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THE USE OF HYDROGEN PEROXIDE AND SILVER NANOPARTICLES IN HORTICULTURE

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ABSTRACT

Both hydrogen peroxide and silver can oxidize organic and inorganic molecules, and this causes them to affect the metabolism of living organisms in many ways. The paper presents examples of the impact of H_2O_2 and silver on stimulating plant growth and development and increasing plants' resistance to biotic and abiotic stresses. The most underlined proposal for application in horticulture is the control of microorganisms during cultivating and storing vegetables, fruits, and flowers, aiming to replace synthetic pesticides. Preparations containing H_2O_2, silver, or both components can be widely used in horticulture for plant protection, in the form of spraying and soaking seedlings, to protect them during the time of storage, for disinfecting tubers, bulbs, and rhizomes before planting, for fogging potatoes and root vegetables during storage, for quick healing of wounds on the roots and aboveground parts of plants, after cutting and in the case of frost damage and injuries caused by winds, for disinfecting seeds, and, as stimulants of plant development and inducers of resistance to biotic and abiotic stresses. However, their practical use depends on obtaining the legislator's consent for their broader use in horticultural production.

Key words: hydrogen peroxide, silver nanoparticles, plant protection, control of microorganisms, plant stimulants, resistance induction

INTRODUCTION

The consistently increasing concern for the quality of plant products and the production environment in agriculture and horticulture has led to the withdrawal of many pesticides from cultivation. Unconventional plant protection products that do not pollute the produce nor the environment or pollute to a much lesser extent are being sought. Among them, some hopes are associated with hydrogen peroxide stabilized with silver, known in Poland as Huwa San TR-50 and Bisteran preparations. Huwa San TR-50, containing 50% hydrogen peroxide and 0.036% colloid silver, was approved for use in Poland by the Minister of Health No. 4236/10 of 2010 as a product for surface disinfection and by the decision of the Minister of Agriculture and Rural Development No. S-253e/17 of 2017 as a plant growth stimulant and for quick healing of plant wounds. Both components, hydrogen peroxide and silver, can negatively affect microorganisms. However, when combined in a formulation, silver is most often considered a factor stabilizing hydrogen peroxide, slowing down its disintegration, thus extending the durability of the preparation. The mechanism of their impact will be discussed separately below.

CHARACTERISTICS OF HYDROGEN PEROXIDE AND ITS INCREASING IMPORTANCE IN THE GLOBAL ECONOMY

Hydrogen peroxide (H_2O_2), identified in 1818 by Louis Thénard (after Linley et al. 2012), is an inorganic chemical substance whose molecule differs from water by an additional oxygen atom. Oxygen is quickly released, oxidizing organic and inorganic molecules and participating in many metabolic changes in the cells of living organisms. H_2O_2 is easily soluble in water. At an average room temperature,

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it forms a thick, colorless liquid, gradually changing color to blue with increasing concentration. Hydrogen peroxide is more stable in acidic and neutral environments than in alkaline. Thanks to its properties, H_2O_2 has several applications in medicine, cosmetics, food, chemistry, paper, and textile industries and is commonly used to disinfect water (Linley et al. 2012). It is considered a "green industrial oxidant" because it leaves no harmful residues and quickly breaks into water and oxygen molecules. The properties of H_2O_2 have resulted in a growing demand for this compound, and its consumption has significantly increased in the last three decades (Ciriminna et al. 2016). Hydrogen peroxide is ubiquitous in the Earth's atmosphere, human and animal organisms, and plants. In plants, it is produced as a byproduct of photosynthesis, photorespiration, fatty acid oxidation, and electron transport in mitochondria (Kuźniak & Urbanek 2000). The interest in using this compound in agriculture is related to its direct effects in controlling harmful microorganisms, modifying the internal resistance of plants to biotic and abiotic stresses, and impacting plant growth. The oxidizing effect of hydrogen peroxide is widely used in the disinfection of water, municipal and industrial wastewater, and in medicine to disinfect damage to the skin, surfaces, rooms (usually in volatile form), and medical equipment, replacing chlorine compounds and formalin. In plant production, H_2O_2 is proposed as a compound to control viral, bacterial, and fungal pathogens and plant parasitic pests, disinfect storage rooms, and protect plant products destined for storage (McDonnell 2014; Anonymous 2015). The liquid form affects easily accessible surfaces, while the gaseous form allows penetration into hard-to-reach places, e.g., in storage rooms.

The constant presence of hydrogen peroxide in the tissues of plants and animals prompted biologists to explain its role in the life of plants and microorganisms, which resulted in many experiments. For example, its participation in plant resistance to pathogens and response to mechanical damage and sudden temperature drops was studied by Juven and Pierson (1996), Orozco-Cárdenas et al. (2001), Baker and Orlandi (1995), and many others.

MECHANISM OF ACTION OF HYDROGEN PEROXIDE AND ITS EFFECT ON MICROORGANISMS AND PLANTS

Hydrogen peroxide plays a vital role in the life of plants because it is an essential participant in metabolism and processes related to plants' response to biotic and abiotic stresses. As a result of the responses associated with the activation of immune functions, there are, among others, reinforced cell walls, enhanced plant growth and development, production of growth regulators, and phytoalexins (Dat et al. 2000; Kuźniak & Urbanek 2000; Quan et al. 2008). In these responses, H_2O_2 is a transmitter of signals initiating or controlling metabolic processes (Cheeseman 2007; Ślesak et al. 2007; Khan et al. 2018). The essence of the molecule is its high reactivity in the cell environment, which results in the formation of reactive oxygen and hydroxyl groups, which are strong oxidants (Linley et al. 2012). The high reactivity of OH radicals in the biological environment causes a cascade formation of other reactive oxygen species (ROS), affecting the chemical transformations of several organic compounds; as a result, the role of H_2O_2 in plant life involves many processes in which it can be formed or broken down, being both a substrate and a signal carrier (Cheeseman 2007; Quan et al. 2008; Petrov & Van Breusegem 2012; Khan et al. 2018). These reactive oxygen compounds can be cytotoxic and genotoxic (Abdollahi & Hosseini 2014; Ahire & Mishra 2022). Too high concentration of reactive oxygen (ROS) leads to oxidative stress that harms plants. Plants have mechanisms to annul excess H_2O_2. Plant cells protect themselves against hydrogen peroxide through catalase activity and, in the event of DNA damage, through its rapid repair. The small dimensions of H_2O_2 particles enable easy penetration through the cell membranes of microorganisms, showing a biocidal effect against a broad spectrum of bacteria, fungi, and viruses, but also against harmful protein particles, such as prions (Żegliński 2006). At a concentration of 3%, this compound is known as hydrogen peroxide, which destroys naked cells, while at higher concentrations of 10–30%, called perhydrol, it also kills bacterial and fungal spores.

The biocidal mechanism consists in the fact that H_2O_2 penetrates cells and is decomposed thereby by protective enzymes, e.g., catalase or reducers, such as metal ions, causing the release of hydroxyl radicals affecting life-critical compounds. The main biocidal effect is lipid membranes' destruction and DNA strands' breaking. Imlay and Linn (1986) believe that H_2O_2 causes DNA damage at lower concentrations, but at higher concentrations, it also damages other cell structures, and the degree of injury is a function of exposure time. Brandi et al. (1991) showed that lower concentrations of this compound caused morphological changes in bacterial cells (e.g., filamentation) and higher cell shrinkage, which may result from damage to internal structures.

Considering the small number of agents approved for plant protection in organic farming and the complete harmlessness of hydrogen peroxide to humans and the environment because there are no harmful residues, only oxygen and water, preparations based on this compound have been introduced for practical use.

EXAMPLES OF THE USE OF HYDROGEN PEROXIDE IN HORTICULTURE

For the decontamination of water, soil, tools, and packaging

Using this compound in gaseous form is more effective than in liquid form, as it disinfects faster at low and high temperatures and, therefore, can be applied in lower concentrations than when administered in liquid form (McDonnell 2014). According to the cited author, using H_2O_2 does not risk harmful microorganisms becoming resistant to it because its action is nonspecific, unlike that of antibiotics. According to Nikkhah et al. (2010), hydrogen peroxide is one of the most commonly used disinfectants for fruit packaging. Linley et al. (2012) indicate the possibility of using the agent for disinfecting tools and water, including drinking water, and in the processing of agricultural products, for disinfecting rooms, greenhouses, plastic tunnels, and storage rooms. As one of the significant advantages of the agent, the authors indicate the safety of its use, the lack of unpleasant odor, and the possibility of using the disinfected rooms soon after treatment. In a concentration from 3 to 6% and the fogging time from 10 minutes to 10 hours, depending on the intensity of contamination and the variety of microorganisms, H_2O_2 can be used to disinfect garden substrates, seedlings, and the root system of fruit and ornamental shrubs and trees.

When decontaminating soil and reusing growth substrates, H_2O_2 decomposes pesticide and herbicide residues by oxidizing them (Newman 2004). Its effectiveness is limited in a high organic substrate. ZeroTol is recommended as a pesticide for use in organic crops and as a sterilizer for the disinfection of greenhouses and gardening equipment to protect plants against bacteria, fungi, and viruses (Miyasaki et al. 1986). Rapid wound healing has been observed using H_2O_2 as a fogging agent on plant material with new lesions. Vänninen and Koskula (1998) showed that H_2O_2 could be used as a spray on seedlings and older cucumber plants grown under mineral wool covers as an algicide against green algae, on which water moths (*Scatella stagnalis*) feed. Reciclean containing H_2O_2 reduced algae growth by 40–60%, thus reducing the number of water moths by 73–92%. The agent also minimized algae growth in the water and on the surface of other substrates, primarily when used in the initial stage of algal development.

According to Japhet et al. (2022), the constant addition of H_2O_2 to water in a closed circuit allows it to water plants, inhibiting clogging and biofilm formation in the irrigation system. Barta and Henderson (2000) proposed the use of H_2O_2 as an alternative to conventional bleach for disinfecting hydroponic plant growth systems. At a concentration of 0.5% it reduces the microbial population to almost zero, but this must be repeated during cultivation. Bosmans et al. (2016) showed that H_2O_2 can effectively reduce biofilm formation by rhizogenic bacteria, but its concentration should be adapted to the particular *Agrobacterium rhizogenes* strain. Parke and Fisher (2012), researching the possibilities to propagate plants with disinfecting water, indicate that, apart from chlorine and copper compounds, hydrogen peroxide is particularly effective when the *Pythium* and *Phytophthora* genera are present in the production environment. Stewart-Wade (2011) suggests the elimination of organic residues from the water used in plant production on which microorganisms live, which makes it possible to almost wholly avoid the development of potential pathogens. Fredrickson (2005) indicated that adding hydrogen peroxide to water used for watering plants removed chlorine and sulfur residues, components of pesticides, and fertilizers from the soil, and the released oxygen caused faster decomposition of dying roots, eliminating potential plant pathogens and algae growth. Consequently, such action of the agent affected the increase in yield and plant quality. The agent is also recommended for treating seeds, soaking or watering seedlings, bulbs, and tubers, and spraying crops in the field and under covers. In a study by El-Mougy et al. (2008), hydrogen peroxide was a helpful agent for postharvest protection of strawberry fruits against *Botrytis cinerea* and disinfection of water, surfaces, and tools.

In the protection of plants against diseases and pests

The first pesticides containing hydrogen peroxide were registered in 1977 (Anonymous 2015). According to the United States Environmental Protection Agency (EPA 2014) and the Canadian General Standards Board (CGSB 2011), by 2014, there were 167 hydrogen peroxide-based agents registered in the US. Currently, many scientific reports prove the effectiveness of H_2O_2 as a biocide.

The protection of plants against diseases and pests as a result of H_2O_2 application is associated not only with the direct attack and destruction of microorganisms by this compound but also with its impact on plant metabolism, which causes the plant to acquire defensive capabilities as a result of hypersensitivity responses and systemic acquired resistance (SAR). Applying H_2O_2 mimics the process generated by plants in response to pathogen attack by rapidly producing reactive oxygen, known as the “oxygen burst” (Lei et al. 2014). The cited authors observed that *Arabidopsis* cells attacked by aphids accumulated H_2O_2, leading to a hypersensitivity reaction and the cells’ death. Numerous papers, including Bestwick et al. (1997), showed the appearance of H_2O_2 at the sites of penetration by bacterial pathogens. Small H_2O_2 molecules, after penetrating cell membranes, quickly decompose into reactive oxygen and water upon contact with biological material and show a biocidal effect on a broad spectrum of bacteria, fungi, and viruses. Baatout et al. (2006) report that H_2O_2 increases the permeability of cell membranes, increasing with the concentration of the compound, and Qin et al. (2011) emphasize its negative impact on the mitochondria of fungal proteins.

In the scientific literature, it is possible to find both reports on experiments with H_2O_2 and recommendations for using it to fight pathogenic microorganisms and parasites. For example, hydrogen peroxide was found effective against several fungal pathogens, such as downy mildew (*Pseudoperonospora cubensis*) on cucurbits (Kuepper 2003), potato late blight (*Phytophthora infestans*) (Kuepper & Sullivan 2004), and apple scab (*Venturia inaequalis*) (Phillips 2005). Hydrogen peroxide has been recommended in organic tomato production (Diver et al. 1995). In addition, H_2O_2 was effective in controlling tomato wilt (*Ralstonia solanacearum*) (Hong et al. 2013), potato blackleg (*Dickeya solani*), pepper golden mosaic (PepGMV) (Mejía-Teniente et al. 2019), European stone fruit yellows (Musetti et al. 2005), the nematodes *Meloidogyne javaica* (Karajeh 2008); Jansen et al. 2002; Bolm et al. 2004), *Bactrocera* spp. flies on *Syzygium samarangense* (Khandaker et al. 2018). Hafez et al. (2020) reported that in an open-field experiment, H_2O_2 limited the infection of pear trees with *Erwinia amylovora*. Thakulla et al. (2022) studied the addition of hydrogen peroxide in the form of ZeroTol preparations (H_2O_2 + peracetic acid) and PERpose Plus (H_2O_2 + hydrogen dioxide) in hydroponic tomato cultivation. They found adverse effects of both preparations on the growth, dry and fresh weight of algae but a positive impact on tomato plants. The US organic farming guidelines allow the use of hydrogen peroxide-based disinfectants, fungicides, and algicides (EPA 2009).

Despite many reports on the positive effect of H_2O_2 on reducing plant diseases, there are doubts about whether this compound can be recommended for large-scale production. Miller (2006) did not recommend H_2O_2 as a biopesticide due to its short-term activity and assumed that this compound could be effectively used only for surface disinfection and water decontamination. However, its use against plant pathogens is risky, especially in field production.

As an inducer of plant resistance to pathogens and abiotic stress factors, in the stimulation of plant growth and development

H_2O_2 participates in plant metabolism in many ways, including activating plant genes related to resistance (Orozco-Cárdenas et al. 2001). An increased concentration of this compound was found in places of infection and damage, where it acts as a direct biocide and a factor accelerating wound healing (Orozco-Cárdenas et al. 2001). This process was observed within 4 hours of leaf tissue damage (Olson & Verner 1993). In addition, more active oxygen in the tissues stimulates metabolism and, depending on the conditions, acts as a transmitter of signals initiating various biochemical reactions. In this role, H_2O_2 activates the processes leading to damage regeneration, stimulating growth, and making plant organisms resistant to biotic and abiotic stresses. These activities are initiated by releasing oxygen, similar to an oxygen burst, which manifests the initiation of plant defense reactions in response to biotic and abiotic stress factors. For example, an increased oxygen concentration was observed in tomatoes at the inoculation site with the fungus *Cladosporium fulvum* (Borden & Higgins 2002). Chen et al. (1993) believe that salicylic acid, as an inducer of plant resistance to adverse environmental factors, limits the activity of catalase, which leads to the accumulation of hydrogen peroxide, thereby enhancing its effect. The consequence is the thickening of cell walls and the activation of genes that can induce systemic resistance of plants to pathogens. For example, H_2O_2 induced the resistance of damaged fruits to infection by *Penicillium* spp. (Janisiewicz et al. 2016). Increased amounts of H_2O_2 accumulated in places of mechanical damage to apples and citrus fruits resulted in injury healing (Macarisin et al. 2011). One of the factors inducing resistance is the stimulation of lignin synthesis, which is deposited in the cell walls of infected plants, preventing further pathogen spread (Ros Barceló 1998; Hückelhoven et al. 1999; Płażek 2011). A similar response was found by Martinez et al. (2000) in the case of cotton infection by *Xanthomonas campestris* pv. *malvacearum*. H_2O_2 stimulates wound healing (Ros Barceló 1998). External application of H_2O_2 in amounts not causing oxidative stress eliminated the toxic effects of stress caused by copper, nickel, salinity, drought, and low and high temperatures, among others. Uchida et al. (2006) demonstrated the biosynthesis of osmoprotectants in plants treated with H_2O_2 in response to abiotic stresses. Szpunar-Krok et al. (2020) reported a positive effect on the efficiency of the potato photosynthetic apparatus using H_2O_2 at a concentration of 1%. Adding H_2O_2 to the soil increased photosynthetic activity and, in the end, the amount of soluble sugars in melon fruit (Ozaki et al. 2009).

Torres et al. (2003), researching the resistance of apples to *Penicillium expansum*, which causes fruit rot during storage, found an increase in the concentration of hydrogen peroxide and reactive oxygen species (ROS) in the harvested fruits as a result of artificial inoculation with the pathogen, followed by an increase in the resistance to it. Bestwick et al. (1997) found a hypersensitive response in lettuce to *Pseudomonas syringae* pv. *phaseolicola* as early as 5–6 hours after cell inoculation with the bacterium. Olson and Verner (1993) and Wu et al. (1997) showed that the agent could make potato plants resistant to several species of pathogens, causing an even several-fold increase in the concentration of salicylic acid in the affected tissues and an increase in the amount of lignin in the shoots and roots. Research by Türküsay and Tosun (2005) showed that using hydrogen peroxide for spraying tomato plants infected with *Clavibacter michiganensis* ssp. *michiganensis* can induce resistance to this bacterium. In a study conducted on cucumbers, Lee et al. (2004) showed that when there was a sharp drop in temperature, hydrogen peroxide accumulated in the cytoplasmic membranes of root cells, and the water uptake by the root system decreased by half. This action of the agent affects limiting the freezing of plants. According to Mejía-Teniente et al. (2019), exogenous application of H_2O_2 induced pepper plant resistance to PepGMV. Borden and Higgins (2002) showed that reactive oxygen species formed in tomatoes infected with *Cladosporium fulvum* strongly limited plant colonization by this pathogen. Price and Lee (1970) found that the bacterium *Lactobacillus plantarum* produced hydrogen peroxide, which strongly inhibited the growth of *Pseudomonas* spp. and other pathogenic bacteria.

Research by Webber et al. (2007) showed that foliar and soil applications of H_2O_2 accelerate the development of the root system and increase the number of nasturtium flowers. The authors believe this is related to eliminating soil pathogens, although more oxygen can accelerate faster root growth. Experiments conducted by Khandaker et al. (2018) showed a positive effect of H_2O_2 on the growth and development of wax apple (*Syzygium samarangense*). Spraying trees with hydrogen peroxide in concentrations from 0.05% to 0.2% at weekly intervals increased the number and size of fruit on the trees, reduced fruit drop, and increased fruit yield and quality. The fruits had a higher content of potassium, anthocyanins, and carotene, on average about 60%, but also a higher amount of flavonoids, sugar, proteins, and antioxidants. In the experiments by López-Delgado et al. (2005), spraying potatoes with hydrogen peroxide from the 3rd to the 10th week of their cultivation increased the thickening of shoots. It caused a significant increase in lignin in the conductive bundles and faster growth of plants. Al-Mughrabi (2010) showed that fogging potato tubers during storage and treating them with H_2O_2 before planting resulted in better germination, increased shoot number, and a higher yield of better quality tubers. On the other hand, Orozco-Cardenas and Ryan (1999) emphasize that an agent present in the conductive bundles can inhibit the movement of harmful viruses, bacteria, and fungi. It is also essential to use H_2O_2 to quickly heal potato tubers or other plants damaged during harvest and transport (Orozco-Cárdenas et al. 2001). In the technology developed by Afek et al. (2000) and Al-Mughrabi (2007), the agent was used to extend potato storage for up to 6 months.

In improving the storage

Hydrogen peroxide has also found valuable applications in spraying, bathing, or gasification in storing vegetables, fruits, and flowers. It is used to remove bacterial and fungal contamination from the surface of fruits and vegetables and as an addition to the solution in which flowers are stored. In the storage of citrus fruit, it was used as a disinfectant, replacing the previously used formaldehyde (Smilanick et al. 2014). Bathing apples in an aqueous solution of 1% H_2O_2 reduced the *Escherichia coli* population (Sapers & Sites 2003). However, the same treatment was ineffective for decontaminating melons, meaning that the concentration and duration of exposure must depend on the fruit surface area and the plant growing conditions (Ukuku 2004). On the other hand, bathing whole melon fruit in 2.5% and 5% solutions effectively removed *Salmonella* spp. from the surface, while in the storage of cut fruit, the fruit had to be bathed whole and then cut. Using H_2O_2 at 1% and 1.5% concentrations on harvested strawberry fruit protected them with a 90% effectiveness against *Botrytis cinerea* and *Rhizopus stolonifer* (El-Mougy et al. 2008). However, hydrogen peroxide can have adverse effects, e.g., browning that reduces the quality of fruit, browning of lettuce leaves, and degradation of anthocyanins in berries (Nikkhah et al. 2010; Pietrysiak et al. 2019). H_2O_2 applied in gaseous form minimized the infection of grapes by *Botrytis cinerea* (Forney et al. 1991). Washing mushrooms in a 5% H_2O_2 solution immediately after harvesting allowed them to be stored at 4°C without reducing their quality (Sapers et al. 2001). Applying H_2O_2 protected peach flesh against darkening, gradually reducing it (Ding et al. 2009). When stored for four weeks, pepper fruits bathed in a 15% hydrogen peroxide solution maintained high fruit quality, reduced weight loss, and minimized putrefaction (Bayoumi 2008).

In potato storage, it is crucial to maintain their good health by minimizing the development of possible pathogens transferred onto the tubers and preventing the growth of sprouts (Afek et al. 2000; Kleinkopf et al. 2003). Research by Afek et al. (2000, 2001) and Al-Mughrabi (2007, 2010) indicates the possibility of using hydrogen peroxide (HPP with an unknown mixture of stabilizers, and StorOx with 27% H_2O_2) to minimize the development of pathogens causing wet rot of tubers (*Erwinia carotovora*), alternariosis (*Alternaria solani*), fusariosis (*Fusarium sambucinum*), powdery scab (*Helminthosporium solani*), dry rot (*Phytophthora erythroseptica*), late blight (*P. infestans*), and *Pythium ultimum*. Gachango et al. (2012) confirmed the effectiveness of H_2O_2 in protecting potatoes against *F. sambucinum*, *P. erythroseptica*, *P. infestans*, and *P. ultimum* in storage. They showed that H_2O_2 completely inhibited the development of *P. infestans*, with an effectiveness of four times greater for *F. sambucinum* and six times greater for *P. ultimum* on tubers, compared to the control. Czajkowski et al. (2013) indicated that among the several agents tested for potato protection against blackleg during storage, only H_2O_2 did not cause toxicity to plants.

MECHANISM OF ACTION OF SILVER AND ITS EFFECT ON MICROORGANISMS

Silver has accompanied man since the dawn of civilization. First, it was used to make jewelry, the contact with which was supposed to guarantee health and vigor (Lara et al. 2011). In ancient Hindu medicine, silver was described as a therapeutic agent in the fight against many diseases. In modern medicine, there is a growing interest in silver as a helpful factor in treating bacterial infections of various origins, especially in the fight against multidrug-resistant bacteria. Silver has an antimicrobial effect both in the ionized form (given in the form of salt) and as nanoparticles (<100 nm). For this reason, it is used more and more commonly to disinfect water and as an additive to fabrics, cosmetics, plastics (Lara et al. 2011). The role of silver in the fight against microorganisms increased after it was possible to produce it in the form of nanoparticles and thus replace the ionic form. According to Durán et al. (2016) and Mufamadi and Mulaudzi (2019), silver nanoparticles can affect bacteria differently. For example, (i) as positively charged, it attaches itself to negatively charged bacterial cell walls, creates pores in them, penetrates inside, and binds to DNA bases, causing damage to their function (inhibition of replication); (ii) produces reactive oxygen (ROS) that changes the permeability of the walls and the outflow of intracellular fluids, leading to cell destruction, causing disruption in respiration, binding to the chromosome and causing its damage; (iii) binds to ribosomes inhibiting protein synthesis. According to Yamanaka et al. (2005), silver ions interact with ribosomes and suppress the expression of enzymes and proteins essential to ATP production, resulting in ATP deficiency. The biocidal effect of nanosilver on viruses is based on the inhibition of nucleic acid replication (Gupta et al. 2018). The hyphae of sclerotia-forming fungi (*Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Sclerotinia minor*) were destroyed by silver nanoparticles (AgNPs), which is manifested as the separation of layers of the hyphal wall and then its complete disintegration, what prevented germination (Min et al. 2009). Thus, silver has a multilateral nonspecific inhibitory effect on microorganisms, interfering in a broad spectrum of biological processes (Pal et al. 2007; Ouda 2014; Kędziora et al. 2016), e.g., it destroys the structure and function of cytoplasmic membranes and inactivates the main essential enzymes. The biocidal mechanisms of silver ions and nanoparticles are similar. However, due to its size, nanosilver has a greater capacity for direct contact with a bacterial cell. It penetrates the cell more efficiently and oxidizes there, gaining biocidal ability. The antimicrobial effectiveness of AgNPs depends on the size and shape of the nanoparticles, the sensitivity of microorganisms to silver (Lara et al. 2011), and the preservation of activity for a longer time than that provided by H_2O_2 (Kędziora et al. 2016). Because nanosilver particles accumulate on the outer membranes of cells (Sondi & Salopek-Sondi 2004; Gajbhiye et al. 2009), it is effective at low concentrations (Jo et al. 2009).

The influence of nanosilver on plants is also multilateral (Mahajan et al. 2022). Silver enters plant cells through pores in the cell walls, both in the roots and leaves, travels inside the cytoplasm, and is incorporated into the metabolism of various organelles (Yan & Chen 2019). Over longer distances, it travels through the xylem, reaching all parts of the plant (Yan & Chen 2019). Depending on the form of silver (ionic or nanoparticle), its concentration, plant growth stage, and environmental conditions, its effects on plants can be positive or negative.

The positive effects include interference in the hormonal balance of the cell, primarily in inhibiting ethylene levels, which delays cell aging (used in storage and in extending the shelf life of flowers). In addition, silver increases the number and size of pores in the roots, which leads to a more efficient water and nutrient uptake and better communication with the microbiome, which increases colonization with nodule bacteria and other beneficial immune-enhancing microorganisms. Thirdly, silver generates ROS, which enables the oxidation of essential elements and increases the antioxidant reserve and the formation of chlorophyll, thus increasing biomass and the amounts of secondary metabolites (Mahajan et al. 2022).

The phytotoxicity of silver manifests itself at the morphological and physiological levels. Silver can inhibit seed germination, chlorophyll and carotenoid synthesis, and root growth, reducing leaf area and biomass production (Yan & Chen 2019). Silver can negatively affect the level of macro- and microelements by allowing them to flow out of the cell. Disturbance of root geotropism and increased reduction of auxin and increase of cytokinin level were found. Depending on the plant and growth phase, exposure to silver deregulates signal genes, increasing and decreasing their expression. The penetration of silver into the cell area leads to various types of chromosomal aberrations in the studied biological systems, such as incorrect orientation in metaphase, spindle dysfunction, fragility, fragmentation, unequal separation to the poles, scattering and lagging of chromosomes, etc. The basis of the mechanism of phytotoxicity is the oxidative stress in cells induced by ROS, increased by the presence of ionic silver released from nanosilver particles or silver compounds. Ionic silver causes an n-fold increase in H_2O_2 production, leading to many disorders in metabolism and also in the course of photosynthesis. The importance of silver to the plant cell has been extensively studied, primarily in laboratory conditions. Its comprehensive effect, including a negative impact on plants and the environment, must lead to its use being precise as to the chemical form, concentration, duration of action, stage of plant development, the intended use of the product, and the type of environment in which it will remain.

The properties of silver nanoparticles allow them to be used for the mineralization of pesticides in water purification. Nanosilver has been used chiefly in medical applications, but many reports apply to agriculture (Manimegalai et al. 2011).

EXAMPLES OF THE USE OF SILVER IN HORTICULTURE

In the stimulation of plant growth and development

Gupta et al. (2018) examined the effect of silver nanoparticles on the growth of rice seedlings. They demonstrated the stimulatory effect expressed as promotion of seedlings length and biomass. Salachna et al. (2019) found that soaking lily bulbs in silver nanoparticle solution stimulated plant growth, accelerated flowering with more flowers, and flowered longer. Prażak et al. (2020) studied the effects of AgNPs on bean seed germination and plant growth parameters at typical and low temperatures. At low concentrations ($0.25\ mg \cdot l^{-1}$), nanosilver stimulated quick and uniform seed germination in the laboratory and positively impacted plant growth and development in the field experiment. A particularly pronounced positive effect was revealed during germination at low temperatures, indicating that nanosilver may activate the tolerance mechanism to environmental stresses. Higher concentrations killed microorganisms but also negatively affected plant growth.

In the fight against pathogens

Kim et al. (2012) tested the *in vitro* sensitivity to AgNPs of several plant pathogens and found that all were sensitive. However, the result depended on the type of fungus and the concentration of the preparation, with the effect increasing with the concentration (up to 100 ppm). Silver administered as $AgNO_3$ or nanoparticles effectively eliminated or reduced the disease on ryegrass leaves, mainly when it was applied before inoculation with the fungus (Jo et al. 2009). Field experiment results of Lamsal et al. (2011) indicate that silver nanoparticles at a concentration of 10 to 100 ppm were more effective than classical fungicides in controlling pepper anthracnose (*Colletotrichum*), mainly when used preventively. In a field experiment, Gado et al. (2016) found that AgNPs reduced the severity of a disease on roses caused by *Phragmidium* spp. by one third. According to Shoaib et al. (2022), using nanosilver to control nematodes such as *Meloidogyne incognita*, *Pratylenchus penetrans*, and *Tylenchulus semipenetrans* is very promising. Al-Azzazy et al. (2019) reported that low dosages of AgNPs reduced the population of phytophagous mites associated with tomato plants in field cultivation.

In addition to the direct impact on microorganisms, silver molecules are involved in inducing the systemic resistance of plants to pathogens (Chen et al. 1993). For example, it was found that silver nanoparticles induced resistance of *Arabidopsis* seedlings to *Pseudomonas syringae* pv. *tomato* (Chu et al. 2012).

In the storage of fruits, vegetables and flowers

Nanosilver has been used to extend the life of cut flowers, and presumably, this is its most indisputable application. Its action is related to the inhibition of the multiplication of microorganisms in the storage solution and in the conductive bundles, through which silver reaches the flower, where it silences the ethylene biosynthesis genes, which are of great importance for the durability of climacteric plants (Basiri et al. 2011; Naing & Kim 2020). The positive impact on the durability of cut flowers has been reported in, for example, carnation (Sedaghathoor 2015), gladiolus (Li et al. 2017), tree peony (Zhao et al. 2018), gardenia foliage (Lin et al. 2019), snapdragon (Rabiza-Świder 2020), peony (Skutnik et al. 2020a), garden cosmos (Skutnik et al. 2020b), *Thalictrum aquilegifolium* (Poniewozik et al. 2020), lisianthus (Skutnik et al. 2021), tuberose (Paul et al. 2021), *Strelitzia* (Thakur et al. 2022), alstroemeria (Anvari et al. 2022), and rose (Ha & In 2022). Nanosilver was mainly applied as pulse treatment at 5–20 mg·l^{-1} for 24 h or as a permanent presence in the liquid in which flower stalks were immersed. Silver has also been recommended as an application on fresh fruits and vegetables to inhibit the reproduction of microorganisms and, as a result, to extend the shelf life (Kale et al. 2021). For example, coating asparagus with a nanosilver solution delayed weight loss, the loss of ascorbic acid and chlorophyll, prevented the growth of microorganisms and, as a result, extended the shelf life by ten days when stored at 2 °C (An et al. 2008). Similar results were obtained for pears and carrots (Fayaz et al. 2009). Motlagh et al. (2013) reported positive effects of storing barberry fruits in plastic bags with the addition of nanosilver.

In *in vitro* plant cultures

Silver in ionic form and as nanoparticles were used to improve the effect of *in vitro* plant cultures (Mahendran et al. 2019; Andújar et al. 2020; Zia et al. 2020). It displays many different functions as an antimicrobial agent and plant growth stimulant. One of the first reports on the effective use of silver (in the form of $AgNO_3$) concerned the elimination of the bacterial vector (*Agrobacterium tumefaciens*) from rose callus. Common antibiotics caused callus blackening and impaired shoot organogenesis. Silver nitrate inhibited the proliferation of bacteria and enabled organogenesis (Orlikowska et al. 1996). Nowadays, many authors recommend the use of nanosilver for the decontamination of initial explants, e.g., *Arabidopsis* seeds (Mahna et al. 2013), nodal segments of rose shoots (Shokri et al. 2015), valerian stems (Abdi et al. 2008), capitulum of gerbera (Fakhrfeshani et al. 2012), *Araucaria* (Sarmast et al. 2011), lemongrass (Salisu et al. 2014), *Pennisetum* (Parzymies et al. 2019), and others. Parzymies (2021) reported that nanosilver particles reduced the contamination of explants but also decreased regeneration and slowed down the growth of *Aldrovanda vesiculosa.* By contrast, Andújar et al. (2020) demonstrated that silver nanoparticles (preparation Argovit™) reduced contamination and enhanced the growth and multiplication rate of *Psidium friedrichsthalianum*. Nanosilver or $AgNO_3$ was added to the medium to protect cultures before contamination and to affect regeneration, multiplication, shoot rooting, and stimulating the production of secondary metabolites (Kim et al. 2017; Mahendran et al. 2019; Mahajan et al. 2022; Tung et al. 2022). Depending on the plant species and goal of *in vitro* culture, the form and concentration of the agent must be determined experimentally. The most effective *in vitro* is its inhibitory action against ethylene, especially in ethylene-sensitive plants such as cruciferous vegetables (Chi & Pua 1989; Akasaka-Kennedy et al. 2005) and potatoes (Perl et al. 1988; Ehsanpour & Nejati 2013).

RESERVATIONS REGARDING THE USE OF SILVER

The antimicrobial effectiveness of silver-containing preparations and their increasingly common use in medicine, plant and animal production, and in water disinfection as an additive to clothing and everyday products raise concerns about its impact on human health and contamination of the natural environment (Jampílek & Kráľová 2018). The ease of penetration into cells, especially in ionic form, and the comprehensive influence on cell metabolism are not limited to harmful microorganisms but all living organisms. Unlike a multicellular organism, a single cell has little chance of surviving the "invasion" of silver. However, considering the ability of this element to accumulate on the surface of cell membranes, its harmfulness to higher organisms should also be considered. Nanosilver is the most dangerous for the liver, which receives all substances that the body considers toxic. However, the lungs, blood tissue, heart, nervous and immune systems may also be at risk (Rezvani et al. 2019).

The widespread use of nanosilver means that it should be used with caution because it finds many ways to the environment, first to the production environment and then to the soil, water, and air, where it moves without restrictions. The long-term impact of this biocidal agent on microorganisms makes it necessary to consider the possibility of immunizing them against it, even though the biocidal mechanism is nonspecific. Graves et al. (2015) reported that *Escherichia coli* may become more tolerant to silver nanoparticles in the 200th generation of these bacteria. Silver also affects plants, and its exact role in plant metabolism has not been precisely determined; therefore, research is ongoing (Bapat et al. 2022). The above considerations should be considered when using silver-based preparations, although there is now a consensus that they are much less harmful than synthetic pesticides (Jo et al. 2008).

PROPOSED USE OF PREPARATIONS CONTAINING H_2O_2 AND SILVER IN HORTICULTURE

H_2O_2 has advantages that make it predestined for application to plants to combat disease-causing agents directly, but its short-lived impact is its disadvantage. It is a contact agent; it can disinfect plant surfaces after application but soon leaves the plant vulnerable to subsequent microbial attacks (Miller 2006). Currently, preparations with hydrogen peroxide also contain a stabilizer that extends its durability. The stabilizer may be a metal; most often, it is silver. However, Martin et al. (2015) believe that the antimicrobial properties of such preparations (e.g., Huwa San TR-50) are mainly due to hydrogen peroxide, and the presence of silver ions allows for more effective action of the agent on microorganisms. Martin et al. (2015) and Alkawareek et al. (2019) believe that the silver in this compound facilitates electrostatic interaction on the surface of bacterial cells, adhering to the cell walls. On the other hand, Chu et al. (2011) think silver nanoparticles are potent bacteria inhibitors with a much broader antimicrobial activity than hydrogen peroxide. Shuval et al. (2009) reported that the combined formulation of H_2O_2 and silver is 100 times more potent and long-lasting than H_2O_2 alone. Although both components are biocidal, combining them in one preparation resulted in a synergistic effect in combating microorganisms (Alkawareek et al. 2019).

In disinfection of surfaces and storage chambers

The preparation with these two components is proposed for disinfecting swimming pool water (Martin et al. 2015), surfaces, and equipment where other agents caused corrosion of metal parts, and for disinfecting rooms without the risk of leaving an unpleasant odor in with (Linley et al. 2012). As a result of a 24-month experiment, it was found that the H_2O_2/silver formulation was thermostable and more effective at higher temperatures, which predestines the use of the preparation for disinfecting hot water. Silver is deposited on the inner surfaces of pipes. The positively charged metal binds the negatively charged bacterial walls, destroying the structure of the membranes, which leads to the penetration of H_2O_2 and the death of bacterial cells (Shuval et al. 2009). Darzi et al. (2020) reported removing CGMMV virus particles from the surface of infected seed boxes in a 1% solution of Huwa San TR 50.

Martin et al. (2015) reported that the agent used to disinfect surfaces as well as to disinfect drinking water eliminated *Legionella* spp., *Escherichia coli*, *Cryptosporidium parvum*, *Giardia* spp., *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Streptococcus aureus*, and others species, without affecting the taste and smell of the water. Wilson (2011) emphasized that Huwa San TR-50 added at the concentration of 200 $ppm \cdot m^{-3}$ disinfected drinking water, eliminating *Legionella* spp. within 24 hours. The authors speculate that this agent added at a concentration of 15–20 ppm can also kill other species of bacteria.

In Poland, research on the activity of Huwa San TR-50 in the disinfection of water intended for irrigation of horticultural crops was conducted by Orlikowski et al. (2017a, b), showing a reduction in the population of pathogenic fungi from the genera *Cylindrocladium*, *Fusarium*, *Phytophthora* and *Pythium*. An example can be an 87.5% reduction in *Phytophthora cinnamomi* by adding 100 ml of the agent per m^3 of water.

Huwa San is also a secondary disinfectant, mainly to eliminate carcinogenic trihalomethanes formed after water chlorination. The World Health Organization allows up to 100 µg of silver per liter in drinking water as the active ingredient of Huwa San TR-50 (Martin et al. 2015).

Huwa San TR-50 at a concentration of 10% was used for surface decontamination of initial pistachio shoot explants. It was less effective than $HgCl_2$ in eliminating surface contaminants and less damaging to the explants (Korkmaz et al. 2022).

In plant protection

Linley et al. (2012) indicate that agents based on silver-stabilized hydrogen peroxide are more and more commonly used as biocides, especially since their environmental toxicity is lower than that of synthetic pesticides. Mahesha et al. (2021), in laboratory tests, studied the effectiveness of preparation based on colloidal silver with H_2O_2 (Alstasan Silvox) against some bacterial and fungal pathogens *in vitro* and its toxicity to young cotton plants and found that toxicity to plants occurred only at a concentration of 10,000 ppm. Linley et al. (2012) emphasize the much higher activity of the gaseous agent because it reaches the smallest nooks and crannies, surrounding the hyphae and spores of harmful microorganisms.

Introducing agents containing hydrogen peroxide and colloidal silver on the Polish market (Huwa San TR-50, Bisteran) resulted in an interest in its use in protecting horticultural plants, especially ornamental ones, against the most dangerous diseases. In studies by Wojdyła (2012, 2016a, b), the use of the agent at a concentration of 0.05% and 0.1% for repeated spraying plants of cissus, geraniums, willows, and roses protected them with an effectiveness of up to 80% against diseases such as powdery mildew (*Oidium* sp. and *Podosphaera pannosa*), rose black spot (*Diplocarpon rosae*), rust (*Puccinia pelargonii-zonalis* and *Melampsora epitea*), and its effectiveness ranged from 60 to 90%. In turn, Włodarek et al. (2016) showed a 60% effectiveness of the agent in protecting brassica vegetables against black cruciferous (*Alternaria* spp.). Research by Orlikowski (unpublished) assessed the effects of the agent on minimizing the occurrence of soil pathogens of the genera *Fusarium*, *Pythium,* and *Phytophthora* in the substrate. Abundant spraying of the infected substrate with the agent at a concentration of 1 and 2%, followed by thorough mixing, resulted in the elimination of these pathogens in approximately 90%. The agent used for once-repeated spraying of poinsettias with the first symptoms of grey mold (*Botrytis cinerea*) reduced the development of the disease by about 90%, while prophylactic spraying of rooted chrysanthemum seedlings with this agent protected them entirely against the occurrence of grey mold. Huwa San TR-50 has also proven to combat grey mold on chrysanthemum micro-seedlings acclimatized in a greenhouse (Orlikowska, unpublished data). The use of the agent in the protection of orchard plants has also been tested. Applied at weekly intervals at a concentration of 0.1% for twice-repeated spraying of plum trees with cankers developing on the trunks resulted in wound healing in about 80% within two months (Orlikowski, unpublished data). Spraying ripening raspberries with the first symptoms of the grey mold with a 0.1% agent almost wholly stopped the further development of the disease (Orlikowski, unpublished data). Włodarek et al. (2015) showed that Huwa San TR-50 used to protect *Brassica pekinensis* against *Erwinia* and *Pseudomonas* resulted in better head quality and enabled extended storage.

Soika (unpublished data) showed that spraying roses four times with Huwa San TR 50 in concentrations of 0.05% and 0.1% resulted in a 95% reduction in the rose aphid population on the shoots. The above scientist sprayed thuja plants with a 0.05% Huwa San TR-50 and observed a 60% reduction in the number of thuja beetles. Alhewairini (2017) showed that spraying cotton with an agent at a concentration of 0.1% resulted in the elimination of aphids (*Aphis gossypii*) in at least 90%. At this concentration, however, the agent did not reduce the honey bee population (*Apis mellifera lamarckii*). Observations carried out in nurseries of fruit and ornamental trees sprayed with 0.1% Huwa San TR-50 for quick healing of wounds showed that the use of the agent resulted in a drastic reduction in the occurrence of the spider mite (Orlikowski, unpublished data).

Most reports on the effectiveness of Huwa San relate to the control of spider mites on various plants: date palm (Alhewairini & Al-Azzazy 2017), *Citrus sinensis* (Al-Azzazy & Alhewairini 2019; Alhewairini 2018), *Solanum lycopersicum* (Alhewairini & Al-Azzazy 2018), olive tree (Al-Azzazy & Alhewairini 2018; Alhewairini et al. 2020), *Vitis vinifera* (Al-Azzazy & Alhewairini 2020). As Wojdyła (2012) showed, Huwa San TR-50 limits the development of foliar pathogens *Podosphaera pannosa*, *Puccinia pelargonii-zonalis*, *Diplocarpon rosae* and *Oidium*, and *Podosphaera pannosa* on rose (Wojdyła 2016a) and *Melampsora epitea* on willow (Wojdyła 2016b). Huwa San TR-50 reduced tomato diseases caused by *Fusarium solani* and *Rhizoctonia solani* (Hamed et al. 2022). This preparation can control *Alternaria* spp. on Chinese cabbage (Włodarek et al. 2016). Grzegorzewska and Kowalska (2013) write about its use in effectively controlling *Sclerotinia sclerotiorum* on carrots. Huwa San 25 inhibited the bacterial disease caused by *Erwinia amylovora* (Hafez et al. 2020) on pear trees while stimulating shoot growth.

The bacterium *Agrobacterium rhizogenes* causes mad root disease, mainly on cucumbers and tomatoes grown in mineral wool (Martin et al. 2015; Bosmans et al. 2016). Depending on the bacterial frequency, losses can reach up to 30%. For the protection of plants against disease-causing bacteria, it is advised to remove diseased plants immediately after noticing the disease and disinfect communication routes, vehicle tires, footwear, and hands with Huwa San TR-50. Vanlommel et al. (2020) reported that immersing mineral wool bricks once in a bath with Huwa San TR-50 before planting tomato plants reduced the occurrence of the bacterium throughout the entire cultivation cycle. However, in greenhouse tomato cultivation in Belgium, it is recommended to use Huwa San TR-50 throughout the cultivation period, primarily to disinfect the irrigation system (Anonymous 2016).

In the stimulation of plant growth

Huwa San TR-50 stimulated tomato seed germination and seedling growth (Türküsay & Tosun 2005). Stępowska's research (unpublished) on the use of the agent in the cultivation of peppers showed that Huwa San TR-50 at a concentration of 0.05%, sprayed five times on plants grown in a polytunnel, increased the chlorophyll content in the leaves. As a result, a significant increase in fruit yield and quality were obtained. Not without significance was the beneficial effect of the agent on the soil and the substrate.

Tests of the agent for registration purposes at the National Institute of Horticultural Research in Skierniewice (2012) showed its high effectiveness in the healing of wounds. Within three months of spraying with Huwa San, the scars on damaged trees healed to about 90%. These data indicate the necessity of using Huwa San TR-50 after pruning fruit trees and shrubs to heal injuries caused by storms, post-frost cracks, and injuries caused by physiological disorders. Its use is also helpful for alleviating damage to root vegetables, brassicas, and onions suffered during harvesting and transport. The agent also has a healing effect on plant organs damaged by pests, e.g., insect caterpillars, aphids, cup mites, and spider mites (Soika, unpublished). In the case of the spider mite inhabiting chrysanthemum leaves, the pest was found dead as early as 6 minutes after spraying the plants with Huwa San TR-50 at a concentration of 0.1% (unpublished data). Wojdyła et al. (2022) showed that immersion of hyacinth tubers before planting stimulated their growth and development.

In the storage of fruit and vegetables

Aharoni et al. (1994) showed that Sanosil-25 containing 48% H_2O_2 and 0.05% silver ions, added to melon fruit baths before storage, reduced the decay caused by *Fusarium solani* and *Alternaria alternata* when incorporated into wax-covering fruits. The same preparation applied as short dipping reduced the decay of eggplant and sweet red pepper fruits caused by *Botrytis cinerea* and *A. alternata* (Fallik et al. 1994). Huwa San TR-50 was effective against *Sclerotinia sclerotiorum* on freshly cut carrot slices (Grzegorzewska & Kowalska 2013). Observations on the harmfulness of nine compounds used to protect potatoes against *Dickeya solani* showed that only Huwa San TR-50 was not phytotoxic to the tubers and strongly limited the development of black leg when tubers were soaked for 15 minutes in the solution (Czajkowski et al. 2013). Therefore, Huwa San can be commonly used to minimize the occurrence and development of pathogens during potato storage (Al-Mughrabi 2010).

The presence of organic residues and minerals introduced into the water with fertilizers favors the development of microorganisms that form the biofilm inside the ducts, whose thickness increases gradually during cultivation. Research by Armon et al. (2000) showed that Huwa San TR-50 at a concentration of 0.003% added to water throughout the plant cultivation period strongly reduced biofilm formation.

CONCLUSIONS

1. Preparations containing H_2O_2 or silver, or both components, could be widely used in horticulture for plant protection, preventively and curatively, in the form of spraying and soaking seedlings before or after digging them up, to protect them during the time of storage, for disinfecting tubers, bulbs, and rhizomes before planting, for fogging potatoes and root vegetables during their storage, for quick healing of wounds on the roots and aboveground parts of plants, after cutting and in the case of frost damage and injuries caused by violent winds, for disinfecting seeds, and, as stimulants of plant development and inducers of resistance to biotic and abiotic stresses.
2. H_2O_2 and silver can be used mainly for disinfecting water, irrigation pipes, storage rooms, greenhouses and polytunnels, pallet boxes, tools, machines, and footwear.
3. Preparations containing H_2O_2, silver, or both components could substitute for synthetic pesticides, especially in sustainable production technology. Due to the possibility of environmental contamination with silver, preparations containing this ingredient should be used cautiously and in accordance with the manufacturer's instructions.
4. To combat microbiological contamination on initial explants *in vitro* propagation and to regulate ethylene action *in vitro*.
5. Nanoparticles of silver could be used to store cut flowers as an agent, eliminating bacteria from the storage solution, destroying internal bacteria, and reducing the concentration of ethylene, which extends flower shelf-life.

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