38-095-370-59-00. E-mail t_kolombar@i.ua*

Among the warehousepests, insects and mites including *Tyrophagus putrescentiae* (Schrank, 1781) (Acari: Acaridae) play an important role. The most effective way to protect products from these pests is their treatment with synthetic pesticides, but most of such chemivals have a negative effect on the human body. The development of an environmentally safe and cost-effective alternative to the fumigation of agricultural products with pesticides is a recent problem. Of the 19 chemicals studied, four exhibited acaricidal effects. Under the influence of essential oils *Lavandula angustifolia* Mill., *Rosmarinus officinalis* L. and *Foeniculum vulgare* Mill., mites stopped their motor activity in the 3rd minute of the experiment, and 75 % of the species representatives died from acetic acid during this time. For 21 days, these oils reduced the number of both adults and eggs, and acetic acid was found to be a fumigant only for adult mites and did not affect the number of eggs. Other researched acids (ascorbic, citric, tartaric and malic) and essential oils (*Mentha piperita* L. and *Eucalyptus globulus* Labill.) did not affect the motor activity of the species representatives. Among the spice repellents, we can note cinnamon and carnation;coriander and ginger exhibited theeffectsof attractants.

Keywords: attractant, repellent, fumigant, acaricide, warehouse protection, mold mite.

Introduction

Losses of products during their storage occur due to physical, physiological and biochemical processes, mechanical damage, and damage caused by diseases and agricultural pests. According to the Food and Agriculture Organization (2018–2020), 9 % of the harvested crop is lost annually under storage conditions due to pest vital activity. Invertebrates including mites play an important role among pests of stocks (Rodriguez, & Rodriguez 1987). *Tyrophagus putrescentiae* (Schrank, 1781) is a species of cosmopolitan mites, a pest of food products especially highin protein and fat (meat, cheese, nuts, seeds, long-term food storage) (Hughes, 1976; Edde et al., 2012; Robertson, 1952), and vegetable and grain crops (Griffiths et al., 1976; Van Hage-Hamstem, & Johansson, 1992).

Together with a related species *T. longior* it is often called mold mite. In the wild, this species is found all over the world within a wide range of habitats. Fang & Zhang (2007) represented an extensive list of occasions where this mite species was found in soil, plants, and flowers, as well as where it was found to be associated with agricultural soils. *T. putrescentiae*is also known as a predator. It attacks the larvae *Lasioderma serricorne* (F.) (Papadopoulou, 2006) and destroys southern corn rootworm eggs (Brust, & House, 1988). *T. putrescentiae* also was defined as the pathogen causing human diseases in different regions of the world (Green, & Woolcock 1978).

The mite *T. putrescentiae* has an elongated, oval body narrowed to gnatosome. Length of female body including gnathosoma 578–700 µm, width 300–350 µm. The male body: 520–630 µm, width 240–310 µm. Four pairs of light brown legs, each with six segments and sensils, the cuticle is smooth, translucent. The mite has four pairs of bristles differ in length. The oral apparatus (gnatosome) is formed by two pairs of modified forelegs: chelicerae and pedipalps. Adult mites and nymphs have 4 pairs of walking legs, and the larvae have three pairs. Each leg consists of a coxa and 5–6 free segments. The larval body length is 220–290 µm, externally the larva differs from an adult stage in the absence of the last pair of legs, sexual apparatus and sexual organ. Nymphs are usually similar to adult mites, but they have an underdeveloped sexual apparatus. Eggsare white and oval in shape, 125–140 µm long × 80–90 µm wide.

Females can lay between 165 and 514 eggs during their lifetime, and population doubling can occur in 2–4 days (Sánchez-Ramos et al., 2007), which leads to large product losses. The egg-to-adult stage takes 1–3 weeks, depending on environmental and nutritional conditions (Kheradmand et al., 2007). Optimal conditions for development are humidity 90 ± 5 % and temperature 30…32 °C. The minimum reproduction temperature is 10.4 °C, and the maximum is 34.8 °C.

Mold mite infestations are very common, but often difficult to detect, except on occasions of severe infestations. *T. putrescentiae* feeds on different fungi including molds (Bahrami et al., 2007) and a number of dermatophytes and yeasts (Duek et al., 2001). In culture, this mite species was also reported to feed on nematodes and other microorganisms (Bilgrami, & Tahseen 1992). Today the role of fungi in the nutritional ecology of *T. putrescentiae* is yet to be fully understood. For example, despite the fact that the presence of molds has been shown to encourage *T. putrescentiae* growth and development, this mite species would develop well on susceptible foods free from mold infestation (Canfield, & Wrenn 2010).

The main ecological factors affecting the mite growth and development were considered to be temperature, food sources, and, in particular, the microenvironment relative humidity (Sánchez-Ramos et al., 2007). Growth and development of individuals can stop with prolonged exposure to low humidity (up to 65 %). Therefore, reducing humidity is one of the key methods of controlling mold mite. To achieve the same goal, freezing, warehouse management, as well as phytosanitary fumigation with phosphine are used (Nayak, 2006a; Eaton, & Kells, 2009; 2011).

It should be noted that representatives of this species are quite resistant. They can remain viable without food at a temperature of 20…25 °C for 31 days, and they do not develop at 6…10 °C (Sánchez-Ramos, & Castañera, 2001), but can survive in the inactive state even at 0 °C (Mueller et al., 2006). In addition, representatives of this species quickly adapt to the chemical compounds used to control them, so the development of new biological pesticides is relevant.

For a long time, methyl bromide was used to treat storerooms and products. It quickly destroys pests (within 48 hours) and has a broad-spectrum action, controlling not only insects and mites, but also nematodes and plant pathogens (Barker, 1967). But this chemical is ozone-depleting, so most countries have banned its use. Numerous studies on alternative pest control methods have been conducted, including studies on *T. putrescentiae* mites. Zhang (2018) and his colleagues suggest carrying out preventive measures and monitoring the number of mites to use fumigation only if their number exceeds a critical threshold; this, accordingly, minimizes the use of harmful chemicals. To get information about the presence and number of mites in food storage rooms, bait traps have been introduced as a new method. Dog food is often used as a bait (Amoah et al., 2016). An effective alternative to methyl bromide is the use of cotton nets with propylene glycol (Campbell et al., 2018). Ham pieces soaked in solutions of either propylene glycol (1,2-propanediol), lard, ethoxyquin or butylated hydroxytoluene are used for priphylactic or significant reducing the mite population growth (Abbar et al., 2016). In another work, ham cubes were coated directly or wrapped in nets saturated with $C_8C_9C_{10}$ with combinations of either soybean oil, xanthan gum (XG) or carrageenan (CG) + propylene glycol alginate (PGA). Soybean oil in combination with various components controlled the growth of *T. putrescentiae* population, but the industrial use of this method still requires a detailed study (Rogers et al., 2020).

Many alternatives have been tested as substitutes for methyl bromide, from physical control methods such as heat, cold, and sanitation, to fumigant substitutes such as phosphine, sulfuryl fluoride, carbonyl sulfide, and others (Fields, & White, 2002; Zhao et al., 2015). The use of extremely low temperatures is an acceptable method for processing meat (Abbar et al., 2016), but it cannot be used, for example, against *T. putrescentiae* in vegetable storage facilities. The same author (2018) recommended a combination of fumigation with high temperatures, but this method is not suitable for all types of products. The control of mites in dry-cured ham by means of physical methods involving light, temperature, microwaves, ionizing radiation and gases at various pressures was studied (Arnau, & Guerrero, 1994).

Hasan et al. compared the effects of methyl bromide and phosphine in 2020 and proposed sulfuryl fluoride as a fumigant in 2021(Hasan et al., 2021). Pirimiphos-methyl, cypermethrin, deltamethrin, chlorfenapyr, and β-cyfluthrin have varying degrees of efficacy against the three mite strains and require additional research (Jitka et al., 2014). Yang et al. (2022) showed the effectiveness of nitric oxide fumigation against all mite stages. Integrated mite control in animal feed storage facilities was proposed. Its strategy involved some processes such as limiting the water content in the processed feed up to 12 %, admixing vegetable oil to some feed, adherence to good hygiene practices into and around the processing and storage facility, and rejection of infested materials at the receiving areas (Nayak, 2006b). But a lot of attention is still paid to the study of the effect of natural components on mites *T. putrescentiae*. Among them, it is worth noting essential oils and plant extracts, such as citronella java, clove bud, clove leaf, lemongrass, nutmeg, oregano, pimento berry, thyme red, and thyme white oils (Kim, 2003), Cnidium officinale (Kwon, & Ahn, 2003), Melissa officinalis oil (Song, & Lee, 2018), Allium sativum (Preisser et al., 2018), Ocimum basilicum, Achillea fragrantissima and Achillea santolina (Al-Assiuty et al., 2019). The purpose of our study was to identify changes in motor activity of *T. putrescentiae* under the effect of essential oils, surfactants, acids and spices with their subsequent use in crop protection during storage.

Materials and methods

The experiment was divided into three steps. The first step was the determination of the acaricidal properties of surfactants, spices, organic acids and essential oils on potato slices. In the study, 5 common household chemicals (Table 3.1, see p. 140), 5 essential oils (Table 3.2, see p. 141), 5 acids (Table 3.3, see p. 142) and 4 types of spices (Table 3.4, see p. 143) were selected.

To carry out the first step, potato samples were taken in storage; the vegetables were examined for the presence of mites *T. putrescentiae*. Special attention was paid to rotten and damaged parts of vegetables. In the presence of an object under study on the potato, thin sections were made and laid out on slides placed at a distance of 5 cm from each other.

The samples were treated with the test chemical, and the motor activity of mites was registered using a Sigeta MB-120 40x-1000x LED Mono microscope (Sigeta, Ukraine). To research the effect of spices on mites, dry parts of plants were mixed with boiled water in a ratio of 1:1. The observation time was 180 seconds, the repeatability was 6 times, and changes in the motor activity of 6 individuals were recorded on each section. The movement of at least one limb for a certain time was taken for motor activity. As a result, chemicals with acaricidal properties against *T. putrescentiae* have been identified.

Table 3.1

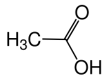
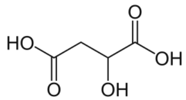
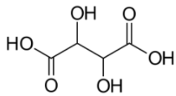
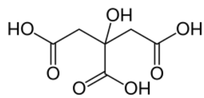
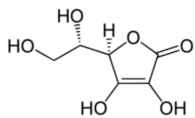
Characteristics of surfactants used during the experiment

Name of the household product	Producing country	Chemical composition
Means for washing glass and mirrors “Galax”	“Galax”, Ukraine	<5 % anionic surfactants, aroma, preservatives
Liquid for disinfection “АХД 2000 expert”	“Lysoform”, Ukraine	propan-1-ol, propan-2-ol, alkyldimethylbenzylammonium chloride, aroma, aqua
Goat’s milk face wash	“Ziaja”, Poland	aqua, cetearyl alcohol, dimethicone, olive oil PEG-7 esters, propylene glycol, shea butter ethyl esters, sodium cocoyl glutamate, tocopheryl acetate, goat milk extract, sodium hyaluronate, carbomer, phenoxyethanol, ethylhexylglycerin, parfum (fragrance), linalool, citronellol, butylphenyl methylpropional, hexyl cinnamal, geraniol, eugenol, benzyl saliccylate, alpha-lsomethyl lonone, sodium hydroxide
Protein shampoo	“HSA Hair Styling Applications SpA”, Italy	aqua (water), sodium coceth sulfate, glycerin, cocamidopropyl betaine, cocobetaine, lauroyl/myristoyl methyl glucamide, hydrolyzed soy protein, hydrolyzed wheat gluten, hydrolyzed corn protein, c13-15 alkane, panthenol, polyquaternium-10, guar hydroxypropyltrimonium chloride, propylene glycol, potassium sorbate, sodium chloride, sodium benzoate, citric acid, parfum(fragrance)
Dishwashing liquid “Fairy”	“Fairy”, United Kingdom	5–15 % – anionic surfactants, preservatives, aroma, linalool

Information was collected from product packaging.

Table 3.2

Acids used in the study of the chemicals' acaricidal properties against *T. putrescentiae*

Name	Name IUPAC	Formula		E number	Solubility in water
		molecular	structural		
Acetic acid	acetic acid	CH_3COOH, $C_2H_4O_2$		E260 (preservatives)	soluble in all proportions
Malic acid	hydroxybutanedioic acid	$C_4H_6O_5$		E296 (preservatives)	558 g/L (20 °C)
Tartaric acid	2,3-Dihydroxybutanedioic acid	$C_4H_6O_6$		E334 (antioxidants)	139.44 g / 100 g
Citric acid	citric acid	$C_6H_8O_7$		E330 (antioxidants)	54.0 % (10 °C) 59.2 % (20 °C) 64.3 % (30 °C) 68.6 % (40 °C) 70.9 % (50 °C) 73.5 % (60 °C) 76.2 % (70 °C) 78.8 % (80 °C) 81.4 % (90 °C) 84 % (100 °C)
Ascorbic acid	l-threo-Hex-2-enono-1,4-lactone or (R)-3,4-Dihydroxy-5-((S)-1,2-dihydroxyethyl)furan-2(5H)-one	$C_6H_8O_6$		E300 (antioxidants)	1 : 3.5

The data was summarized on the basis of publicly available sources.

Table 3.3

Characteristics of essential oils used in the study

Substance	Plant	Chemical composition, compounds (concentration, %)	ISO	References
Lavender oil	*Lavandula angustifolia* Miller, 1768	caryophyllene (24.12), β-phellandrene (16.00), 1,8-cineole (15.69), terpinen-4-ol (9.57), α-terpineol (6.00), borneol (5.07), santalene (4.50), D-limonene (2.10)	3515	Jianu et al. (2013)
Tasmanian bluegum oil	*Eucalyptus globulus* Labillardière, 1861	1,8-cineole (76.65), α-pinene (5.65), α-terpineolacetate (4.85), alloaromadendrene (3.98), α-terpineol (1.96)	770	Chalchat et al. (1995), Abdossi et al. (2015)
Peppermint oil	*Mentha piperita* Linnaeus, 1753	menthol (53.28), menthyl acetate (15.10), menthofuran (11.18), 1,8-cineole (6.69), neomenthol (2.79), menthone (2.45), (Z)-caryophyllene (2.06), germacrene D (2.01)	856	Andoğan et al. (2002), Saharkhiz et al. (2012)
Rosemary oil	*Rosmarinus officinalis* Linnaeus, 1753	piperitone (23.70), α-pinene (14.90), linalool (14.90), 1,8-cineole (7.43), camphor (4.97), borneol (3.68), camphene (3.33), bornyl acetate (3.08), β-caryophyllene (2.68), myrcene (2.07)	3053	Özcan, & Chalchat (2008), Gachkar et. al. (2007)
Foeniculum oil	*Foeniculum vulgare* Miller, 1768	estragole (64.80), anethole (12.24), limonene (7.81), methyl chavicol (5.61), fenchone (3.12), α-pinene (1.30)	17412	Dadalioğlu, & Evrendilek (2004)

In the second step, potato slices with mites present on them were treated with four chemicals that showed the greatest effectiveness in the previous step of the study. The samples were placed on slide glasses located at a distance of 1 cm from each other in a plastic container. The container was placed to a storage facility for 21 days where potatoes were stored at a temperature of 12...18 °C and relative humidity of 70 %. The second step of the experiment demonstrated the effect of acaricidal chemicals on the offspring of mites *T. putrescentiae*.

Table 3.4

Characteristics of the spices used in the experiment

The name of the spice	Plant	Chemical composition	References
Coriander	seeds *Coriandrum sativum* L., 1753	petroselinic acid, linoleic acid, stigmasterol, β-sitosterol, Δ5-avenasterol, campesterol, phosphatidylcholine phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine	Ramadan, & Mörsel (2002)
Ginger	root *Zingiber officinale* Roscoe, 1807	zingiberene, valencene, β-funebrene, selina-4(14),7(11)-diene, citronellyl n-butyrate, β-phellandrene, camphene, α-pinene	Sharma et. al. (2016)
Carnation	buds *Syzygium aromaticum* (L.) Merr. & L. M. Perry	eugenol, eugenol acetate, quercetin, β-caryophyllene	Razafima-monjison et. al. (2014)
Cinnamon	bark *Cinnamomum verum* J. Presl, 1825	(E)-cinnamaldehyde, linalool, β-caryophyllene, eucalyptol, eugenol	Behbahani et. al. (2020)

The third step was the determination of the attractant and repellent properties of the studied chemicals. Thin slices of potato with mites were placed on slide glasses, 1 ml of the chemical was dripped next to the slice at a distance of 5 mm using a pipette, and the motor activity of mites has been monitored for 3 minutes. The experiment was repeated six times for data reliability. The data in the table was presented as x ± SD.

Results

As a result of the first step of the study, chemicals that have an acaricidal effect on *T. putrescentiae* representatives were identified.

Among the 19 chemicals, 4 of them showed the greatest efficacy against mites. Reduced motor activity due to the effect of essential oils *Lavandula angustifolia* Mill., *Rosmarinus officinalis* L. and *Foeniculum vulgare* Mill. occurred even in the first minute of the experiment (Table 3.5, see p. 145), and already on the third minute the mites stopped moving (Fig. 3.1). Under the effect of acetic acid, almost 75 % of the mites stopped their moving within three minutes of observation. Other chemicals changed the intensity of movement of the species representatives (Table 3.6, 3.7, see p. 145), but did not result in a complete cessation of activity or death. In addition, it should be noted that in the study with the "Fairy" dishwashing detergent, the motor activity of mites was not observed for some time, but it was restored after 40 seconds of the experiment.

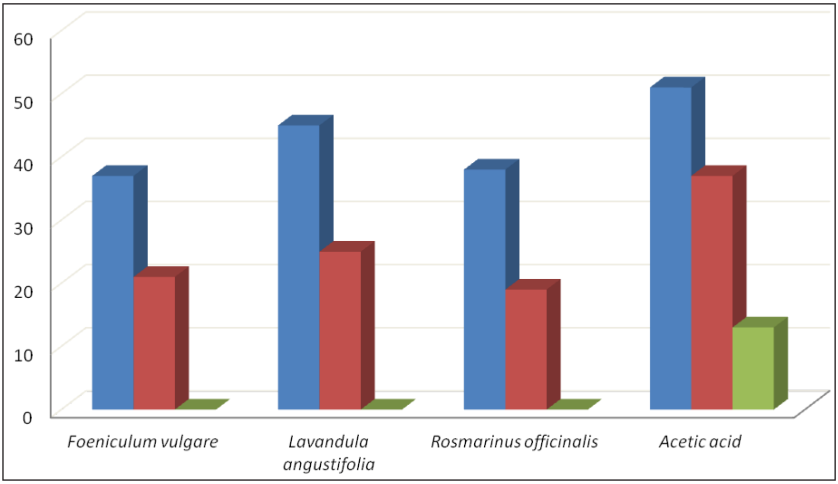


Fig. 3.1. Effects of essential oils *Lavandula angustifolia* Mill., *Rosmarinus officinalis* L. and *Foeniculum vulgare* Mill and acetic acid on motor activity of mite *T. putrescentiae*. Abscissa axis: essential oils and acetic acid that exhibited acaricidal properties, ordinate axis: number of movements, blue column: number of movements of legs before treatment, red column: number of movements in the 1st minute after treatment, green column: number of movements in the 3rd minute of the study

Table 3.5

Effect of essential oils on *T. putrescentiae* motor activity ($x \pm SD$)

Name of essential oil	Number of movements of legs before treatment	Number of movements of legs after treatment*	F, at $F_{0.05} = 4.96$	P
Foeniculum vulgare	36.5 ± 14.9	21.0 ± 9.1	4.71	0.055
Lavandula angustifolia	75.3 ± 9.0	86.2 ± 6.9	5.50	0.041
Rosmarinus officinalis	62.5 ± 14.0	18.7 ± 3.7	42.99	4.9×10^{-5}

Note: * – observations made for 60 seconds.

Table 3.6

Motor activity of *T. putrescentiae* under the influence of aqueous solutions with spices ($x \pm SD$)

Nameof spice	Number of movements of legs before treatment	Number of movements of legs after treatment*	F, at $F_{0.05} = 4.96$	P
Coriandrum sativum	43.8 ± 6.6	50.8 ± 7.0	0.50	0.496
Cinnamomum verum	43.8 ± 6.6	50.8 ± 7.0	8.28	0.016
Zingiber officinale	54.3 ± 10.9	42.2 ± 11.1	3.68	0.084
Syzygium aromaticum	51.7 ± 6.2	40.0 ± 9.1	6.75	0.027

Notes: see Table 3.5.

Table 3.7

Effect of household chemicals on motor activity of *T. putrescentiae* ($x \pm SD$)

Name	Number of movements of legs before treatment	Number of movements of legs after treatment*	F, at $F_{0.05} = 4.96$	P
Means for washing glass and mirrors	47.2 ± 10.8	38.2 ±13.7	1.59	0.236
Liquid for disinfection	23.2 ± 7.6	40.8 ± 8.0	14.44	0.003
Protein shampoo	51.0 ± 11.7	37.7 ± 9.9	4.53	0.059
Dishwashing liquid	43.5 ± 10.0	36.5 ± 9.0	1.61	0.233
Goat's milk face wash	37.2 ± 6.2	45.0 ± 6.4	4.69	0.056

Notes: see Table 3.5.

Based on the results of the second step of the experiment, it can be concluded that none of the 4 chemicals (selected on the basis of the first step results) destroyed 100 % of mites, although it reduced their number. Essential oils reduced the number of both adult mites and their eggs, but acetic acid was found to be a fumigant only for adult mites and does not reduce the number of eggs (Fig. 3.2).

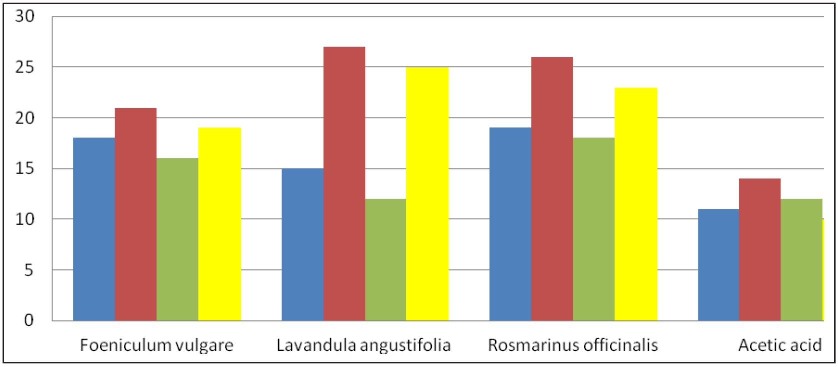


Fig. 3.2. Effect of organic acid and essential oils on mite *T. putrescentiae* activity on potato slices. Abscissa axis: compounds used that exhibited acaricidal properties; ordinate axis: the number of mites and eggs on the 1st and 21st day of treatment, blue column: 1st day of observation (eggs), red column: 1st day of observation (adults), green column: 21st day of observation (eggs), yellow column: 21st day of observation (adults)

The results of the last step of the study showed that all acids (malic, tartaric, citric, ascorbic) did not affect mites,except acetic acid. Surfactants as a component of liquid for disinfection, means for washing glass and mirrors and dishwashing liquid can be identified as repellents; but goat's milk face wash fragrance and shampoo attracted mites. As already determined at the beginning of the study, essential oils *Lavandula angustifolia* Mill., *Rosmarinus officinalis* L. and *Foeniculum vulgare* Mill acted like fumigants, and others (*Mentha piperita* L. and *Eucalyptus globulus* Labill.) did not affect mites *T. putrescentiae*. Among the spice repellents, we can note cinnamon and carnation; coriander and ginger exhibited attractant properties.

Discussion

At the beginning of the new crop storage season, storage facilities need to be thoroughly cleaned out of vegetable residues and debris, ventilated and disinfected with sulfur gas from pests, and all storage furniture and equipment need to be wiped with a 1 % formalin solution, thereby preventing the spread of new bacteria. In order to protect fresh products and extend the shelf life, it is necessary to comply with sanitary standards, constant temperature and humidity, which should be different at different times of the year. To protect against pests, fumigation is used as the most effective method of controlling parasites and fungal infections that appear on products during storage. Pesticides toxic to humans and the environment are often used as fumigants. Therefore, the search for an environmentally safe and cost-effective alternative is the biggest challenge for scientists.

One of the most common pests in European storage facilities is the mite *T. putrescentiae*. In addition to storage areas, mites of this species can be found in old hay, bird nests, and animal enclosures (Solarz et al., 2004; Solarz, & Pająk, 2019). *T. putrescentiae* has been shown to attack L. serricorne, the stored product pest (Papadopoulou, 2006), and to be an effective egg-eating predator of the field pest southern corn rootworm, Diabrotica undecimpunctata Howard (Brust, & House, 1988). This mite species has also been recorded to predate on some nematode species such as Meloidogyne javanica (Treub), and Aphelenchus avenae Bastin (Bahrami et al., 2007; Kheradmand et al., 2007). Besides damage to stored products and predation on other insects, *T. putrescentiae* was considered to be a species essential for health and medicine. This mite can cause allergies that affect human skin and breathing. Thus, *T. putrescentiae* was found to be allergic mite predominant in dust and debris samples from several coalmines (Solarz, & Solarz, 1996).

First of all, preventive measures should be considered in the fight against mites, that would contribute to the better preservation of agricultural products. They should be based on the peculiarities of distribution, development, and reproduction of the species representatives and depend on the conditions and type of product. These are agrotechnical measures that prevent mite infestation at the stage of growing and collecting products. These measures include winter tillage with the destruction of weeds and planting pest-resistant crop species. In areas of storage and transportation of stocks, compliance with sanitary and hygienic requirements are provided, and control over compliance of production and warehouse premises with food storage standards, rational arrangement of warehouse and production premises, keeping the premises and vehicles clean, destruction of garbage and waste after each new season, timely repair of the warehouse to eliminate cracks, monitoring the product condition at the beginning of storage and during the season, compliance with the rules for preparing warehouses for receiving food products, regular monthly entomological surveys of stocks, as well as storage of products infected with mites in a separate room are ensured (Oksentiuk, 2020).

Ventilation of premises also has an impact on mite infestation. Quite effective in the fight against mold mites is the use of a thermal method: heating or cooling products to temperatures critical for the development of mites (below 13 °C or above 35 °C). In the case of grain, it can be heated even to 50…55 °C. Among the chemical control measures, gasification (fumigation) and aerosol disinfection should be noted. The first of them is applied only if the room is sealed and remote from residential objects. Methyl bromide is one of the most effective acaricidal agents. For unsealed rooms, aerosol treatment of adjacent territories, equipment and external walls with an emulsion of 3 % green oil concentrate is often used. The method of treatment with a caustic soda solution is less effective.

An important regulator of the mite number is a biological method of control. It includes both the use of biological agents (predators, parasites) and plant origin fumigants. For example, acorologians back in 1959 proposed methods of biological control with the use of a predator *Cheyletus eruditus* (Schrank) (Žďárková, 1967). This mite is a common companion of acaroid mites, and this predator is able to completely destroy the pests present in the substrate within a short period of time. In 2016, experts from China investigated the possibility of using representatives *Neoseiulus barkeri* (Acari: Phytoseiidae) in the protection of stocks from *T. putrescentiae*. In addition, the work indicated that the photoperiod of 12:12 has been considered optimal for the development and reproduction of *N. barkeri* fed on *T. putrescentiae*, and for this reason *N. barkeri* may be used most efficiently as a biological control agent under this regime (Zou et al., 2016). Husseini et al. (1993) demonstrated potential opportunities of *Orius majesculus* Reut. (Het., Anthocoridae) as a biocontrol agent for mold mites. The work of Zhang et al (2022) described the conditions for breeding a predator *Lasioseius japonicus* (Acari: Blattisociidae) to protect stored food products from mites.

Increasingly, researchers pay attention to the use of environmentally friendly substances to reduce the number of pests in agricultural products. Among these chemicals are essential oils extracted from plants. Essential oils are volatile mixtures of hydrocarbons with different functional groups, and their repellent effect is associated with the presence of monoterpenes and sesquiterpenes. Individual compounds present in these mixtures with high repellent activity include alpha-pinene, limonene, citronellol, citronellal, camphor and thymol. Finally, although from an economical point of view synthetic chemicals are still more frequently used as repellents than essential oils, these natural products have the potential to provide efficient and safer repellents for humans and the environment (Nerio et al., 2010). Macchioni et al. (2002) showed the efficiency of such components of essential oils extracted from 4 Pinus species (*P. pinea* L., *P. halepensis* Mill., *P. pinaster* Soil in Ait., and *P. nigra* Arnold) as 1,8-cineole and limonene. Yang, & Lee (2013) investigated verbenone structural analogues extracted from *Artemesia aucheri* as natural acaricides against *T. putrescentiae* (Article). In the present Park et al. (2017) evaluated the potential abilities of *Myosotis arvensis* oil-derived 3-methylbenzaldehyde and its structural analogues to act as a new acaricide and mite kit (mite color deformation) in relation to *T. putrescentiae* (Schrank). The acaricidal effects of *Eugenia caryophyllata* bud oil compounds were studied (acetyleugenol, β-caryophyllene, eugenol, α-humulene) (Kim et al., 2003). During our experiment with buds of *Syzygium aromaticum*, the mites did not die, but ran away. This may be due to the use of dry buds mixed with water immediately before the experiment itself, rather than essential oil. *Thymus vulgaris* (Jeong et al., 2008); *Foeniculum vulgare* (Lee et al., 2006); *Eucalyptus dives*, *Melaleuca leucadendron* and *Leptospermum pertersonii* (Song et al., 2016); *Cinnamomum zeylanicum* and *Eugenia uvalha* (De Assis et al., 2011), and others are among the plants whose essential oils are suggested to be used in the fight against mold mite.

Mueller et al. (2006) in their work examined the effects of two liquid cleaning agents on mites, but the results showed no response to them in mites. Among household chemicals in our study, three substances were identified as repellents, and two as attractants of mites, which does not exclude the possibility of using surfactants in the control of *T. putrescentiae*, but requires additional detailed study.

Conclusion

Of the 19 chemicals studied, 4 exhibited acaricidal effects. Namely, mites stopped motor activity under the effect of essential oils *Lavandula angustifolia* Mill., *Rosmarinus officinalis* L. and *Foeniculum vulgare* Mill. in the 3rd minute of the experiment, and 75 % of the species died of acetic acid during this time. For 21 days, these oils reduced the number of both adults and eggs. Therefore, acetic acid was found to be a fumigant only for adult mites and did not affect the number of eggs. Other acids (ascorbic, citric, tartaric and malic) did not affect the motor activity of mites. Surfactants as a component of liquid for disinfection, means for washing glass and mirrors and dishwashing liquid can be identified as repellents; but goat's milk face wash fragrance and shampoo attracted mites. By the action of essential oils *Mentha piperita* L. and *Eucalyptus globulus* Labill., the motor activity of mites has not changed significantly. Among the spice repellents, we can note cinnamon and carnation; coriander and ginger exhibited attractant properties.

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