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BIOCHEMICAL AND PHYSIOLOGICAL MECHANISMS INVOLVED IN PLANT DEFENSE

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ABSTRACT

Plants, being autotrophic organisms fixed in the soil, cannot move to avoid stress factors. However, they have developed during evolution a series of effective means of defense, both against biotic factors and abiotic (drought, radiation, extreme temperatures).
Plants of the species Alnus glutinosa and Fraxinus excelsior were studied to observe how the hydrogen peroxide content changes under the influence of thermal stress (high temperatures). An increase in peroxide content was found at high temperatures, a greater difference compared to the control observed in Fraxinus excelsior, which is better adapted to high temperatures. The salicylic acid content determined in the species Salix alba and Filipendula ulmaria varied depending on the age of the plants and climatic conditions, being higher in young plants and organs and under conditions of thermal and water stress.

INTRODUCTION

Naturally, plants have a variety of biochemical and physiological mechanisms that allow them to recognize and respond to a wide range of biotic (pathogens, insects) or abiotic (drought, extreme temperatures, pollution, ultraviolet radiation) stress factors.

The study of these mechanisms has a particular scientific relevance, as it allows the identification of key processes involved in the natural resistance of plants, the discovery of possible biochemical stress markers, useful in monitoring plant health, the development of modern plant protection strategies, based on the stimulation of plant immunity (through biostimulators, resistance inducers, etc.). Finally, knowledge of these methods can be useful in the cultivation of food and ornamental plants, contributing to reducing dependence on synthetic phytosanitary products.

In addition, this topic has an interdisciplinary character, being at the intersection of plant physiology, biochemistry, biotechnology and plant protection, which makes it extremely current and relevant for contemporary research.

Therefore, the investigation of biochemical and physiological defense mechanisms contributes not only to the advancement of fundamental knowledge in plant biology, but also to their practical application - in genetic improvement, in integrated crop management and in the creation of more resilient and environmentally friendly cropping systems.

One of the most important defense barriers of the plant is the epidermis, covered on the outside by a protective layer called the cuticle.

The epidermis does not have chloroplasts, but contains specialized cells (stomata), which regulate gas exchange and can control transpiration depending on environmental conditions (Mauseth 2014).

They often present trichomes, hairs of different types, which act as a mechanical defense: tector trichomes which protect against water loss and reflect excessive light; glandular trichomes which secrete sticky or toxic substances, which can immobilize or repel insects (Fahn 1990).

Sclerenchyma and collenchyma tissues provide mechanical strength and help the plant defend itself from external forces (Raven et al. 2005).

Sclerenchyma has thickened and lignified cell walls, difficult to penetrate by insects or pathogens. Collenchyma, although more flexible, provides protection to young stems and leaves.

Some plants form specialized tissues around wounds or infected areas in response to the entry of pathogens: the formation of callus, a regenerative tissue that insulates the affected area; lignification or suberification of cell walls in areas of infection, to limit the spread of pathogens (Mauseth 2014).

Plants that grow in dry environments or in areas where there are many herbivores may have tough leaves, with spiny edges or heavily cutinized leaves, difficult to consume or digest. This is also a passive, morphological defense.

The morpho-anatomical defense mechanisms of plants reflect their remarkable evolutionary adaptations to varied environments and stress factors. Through modifications of the external and internal structure, plants manage to maintain their integrity and functionality, without the need for mobility. These mechanisms, together with the biochemical ones, form a complex and efficient protection system, essential for the survival of plant species (Ciobanu 2009).

Physiological defense methods are very complex and varied.

Salicylic acid is a key chemical in the activation of systemic acquired resistance (SAR). After a local infection, the concentration of salicylic acid increases, which causes the expression of PR (pathogenesis-related) genes, which help protect the entire plant against other pathogens (Walters 2015).

Ethylene and jasmonic acid are two substances involved in the response to insect and necrotrophic pathogen attack. They activate the synthesis of defensive proteins, such as proteinase inhibitors or antimicrobial compounds (Van Loon et al. 2006).

Phytoalexins are antimicrobial compounds synthesized de novo by plants in response to infection. Examples include resveratrol (in grapevines), capsidiol (in peppers), rishitin, and lubimin. These substances inhibit the growth of fungi and bacteria, thus contributing to plant immunity (Taiz et al. 2015).

Studies on the signaling pathways that control the induction of camalexin, the main phytoalexin in *Arabidopsis,* particularly highlight the complexity of these regulatory mechanisms, which in turn depend on the infecting pathogen (Ahuja et al. 2012).

During pathogen attack, plants rapidly produce reactive oxygen species, such as hydrogen peroxide, superoxide and hydroxyl radicals. These molecules play a role in destruction of pathogen cells, strengthening the cell wall by depositing lignin, and activating defense genes. This oxidative burst is one of the fastest physiological responses of the plant during an attack (Pieterse et al. 2012).

During interaction with pathogens, plants can alter the pH of the apoplastic (extracellular) space, creating an unfavorable environment for the development of pathogens. This mechanism is also associated with the activation of peroxidase enzymes (Walters 2015).

The hypersensitivity reaction involves programmed cell death around the site of infection to isolate the pathogen and prevent its spread. This reaction is rapid and involves complex physiological mechanisms, including signaling through reactive oxygen species and production of salicylic acid (Agrios 2005).

Plants can also produce substances that inhibit the digestive enzymes of insects or pathogens: proteinase inhibitors that block the activity of proteolytic enzymes in the insect gut, reducing protein digestion, and lectins, proteins that bind to carbohydrates on the surface of microbial cells, disrupting cellular processes (Pichersky & Gershenzon 2002).

Glucosides are essential components of the chemical defense system of plants, providing protection against a wide range of pests and pathogens. Their diversity and specificity allow plants to adapt and survive in varied and often hostile environments. The study and understanding of these compounds can contribute to the development of more effective and environmentally friendly plant protection strategies (Agrios 2005). They are often stored in inactive form and become toxic only when the plant is attacked (Walters 2015).

Alkaloids can act as defense compounds in plants, being efficient against pathogens and predators due to their toxicity. Fast perception of aggressors and unfavorable environmental conditions, followed by efficient and specific signal transduction for triggering alkaloid accumulation, are key steps in successful plant protection (Matsuura & Fett-Neto 2017).

Purine alkaloids also act as a natural insecticide and inhibit the germination of seeds of other species (allelopathy) (Taiz et al. 2015).

Quinoline alkaloids like quinine, found in *Cinchona officinalis* provides protection against insects (such as mosquitoes that transmit malaria) (Bruneton 1995).

Isoquinoline alkaloids (morphine, codeine, papaverine) and have a rol in antimicrobial and antiparasitic protection (Dewick 2002).

Tropane alkaloids (atropine, scopolamine) discourage consumption of the plant due to toxic (anticholinergic) effects (Evans 2009).

Isoprene is emitted by tree leaves (*Populus sp., Quercus sp*.) and plays an important role in protection against heat stress (Dudareva et al. 2013).

MATERIAL AND METHODS

The experiments used plants belonging to the species *Fraxinus excelsior* and *Alnus glutinosa*. The determinations were carried out in June in the morning at a temperature of 25 degrees Celsius and later at temperatures of 31 and 37 degrees Celsius. To observe how the hydrogen peroxide content varies, this compound was determined shortly after the leaves were detached from the plants.

The determination of H_2O_2 in leaves was performed by the colorimetric method with iodine (Zhou et al. 2016).

Hydrogen peroxide present in plant tissue oxidizes potassium iodide (KI) to iodine (I_2). The amount of iodine formed can be measured titrimetrically with sodium thiosulfate ($Na_2S_2O_3$).

Weigh 1–2 g of fresh leaves. Grind them well with a little cold distilled water to prevent the degradation of H_2O_2. Filter to obtain a clear extract. Add a few milliliters of 1–5% KI solution to the extract, then add acetic acid to maintain the acidic environment. H_2O_2 will oxidize the iodide to iodine, resulting in a yellow-brown color. The absorbance at 390– nm (yellow-brown color of iodine) is measured spectrophotometrically and compared with a calibration graph obtained with standard H_2O_2 solutions. H_2O_2 is very reactive and unstable, so these values are indicative and represent the "instantaneous" concentration measured immediately after collection.

The salicylic acid content was determined in the species *Salix alba* and *Filipendula ulmaria.*

Most of the active salicylic acid comes from the hydrolysis of salicin, so the free salicylic acid values are low compared to salicin.

A classic application for determining the amount of salicylic acid in plant branches is colorimetric spectrophotometry, using the reaction with $FeCl_3$. Young branches or green leaves are harvested. Small pieces are cut and dried at low temperatures (40–50 °C) to avoid degradation of salicylic acid. The dried sample is ground into a fine powder. Weigh a precise amount of powder (1 g). Add 80% ethanol in a volume of 10–20 mL. Leave to macerate for 24 hours, then filter the solution to remove solid residues. Take a fixed volume of the extract (1 mL).

After adding 1 % $FeCl_3$, it is observed that salicylic acid forms a colored complex (violet or blue green) with Fe^{3+}. The absorbance of the solution is measured at λ = 540 nm using a spectrophotometer. A calibration curve is then prepared with standard solutions of salicylic acid of known concentrations. To calculate the concentration, the absorbance of the sample is compared with the standard curve to determine the concentration of salicylic acid in the extract. This is related to the dry weight of the sample to obtain the amount of salicylic acid in mg/g.

RESULTS AND DISCUSSIONS

Hydrogen peroxide content

Determination of the hydrogen peroxide content in the species *Fraxinus excelsior* and *Alnus glutinosa* indicated significant variations throughout the day, depending on the air temperature. Thus, if in *Fraxinus excelsior* at a temperature of 25 degrees Celsius the content was 8 μmol/g, at 37 degrees Celsius it was almost 10 times higher, with a value of 76 μmol/g.

In *Alnus glutinosa*, the differences were smaller, with a content of 10 μmol/g in the morning and 34 μmol/g at 37 degrees (Graph 1).

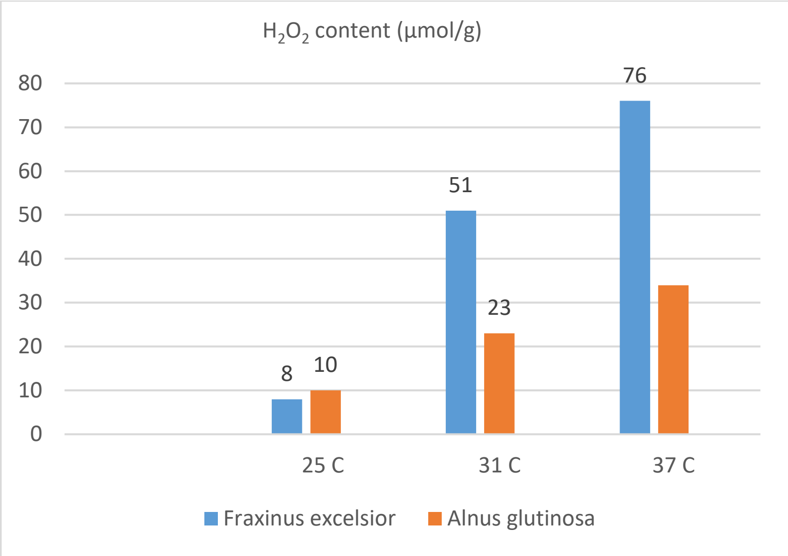
Fraxinus excelsior is much better adapted to drought and high temperature conditions, and it is very possible that the production of peroxide in larger quantities has an important factor in this resistance.

At the same temperature (31 degrees) young leaves of both species have a higher hydrogen peroxide content than mature leaves (Graph 2).

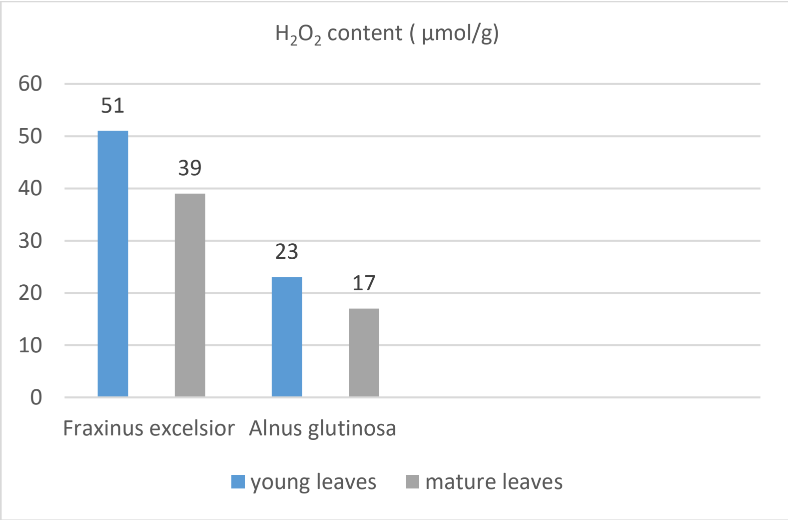
Salicylic acid content

Salicylic acid was determined both in the leaves and in branches of the two species, *Salix alba* and *Filipendula ulmaria* in two different periods: May and August.

Among the plant organs, the branches have a higher level of salicylic acid, with the highest value in *Salix alba* (0.18 g/100 g dry matter) (Graph 3).



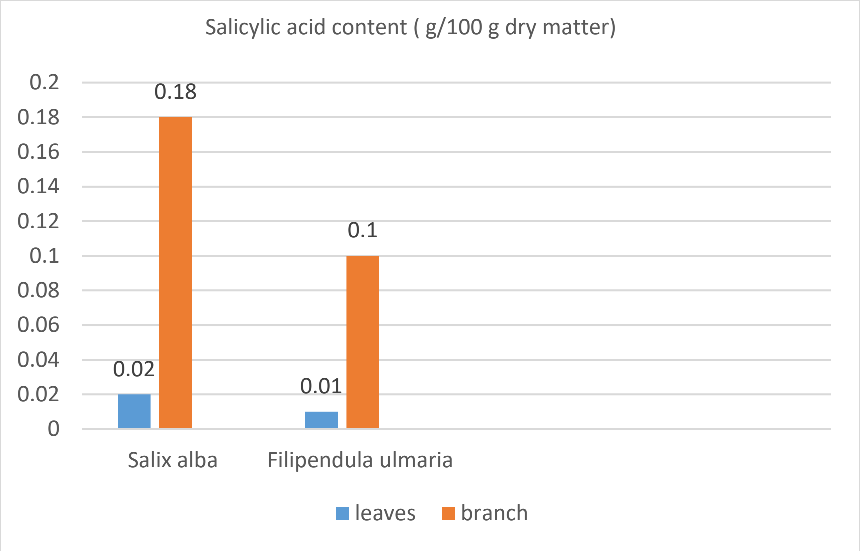
Graph 1. Hydrogen peroxide content of *Fraxinus excelsior* and *Alnus glutinosa* leaves at different temperatures



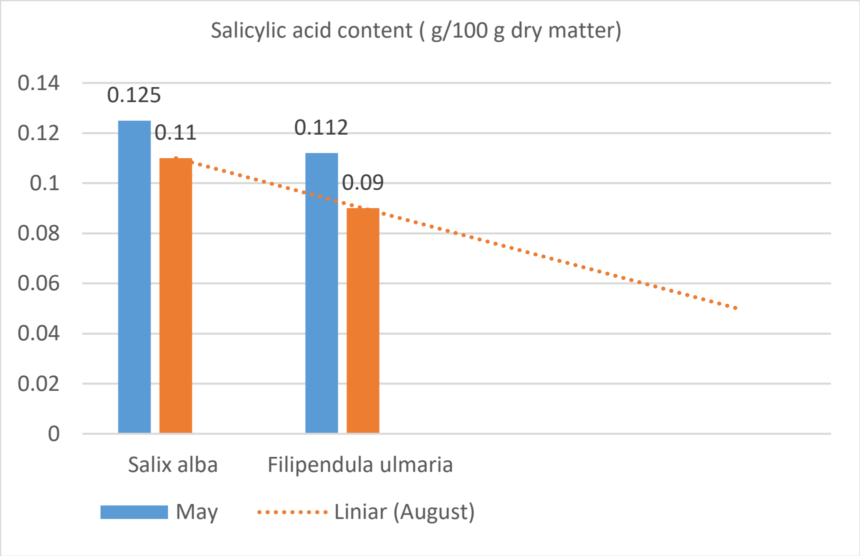
Graph 2. Hydrogen peroxide content in mature and young leaves of *Fraxinus excelsior* and *Alnus glutinosa*

From graphic 4 data it appears that this compound is found in both species in greater quantities during the transition period from spring to summer and decreases towards the end of summer.

The determinations carried out on mature and young branches indicated a higher content in the organs that are in the growth stage (Graph 5).

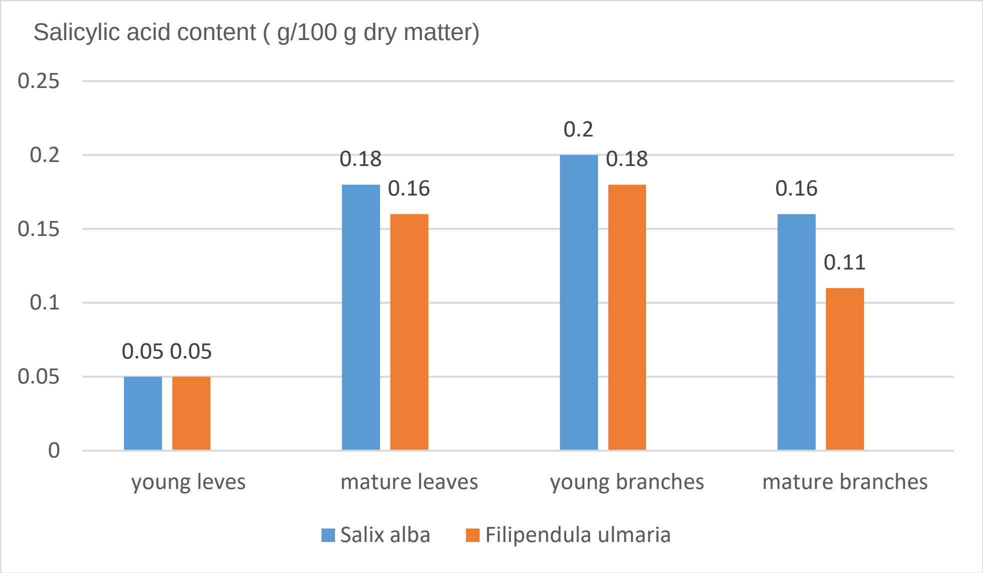


Graph 3. Salicylic acid content in leaves and branches of *Salix alba* and *Filipendula ulmaria*



Graph 4. Salicylic acid content in *Salix alba* and *Filipendula ulmaria* in May and August

Given the multifaceted roles of salicylic acid in plant responses to biotic and abiotic stresses, the underexplored diversity in salicylic acid biosynthesis and signaling presents a challenge to translational research for crop improvement in the face of climate change (Ullah et al. 2023).



Graph 5. Salicylic acid content in young and mature organs of the species *Salix alba* and *Filipendula ulmaria*

Recent comparative analyses measuring phenolic/salicylate compounds in different species/clones report large differences: for example, analyses on related species have found free salicylic acid at levels of the order of 0.1 mg/g for some species (values for *Salix alba* may be similar or lower), and salicin usually much more abundant (mg/g in leaves/bark). These studies highlight the strong variability between species and clones (Zombori et al. 2025).

Most recent work shows that free salicylic acid occurs in relatively small amounts in plant material; instead, there may be salts/hexosides or precursors (salicin, methyl salicylate) that can generate salicylic acid through chemical or metabolic processes. Many analyses report the total salicylate content rather than a fixed number of mg/g for free salicylic acid (Stawarczyk et al. 2021).

CONCLUSIONS

Hydrogen peroxide and salicylic acid are key components of the plant defense system, acting both locally and systemically. They participate in increasing plant resistance to abiotic stress.

Large amounts of salicylic acid are found in the species *Salix alba* and *Filipendula ulmaria.*

Young plant organs (branches, leaves) accumulate larger amounts of salicylic acid mainly in spring and early summer.

Hydrogen peroxide is synthesized in large quantities during periods of stress and shows higher values in young plant branches.

Fraxinus excelsior produces higher amounts of hydrogen peroxide under heat stress conditions and is better adapted to these conditions.

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