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Contact information:
George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș
Gheorghe Marinescu street no. 38, Târgu Mureș, 540139, ROMANIA
Phone: +40-265-21 55 51, fax +40-265-21 04 07
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ASCORBIC ACID MITIGATES DICHLORVOS- EVOKED HIPPOCAMPAL DEGENERATION IN MALE WISTAR RATS

Charles A. OYINBO[1*], Domotimi G. DOUTIMIFI[1]

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

*Correspondence:
Charles A. OYINBO
charles.oyinbo@ndu.edu.ng

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Abstract: Dichlorvos, a widely used pesticide, poses significant neurotoxic risks. This study examined the protective potentials of vitamin C on dichlorvos (DV)-induced hippocampal damage in Wistar rats. Five groups of rats were exposed to different aqueous dilutions of DV aerosol in a chamber for 4 hours daily over 21 days. Three groups were co-administered vitamin C (160 mg/kg) daily. Histological examination revealed that rats exposed to DV dilutions exhibited hippocampal damage characterized by pyknosis and structural alterations in the cornu ammonis and dentate gyrus. The Y-maze and novel object recognition tests revealed impaired short-term spatial (SM) and non-spatial memory (NSM). However, vitamin C supplementation ameliorated the extent of neurodegeneration in the hippocampus and the spatial and non-spatial cognitive deficit levels. The amelioration in NSM function was remarkable; there was no statistical difference between the control and vitamin C-supplemented groups. In contrast, there were significant differences between the supplemented groups and the rats exposed to DV without vitamin C supplementation. However, the ameliorative effect of vitamin C on SM impairment seems less pronounced; there was a statistical difference between the control and the supplemented groups and between the supplemented groups and the rats exposed to DV without supplementation. These findings highlight the neurodegenerative and apoptotic effects of dichlorvos on the hippocampus and suggest a potential benefit of vitamin C supplementation in mitigating the neurotoxic effects of DV.

Keywords: neurodegeneration, apoptosis, hippocampus, neuroprotection

1. Introduction

Dichlorvos is a widely used pesticide in less developed countries because of its potency and low cost (Nwankwo et al., 2019). It is a chemical classified (class 1B) by WHO as highly hazardous (WHO, 1992). It is an organophosphorous, colourless, aromatic compound. Furthermore, research indicates that undue exposure may cause damage to many organs, including the brain (Mostafalou and Abdollahi, 2017). The harmful effects of DV in most organs are linked to the production of free radicals, which impair mitochondrial function and cause inflammation and cell death. This cascade causes hepatocyte death and liver dysfunction (Saka et al., 2025). The consequences in the kidneys include renal tubular and glomerular necrosis, resulting in kidney damage (Adeoye et al., 2022). Reports

also suggested that this cascade triggers cardiomyocyte necrosis and cardiac impairment (Salem et al., 2023), a reduction in the mature spermatocyte population and vascular congestion in the testis (Saka et al., 2024). Neuronal damage coupled with neurological deficit have been reported in dichlorvos (DV) exposure (Imam et al., 2018). Certain in vivo studies have associated DV with oxidative stress, cognitive impairment, and hippocampal damage, leading to memory consolidation deficits (Farkhondeh et al., 2020; Anderson et al., 2023). Studies show that the hippocampus, a critical structure for memory formation, is particularly vulnerable to the neurotoxic effects of DV (Huang et al., 2022; Ommati et al., 2024). This is due to its high metabolic rate, a relatively weaker blood-brain barrier in its location, and its unique plasticity necessitated by its crucial role in learning and memory, which makes it susceptible to changes caused by various insults, including toxins like organophosphate (Davidson et al., 2024). According to Mostafalou and Abdollahi (2023), dichlorvos causes damage by blocking acetylcholinesterase activity in an irreversible manner, which leads to acetylcholine buildup in the synaptic cleft and excessive stimulation of cholinergic receptors. This can cause death, paralysis, or cognitive impairment (Saravanakumar et al., 2023).

Rajak et al. (2022) also demonstrated that DV induces neuroinflammation and oxidative stress, which damages hippocampal neurons. Consequently, this disruption of hippocampal function causes impairments in spatial and non-spatial recognition memory (Chauhan et al., 2021). Chronic exposure to DV has been implicated in neurodegenerative disorders and neuropathological lesions in the neocortex, hippocampus and cerebellum. These lesions were attributed to increased neuronal death (Yu et al., 2021; Sarailoo et al., 2022). Chronic DV exposure causes neuropathy affecting central and peripheral nervous system axons, potentially leading to paralysis (Uwaifo and John, 2020; Ranjan et al., 2022).

Apart from its agricultural use, it is also used as an aerosol spray for insect control in public institutions like schools, hospitals, and offices in developing counties (Clesceri et al., 2021; Mendoza et al., 2023).

Additionally, it is used as an anti-helminthic agent for pigs, horses, and dogs in veterinary practice and against crustacean ectoparasites that infect fish in aquaculture (Martins et al., 2021; Pattanayak et al., 2024). Recent studies, however, indicated that only a small proportion of the dichlorvos used reaches the targeted pests; over 90% go into soil and water as contaminants (Bankole et al., 2020; Kiruthiga, 2021), causing additional health risks. However, ascorbic acid, a natural antioxidant, has been shown to mitigate oxidative stress and improve cognitive function (Raeeszadeh et al., 2022). However, its potential protective effects against dichlorvos-induced toxicity on hippocampal structure and memory consolidation remain unclear. The primary endeavour of this study was to examine the protective effects of ascorbic acid against dichlorvos-induced toxicity on hippocampal structure and memory consolidation (**Fig. 1.**).

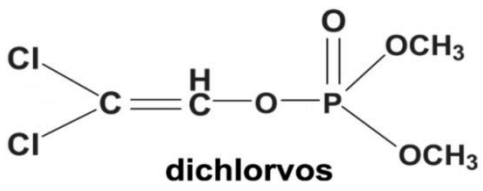

Fig. 1. Structural formula of Dichlorvos (Okoroiwu and Iwara, 2018)

2. Materials and methods

Animal acclimatization and handling

Twenty-five young adult (7 weeks old) male Wistar rats (180-200 g) were sourced from the pharmacology department's animal unit at Niger Delta University, Nigeria. We randomly assigned them to five groups (n = 5 per group) and housed them in a PVC cage (40 cm × 40 cm × 20 cm). Before beginning the experiment, they spent 21 days acclimating to standard laboratory conditions in the research vicinity, in 12 hours of daylight and 12 hours of darkness. Access to regular rat chew and water was unrestricted. All protocols in this study were approved by the College Ethics Committee, CHS, Niger Delta University (Ref No. 02-0852024/080).

Administration schedule

Dichlorvos (DV) concentration and administration followed previously established protocols (Ogunsola et al., 2019; Hart and David, 2022). The lethal inhalation concentration of DV was determined to be 50 ml per 50 ml of distilled water. Following these methods, the groups were treated as detailed (**Table 1**) for 21 days.

Table 1. Group and treatment regimen

Group (GP)	Amount DV in distilled water (v/v)	Vitamin C per oral supplementations
1	Nil	nil
2	40/60	160 mg/kg
3	10/90	160 mg/kg
4	20/80	160 mg/kg
5	40/60	160 mg/kg

Dichlorvos (DV) exposure

Rats were exposed to DV vapour in a partially ventilated PVC box. Each group was provided a box measuring 40 cm × 30 cm × 20 cm. Each box featured a perforated lid containing ten 2-cm-diameter ventilation apertures. Within each box was placed a cylinder (10 cm height × 10 cm diameter) containing cotton wool soaked with the appropriate DV solution for the particular group (**Table 1**). Groups 2-5 rats were introduced into the DV-inhalation boxes and were exposed to DV vapours emanating from this setup for four hours daily for 21 consecutive days.

Novel object recognition test (NORT)

Short-term non-spatial memory (NSM) was assessed through the novel object recognition as previously described (Lueptow, 2017). Briefly, a 40 cm × 40 cm transparent glass box was used for the novel object recognition test. This test is predicated on rodents' proclivity to naturally explore unfamiliar objects for longer periods than the accustomed ones. During the training phase, they were familiarised with the test box. Two identical objects were placed at equal distances within the arena, and each rat was allowed to explore for 5 minutes and then returned to the home cage. Thirty minutes later (retention interval), the rat was reintroduced and allowed to walk around the test arena with a familiar object (FO) and a new one (NO) for 5 minutes to test short-term non-spatial recognition memory. The objects and the test box were cleared with 70% alcohol after each test. We assessed cognitive deficit by analysing object

discrimination abilities during both the training (T1) and testing (T2) phases. The discrimination index (DI) was calculated using the formula (1):

$$DI = \left(\frac{Time\ spent\ with\ NO}{Time\ spent\ with\ NO + Time\ spent\ with\ FO}\right) 100 \ldots\ldots\ldots(1)$$

Where FO (familiar object) and NO (novel object)

This metric quantifies the preference for the novel object, providing an indicator of memory and discrimination ability.

Y-Maze test

This test was also to measure the short-term spatial memory of the animals by recording the number of triads and arm entries to calculate the proportion of alternation (Kraeuter et al., 2019). Using a wooden constructed three-arm Y-maze, 50 cm each, 12 cm wide, and standing 22 cm from a flat surface. Each animal spent five minutes exploring its various arms after being carefully positioned in the middle of the maze. By keeping track of the frequency of appropriate sequences between the arms, such as ABC, ACB, BCA, BAC, CBA, or CAB, the percentage of proper alternations was calculated. This test evaluates the animal's capacity for spatial memory and judgment. Analysis of correct alternation was estimated as previously described (Edem et al., 2022, formula 2). Briefly, the correct entries into all three arms without repetition, e.g., ABC, BCA, CAB percentage is:

$$\%Altern. = \left(\frac{Number\ of\ unique\ triads\ (e.g., ABC, BCA, CAB)}{Total\ arm\ entries - 2}\right) 100 \ldots.(2)$$

Histological analysis

Following euthanasia with chloroform, rats were perfused with 10% formal saline, after which their brains were extracted. Coronal sections of the brain at the medial temporal lobe were then isolated for hippocampal morphology using routine haematoxylin and eosin staining (Szunyogova and Parson, 2016). Photomicrographs were captured using a Micro Video Capture^{R} camera mounted to an Olympus BH2 BHT microscope.

Statistical analysis

A one-way analysis of variance followed by the Tukey post hoc comparison test was used to evaluate the significance of differences between groups using GraphPad Prism 5 (San Diego, USA). Data are presented as the mean ± standard error of the mean (SEM). A p-value of ≤ 0.05 was considered statistically significant.

3. Results

Behavioural analysis

The cognitive functions of short-term non-spatial memory and short-term spatial memory were evaluated using the novel object recognition test and Y-maze test, respectively. Exposure to DV (group 2) resulted in significant decrease in DI compared to control ($p < 0.01$) (**Fig. 2.**). Conversely, co-exposure to DV and supplementation with vitamin C (VC) (groups 3-5) significantly enhanced DI relative to the DV-only group ($p < 0.01$). Note that VC supplementation had no discernible effect on DI compared to controls ($p < 0.1$), indicating that VC intervention improves short-term non-spatial memory. Values are mean ± SEM, * $p< 0.01$, Tukey post hoc, n = 5 per group.

DV-exposed rats in groups 2, 4 and 5 showed a significant decrease in the mean SA score compared to the control and group 3 rats (**Fig. 3.**). No significant difference is observed between the control and group 3. Groups 4 and 5 rats showed a significant difference in mean SA score compared to group 2. This indicates that vitamin C intervention is more effective at lower doses of DV exposure. Values are mean ± SEM, * $p< 0.01$, β $p < 0.001$, Tukey post hoc, n = 5 per group.

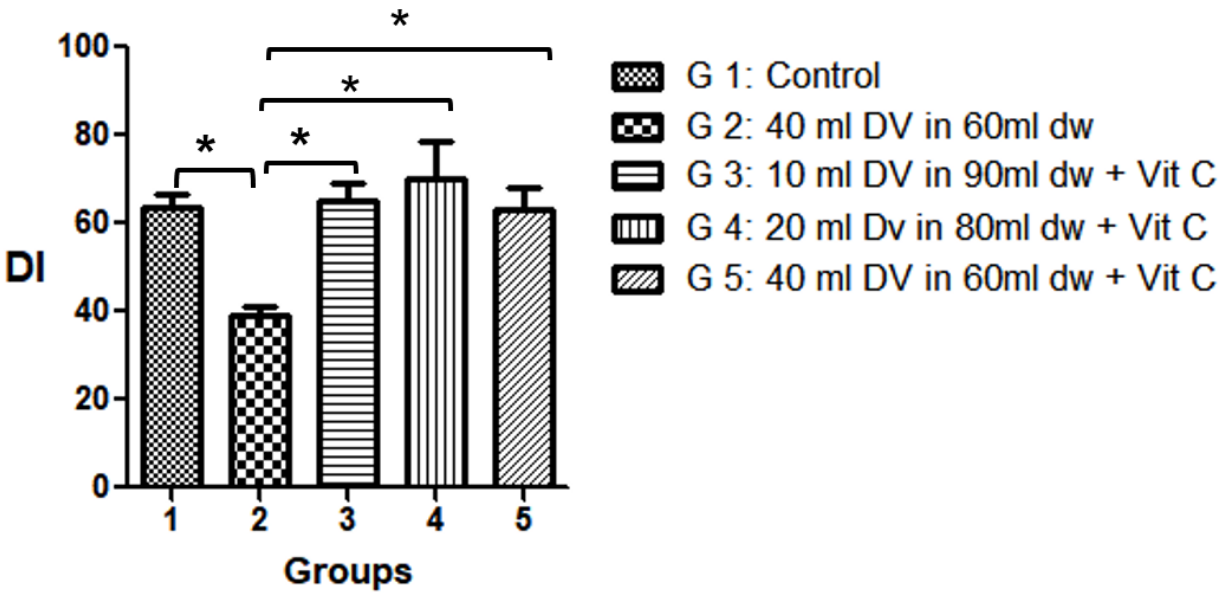

Fig. 2. Novel Object Recognition (NOR) Test: Mean Discrimination Index Scores.

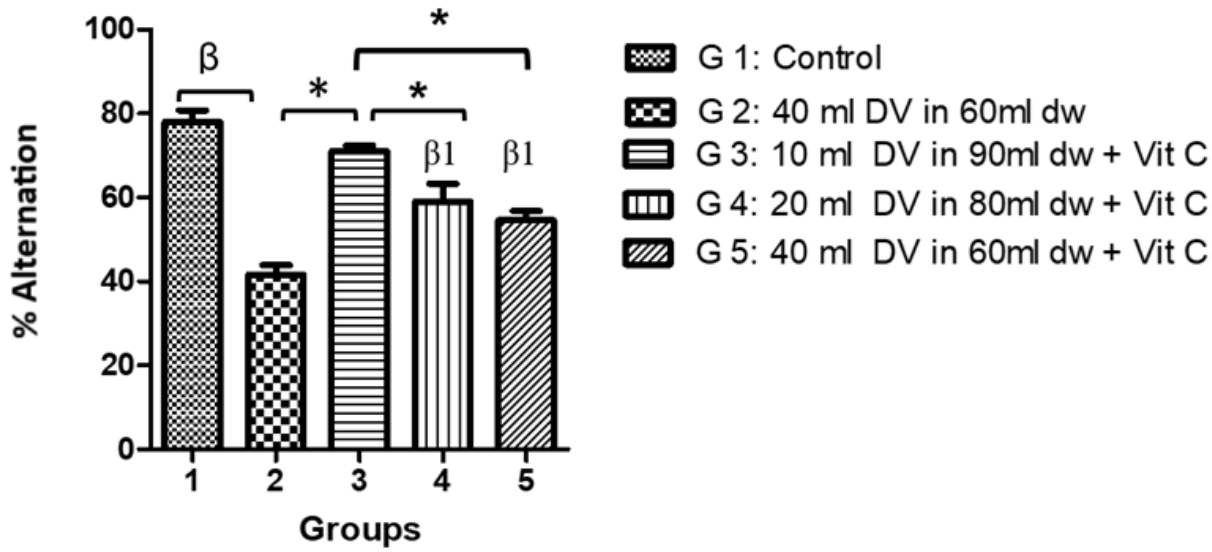

Fig. 3. Y-maze test: Mean Percentage Spontaneous Alternation

Histological analysis (Light-microscopic)

Results show the consequences of DV exposure in the dentate gyrus, CA3 and CA1 regions of the hippocampus (**Figures 4A, 5A, and 6A**).

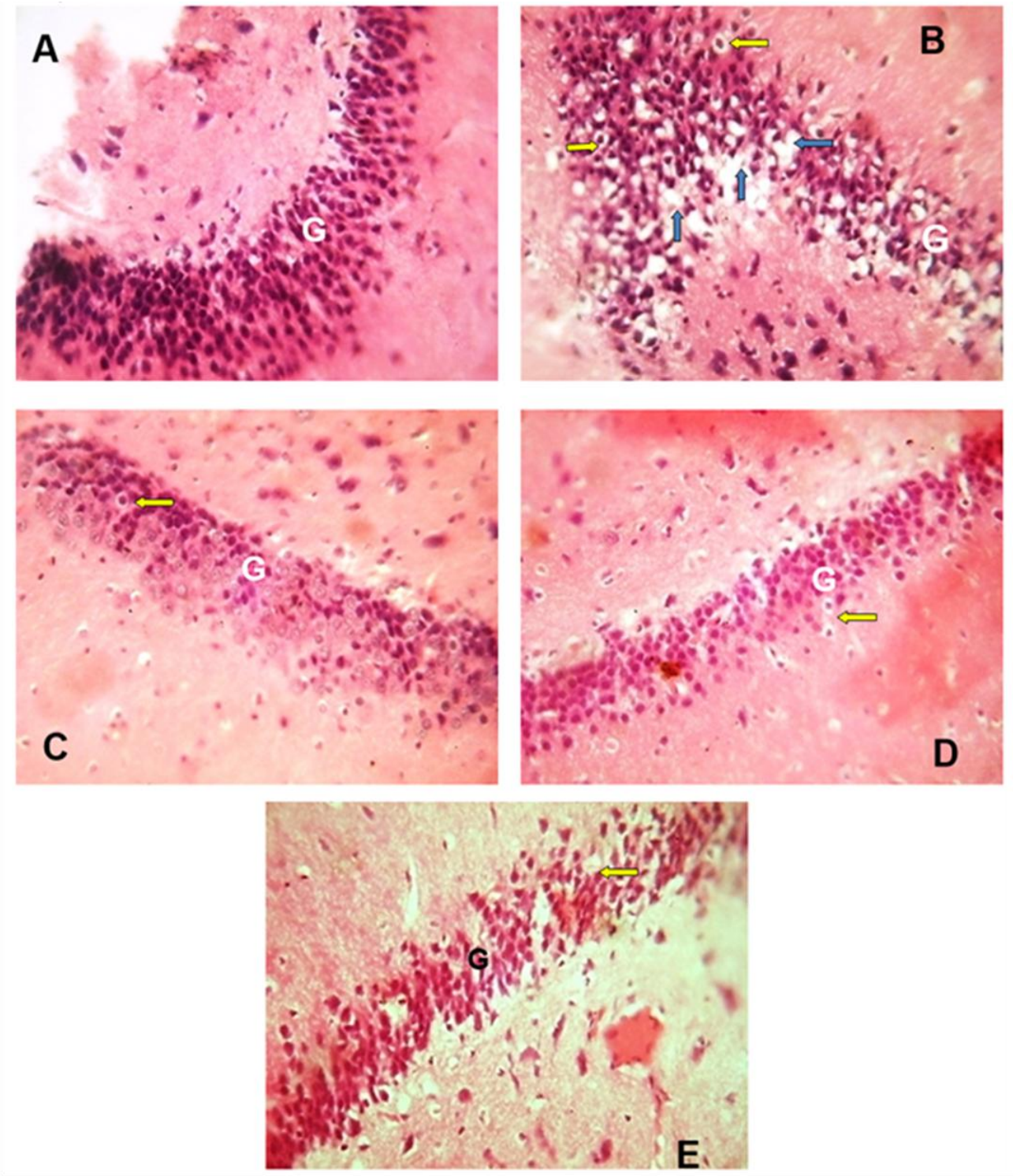

Fig.4. Dentate gyri of representative rats: Rats exposed to DV only (**B**), showed vacuolation (blue arrows) and numerous apoptotic degenerative neurons (yellow arrows). Note the relative reduction in the cell population in the granular layer compared to the control (**A**). Also, observe that the neurons in the G layers are relatively more in rats treated with vit-C (**C, D, and E**) compared to group 2 (**B**). Note the absence of vacuolation in rats that received vit-C. G, granular layer. H & E, mag. x 400.

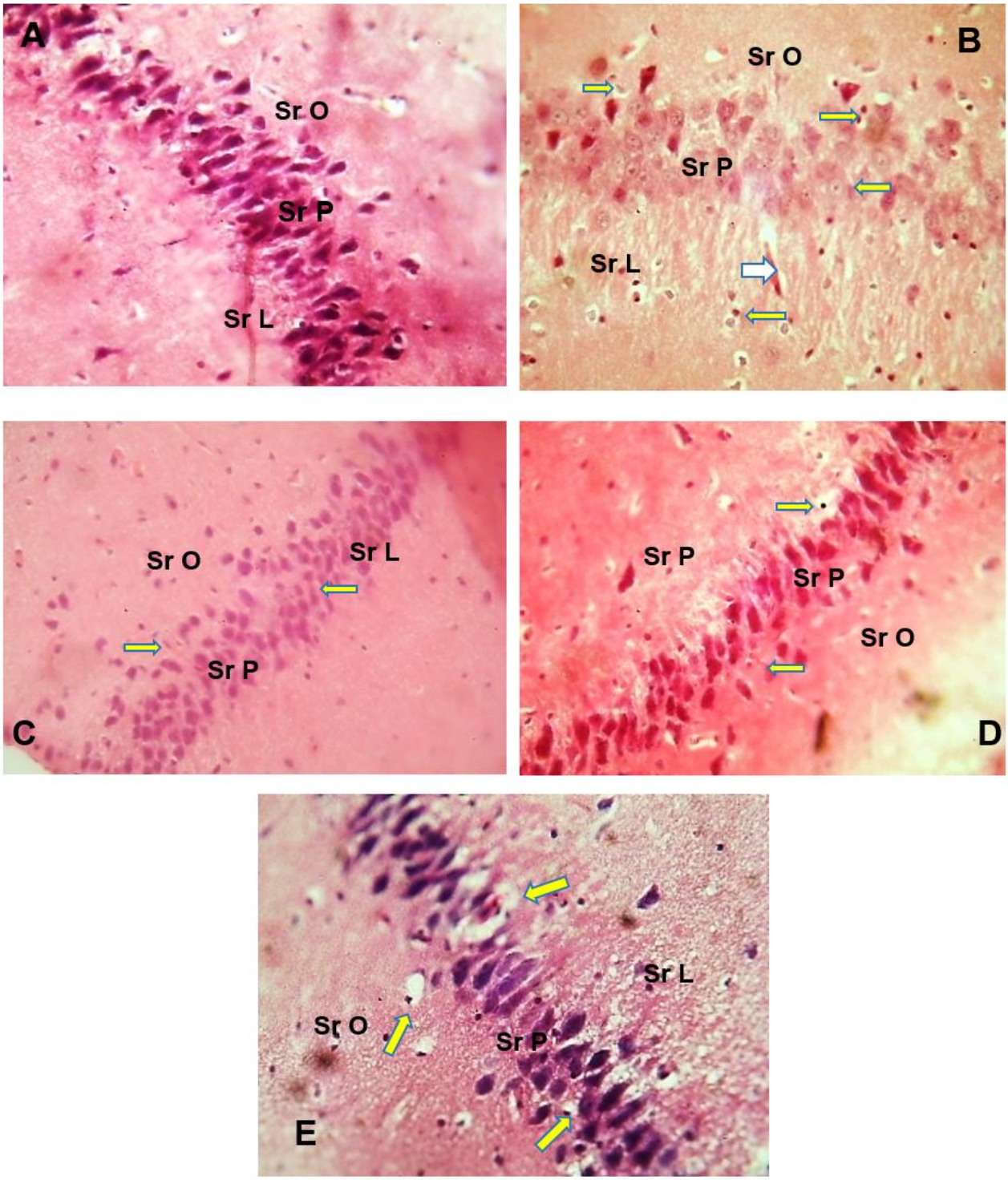

Fig. 5. CA3 regions of representative rats: DV exposure caused widely spread apoptotic (pyknotic) neurons (yellow arrows) and reduced neuronal density. Group 2 rats (**B**) also showed inflammation with loss of cyton and capillary dilation (white arrow). Rats that received vit-C (**C, D, and E**) showed no evidence of inflammation or loss of cyton. Widely spread pyramidal neurons were observed in group 2 because of cell death. Pyknotic degenerative changes were barely observed in GPs 3, 4 and 5 (**panels C, D and E respectively**) compared to GP 2 (**B**). StO, stratum oriens; StP, stratum pyramidales; StL, stratum lucidum. H & E, mag. x 400.

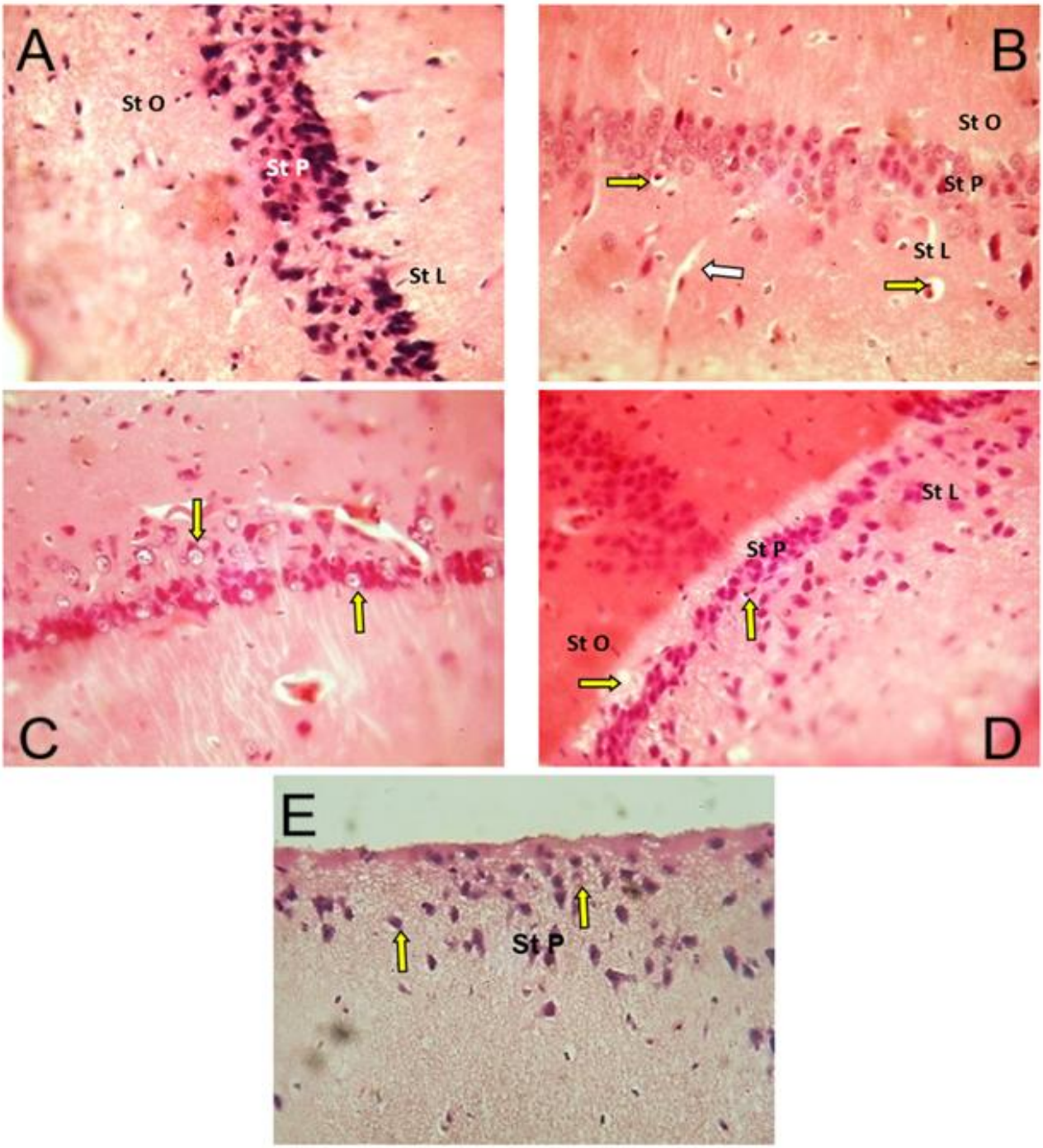

Fig. 6. CA1 regions of representative rats: Control (**A**) shows normal neuronal morphology in 3 strata. Rats exposed to 40 ml DV (**B**) showed loss of pyramidal cyton in the stratum pyramidales, widely spread pyknotic neurons (yellow arrows), dilated capillaries (white arrow), reduced neuronal density, and diffused vacuolation and inflammation. Rats that received vitamin C supplementation showed fewer pyknotic cells and degenerating neurons (**C, D, and E**). StO, stratum oriens; StP, stratum pyramidales; StL, stratum lucidum. H & E, mag. x 400.

4. Discussions

This study examined the neurotoxic effect of subacute DV inhalation on rat hippocampi and the potential of vitamin C to mitigate these effects. This work demonstrated the neurodegenerative consequences of dichlorvos on the hippocampus and the impact of ascorbic acid (vitamin C) on DV neurotoxicity (Figs. 2–6). The neurodegenerative changes observed were vacuolation, inflammation and apoptosis in the dentate neurons and pyramidal neurons (Figs 4B, 5B, and 6B); these findings are consistent with previous reports that align similar histopathological alterations in response to DV insults (Owoeye et al., 2014). This result also supported those of Abdel-Aziz et al. (2022), who demonstrated that vitamin supplementation lessened the extent of damage caused by dichlorvos-induced cytotoxicity in the rat hippocampus. Studies suggest that the pathophysiology of DV-elicited cytotoxicity is through phosphorylating the active centre of brain acetylcholinesterase, causing irreversible inhibition of the enzyme, resulting in acetylcholine building up in synapses and disrupting nerve function (Okoroiwu and Iwara, 2018; Mostafalou and Abdollahi, 2023). Dichlorvos, like other organophosphate insecticides, is known to trigger the production of reactive oxygen species (ROS) within the exposed organisms that result in oxidative damage, which can trigger cell, tissue, and organ damage (Farkhondeh et al., 2020). According to Sharma and Singh (2012), oxidative damage may be the cause of DV neurotoxicity, which results in necrotic cell death; however, we also observed apoptosis (Figs. 4, 5, and 6) characterised by nuclear condensation (Elmore et al., 2016), a fact that was missed out on in previous studies (Owoeye et al., 2014; Abdel-Aziz et al., 2022; Hart and David, 2022).

We found that giving ascorbic acid (vitamin C) to DV-exposed rats (DV + Vc) reduced hippocampal lesions compared to animals exposed to DV without supplements. The granule and pyramidal cells of the three regions showed mild to moderate neurodegeneration; this is consistent with the study of Owoeye et al. (2014), who reported partial amelioration of hippocampal lesions by vitamin C. Reports also suggest that ascorbic acid acts as a free radical scavenger (Gegotek and Skrzydlewska, 2022; Hart and David, 2022); thus, it is plausible that it reduced such oxidative reactions due to DV exposure (Sharma and Singh, 2012; Farkhondeh et al., 2020). Research has shown that hippocampal damage can have extensive effects on cognitive and emotional processes, as the hippocampus is a critical structure within the limbic system, playing a key role in learning, spatial cognition, emotion, social processing, and motivation (Somogyi, 2010; Todorov et al., 2019).

The results from this study on NORT and Y-Maze experiments show that animals in group 2 exhibited worse cognitive impairment after being exposed to dichlorvos only (**Figures 2 and 3**). These findings suggest that dichlorvos has a deleterious impact on learning and memory. Previous reports have indicated that memory decline results from neuronal cell loss along the intrinsic hippocampal pathway (dentate gyrus → CA3 → CA1 → subiculum) (Hainmueller and Bartos, 2020; Bartos, 2023). Neuronal loss may result in a decreased efficiency of neuronal receptivity, which could impair the encoding and consolidation of memories within the hippocampus. This could undermine the memory decline observed in rats treated with DV in that it causes neuronal cell loss in the dentate, CA3, and CA1 regions (4B, 5B, and 6B), thereby promoting memory deficit. Ascorbic acid directly affects the activity of neurotransmitters by modulating

their binding to their receptors and preventing neurodegeneration (Covarrubias-Pinto et al., 2015). Additionally, vitamin C promotes neuroplasticity, which improves the brain's capacity to reorganise and adapt in response to learning and memory formation and also in the development and strengthening of synapses, which facilitate signal transmission (Moretti and Rodrigues, 2022). Thus, in this study, supplementation with ascorbic acid following dichlorvos inhalation (DV + Vc groups) showed improved memory indices (figs 2 and 3). This study reveals that subacute DV inhalation adversely affects memory, which may be partially attenuated by ascorbic acid. However, although ascorbic acid showed some protective effects against dichlorvos-induced spatial and non-spatial memory impairments, the incomplete recovery, notably at higher doses, implies persistent damage to memory formation mechanisms.

This study provides conclusive evidence that vitamin C supplementation can effectively mitigate the detrimental effects of dichlorvos on the hippocampus of Wistar rats. Consistent with previous findings (Akamo et al., 2025), dichlorvos exposure led to pronounced neuronal degeneration within the hippocampus, characterized by a significant increase in apoptotic neurons and a corresponding decrease in neuronal density. This was reflected in the poor performance observed in both the Y-maze and novel object recognition tests (figures 2 and 3), which indicate cognitive deficits (Mostafalou and Abdollahi, 2023). However, co-administration with vitamin C significantly attenuated these obtuse effects. Vitamin C treatment was associated with improved performance in both behavioural tasks, suggesting a significant restoration of cognitive function in the treated rats. This could be plausible due to vitamin C's ability to reduce ROS assault since ROS-induced damage is primarily the mechanism of OP-triggered cellular injury. There was considerably less neuronal damage in the vitamin C-treated rats compared to DV-exposed rats without vitamin C supplementation, suggesting that vitamin C reduces the neurotoxic effects of DV, potentially protecting neuronal integrity and lowering the risk of neurodegeneration.

Conclusions

Despite the valuable insights obtained from this study, several limitations require consideration. While our T-maze and NOR tests could only assess certain critical aspects of cognition, specifically spatial working memory and recognition memory, other aspects of cognition, such as spatial reference memory, executive functions, attention, and cognitive flexibility, remain unexplored in this study. Future studies could benefit from incorporating more comprehensive cognitive tests, such as the Morris water maze, Barnes maze, and the radial arm maze, to provide a more nuanced understanding of how undue dichlorvos exposure impacts cognitive function. It would be desirable if these assessments were automated. Software-automated tests reduce animal handling and stress while increasing researcher efficiency and productivity by expediting data collection, analysis, and interpretation. Nevertheless, our findings suggest that vitamin C may play a neuroprotective role against hippocampal degeneration and cognitive impairment induced by organophosphate-based insecticides in rats.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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INSECT-BASED PROTEINS: NUTRITIONAL BENEFITS, HEALTH EFFECTS, AND CONSUMER ACCEPTANCE

Cristina FILIP[1], Amalia PUȘCAȘ[1*], Tudor-Ionuț ISTRATE[1], Amelia TERO-VESCAN[1]

[1]Department of Biochemistry, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, 38th Gh. Marinescu Street, Târgu Mureș, Romania, 540139

*Correspondence:
Amalia PUȘCAȘ
amalia.puscas@umfst.ro

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Abstract: The excessive consumption of red meat in economically developed countries has been linked to obesity and the onset of inflammatory metabolic disorders responsible for chronic conditions including diabetes, different types of cancer, chronic pulmonary disease, cardiac and vascular diseases. As an alternative, insects, approved for consumption, are recognized as a sustainable, nutritional, rich source of protein due to their high protein concentration, amino acids profile and significant levels of vitamins and minerals. Despite these benefits, their acceptance as food remains low, influenced by cultural, psychological, and sensory factors. This review explores the quality of proteins that can be found in insects compared to plant-based alternatives, emphasizing their digestibility, amino acid profile, and sustainability. Furthermore, the bioactive properties of insect proteins, including their anti-inflammatory and immunomodulatory effects, are examined, highlighting their potential role in metabolic regulation and disease prevention. Additionally, the impact of chitin and other derivatives on the function of gut microbiota is discussed, along with their prebiotic potential. Finally, the legal frameworks governing the acceptances of insects that cand be used in human nutrition are reviewed, showcasing regional differences in their acceptance and regulation. Given the growing need for alternative protein sources, edible insects present a promising yet underutilized solution, requiring further research and public awareness to enhance their integration into global diets.

Keywords: edible insects, insect protein, anti-inflammatory effect, consumer acceptance, sustainability

1. Introduction

In economically developed countries, excessive consumption of products like meat, mainly red meat and meat products is directly related in the increase incidence of obesity but also to the emergence of an inflammatory metabolic state responsible for the onset and progression of long-term illnesses including type-2 diabetes, cancer, chronic pulmonary disease and cardiovascular diseases (Chavda et al., 2024) (Wang et al., 2022). In this context, the incorporation in the diet of new approved protein sources, such as plant-based meat (PBM) analogues or insect protein powder could bring real health benefits due to the content of proteins, vitamins or minerals (Jankowski et al., 2025). As well, the incorporation of alternative protein sources in human diet may be considered a sustainable option due to the reduction of ecological footprint, water consumption, pollution and

waste (Lange and Nakamura, 2023). The main advantages of using edible insects in human nutrition are shown in **Figure 1.**

There are extensive studies in the literature that aim to identify the reasons why different individuals adopt alternative diets that do not use animal products. Among these are: animal welfare, health, environment/sustainability, religious or economic reasons (Rosenfeld and Burrow, 2017). A 2022 report by a non-profit organization based in the United States that promotes vegetarian and vegan lifestyles through research shows that in the US 63% of adults always/sometimes eat vegetarian food, 29% vegan, and only 37% claim to never eat vegan/vegetarian food. Among them, most are women aged 18-34, motivated primarily by health concerns, followed by considerations for animal welfare and taste. Other influencing factors include cost, ethical beliefs, and environmental impact. (Stahler, 2020). In the UK, the Vegetarian Society reports that 4.5% of the population is vegetarian/vegan and meat consumption has decreased by 17% in 2019 compared to 2008 (Stewart et al., 2021). In 2024, the World Population Review analyzed dietary behavior in 45 countries on 6 continents and showed that worldwide the largest number of vegetarians is found in India (approx. 24%), mainly based on cultural reasons, the number of vegetarians is increasing in countries such as Germany (10%) or Sweden (12%), while in Latin American countries, Argentina (12%) and Brazil (14%) a real shift in dietary preferences has taken place (World Population Review, 2024).

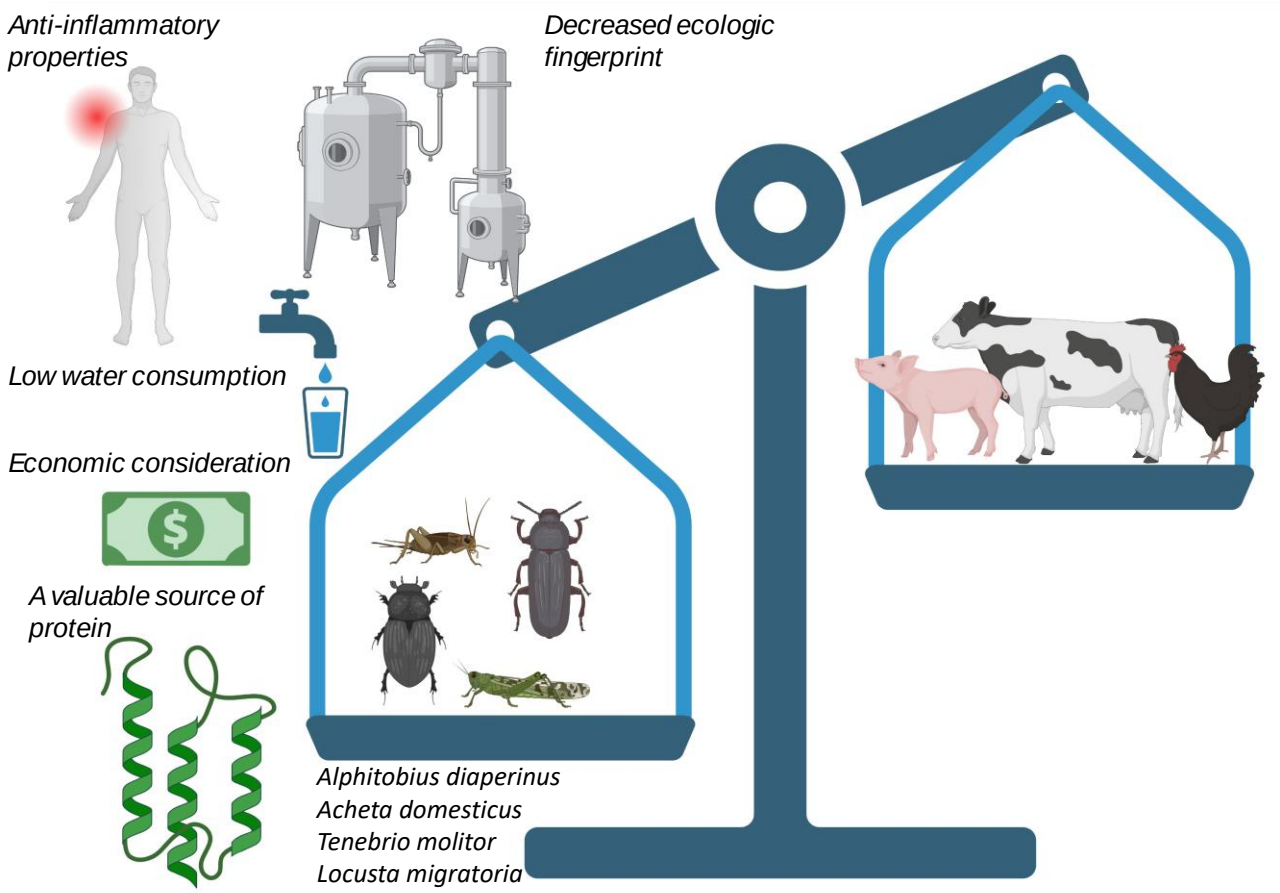

Fig. 1. The most important advantages of using edible insects (Created in https://BioRender.com)

The use of insect powder in food is currently legal in 120 countries, but despite the nutritional benefits (high content of proteins, unsaturated fats, minerals and vitamins) the degree of acceptance is low (Gedrovica, 2019, Bengtsson and Wendin K, 2023) the reasons being social, cultural, disgust and neophobia (Wendin and Nyberg, 2021). The regulations governing the use of edible insects vary across different regions. In EU member states, Regulation (EU) 2015/2283 classifies edible insects as farm animals, with approved species including house cricket (*Acheta domesticus*), the lesser mealworm (*Alphitobius diaperinus*), yellow mealworm (*Tenebrio molitor*), and migratory locust (*Locusta migratoria*) In the USA and Canada, the Food and Drug Administration (FDA) does not explicitly authorize insects for human consumption; nevertheless, the FDA allows insects for human consumption under certain conditions, mainly through regulations related to food safety and the correct labeling of products containing them. In African countries, the consumption of insects is practiced but legislation is scarce. In Asia, China, Japan, and Thailand, the consumption of insects is practiced but they are not included in the Catalogue of Edible Insect Resources (Li et al., 2023).

2. Consumer Acceptance of Insect-Based Foods: Gender Differences, Cultural Barriers, and Sensory Perceptions

A meta-analysis of 119 studies on gender differences in insect acceptance shows that, in most cases (71.2%), males accept insects as food. In contrast, 34.6% of studies indicate that gender has little influence on this acceptance (Kröger T et al., 2022).

An article published in 2024 shows that in Slavic countries, compared to countries where insects are considered a traditional food source, traditions, symbolism and religious beliefs promote aversion to entomophagy, and understanding these cultural barriers can contribute to developing strategies for the recognition of insects as a viable alternative protein source. (Orkusz and Orkusz, 2024).

A questionnaire-based study conducted in Sweden on 123 physically active healthy individuals showed that the extent to which insects are accepted as an alternative protein source is 35–40%. A different study examining perceptions of entomophagy in Western countries also attributes the low acceptance of food derived from insects to sensory properties associating the taste with that of spoiled food, mainly due to the fat content. (Ribeiro et al., 2024).

A study on the acceptance of insect consumption in Italy found that only 38% of respondents believe that insect-based foods could be part of the Italian diet, while 32% completely reject this idea. A study investigating the acceptance of insect consumption in Italy revealed that only 38% of respondents consider insect-based foods a possible part of their diet, whereas almost an equal number of respondents firmly oppose the idea of consumption. However, acceptance of insects as a food ingredient is higher when they are included in processed products, such as salty snacks, where they are not easily visible (Toti E et al., 2020).

3. Edible Insects as a Sustainable Protein Alternative: Nutritional Benefits, Health Impacts, and Microbiome Influence

3.1. Protein Quality and Sustainability: Edible Insects vs. Plant-Based Alternatives

Plant-based proteins have a low nutritional value due to their deficient content in certain essential amino acids and the presence of antinutritional factors like inhibitors specific to proteases, phytates, polysaccharides different from starch, and phenolic compounds, which limit nutrients absorption. Paradoxically, the

removal of these components involves industrial processing that limits their sustainability (Duque-Estrada and Petersen, 2023). Insects approved for consumption are regarded as a significantly more resource-efficient source of protein than both animal and plant-based alternatives. (Aguilar-Toalá et al., 2025). The amount of protein present in the species approved in the EU is: *Tenebrio molitor* 50.32 ± 0.21% of dry mass (rich in essential amino acids including methionine), *Locusta migratoria* 69.80 ± 0.30% of dry mass (Oh and Kim, 2025), *Alphitobius diaperinus* 57.67 % of dry mass (Rumbos et al. 2019) and *Acheta domesticus* 64.3% of dry mass (Quinteros et al., 2022).

A 2024 study shows that the protein percentage in edible insects is between 67.49 - 72.36% of dry mass, with a high content in leucine (Lampová et al., 2024). In this study a specific scientific metric score was used to evaluate protein quality which offers insight into protein quality, specifically the adequacy of essential amino acid content The DIAAS, the digestible indispensable amino acid score, values determined were the highest in yellow mealworms, with values up to 103.32 ± 10.11% followed by house crickets with an average of 96.61 ± 8.86% and 89.71 ± 9.22% for migratory locusts highlighting a high to superior protein quality and quantity for the insect used in the study (Pan et al., 2022).

3.2. Anti-Inflammatory and Immunomodulatory Potential of Insect-Derived Bioactive Compounds

Even though numerous studies show that insects are a potential source of compounds with antioxidants, insulin regulator effects on glucose and lipid metabolism, anti-inflammatory, immunomodulatory, hypotensive, antibacterial, and cardiovascular risk reduction (Acosta-Estrada et al., 2021, Cunha et al., 2023), these effects are still controversial. Even though the anti-inflammatory and immunomodulatory effects of insects are not fully demonstrated by in *vivo* studies, it is unanimously accepted that the prevention of inflammatory diseases is a socio-economic goal in developed countries. A 2024 review article introduces the term immunonutrition associated with biopeptides isolated from edible insects with the role of preventing macrophage stimulation resulting in the production of pro-inflammatory mediators (Rivero-Pino et al., 2024).

A 2018 study showed a decrease after 14 days period in plasma levels of cytokine inflammatory mediator TNF-α of cricket powder-based meals compared to isocaloric placebo meals (Stull et al., 2018). Chitin and chitosan exhibit anti-inflammatory properties by suppressing the NF-kB signaling pathway, which regulates the activation of B-cells, and by influencing the secretion of interleukin (IL)-10 (Acosta-Estrada et al., 2021). In a study from 2017 examining the impact of thermal processing (grilling, boiling) of protein hydrolysate isolated from insects (*Tenebrio molitor* and *Gryllodes sigillatus*) on the antioxidant and anti-inflammatory properties of them shows that the thermal processing has a beneficial effect by increasing the cyclooxygenase-2 (COX-2), the antioxidant effect and the inhibitory effect of lipoxygenase. (Zielińska et al., 2017).

Several peptides obtained by hydrolysis of insect proteins have been described as playing a role in modulating inflammatory processes. Thus, a study conducted in 2021 shows that the peptide Ala-Gly-Leu-Gln-Phe-Pro-Val-Gly-Arg (AGL9), isolated from the protein hydrolysate of *Allomyrina dichotoma larvae* administrated for five weeks to mice with hepatic steatosis unrelated to alcohol consumption resulted in the stabilization of serum levels of adiponectin, hepatic transaminases, lipoprotein profile levels such as triglycerides (TG), total cholesterol (CHOL),

high-density lipoprotein (HDL), very low-density lipoprotein (VLDL) and leptin by suppressing the AMPK/Nrf2 signaling pathway (Fan M et al., 2021). Peptides BPP-21 and BPP-22, isolated from honeybee pupae, significantly increased body weight gain and macrophage phagocytosis, while simultaneously reducing serum levels of the cytokines IL-2 and interferon (IFN)-γ, as well as immunoglobulins (IgA, IgG, and IgM). These effects were observed *in vitro* studies on RAW 264.7 cells and were associated with the stimulation of extracellular signal-regulated kinase (ERK) and p38 phosphorylation, which are involved in the modulation of the MAPK signaling pathway (Chen et al., 2022).

Regarding studies on the anti-inflammatory role carried out *in vivo*, in humans, there are no studies in the literature that explain the fate of chitin and biopeptides obtained by protein hydrolysis in the human body (Rivero-Pino et al., 2024).

3.3. Modulation of the gut microbiota

Studies in the literature show that chitin, chitosans and low molecular weight chitooligosaccharides from insects and crustaceans have a prebiotic effect. Fungal chitins favor the growth of *Bifidobacterium species*, but the effect of insect chitin on the gut microbiota remains mainly unclear and is limited by the availability of sufficient quantities to be used in preclinical and clinical studies (Stull and Weir, 2023).

To date, only a few studies in humans, animals and *in vitro* models have investigated the prebiotic effect of whole insects (25 g/day cricket providing an equivalent of 2 g chitin/day), demonstrating that they can promote the proliferation of beneficial bacteria such as *Faecalibacterium*, *Lactobacillus* and *Bifidobacterium*, but it could not be clearly established whether these effects are exclusively attributed to chitin or its derivatives (Stull et al., 2018).

Insect chitin can influence the intestinal microbiota by inhibiting the growth of some bacteria, both pathogenic and probiotic, such as *Lactobacillus spp.*, *Clostridium perfringens*, *Escherichia coli*, *Vibrio cholerae* and *Salmonella typhimurium*, effects observed both in human studies and *in vitro* experiments (Lopez-Santamarina et al., 2020; Gil et al., 2004).

On the other hand, insects can be infested with specific bacteria that populate the insect exoskeleton, mouthparts and intestine, with potential pathogenic effects in humans, such as: *Escherichia coli*, *Salmonella*, *Clostridium perfringens*, *Enterobacteriaceae*, etc. Therefore, legislation in the US and Europe prohibits the consumption and marketing of insects collected in the wild, only those originating from insect farms (Aguilar-Toalá et al., 2022).

Conclusions

Edible insects represent a promising alternative protein source with significant nutritional, environmental, and health benefits. Their high protein quality, rich amino acid profile, and bioactive compounds suggest potential advantages over both traditional meat and plant-based proteins. Additionally, their low ecological footprint makes them a sustainable solution for addressing global food security challenges. However, their acceptance remains limited due to cultural barriers, sensory perceptions, and food neophobia. Further research is needed to optimize processing techniques, improve consumer perception, and assess the long-term health effects of insect-derived proteins. Public education and regulatory support will contribute significantly to promoting entomophagy as a viable and sustainable component of future diets.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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THE RELATION AMONG SOIL CONDITIONS, LICHEN GROWTH AND PRECIPITATION UNDER SUBALPINE CONIFEROUS FOREST IN NORTH ITALY

Polina LEMENKOVA*

[1]Alma Mater Studiorum – University of Bologna, Department of Biological, Geological and Environmental Sciences, Via Irnerio 42, IT-40126 Bologna, Emilia-Romagna, Italy

*Correspondence:
Polina LEMENKOVA
polina.lemenkova2@unibo.it

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Abstract: Boreal coniferous mountain forests mitigate climate through biophysical and biochemical processes, especially water balance. The link between forests and climate includes direct and nonlinear interactions with atmospheric composition, hydrologic cycle, and water balance. At the same time, forests are fragile ecosystems with high importance as water sources and climate at local and regional levels. Monitoring forest enables to predict consequences of climate change. In this study, the boreal forests were investigated to simulate climate cooling and warming. The area is located in the subalpine mountain forests of South Tyrol. Methods include statistical investigation, eddy covariance assessment of evapotranspiration, water discharge and fog interception. The dense, old-growth forest (>200 years old) was compared in sections with young patches (<30 y. o). Technical instruments included tree transpiration sensors, phenocam images, throughfall and stemflow gauges, water discharge measurements, soil moisture sensors and epiphytes quantification. Despite the importance of coniferous forests, the effect of boreal forests on climate processes is not sufficiently studied. While previous studies measured different components of the water balance, little is known about the frequency and influence of fog in water balance. To fill in this gap, this study presented the investigation on the relationships between water balance, forest age and structure in the Alps of north Italy.

Keywords: coniferous forests, ecology, parametrization, modelling, biosphere, canopy interception, fog

1. Introduction

Climate change affects forest community and vegetation ecosystems worldwide (Schneider, 1992; Klaučo et al., 2013, 2017; Wong et al., 2010; Lemenkova, 2022a; Hasanbegović et al., 2024). Concern about global warming and its effects on forest vegetation is widespread (Rodriguez-Franco & Haan, 2015; Stolte, 2001; Park et al., 2014). Many discussions have covered a wide range of topics regarding vegetation and land cover change under the effects of climate (Wang et al., 2025a; de la Huerta-Schliemann et al., 2025; Liu et al., 2025; Lemenkova 2024a, 2025a; Chia et al., 2014; Silapaswan et al., 2001). Nevertheless, little is known about the effects of the forest structure and lichen communities on climate warming. The underlying mechanisms that influence

hydrological water cycle and tree composition in alpine forest ecosystems (tree age and height) is still unknown.

The ability to predict future consequences of climate change depends on thorough investigation of hydrological relationships and their modelling using advanced tools (Addae and Dragićević, 2022; Ni et al., 2024; Lemenkova, 2019a, 2019b; Addae et al., 2024). Biophysical relationships between forest and environment can enhance or diminish negative climate effects (Descals et al., 2023; Brašanac Bosanac et al., 2024; Thien and Phuong, 2023).

Coniferous forests are under pressure from global climate change and external forces (Lee et al., 2014; Diem, 2003). Specifically, mountainous forests mitigate warming through evaporation and cooling (Dimitrijević et al., 2023; Lemenkova 2022b; Huo et al., 2009). To evaluate current state of forests, the interdisciplinary research is required which uses advanced methods of modelling data (Lunnan et al., 2004). Boreal forests have significant biophysical effect on the biomes of Europe. This includes the impacts on annual mean temperature, precipitation and mitigation of climate extremes (Boisvenue et al., 2012; Cheng et al., 2015). Loss of boreal forest affects glaciation and increases warming. Through absorbing water by canopy and returning it to the atmosphere as vapor, mountain forests have a significant impact on water balance. This maintains water cycle and mitigates contrasts in temperature and precipitation. Besides, infiltration is also increased and runoff is decreased in mountain forests. The relevance of fog for water balance of temperate mountains of Alps is still unknown.

Forests in the Alps are influenced by convective clouds appearing over mountain peaks in the summer, and thermal inversions, which lead to higher fog occurrence in valleys, in the winter. These high variations in cloudiness at small scales affect evaporation fluxes (Barr et al., 2021; Pattey et al., 1997). Estimation of these fluxes includes large uncertainties and is difficult to interpret (Wang et al., 2007; Beverland et al., 1996). To better evaluate such relationships, diverse modelling techniques to evaluate interacting climate factors and environmental variables should be considered (Barr et al., 1997; Lemenkova, 2019c, 2024b; Krstić 2024; Wang et al., 2025b; Ioniță et al., 2025). Modelling techniques investigate the impacts of global change to identify feedbacks from climate processes on ecosystems and the potential of forests to mitigate climate change (Pawendkisgou and Yaméogo, 2023; Lemenkova 2025b; Ozdemir and Abdikan, 2025).

The amount of water intercepted by the forest canopy varies with age, structure and leaf type (Hadiwijaya et al., 2021; Park and Hattori, 2004). Also, it depends on presence of mosses and epiphytes which may intercept water (Węgrzyn et al., 2012). Specifically, lichens and epiphytes store water inside the forest, sustain evapotranspiration, decrease Bowen ratio, and increase air humidity (Rouse & Kershaw, 1971). In this way, forests decrease water pressure deficit and create little water stress to leaves and epiphytes (Valladares et al., 2016; Jongman & Korsten, 2017). In temperate mountain forests needles sustain for years and epiphytes develop on boles and branches of old trees (Dettki et al., 2000; Giordani, 2012). In aged forests, lichens continue to grow on old plants and increase interception capacity, when stand leaf area index (LAI) has reached maximum. In this way, lichens and age of forests contribute to water balance and regulate climate parameters, which has also been reported earlier (Errington et al., 2022; Kentjens et al., 2023).

2. Materials and methods

The experiment was conducted in South Tyrol, Italian Alps. The catchment has an area of 0.44 km^2. The water basin was measured on local DEM using ArcGIS. The tree layer (diameter at breast height (DBH) >5 cm) consisted of 85% spruce [*Picea abies* (L) Karst.], 12% Swiss stone pine (*Pinus cembra* L.), and 3% European larch (*Larix decidua* Mill) trees. Scots pine (*Pinus sylvestris* L.) and European rowan (*Sorbus aucuparia* L.) were also presented occasionally. The dominant tree height was ca. 29 m. The understory consisted of alpenrose (*Rhododendron ferrugineum* L.) and blueberry (*Vaccinium myrtillus* L.). Intervening grasslands were dominated by wavy hair-grass [*Deschampsia flexuosa* (L.) Trin]. The forest is of natural origin and managed for wood production. The soil has developed above a layer of glacial till, with a depth of approximately 1 m, placed on top of a porphyry bedrock. The soil was classified as Haplic Podzol according to the FAO soil taxonom and on average consisted of 49% sand, 39% silt, and 12% clay.

We aimed to understand the potential influence of fog and tree age and the associated abundance of lichens on water balance. To estimate water balance, the relative contribution of the water components in the balance at the catchment level was measured. Fog had a noticeable impact under certain meteorological conditions. We assume that older trees have higher interception capacity and lower throughfall rates than younger stands. This is because of the epiphytic lichens in older forest. Positive feedback is established between the tree age and evaporation fluxes. To estimate fog water, it was derived from the comparison of the gross and net precipitation.

The traditional harvest method creates small gaps, ca. 50 m wide, and involves the thinning of trees (Connell et al., 2004; Jonsell & Rubene, 2024). The diameters and heights of trees were measured regularly following existing methods (Zhang et al., 2015; Stepper et al., 2016), while the diameter of a subset of trees is measured annually by manual dendrometers. The inventory (tree height, size and position) was performed using TruPulse sensor. In addition, tree size was assessed using a laser technique. The forest has heterogeneous vegetation structure, with even-aged groups at a larger scale. First group consists of old spruce trees with an age of ca. 200 years; second group consists of young, ca. 30 years-old trees were present at the study site. In both stands, parts of the living crown often reached the ground.

Water partitioning and water balance at catchment level

The water partitioning at catchment level was estimated considering existing similar works (Skalova et al., 2002; Shen et al., 2013) for old and young forest (‘of’ and ‘yf’) for five months. Total precipitation (P) was split into rain (Pr, mm) and mixed precipitation (Pm, mm) (only 9 days with fog-only), canopy interception (I, mm) was calculated as P - throughfall (Tf) - stemflow (Sf):

$$P = Pr + Pm + F = Tf + I + Sf \quad (1)$$

Total forest evapotranspiration (ET_EC) was considered to be the sum of tree transpiration (T), evaporation of intercepted water (I), transpiration of the understory (Tu), and soil evaporation (Es).

$$ET_{EC} = T + I + Tu + Es \quad (2)$$

ET_EC was measured for the whole forest using existing techniques of eddy covariance (Rummel et al., 2002; Kang et al., 2025; Yan et al., 2023), T for trees of the two stands using

sap flow sensors, and I was equal to tree interception (as intercepted water will eventually evaporate) calculated from precipitation balance.

The sum of understory evaporation plus soil evaporation (Esu) was calculated as the residual of ET_EC - T - I.

$$Esu = ET_{EC} - T - I \quad (3)$$

Discharge (DC) and change of soil moisture (dSWC) were measured using existing techniques (Wu et al., 2019) for the whole forest.

The annual water balance was calculated following Eq.1,

$$Pr + Pm = ET_{EC} + dSWC + DC + DPe \quad (4)$$

where all acronyms are listed above except for DPe, which is deep percolation. All these terms were expressed in mm (1 mm = 1 kg m^{-2} of water).

To understand fog occurrence, meteorological conditions were compared during periods with dry conditions (dry), fog (<1 km visibility) and rainfall. These included relative humidity, TºC, radiation, wind speed and direction, ratio of diffuse to total global radiation and VPD. The influence of aged tree in water balance was evaluated by estimating and comparing water interception, epiphyte composition and TºC at different heights of canopy forest. The role and contribution of fog were estimated by its impact on throughfall. A linear regression equation was determined between throughfall and precipitation rate for mixed precipitation and rain-only events for both stands. These gave a predicted throughfall for a precipitation event with rain-only and with fog (mixed). Fog contribution to throughfall for mixed precipitation days was estimated as the difference between measured throughfall and the contribution of rain to throughfall calculated as the product of precipitation and the slope of the throughfall to precipitation equation from rain-only events. Additional measurements at soil level were performed in an old and young forest, in which soil moisture was measured. Water discharge was estimated at catchment level.

Evapotranspiration and tree transpiration

Evapotranspiration (ET_EC) was calculated from eddy covariance data following the ICOS setup and elaborated with Eddypro. Based on monthly energy balance ratio, we estimated the systematic errors in the eddy covariance measurements for LE+H as 17.6%. Given a Bowen ratio of ca. 1 in summer, this can be assumed to be half of this value in LE (8.8%). The random error of EC and the other components of the water balance was calculated statistically. Tree transpiration was estimated by measuring sap flow of five spruce trees with a DBH ranging from 23 cm to 57 cm. The sap flow measurements of up to 10 trees showed a representative behaviour of these trees for their size classes. To minimize the errors caused by incoming shortwave radiation, one sensor of the tissue heat balance sensor was installed at the north side of the trees. The measuring system provided sap flow rates integrated for the whole sapwood depth per unit trunk circumference (kg h^{-1} cm^{-1}). Tree transpiration was calculated by dividing sap flow by the projected crown area of the respective tree, thereby converting units from kg per tree to mm. The projected crown area was estimated from mean crown radius in the four cardinal directions. The average tree transpiration of the two smaller trees (DBH 23 cm and 32 cm) represented young stand, while the average of the larger trees (DBH 43 cm, 50

cm, and 57 cm) – old stand. All trees were dominant or codominant.

Local trees and spruces were characterized by an almost columnar shape. This shape and the high LAI (4.74 ± 0.88 for the 200-y.o. stand and 4.65 ± 0.86 for the 30-y.o. stand, as measured by hemispherical images), created microclimatic conditions in the crown, favouring lichen growth. Precipitation below the canopy as throughfall was measured with sixteen manual rain gauges, arranged in two groups of eight in the two main forest formations, the 200-y.o. section and the 30-y.o. section. These pluviometers, with a 10-cm diameter orifice, were arranged in rows with a 5-m distance between each pluviometer and data were recorded almost on a weekly basis. Six tipping bucket pluviometers arranged 5 m apart in two groups of three recorded the below canopy precipitation at a 10-min resolution.

To increase collection representativeness, funnels with a diameter of 30 cm were placed above the orifice of each pluviometer. The periods characterized by dry conditions, with fog and with rain, were compared. These included both observation and prediction periods to assess the accuracy. The number of days characterized by dry conditions, fog, precipitation (rain or snowfall), and mixed precipitation were calculated.

Water storage capacity of lichens

The role of lichens in water balance was assessed by measuring air temperature and humidity inside the crown and the water storage capacity of the lichens at two different tree heights. The water storage capacity of lichens was assessed in spring on tree with height of 28 m and a DBH of 53 cm which is representative of the old stand. To estimate the lichen weight, the tree was divided into 3-m sections. In each of them, all the branches were counted and all lichens present above a single branch were collected, together with the lichens growing on half of the main stem. In the laboratory, the lichens were first wetted until water saturation. They were dried in an oven at 45 °C until a constant weight was achieved and then weighed to assess their dry weight.

3. Results and discussion

From January until August, the total fog frequency was 296.5 h during 72 days with fog and 37 days with mixed precipitation (109 days; 30% of days were foggy and 15% had mixed precipitation). Evaluated water components at canopy level are summarized in **Table 1**. The average monthly precipitation (monthly and max daily sum) and temperature (monthly and daily average) and for the long-term period are presented in **Fig. 1**.

The average monthly precipitation as a monthly sum and maximum daily sum, showed the importance of convective rainfall events in the warm summer, while cold winters were dry. The precipitation was in accordance with the 20-year average. Also, air temperature deviated from the 20-year average in 2019, mainly during the first half of the year: February and June were too warm and May was too cold. Fog occurred for 241 h during 9 days with fog (5%) and 45 days with mixed precipitation (27%) within a period of 167 days (from late spring to autumn). The data are based on daily measurements divided into dry and precipitation periods. Precipitation measured inside and outside the forest (minor amount of precipitation during “dry” period because periods were defined based on the outside climate station alone), throughfall and stemflow measured with automatic tipping gauges, storage/interception calculated as P - Tf – St. Average values of radiation, relative humidity and vapour pressure deficit (VPD) during days with precipitation, fog and mixed precipitation were calculated to predict fog occurrence.

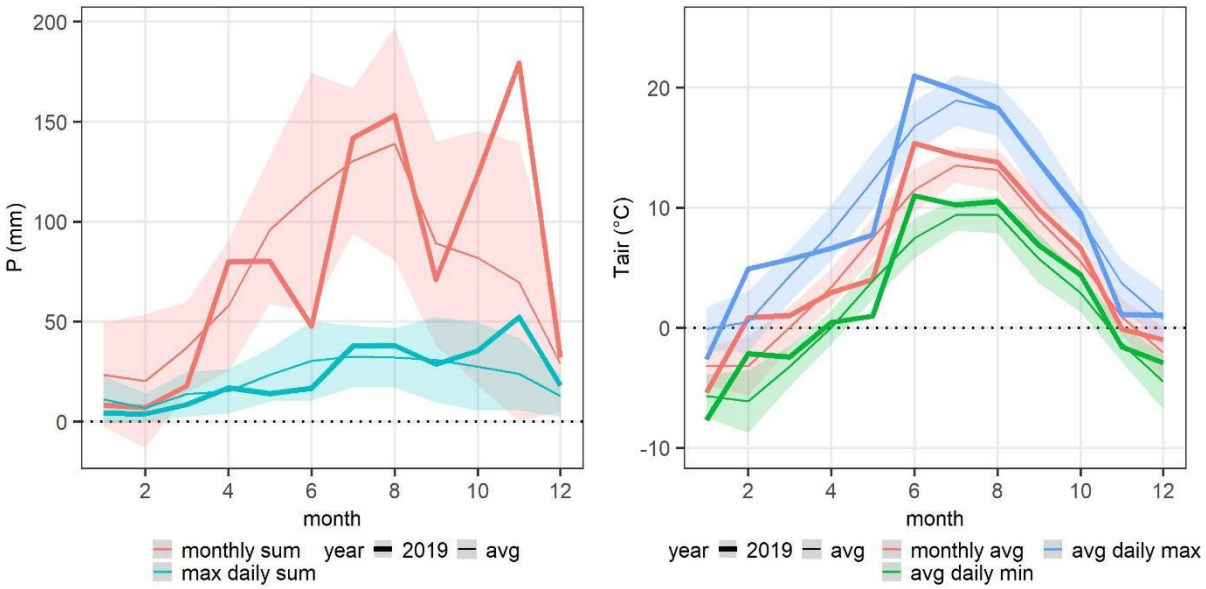

Fig. 1. Average monthly precipitation and temperature and for 2019 and long-term period

Table 1. Precipitation, evapotranspiration, throughfall, stemflow and interception

Period	Days	P	ET	Throughfall	Stemflow	Interception
young forest						
dry	78	1.3 ± 1.8	350	2.6 ± 3.3	0.0	-1.3 ± 3.3
fog	8	0.1 ± 0.2	26	0.2 ± 0.0	0.0	-0.0 ± 0.2
mixed fog+P	42	460 ± 35	110	292 ± 26	1.0	167 ± 36
rain/snow only	34	132 ± 21	147	47 ± 11	0.1	85 ± 19
old forest						
dry	78	1.3 ± 1.8	350	0.6 ± 0.2	0.0	0.7 ± 1.4
fog	8	0.1 ± 0.2	26	0.1 ± 0.0	0.0	0.1 ± 0.2
mixed fog+P	42	460 ± 35	110	216. ± 11	0.8	242 ± 29
rain/snow only	34	132 ± 21	147	36 ± 3	0.2	97 ± 16

The ratio of total to global radiation (ratio Rg dif/Rg tot) was lower during dry periods when total Rg was high. On the contrary, diffuse Rg was less affected by rain and fog. As expected, VPD was higher and relative air humidity (RH) lower during periods with dry conditions compared to periods with fog and precipitation. Data variability was higher within the Rg dif/Rg tot ratio than within the RH and VPD. These results highlight the importance of days having both fog and rainfall (mixed precipitation). Previous observations over 254 days were with cloudy conditions (70%) in South Tyrol but did not obtain fog or

mixed precipitation. Days with fog (defined as days with fog and days with mixed precipitation) may account for 45% of cloudy days in 2011, **Table 1.**

Meteorological conditions during days with rain, fog, and mixed precipitation

Data variability is higher within the Rg dif/Rg tot ratio than within the RH and VPD. The air temperature (T) and VPD were overall lower during foggy and wet days and increased during the first dry days, but decreased again towards the end of the measuring period, **Fig. 2**. When accounting for the interception, tree transpiration contributed slightly less to ET than the soil and understory E(T), while there is a contribution of the understory that accounts for 10–70% of the total transpiration. Discharge (DC) and the change in soil moisture (dSWC) completed water balance, as both were measured for the entire forest, the same values were used for both stands. Both DC and dSWC were of minor importance compared to T and Esu. Most of the discharge occurred during snowmelt in spring before the start of the measurement period. Changes in soil moisture are important in water balance and were levelled out over the entire measuring period. Average values of radiation, relative humidity and vapour pressure deficit (VPD) during days with precipitation, fog and mixed precipitation were calculated to predict fog occurrence in 2019. The ratio of total to global radiation (ratio Rg dif/Rg tot) was lower during \dry periods when total Rg was high. On the contrary, diffuse Rg was less affected by rain and fog. As expected, VPD was higher and relative air humidity (RH) lower during periods with dry conditions compared to periods with fog and precipitation. Relative humidity exhibited the opposite pattern of T and VPD.

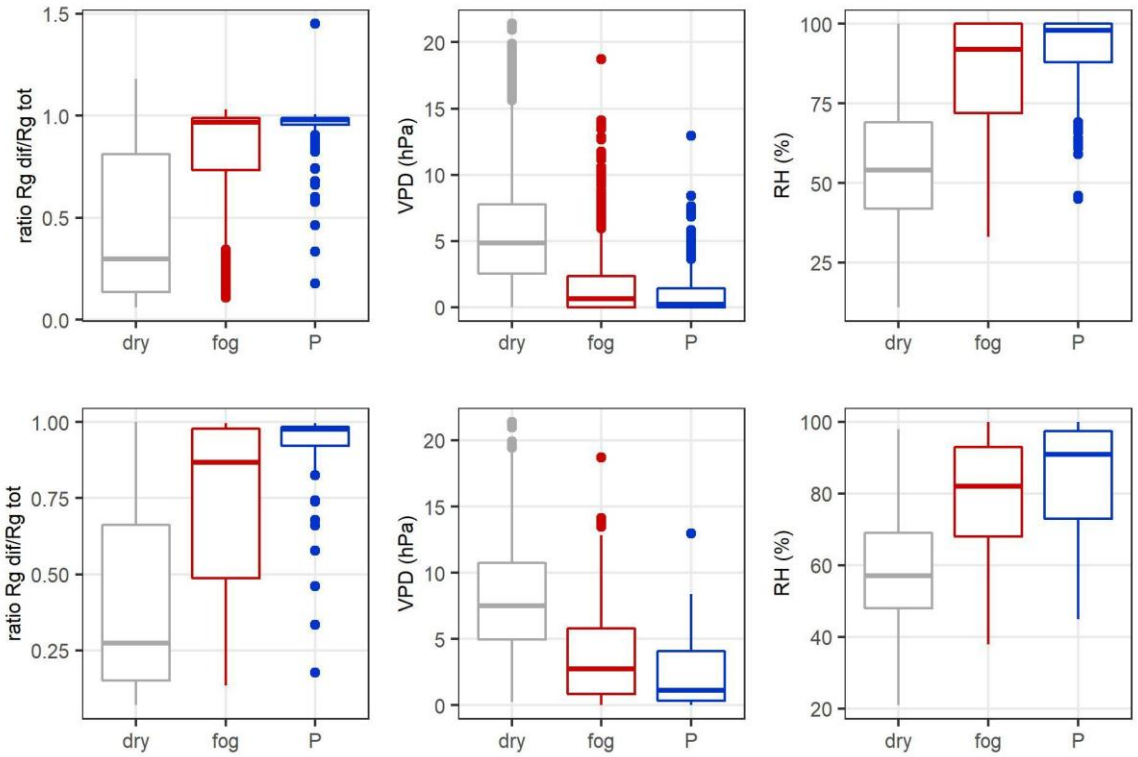

Fig. 2. Meteorological conditions (ratio of diffuse to total global radiation, vapor pressure deficit VPD, and relative air humidity RH) during periods with fog (less than 1 km visibility), precipitation, and dry conditions (dry) in winter (top row) and spring (bottom row)

The decrease in temperature was observed during the day but an increase during the night due to fog in long-term data, with different night sensitivity of temperature.

Uncertainties regarding the ability of sap flow measurements to predict absolute values led to an underestimation of tree transpiration.

Time course of radiation (total radiation top-left, diffuse radiation top-center, ratio of diffuse to total radiation top-right), air temperature (bottom-left), air humidity (bottom-center) and vapor pressure deficit (bottom right) during a foggy period in in late spring, end of May, the start of June (a.) and end of October, the start of November (b.) are presented in **figure 3**.

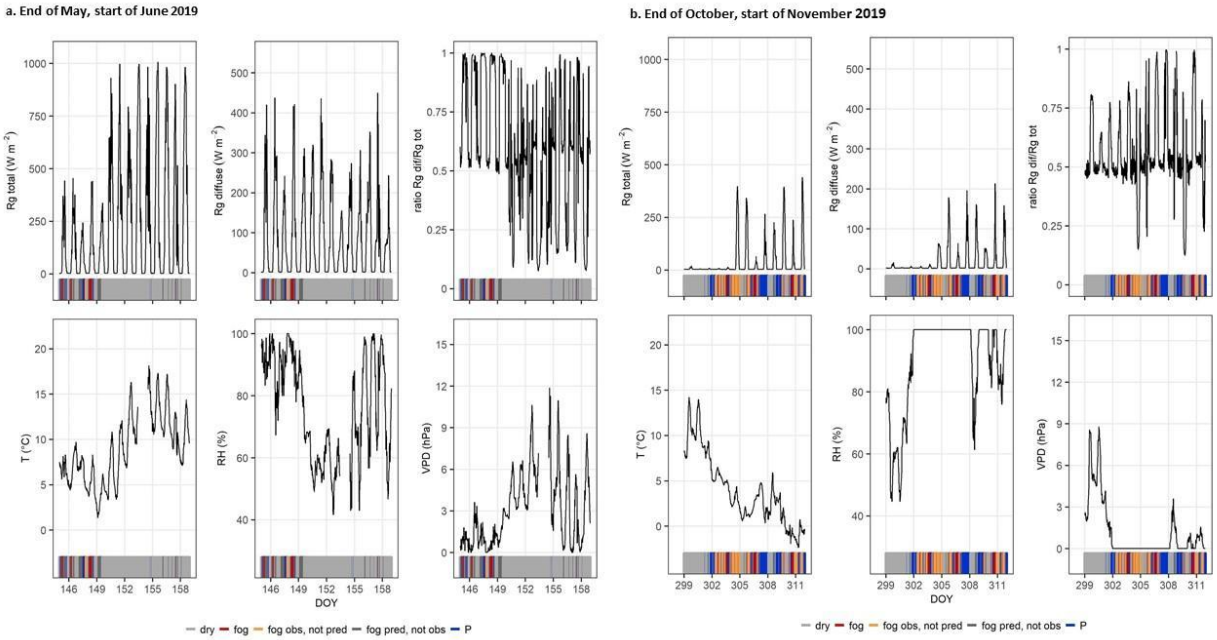

Fig. 3. Statistical analysis on data showing radiation, air temperature, humidity, and vapor pressure deficit (bottom right) during a foggy period, 2019

Canopy evapotranspiration and tree transpiration

Even though the precipitation totals were below the long-term average, rainfall events occurred quite regularly during the entire measuring period. The transpiration of the old (Tof) and young stand (Tyf) calculated from sap flow measurements was low compared to canopy evapotranspiration from eddy covariance. The time courses of T and ET corresponded to weather conditions and an overall decrease. This could be an effect of fog suppressing ET as observed in cloud forests, but also because days with mixed precipitation occurred more often in autumn when ET is lower because of lower temperature and phenology, **Fig. 4**. The Tyf was higher than Tof, as a smaller projected crown area and higher tree density in the young stand more than compensated for the higher sap flow of large single trees in the old stand. Consequently, the regression line of Tyf to ET had a higher slope than that of Tof to ET, for both forest types. The R^2 of the correlation of T and ET was higher than 0.9. Neither ET nor T correlated with P. By splitting the days into dry, mixed precipitation (fog and precipitation), and with rainfall-only, it was found that, not

surprisingly, ET was higher during dry days (55% of total ET in 48% of days) and was suppressed especially in days characterized by the presence of mixed precipitation (17% of total ET in 26% of days). This could be an effect of fog suppressing ET as observed in cloud forests, but also because days with mixed precipitation occurred more often in autumn when ET was generally lower because of both lower temperature and phenology (senesced grasses).

Though the water input through fog-only events at our site remained unknown, fog clearly contributed in mixed fog and rain precipitation when it was estimated to cause 24% of additional throughfall compared to rain only events. Also important, fog was not considered in past studies. Thus, our current findings reveal fog as the missing tie to understand not only soil water recharge during days with mixed precipitation but the decrease of evaporative conditions during dry periods in the studied Alpine ecosystem. The measuring period (from 2019-5-30 until 2019-11-07) did not cover the entire year, the water balance for this period was not closed, with lower inputs (P = 593.6 ± 55.4 mm) than outputs (ET_EC + DS + dSWC = 689.6 ± 70.7 mm).

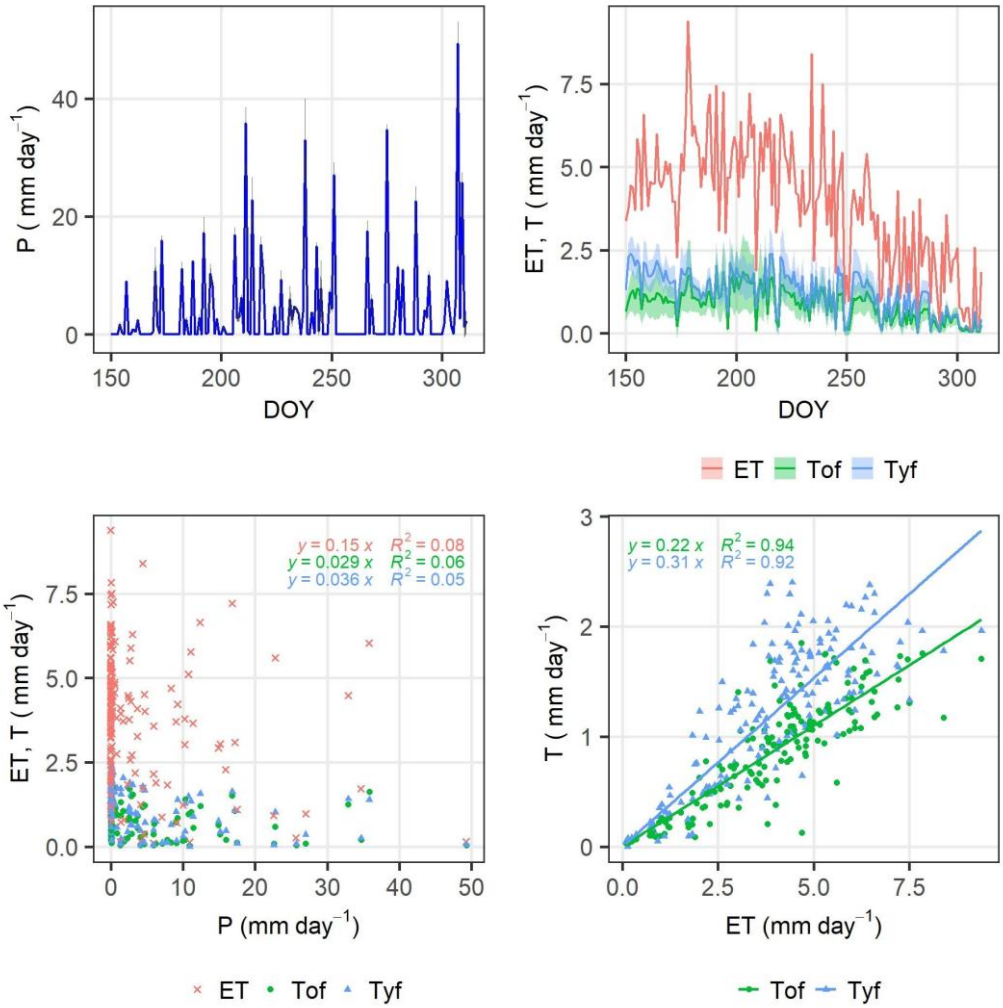

Fig. 4. Daily precipitation (P) and daily evapotranspiration measured with eddy covariance (ET) and daily transpiration for the old (Tof) and young (Tyf) forest upscaled from sap flow measurements (top right)

The frequency of fog events was quantified in a subalpine coniferous forest to assess the hydrological balance at basin and canopy scales. The difference between water input in rain and snow forms (fog not included) and water output as evapotranspiration and water discharge, plus the variation in the soil water content, was 25 mm, within the uncertainty range of the measurements.

Water balance was almost closed for the entire 2019, with a difference of 25.4 mm between the input (P = 985.6 mm ± 82.3 mm) and output [ET_EC (804.9 mm ± 70.7 mm) + DS (167.0 mm ± 0.1 mm) + dSWC (40 mm ± 1 mm) = 1,011.9 mm ± 103.7 mm]. Deep percolation is reported to represent a large component of the hydrological balance at some sites, when water basin lays over a fractured bedrock. In this case, deep percolation is of minor importance.

The results demonstrated that mountain forests have a high capacity to influence the water balance by intercepting water through the canopy and releasing it back to the atmosphere as vapour, which keeps the water cycle running. Additionally, mountain forests reduce runoff and increase infiltration. Warmer temperatures may have very distinct consequences for alpine forests, such as an increase in radial growth, which depends on water availability to compensate for the increased evapotranspiration.

The relevance of interception capacity was high when precipitation was low. Water was used to refill the canopy and soil reservoirs, without being lost as runoff. Large amount of water intercepted by the canopy represents most of the precipitation in the old stand, locally re-emitted as evaporation without stomatal control. It was shown that part of this water and fog can be directly taken by the plant for its needs. Apart from the physiological aspect of water use by the plant, the capacity of the intercepted water to act as a climate regulator at a local scale and the mesoscale is climatologically relevant. One mm of water at 20°C represents 44.2 W m^{-2} of latent heat, which is emitted in place of sensible heat, thereby reducing the temperature and increasing the availability of water vapour in the air. The role of fog is to sustain positive feedback in the water cycle. It favors the presence of dense vegetation and lichens, and increases water vapour. As there is a reduction of water vapour in the air, the presence of old-growth vegetation represents a critical element for climate regulation in the Alpine region. This evidence proves the capacity of forests to regulate extreme heat conditions.

Conclusions

This paper demonstrated that forests serve as climate regulators through balancing water cycle through precipitation and evapotranspiration. Moisture availability needs to be considered to predict the consequences of climate change. Forest overuse and degradation can lead to environmental problems: soil erosion, landslides, rockfalls, increased water runoff or reduced water storage. Other problems may include the drying of springs, and biodiversity loss, and have severe impacts on livelihoods. Understanding water distribution in forests and its consequences is important for land-use management, water policies, and modelling climate system. Modelling enabled to quantify the capacity of the forest to intercept water in canopy. The estimate of this capacity was provided in the two different forest stands, a 200-year-old and a young, 30-year-old stand. The higher water storage capacity of the old stand did not depend on the LAI, which was identical in both stands, but on the other structures, mainly epiphytes. Such organisms, typically represented by filamentous lichens, such as *Evernia divaricata* and *Pseudevernia furfuracea*, were relevant for

water cycle in the old section only and had a water-holding capacity of 0.6 mm for each precipitation event.

Combined with rainfall the same day, as mixed precipitation, fog contributes to higher throughfall, which increases net precipitation (soil water recharge) and evaporative conditions inside the canopy. Fog plays important role in water balance during days with mixed precipitation, maintaining high relative humidity inside the dense coniferous crowns of forest. This helped trees to maintain large leaf area, and the filamentous lichens to grow in the upper canopy. These two features led to a large capacity of the crown, particularly in the mature coniferous forest, to intercept liquid precipitation. Thus, it released only a small amount of precipitation to the soil and eventually to runoff, sustaining local ET with an associated reduction of the sensible heat flux.This research contributes to hydrological analyses and confirms that indicates that natural forests play a key role in dampening heat extremes above vegetated terrestrial ecosystems. It attributes to fog and cloudiness the role of linkage in positive feedback between forests and climate.

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HISTO-ANATOMICAL STUDY OF THE VEGETATIVE ORGANS OF *HERACLEUM SPHONDYLIUM* SSP. *SPHONDYLIUM*

Erzsébet DOMOKOS[1*], Béla DARKÓ[2], Eszter LACZKÓ-ZÖLD[3]

[1]Department of Horticulture, Faculty of Technical and Human Sciences Târgu Mureș, Sapientia Hungarian University of Transylvania, Romania
[2]Department of Botany, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania
[3]Department of Pharmacognosy and Phytotherapy, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania

*Correspondence:
Erzsébet DOMOKOS
domokos.erzsebet@ms.sapientia.ro

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Abstract: This study presents the first comprehensive histo-anatomical characterization of the root, stem, and leaf of *Heracleum sphondylium* ssp. *shpondylium*. Transverse sections revealed key structural features across all organs. The root exhibited a circular outline and secondary growth, with a periderm composed of phellem, phellogen, and phelloderm, a cortex containing aerenchyma and secretory canals, and a wide zone of secondary phloem and xylem, both traversed by medullary parenchyma. The stem displayed a layered organization from epidermis to central pith, including a cortex with collenchyma and secretory canals, and collateral vascular bundles forming a ring that defined the transition to the central cylinder. The dorsiventral leaf anatomy featured a two-layered palisade parenchyma on the adaxial surface and spongy parenchyma on the abaxial side. Secretory canals were consistently present in the cortex and vascular regions of all organs, and long, simple, unicellular trichomes with a basal crown of thick-walled cells were found on the leaf surface. These anatomical structures, particularly the secretory canals and trichomes, likely play a key role in the biosynthesis and release of furanocoumarins.

Keywords: *Heracleum sphondylium* ssp. *sphondylium*., hogweed, secretory canals, trichomes, plant histo-anatomy

1. Introduction

The genus *Heracleum* is represented in Romania by *H. carpaticum* (endemic in Europe), *H. palmatum* (endemic in the Romanian Carpathians), and *H. sphondylium* with three subspecies: *montanum* (European alpine), *sphondylium* (Eurasian), and *sibiricum* (European). The hybrid *H. x rodnense* (*H. carpaticum* x *H. sphondylium* ssp. *sphondylium*) is present in the Rodna Mountains. There have also been reports in Romania of two species escaping from cultivation: *H. mantegazzianum* and *H. sosnowskyi* (Sârbu et al., 2013).

H. sphondylium ssp. *sphondylium*, commonly referred to as hogweed or brânca ursului, is a biennial or perennial plant. It has white, orange-bruising roots. In the first year, a taproot is present. As it ages, a network of external vegetative meristems and related secondary taproots grows, expanding the root crown. Therefore, the root expands and thickens considerably with the years. The plant has a hollow stem with a furrowed surface, reaching a height of 50-200 cm. The leaves have 5-7 broad, lobed, and toothed segments and a less swollen vagina. The white or rarely pink flowers are 3 to 10 mm in size and grow in large umbels (up to 15 cm in diameter) with 12 to 25 rays. The outer flowers of the umbellet are zygomorphic with highly uneven petals, of which the larger ones are directed outwards. The tiny fruits are schizocarps (of 2 mericarps), which can grow up to 1 cm in length. Fruit shape can be from elliptical to rounded, winged, and flattened. Each mericarp has six resin canals (approximately as long as the fruit), two on the inner or commissural face and four on the outer, dorsal face (Sheppard, 1991; Sârbu et al., 2013; Benedec et al., 2017; Matarrese et Renna, 2023).

Furocoumarins (bergapten, isopimpinellin, and heraclenin) and essential oil were found in the seeds and roots of different *H. sphondylium* subspecies. Monoterpenes, sesquiterpenes, and phenyl-propanoids composed manly the extracted essential oils (Matarrese et Renna, 2023).

Regarding the phenolic composition, high levels of rutin were described by Benedec et al. (2017) in the leaves and flowers of the *H. sphondylium* ssp. *sphondylium*. In terms of other flavonoids, roots contain ferulic and chlorogenic acids, leaves have quercitrin, and flowers contain quercetin.

It was found that the essential oil from seeds of *H. sphondylium* ssp. *ternatum* is rich in 1-octanol and octyl butyrate and presents cytotoxic activity against tumor cells (Maggi et al., 2014). Other studies reported the antibacterial, antifungal, and antioxidant biological activities of the different plant extracts (methanol, ethanol, and aqueous) from *H. sphondylium* (Matarrese et Renna, 2023). Furthermore, the vasorelaxant property of the dichloromethane extract was also revealed (Senejoux et al., 2013). The fruit of *H. sphondylium* ssp. *montanum* had a yield of the essential oil of 4.39%, while the root presented a yield of 0.22%. However, the aerial parts of the same taxa had a noticeably lower yield 0.19%. The primary chemicals found in *H. sphondylium* ssp. *montanum* roots essential oil was composed of myristicin (13.8%) and octyl acetate (57.1%), while the fruits showed a rich profile, with myristicin (8.9%) and octyl acetate (73.7%) predominating. The aerial portions, on the other hand, showed a balanced distribution, with the primary essential oil chemicals being octyl acetate (56.1%), myristicin (24.2%), and hexyl butyrate (4.1%) (Kose et al., 2025).

Regarding the histo-anatomical structure of the organs in the case of *H. sphondylium*, Bradley and Fell (1966) described the tissues of the ripe fruits, while Laczkó-Zöld et al. (2023) presented the secretory tissues from various phases of fruit maturity. Bicchi et al. (1990) showed that the two different types of secretory structures (secretory canals and vittae) in *H. sphondyhum* ssp. *sphondylium* fruits store different chemicals, highlighting that chemical variations are linked to histological diversity. Arora et al. (1982) described the characteristics of the leaf epidermal trichomes in *H. sphondylium* using a scanning electron microscope (SEM).

Given that other descriptions of the anatomy of *H. sphondylium* have not been found in the scientific literature, the aim of the study was the detailed anatomical characterization of the vegetative organs of *H.*

sphondyhum ssp. *sphondylium*. This microscopic investigation is the initial phase of a larger project that aims to clarify the possible structures involved in bioactive substances secretion in case of this taxa.

2. Materials and methods

H. sphondylium ssp. *sphondylium* plants were harvested from the spontaneous flora in Sovata area, Romania (N46° 39.010' E24° 58.603'), and after identification, a voucher specimen (Voucher number: FS/1607/2022) was stored at the Department of Pharmacognosy, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureş, Romania.

Before section preparation, root, stem, and leaf samples were preserved in 60% ethanol for the anatomical study. According to Tanase et al. (2017), ruthenium red and iodine green were used to stain rehydrated plant parts. A Canon EOS 250D (Canon Inc., Japan) camera and a light microscope (Ceti Topic-T, Belgium) were used to capture photographs.

3. Results and discussion

Root structure

The cross-sectional outline of the *H. sphondylium* root was circular (**Fig. 1 A, B, and C**), and the root displayed a secondary structure.

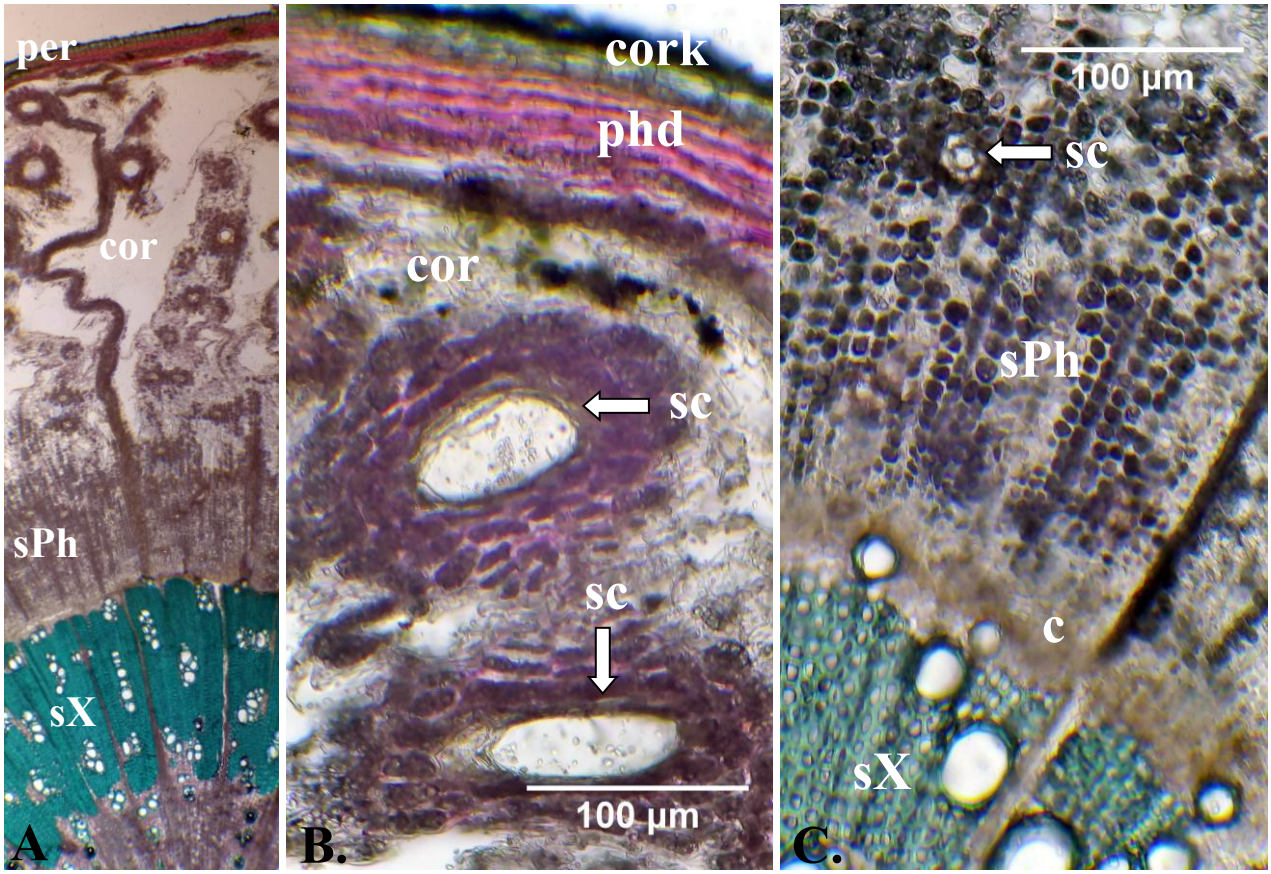

Fig. 1. The root structure of *Heracleum sphondylium* ssp. *sphondylium*: A. general view; B. the secondary cortex and the primary cortex with the secretory canals; C. the secondary phloem with secretory canals and the secondary xylem.

Abbreviations: per-periderm; **cork**-phellem; **phd**-phelloderm; **cor**-cortex; **sc**-secretory canal; **sPh**-secondary phloem; **c**-cambium; **sX**-secondary xylem (photographs by Erzsébet Domokos).

The following tissues, were identified, arranged from the outside of the root to the inside: the periderm or secondary cortex, which is made up of the phellogen, presented a thick layer of suber (phellem) and tabular cork cells (phelloderm); the cortex, which contained numerous secretory canals and aerenchyma; a broad zone of secondary phloem, also containing secretory canals (considerably smaller in diameter than those found in the cortex); and secondary xylem, approximately equal in width to the secondary phloem. Both the secondary phloem and xylem were radially traversed by medullary parenchyma. Similar root structures were described by Betekhtina et al. (2018) for *H. sosnowskyi* and Lee (1965) for *H. mantegazzianum*.

Stem structure

The stem has a circular-costate cross section. The early stages of transition between primary and secondary structures were visible (**Fig. 2 A, B, and C**). The following primary tissues could be identified from the outside to the inside of the stem: the epidermis, composed of large cells covered by a cuticle; long, irregularly distributed unicellular trichomes on the epidermis; the thin cortex with thick layers of collenchyma beneath the epidermis, next to the ribs; cortex containing also a few secretory canals above the vascular bundles; the collateral vascular bundles of various sizes arranged in a circle, demarcating the boundary between the cortex and the central cylinder; numerous secretory canals within the central cylinder (**Fig. 3**); and the central pith (central cylinder which lacks medullar parenchyma).

Leaf structure

The leaf cross-section at the level of the midrib presented the following structure (**Fig. 4. A and B**): an epidermis composed of polygonal and isodiametric cells covered by a cuticle; hypodermic collenchyma cords; secretory canals located both above and below the collateral vascular bundles, as well as within the fundamental tissue. The mesophyll is characteristic to the dorsiventral leaf, with a palisade parenchyma (columnar cells in two layers) located on the adaxial (upper) surface of the leaf, while spongy parenchyma on the abaxial (lower) side (**Fig. 5.**). Long trichomes were observed on the adaxial epidermis. The shape and the structure of the trichomes were in accordance with the description of Arora et al. (1982): simple, unicellular, and have 10-12 tiny and thick-walled crown cells surrounding them at the base.

According to Weryszko-Chmielewska and Chwil (2017), scanning electron microscopy examinations in case of *H. sosnowskyi* stem and leaves, showed that furanocoumarin crystals were present on the surface of the trichomes and other epidermal cells, but also in parenchyma cells. Additionally, they discovered that lipids, essential oil, polysaccharides, tannins, and furanocoumarins are present in these trichomes. The majority of furocoumarins are released by trichomes and epidermal cells. The release is concentrated in subepidermal cells and on the surface of trichomes. Additionally, the furanocoumarins are extruded from epidermal cells and go through the cuticular layer, forming crystals on the epidermis. Strong fluorescence from the trichomes, which are most prevalent on the abaxial leaf surface, suggests that furanocoumarins are present in their secretions (Weryszko-Chmielewska and Chwil, 2014; Bruni et al., 2019).

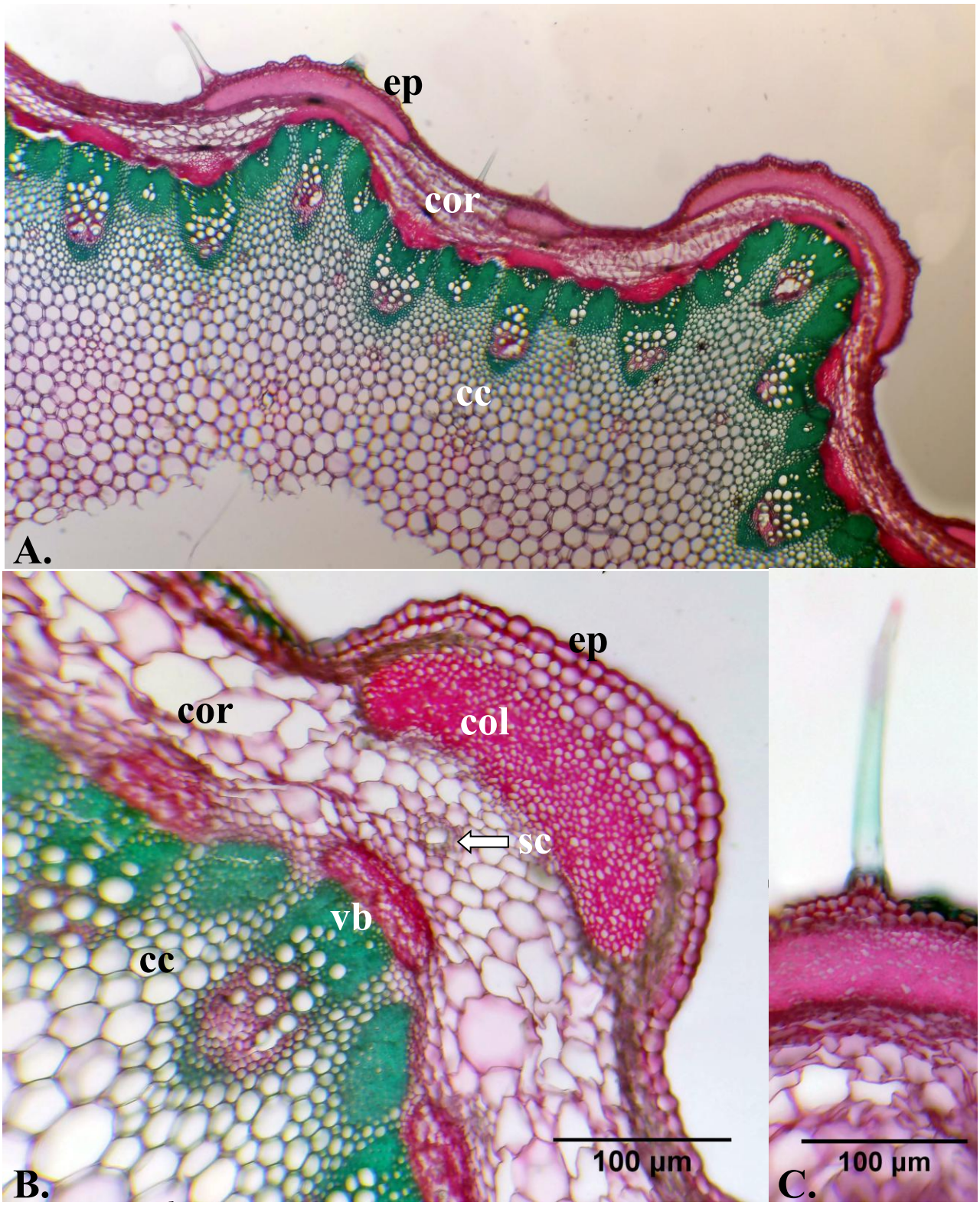

Fig. 1. The stem structure of *Heracleum sphondylium* ssp. *sphondylium*: A. general view; B. the epidermis, the cortex, and the vascular bundles from the central cylinder; C. unicellular trichome on the stem epidermis surrounded by crown cells

Abbreviations: ep-epidermis; **cor**-cortex; **cc**-central cylinder; **col**-collenchyma; **sc**-secretory canal; **vb**-vascular bundle (photographs by Erzsébet Domokos).

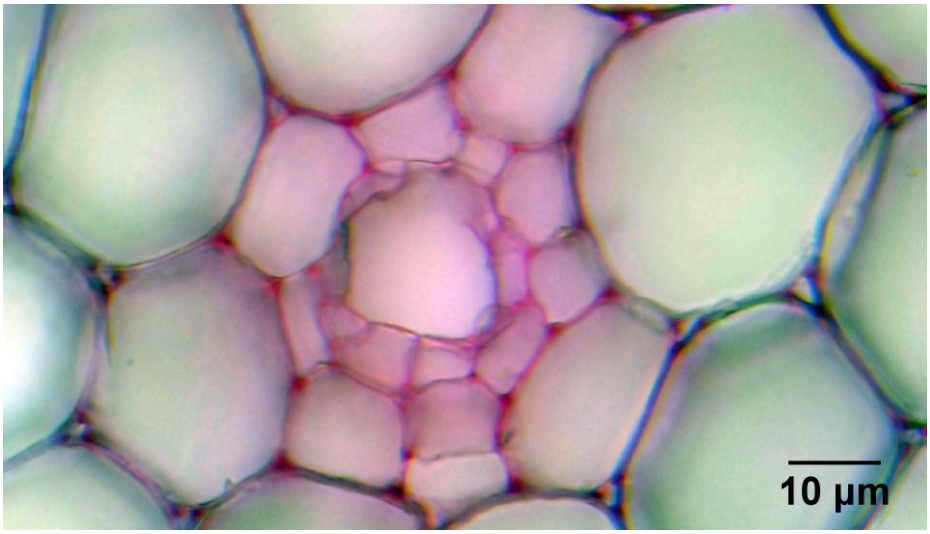

Fig. 3. Secretory canal with epithelial cells from the stem's central cylinder in case of *Heracleum sphondylium* ssp. *sphondylium* (photograph by Erzsébet Domokos).

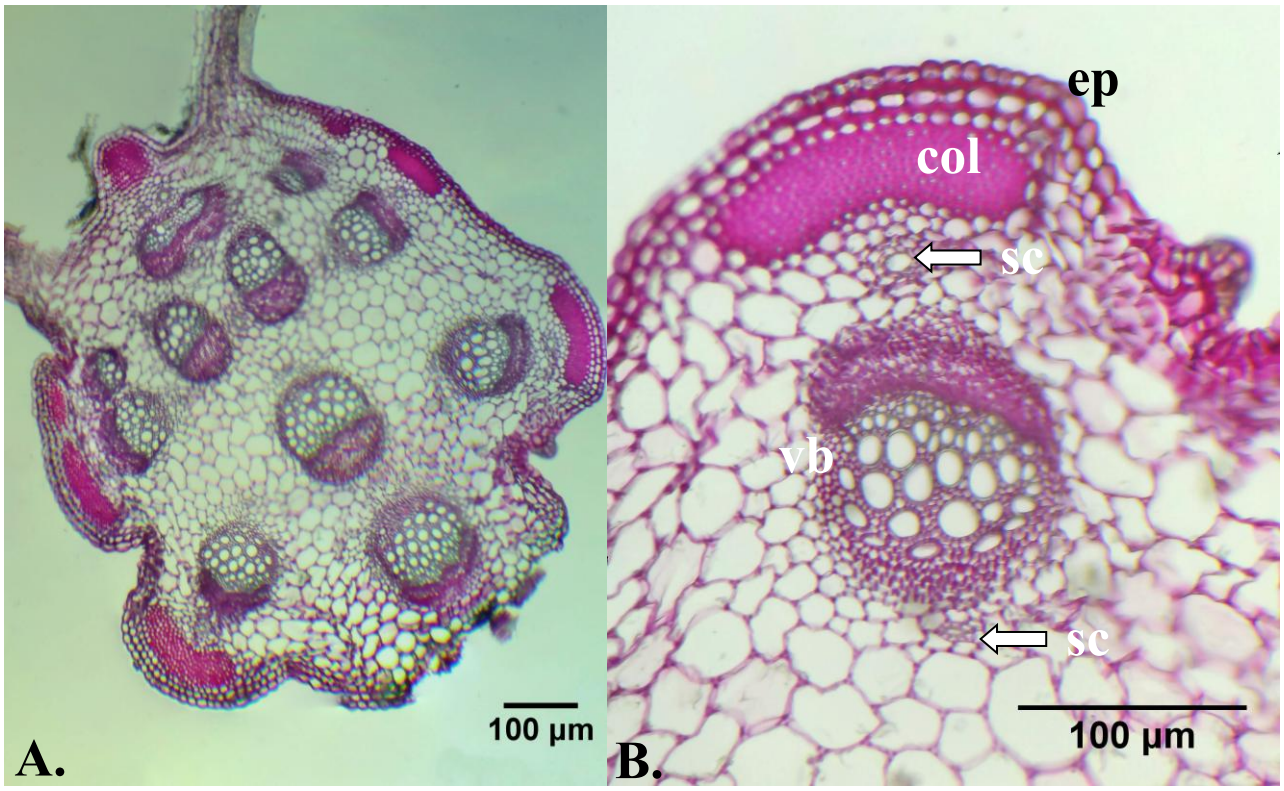

Fig. 4. Leaf cross-section at midrib in case of *Heracleum sphondylium* ssp. *sphondylium*: A. general view; B. secretory canals near the vascular bundle.

Abbreviations: ep-epidermis; **col**-collenchyma; **sc**-secretory canal; **vb**-vascular bundle (photographs by Erzsébet Domokos).

Fig. 5. Dorsiventral leaf structure of *Heracleum sphondylium* ssp. *sphondylium* with a large unicellular trichome on the lower epidermis (photograph by Erzsébet Domokos).

Conclusions

This study provides the first detailed histo-anatomical description of the root, stem, and leaf of *Heracleum sphondylium* ssp. *sphondylium*. It was found that secretory canals are present in the cortex and secondary phloem of the root, in the cortex and parenchymal tissues of central cylinder from the stem, while in the leaf these secretory structures are located near the vascular bundles and in the fundamental parenchyma. These secretory tissues, along with the trichomes on the stem and leaf surfaces, likely play an important role in the production and secretion of furanocoumarins.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Acta Biologica Marisiensis

HISTORICAL EVOLUTION AND PRESERVATION OF THE LIPTHAY MANSION PARK IN LOVRIN

Noémi Melitta HEGEDÜS[1*]

[1]Department of Horticulture, Faculty of Technical and Human Sciences Târgu Mureș, Sapientia Hungarian University of Transylvania, Romania

*Correspondence:
Noémi Melitta HEGEDÜS
hegedusnoemi@yahoo.ro

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Abstract: The Lipthay Mansion in the village of Lovrin, once owned by the Lipthay noble family, is a representative example of architectural and landscape heritage in Banat, Romania. Within the broader regional context, where over 70% of historical landscapes have been significantly altered or completely destroyed during the political transformations of the 20th century, this site stands as an exceptional example of preservation and historical continuity. This study analyzes the evolution of the mansion and its park based on historical research, topographic maps, cadastral surveys, and direct observations of the current state of the estate. The research examines and documents how the site has maintained its spatial integrity and original boundaries despite significant political and social changes in the region, while most similar properties have been severely damaged or fragmented. The study identifies factors contributing to the preservation, including the estate's integration into the urban fabric of Lovrin. The findings contribute to broader understanding of heritage conservation practice in the Banat region and offer recommendations for preservation plans that respect historical authenticity while accommodating contemporary needs of the local community and strengthening local identity.

Keywords: historical garden research, landscape conservation planning, cultural heritage preservation, architectural restoration, Banat historical landscapes

1. Introduction

The historical gardens and parks of Banat represent a significant but increasingly threatened cultural heritage resource within Romania. According to comprehensive surveys conducted between 2010-2017, more than 70% of historical gardens in the region have been either significantly altered, fragmented, or completely destroyed during the political transformations of the 20th century (Ciobotă, 2012). Of the 40 documented historical and non-historical gardens in the region, only few maintained their original boundaries, and merely 3 preserved significant historical landscape elements (Harhoiu, 2015; Hegedüs, 2018). The Lipthay Mansion and its surrounding park in Lovrin stand out as an extraordinary example among these few preserved estates due to several distinctive characteristics: its complete retention of original spatial boundaries, preservation of key

architectural features including facade arrangements and portico, maintenance of historic vegetation patterns, and successful integration into the evolving urban context of Lovrin.

The Lipthay family, one of the oldest noble families in Liptó County (now Slovakia) with a lineage dating back to the 13th century, acquired the Lovrin estate in 1772. Under Frigyes Lipthay's leadership in the early 19th century, the mansion was significantly expanded and the surrounding landscape transformed according to the aristocratic design principles of the era (Lotreanu, 1935). This included the creation of a formal avenue in the western village part and the development of a sophisticated park with circulation patterns, vegetation arrangements, and spatial hierarchies that reflected contemporary landscape design practices. The estate thus offers a rare and intact example of the functional-spatial structure that characterized noble residences in 19th century Banat.

This research aims to accomplish two primary objectives:

1. To document and analyze how the Lipthay Mansion and park have maintained their spatial integrity and character-defining features despite political and social transformations that destroyed similar properties, identifying the specific factors that enabled this exceptional preservation
2. To assess the current condition of architectural and landscape elements, developing a detailed inventory of preserved historical features that can inform conservation decisions

These objectives address a critical gap in heritage conservation practice in Romania, where there are few successful models for integrating historical properties into contemporary contexts without compromising their integrity. By examining the Lipthay estate's remarkable continuity—evident in historical maps from the Josephine Topographic Survey (1769-1772) through present-day cadastral records—this research provides valuable insights for planners, designers, and conservation specialists working in similar contexts.

Unlike properties such as the Csekonics estate in Zsombolya, the severely altered Mocioni Mansion in Foeni, or the fragmented Ambrózy estate in Timișoara (Hegedüs, 2018; Kovács, 2018), the Lipthay estate offers a rare opportunity to study the initial functional-spatial organization of a noble residence in its near-complete form. The study systematically analyzes circulation patterns, vegetation structures, spatial hierarchies, and the relationship between built and natural elements that characterized the original design. This analysis reveals sophisticated design principles that can inform contemporary landscape architecture practice in the region, particularly for projects involving historical properties.

The findings of this research have direct applications for current planning and design practice in Lovrin and throughout the Banat region. By demonstrating how the Lipthay estate's integration within Lovrin's urban fabric has contributed to its preservation, this research provides valuable lessons for community planners seeking to incorporate heritage resources into contemporary development while strengthening local identity and cultural continuity.

Through this multifaceted analysis of the Lipthay Mansion and park, this study aims to contribute not only to heritage conservation scholarship but also to active planning and design practice that thoughtfully balances preservation and adaptation in historical landscapes.

2. Materials and methods

This study employs both historical analytical methods and conservation planning approaches within the framework of cultural landscape conservation as defined by the European Landscape Convention (European Council, 2000). Data collection combined historical document analysis of archival sources documenting ownership changes and interventions, cartographic comparison of maps from 1769-2024 to identify spatial evolution, field observation documenting current architectural and vegetation conditions, comparative analysis with other Banat gardens, and stakeholder consultation. Building on historical analysis, a values-based conservation approach (Ciobotă, 2012) was applied to identify character-defining features, assess significance, evaluate conditions, and develop treatment recommendations based on international conservation principles.

3. Results and discussion

Geographical and Historical Context

Lovrin is located in Timiș County, Romania, along national road DN6. The Lipthay Mansion sits in the village center with frontage on both the national road and a park behind the mansion. This location has remained consistent throughout history according to historical maps (**Fig. 1.**).

From Lovrin's first documentary mention in 1466 until the Lipthay family's acquisition in 1792, the village experienced tumultuous history including invasions and repopulations. In 1777, the family received the estates from Emperor Leopold II, and in 1817, Frigyes Lipthay began improvements, creating an avenue in the western village part and developing the mansion's park comparable to Timișoara parks (Borovszky, 1911; Lotreanu, 1935; Munteanu, 1998).

Fig. 1. Ortofotography of Lovrin (2024), showing the Lipthay Mansion complex boundaries (white contour). The preservation of original dimensions is rare among regional historical estates, particularly given the widespread subdivision during post-communist era.

Spatial Evolution and Urban Context

The village of Lovrin developed along a west-east direction following the national road. The mansion's location near the center, towards the eastern side, opens onto both the national road and a park extending to a parallel street. The mansion is bordered by the Roman Catholic Church to the west and villagers' houses to the east.

This strategic position has remained unchanged through documented periods of settlement development, beginning with the second military topographic survey of the Habsburg Empire. The site has a regular rectangular shape distinguishable in older representations from the Franciscan Topographic Map onward (**Fig. 2. A, B**). This spatial continuity is significant as it demonstrates how the estate's integration into the urban fabric has facilitated preservation.

The site has constant flat topography with no significant slope, typical for the village. Two distinct landscape zones are identifiable: the front vegetal area serving as buffer between mansion and road (appearing only on later maps), and the rear park area currently populated with tall vegetation, denser toward the mansion's back façade and thinning toward the northern boundary (**Figures 3-4**).

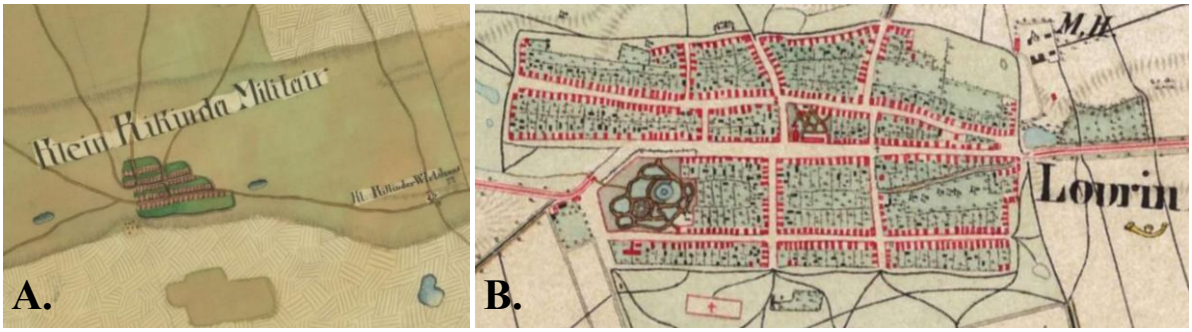

Fig. 2. A. The Josephine Topographic Map from 1769-1772 (source: hungaricana.hu), showing early settlement before mansion construction; **B.** The Second Topographic Survey (1806-1869) (source: hungaricana.hu), first clearly documenting the mansion and landscape with visible paths and vegetation patterns.

Fig. 3. A. The Franciscan Iozefinian Topographic Map of 1869-1887 (hungaricana.hu); **B.** The Kingdom of Hungary Map of 1869-1887 (hungaricana.hu)

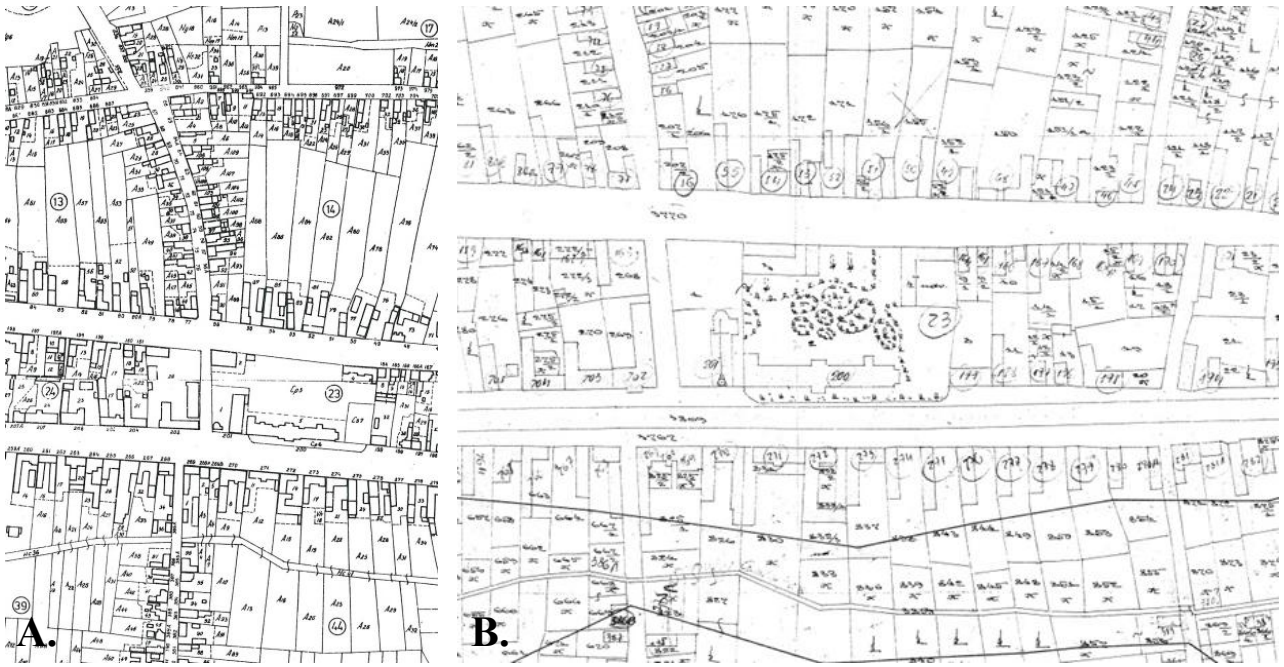

Fig. 4. Fragment of cadastral map from 1899 (The Office of Land Registry and Real Estate Advertising Timiș), providing detailed information about property boundaries (**A.**), vegetation patterns, and built structures that have informed our understanding of the estate's historical organization and subsequent preservation (**B.**).

Functional Zoning and Spatial Organization

The second military topographic survey of the Austrian Empire signals both the functional zoning of the site and the presence of major constructed and landscaped elements on the site. On the southern side of the site is the mansion, while the rear and lateral areas contain auxiliary buildings serving complementary functions necessary for the noble estate.

Regarding the arrangement of major elements on the site, the cadastral map of 1899 shows changes in the rear area of the estate compared to earlier topographic maps. The representative alleys depicted in the topographic maps disappear, and the cadastral map instead shows areas with dense vegetation arranged in a circular pattern, suggesting intentional landscape design with tall vegetation and "alleys" or paths that were planned and can still be identified today. This evolution reflects changing landscape tastes and functions, yet remarkably, the basic spatial organization has been maintained (**Figures 5-6**).

At present, while the original functional zoning is no longer strictly observed, the spatial organization remains remarkably intact. The mansion has been currently renovated, with efforts made to respect its historical character.

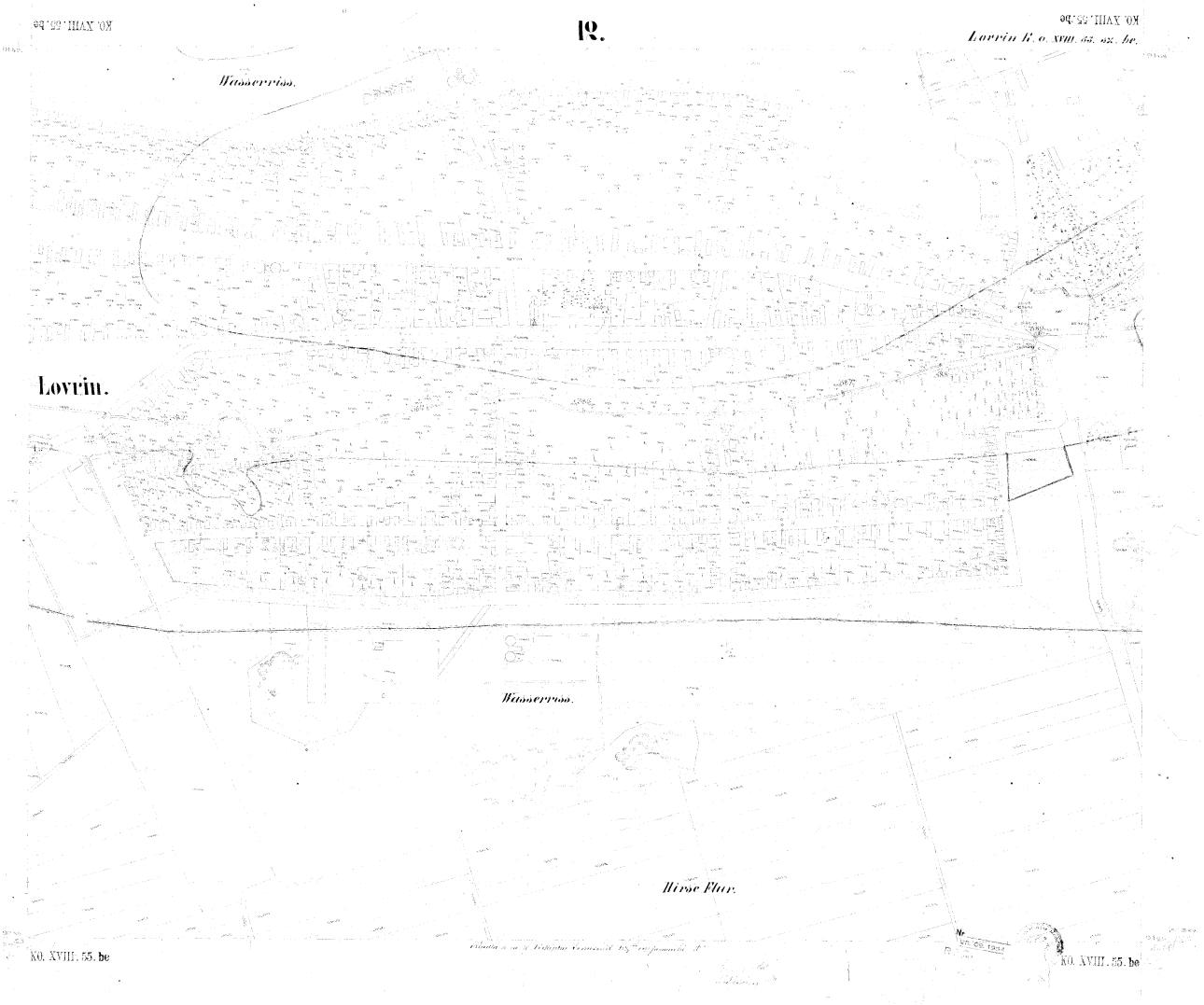

Fig. 5. Fragment of cadastral map from 23.09.1954 (The Office of Land Registry and Real Estate Advertising Timiș) documenting the estate during the early communist period, a time when many similar properties were significantly altered. The retention of the original boundaries during this period was crucial for the site's long-term preservation.

Fig. 6. Fragment of cadastral map from 1988, Scale 1:2000 (The Office of Land Registry and Real Estate Advertising Timiș), showing minimal changes to the estate's organization in the late 20th century, further supporting the site's value as an example of exceptional preservation in a period of rapid change.

Access Points and Circulation Patterns

Access to the site is from the eastern side of the estate, specifically from the right side of the mansion. Currently, access from the left side of the mansion is not possible, as the mansion is adjacent to the property boundary, next to the Roman Catholic Church.

It is assumed that this property boundary, which separates the mansion from the church, did not exist when the mansion was originally built, so access could have been possible in a circular fashion from both sides of the mansion. This assumption is supported by the second topographic survey, where the position and rhythm of the alleys seem to correlate with the location of the church and the space that existed inbetween, approximately identical in width to the space on the right side.

On the other hand, the existence of a possible access from the north, serving the auxiliary buildings and staff, is not excluded. Currently, such access exists in the form of a pathway linking the northern side of the site to the mansion's park. It is uncertain whether this secondary access represents the remains of a former arrangement, as there are constructions shown on the rear side of the site in the second and third topographic surveys, which later disappear from the cadastral maps of 1899. These changes in circulation patterns reflect the evolving functional needs of the estate over time, while still maintaining key historical pathways.

The park's alleys are still visible today. One can observe the main pedestrian and vehicular paths leading to the mansion's access, located on the rear façade, oriented toward the park and characteristic of the period in which the mansion was built. Access to the mansion can also be made from the main street, as the building has an entrance on the main façade, but the fence in front of the building prevents direct access from the street. This dual access system is a significant preserved feature that

illustrates historical approaches to estate planning and social hierarchy.

Architectural Features and Preservation

Both the second and third military topographic surveys, as well as the map of the Kingdom of Hungary, show the mansion with its rectangular plan, without any changes to the layout over time. The mansion has not undergone additions or extensions, thus maintaining its original form both in terms of its floor plan and volume. This architectural continuity is exceptional in the region and contributes significantly to the site's heritage value.

Regarding the façades and openings, the alternation between solid and void is rhythmically executed, with equal intensity toward both the national road and the park, although the significant visual opening, intentionally designed, was oriented toward the park (**Figures 7-10.**). The configuration of the openings suggests the existence of direct connections between the interior and exterior through doors, as well as visual connections facilitated by large windows oriented toward both the park and the public space, toward the main street that passes in front of the building. These design elements demonstrate sophisticated architectural principles that integrated the building with its surrounding landscape.

Fig. 7. The main facade of the Lipthay mansion in Lovrin (2016). This photograph documents the condition before recent renovation efforts, showing the classical architectural elements and the symmetrical organization of openings that have been preserved throughout the building's history.

Fig. 8. The main facade of the Lipthay mansion in Lovrin (2023), after renovation. The preservation of original architectural details during renovation work demonstrates increasing recognition of the site's heritage value and the implementation of appropriate conservation approaches.

Fig. 9. The rear facade with main access to inner courtyard (author's photo, **A.**) compared with 1903 postcard (**B.**), illustrating remarkable preservation of architectural features over more than a century

Fig. 10. Secondary access on the rear side of the property (**A.**) and the organization of existing pathways (**B.**) (the author's photos)

Conservation Significance and Planning Recommendations

The historical gardens of Banat have suffered extensively during political transformations. During the study of heritage-protected and non-heritage gardens in the Banat region, the Lovrin garden presents a unique case where no drastic area reduction or significant landscape modification occurred over time. Within this context, the Lipthay Mansion park represents an exceptional preservation case.

Unlike the nearby destructurated estates, the Lipthay Mansion and park offer a rare opportunity to understand design principles of 19th century noble estates (Kovács, 2018). The site remains one of the few gardens in the region that has not undergone original area changes, appearing identical in size to its original form.

Analyzing the garden, the current condition assessment identified conservation issues witch, are requiring attention: monumental chestnut trees showing disease signs requiring arboricultural care, historical pathways suffering erosion, historical viewsheds obscured by unmanaged vegetation growth, and lack of interpretative elements communicating historical significance.

Based on site characteristics and regional context, recommended conservation approaches include: a preservation zone for immediate mansion surroundings, a rehabilitation zone for the main park area

preserving character-defining features while allowing compatible community uses, and a restoration zone for specific elements like the historical pathway system.

Implementation strategies include enhancing the site's community role through educational programming, developing a cyclical maintenance program, formalizing boundary protection, and prioritizing interventions beginning with the most vulnerable elements.

The climbing plants have disappeared from the mansion's walls, but decorative low vegetation can still be observed along the alley parallel to the mansion's northern side. These detailed elements of the landscape design reveal a sophisticated approach to integrating architecture and plantings that deserves recognition in conservation efforts. The historical photographs (**Fig. 11**) provide valuable documentation of the estate's condition during the mid-20th century, revealing both preserved elements and changes that occurred during the communist period, offering important insights for contemporary conservation approaches.

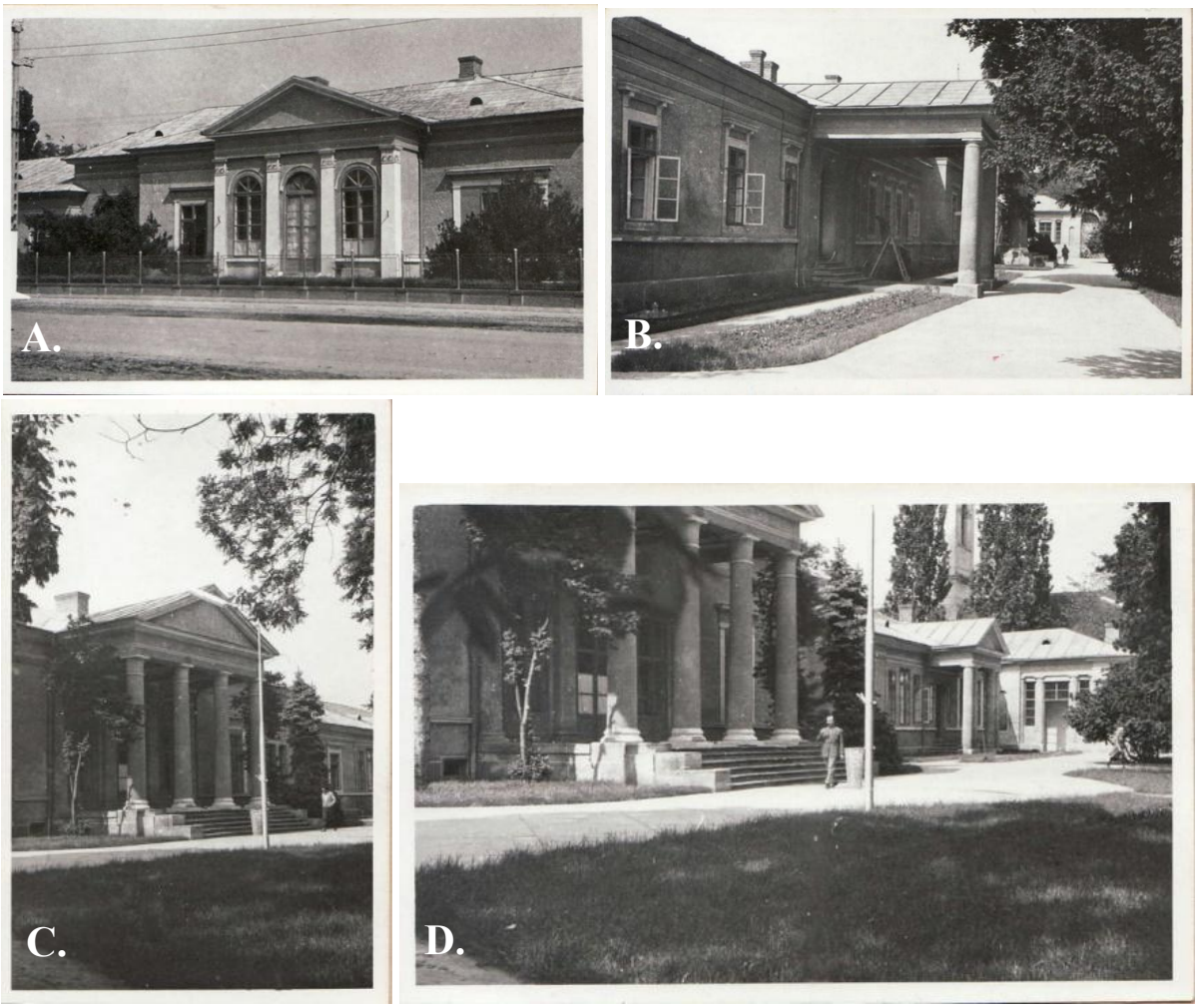

Fig. 11. Vintage images of the main facade and rear facade from 1965 (Photos: Velescu Oliver)

The cadastral map from 1899, as well as the map from 1954, do not make exact references to the arrangement of the rear park, but they do indicate it graphically as a distinct area. This consistent documentation of the park as a defined space has likely contributed to maintaining its boundaries over time, unlike

many similar properties that were subdivided or repurposed.

On the site, a few monumental chestnut trees can be observed. These chestnuts may be of considerable age, but their exact age cannot be determined at this stage of the research. These mature specimens represent living botanical heritage and provide continuity with the site's historical character, warranting special protection in conservation efforts.

In terms of ornamental landscape objects, no specific historical items or park furniture have been identified. However, it is worth mentioning the memorial stone of Horia N. Groza. This stone is located on the right side of the site, in immediate proximity to the mansion, near the main access to the estate. Its position is designed so that it remains visible from the main road. The addition of this commemorative element represents a later layer of cultural significance that has been integrated into the historical landscape without compromising its integrity.

Regional Context and Comparative Significance

The historical gardens of Banat have suffered extensively during the political transformations of the 20th century. The historical gardens in the region were documented (Hegedüs, 2018), of which only few maintained their original boundaries, and merely 3 preserved significant historical landscape elements (Hegedüs, 2018; Harhoiu, 2015). Within this context, the Lipthay Mansion park represents an exceptional case of preservation, maintaining both its original spatial boundaries and key design features despite the tumultuous history of the region.

The Lipthay Mansion and its park offer a rare opportunity to understand the design principles, spatial relationships, and planting strategies that characterized noble estates in 19th century Banat (Hegedüs, 2018; Kovács, 2018). This exceptional state of preservation elevates the site's significance beyond its architectural merits to a position of regional importance for landscape heritage.

Current Condition Assessment

The Lipthay Mansion park remains one of the few gardens in the region that has not undergone changes in the size of its original area. It appears identical in size to its original form today. This spatial continuity also holds true for the built environment, as no significant modifications have been observed over time. The initial image of the historic garden is still discernible in the current arrangement, which shows visible traces of historical design in terms of vegetation patterns and pathway organization.

However, the current condition assessment identified several conservation issues that require attention. The monumental chestnut trees show signs of disease and require professional arboricultural care. Historical circulation routes are visible but suffering from erosion and lack of proper drainage. Some historical viewsheds between the mansion and key landscape features have become obscured by unmanaged vegetation growth. Additionally, the site lacks educational components that would communicate its historical significance to visitors.

Conservation Planning Framework

The conservation approaches recommended based on the site's specific characteristics and regional context include a preservation zone for the immediate surroundings of the mansion, including the formal entrance area and portico connection to the park, which should be subject to the highest level of preservation, maintaining all original materials and spatial relationships. A rehabilitation zone for the main park area that would benefit from a rehabilitation approach

that preserves character-defining features while allowing compatible uses that support community engagement, and a restoration zone where specific elements, such as the historical pathway system and selected planting arrangements documented in historical photographs, should be considered for accurate restoration based on historical evidence.

Conclusions

The Lipthay Mansion in Lovrin represents a remarkable example of the preservation of a historical estate in Banat, Romania, that has remained nearly unchanged over the decades despite profound social and political transformations that have devastated similar sites throughout the region. This exceptional case of continuity offers both scholarly insights into 19th century landscape design practices and practical lessons for contemporary conservation planning in Central Europe.

The research demonstrates that the Lipthay estate's significance extends beyond its architectural merits to encompass historical value as a tangible connection to the aristocratic landscape tradition of Banat and the cultural influence of the Lipthay family, design value as an example of integrated mansion-park design principles characteristic of 19th century landscape architecture, rarity value as one of very few intact historical gardens remaining in the region, and educational value as a resource for understanding historical spatial planning, plant selection, and construction techniques.

This research has documented several key factors that have contributed to the site's exceptional preservation, including the estate's consistent boundaries and spatial organization from the 19th century to the present, the retention of key architectural features, particularly the facade arrangements and portico, the preservation of major landscape elements, including mature trees and historical pathway configurations, and the site's integration within the urban fabric of Lovrin, which has provided context and protection.

Compared to other similar estates in the region that have undergone fragmentation, inappropriate modifications, or complete abandonment, the Lipthay Mansion offers an important case study in heritage continuity. Its preservation provides valuable insights for conservation strategies for other historical landscapes in Romania.

This study demonstrates the critical need for an integrated approach to historical landscape conservation that combines rigorous historical research to understand the site's evolution and significance, technical condition assessment to identify conservation priorities, stakeholder engagement to ensure community support and sustainable management, and appropriate design interventions that respect historical authenticity while accommodating contemporary needs.

The conservation planning recommendations presented in this study offer a framework not only for the preservation of the Lipthay Mansion and park but also a methodological approach applicable to other threatened historical landscapes in the region. By implementing these recommendations, the site can serve as a model of heritage conservation that balances preservation with community engagement.

The Lipthay Mansion and park represent not only architectural and landscape heritage, but also embody cultural memory and regional identity that deserve protection for future generations. Their preservation and thoughtful integration into contemporary community life would contribute significantly to cultural continuity and place-based identity in a region that has experienced substantial loss of its built heritage.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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16. Photo archive of the National Institute of Heritage. Photo: Velescu Oliver (1965)

„George Emil Palade" University of Medicine, Pharmacy,
Science and Technology of Târgu Mureş
38 Gheorghe Marinescu Street, Târgu Mureş, 540139, ROMANIA
Telephone: +40-265-21 55 51; fax:+40-265-21 04 07

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