

## Research topics related to the American pokeweed (*Phytolacca americana* L.) in scientific articles from SCOPUS and Web of Science databases

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**Abstract.** American pokeweed (*Phytolacca americana* L.) is a perennial plant belonging to the Phytolaccaceae family. The plants are showy, can exceed 2 m in height, and have a characteristic reddish discolouration of the stems and other organs. Due to its unique properties, *P. americana* has found many applications in biotechnology, medicine and various industries. It is native to North America, but has already occurred in Asia and Europe. In 2013, it was recorded in Poland and is already known from many sites. The aim of this paper is to show the main research topics related to this species, selected on the basis of scientific articles from SCOPUS and Web of Science databases. Due to easier access to the plant in Poland and Europe, it may become a subject of increased scientific interest and such an overview may facilitate the design of new research topics and the establishment of collaborations. The material covered 209 articles and is divided into chapters on the active substances of *P. americana*, the possibilities of its use in medicine, the physiology and ecology of the plant, the importance for agriculture, as well as issues outside this field.

**Keywords:** agriculture, American pokeweed, biotechnology, industry, medicine, *Phytolacca americana*

### INTRODUCTION

American pokeweed (*Phytolacca americana* L.) is a perennial plant native to North America. It has reached Asia and Europe, in Poland it was recorded in 2013 in the gmina Golezów in the Śląskie voivodeship (Chmura, 2016). According to the i-Naturalist portal and literature (Kopij, 2023) its stands are also found in other regions (*Phytolacca*..., n.d.). It also occurs in large numbers in the area of the experimental station of the Institute of Soil Science and Plant Cultivation – State Research Institute located in Lubelskie voivodeship. *P. americana* plants are easy to find due to their large size and characteristic reddish colouring, particularly well visible on the stem and inflorescence axis. A detailed botanical description of the plant can be found, for example, in the study by Balogh and Juhasz (2008). Pokeweed can rapidly expand its range

and is considered an invasive plant in many countries. As a species that is new to colonised areas, it may be of interest to both scientists and the public.

When looking for information on a particular plant species, a variety of sources can be used, both popular and scientific. A good choice is scientific articles from recognised bibliographic databases, which are carefully checked by expert reviewers before publication. The size of the publication pool depends mainly on the importance of a given plant for humans – the possibility of its use in medicine, industry or agriculture, potential harm (toxicity, weed infestation of crops) (Arabas, Książkowski, 2022; Baars et al., 2020; Święcicki, Surma, 2021). The characteristic features of the plant that distinguish it from others and attract attention, such as its size, scent, colour, ornamental flowers or fruit, may also have some influence. The type of information available also depends on the length of the po-





Figure 1. *Phytolacca americana* plants near the hop plantation in IUNG-PIB experimental station in Puławy.

Figure 2. *Phytolacca americana* plants in forest clearing.

Figure 3. Flowers of *Phytolacca americana*.

Figure 4. Stem and leaves of *Phytolacca americana*.

Figure 5. Fruits of *Phytolacca americana*.



tential study period. In areas of native occurrence, studies are usually available on the potential for use or cultivation, on ways to control populations, on its place in the ecosystem. Reports on the role in tradition and folk medicine may also be numerous. In contrast, in areas where the plant has emerged relatively recently, research focuses on the plant potential to affect native biodiversity, how and under what conditions it disseminate into new areas, potential threats and opportunities (Domaradzki et al., 2013; Urbisz et al., 2013). Information on a particular plant species may also come from articles that do not deal directly with it, but describe experiments in which it is, often one of many, an object of general research on physiology, biochemistry, genetics or ecology.

Due to its unique properties, American pokeweed has found applications in various industries, medicine and biotechnology, but many issues still need to be worked out. Also, its potentially detrimental impact on biodiversity as an invasive plant prompts research into its biology.

The aim of this paper is to present the research topics related to this species to date, which will at the same time help to identify the scope of issues not yet published in this context, with reference to selected literature bases.

#### MATERIAL AND METHOD

Scientific articles from SCOPUS and Web of Science databases, recognised as references by the Polish Minis-

try of Science and Higher Education, were analysed. All records returned in response to the query [*“Phytolacca americana”*, no time limit, open access publications, no review papers] were included. After analysis, articles in which *P. americana* appears only in literature references were excluded. On the basis of a review of the items selected in this way, research topics were separated: active substances, potential for use in medicine, physiology, ecology, importance in agriculture, other topics, and individual citations were assigned to them. The general description of the plant also includes items from outside this resource. The study is illustrated with photographs of the plant taken in the Lublin region (Figures 1–5).

#### DESCRIPTION OF THE RESOURCE

A total of 209 items of literature were selected for the topic review. In the analysis of the characteristics of this resource, additional limiting factors – the availability of electronic versions of the articles and the adopted open access condition of the publication, narrowing the selection, especially of older works – should be taken into account.

The oldest publication from the analysed resource dates back to 1958, its coverage includes articles available in both databases until September 2024. An increasing trend can be observed in the number of items on the American pokeweed (Figure 6), with particularly rapid growth seen in recent years. The largest number of articles comes from

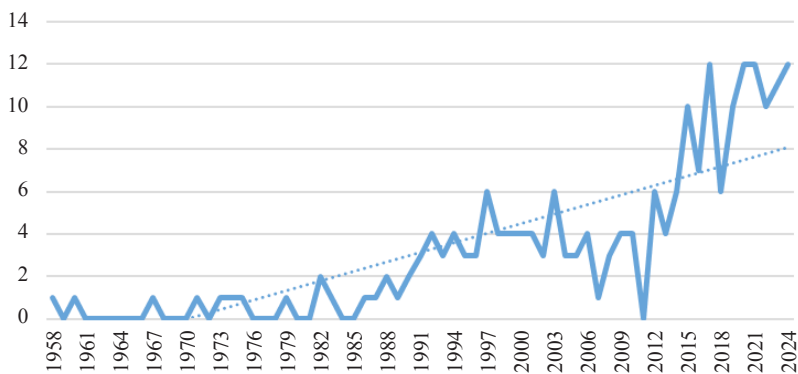


Figure 6. Number of articles related to *Phytolacca americana* in SCOPUS and Web of Science databases per year.

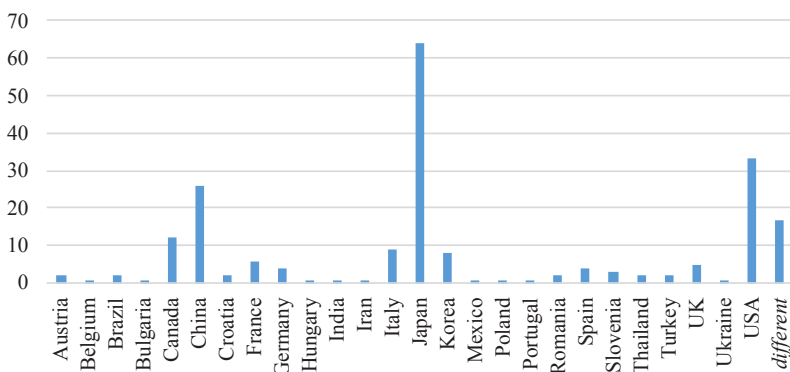


Figure 7. Number of articles related to *Phytolacca americana* in SCOPUS and Web of Science databases acc. to author affiliation.

authors affiliated with Japan, followed by the USA and China (Fig. 7). It should be noted, however, that in publications from Japan and the USA, about 60% are articles published since 2000, while all Chinese records belong to this group. Most articles are on the biochemistry of *P. americana*, mainly on the properties of ribosome-inactivating proteins and the plant's ability to glycosylate various organic compounds. Recent years have seen an increase in papers on the ecology of pokeweed and its invasive potential.

#### ACTIVE SUBSTANCES OF *PHYTOLACCA AMERICANA*

The active compounds of *P. americana* were described in the order determined by the number of publications. Most articles were devoted to ribosome-inactivating proteins and glucosyltransferases. Other enzymes, dyes and compounds from other biochemical groups were also of interest to researchers. Some of the active substances are included in the following chapter on medical applications.

*P. americana* produces **ribosome inactivating proteins** (RIP) of eukaryotes and (in higher concentrations) prokaryotes, mainly by depurination (cited by Barbieri et al., 1992), and they also have antiviral activities. Different proteins with such properties have been isolated from aboveground parts of the plant. They are synthesised in the cytoplasm and deposited in the cell wall (cited by Di, 2016). Day et al. (1998) found that RIP of *P. americana* under certain conditions can also affect DNA. RIP proteins have become the subject of numerous studies, both basic and applied.

The structure of *P. americana* RIP (Ago et al., 1994), the possibility of their hybridisation with RIPs from other plants (Hassan et al., 2018, Hassan et al., 2020, Islam et al., 1991, Kung et al., 1990), and with gonadotropin-releasing hormone to facilitate entry into cancer cells (Yang et al., 2003) have been determined. The genetic basis for the production of these proteins has also been studied (Liu et al., 2000; Neller et al., 2019; Poyet, Hoeveler, 1997; Poyet et al., 1994; Rajamohan et al., 2000).

As these proteins are cytotoxic, their effects on various organisms have been checked (Chaddock et al., 1994; Dore et al., 1993, Hartley et al., 1991, Hudak et al., 2002), including effects on plant ribosomes (Taylor, Irvin, 1990). Watanabe et al. (1997) investigated the effect of RIP of *P. americana* on *Tobacco mosaic virus* (TMV). They found that these proteins could not enter healthy cells, but entered infected cells and stopped virus proliferation by inactivating ribosomes.

The possibility of obtaining and the properties of RIP from different parts of the plant have been studied (Barbieri et al., 1982; Park et al., 2002; Poyet, Hoeveler, 1997; Stirpe et al., 1996). The mechanism of action of RIP of *P. americana* has been the subject of numerous experiments (Barbieri et al., 1992; Irvin, Aron, 1982; Parikh et al., 2002; Prestle et al., 1992; Rajamohan et al., 2001; Ready et al.,

1986; Tourlakis et al., 2010; Tumer et al., 1997; Wang, Tumer, 1999), also studied on the basis of interaction with *Turnip mosaic virus*, which inactivates RIP (Domashevskiy et al., 2012).

RIP of *P. americana* have great potential to destroy viruses and cancer cells, so methods are being developed for their detection (Li et al., 2012; Ready et al., 1986), extraction from plant cell cultures and from modified organisms (*Escherichia coli*, yeast) and purification (Barbieri et al., 1982; Barbieri et al., 1989; Honjo, Watanabe, 1999; Hur et al., 1995; Kataoka et al., 1993; Schönfelder et al., 1992; Smirnov et al., 1997).

The antiviral properties of *P. americana* have been found for, inter alia, *Brome mosaic virus* (Gandhi et al., 2008; Picard et al., 2005), *Zucchini yellow mosaic virus* (Sipahioğlu et al., 2017, Tumer et al., 1997), SARS-CoV-2 (after hybridisation) (Hassan et al., 2020), HIV-1 (Zhabokritsky et al., 2014). Studies have also investigated virus resistance in transgenic plants producing *P. americana* RIP (Lodge et al., 1993).

**Enzymes.** Glycosylation of hydrophobic organic compounds can increase their water solubility and absorption from the gastrointestinal tract, which improves their pharmacological properties and biological activity (Shimoda et al., 2020). The use of natural enzymes for this process increases its efficiency and speed. The potential for using enzymes found in *P. americana* cells for glycosylation has been the subject of numerous experiments (Table 1). Other enzymes produced by *P. americana* – proteases, chitinases, phosphodiesterases – are also the subject of scientific studies.

Ozaki et al. (2012), Maharjan et al. (2020, 2022) and Shimoda et al. (2021), studied the crystal structure of *P. americana* glucosyltransferases in different substrate systems and the conditions of enzyme activity. Noguchi et al. (2009) and Iwakiri et al. (2013) demonstrated the specificity of glucosyltransferases towards selected substrates.

Ukita et al. (1973) studied the pH-dependent activity of 5-phosphodiesterase produced in *P. americana* cell cultures. This enzyme degrades RNA into nucleoside monophosphates, some of which have flavour-enhancing properties.

Uchikoba et al. (1998) showed that proteases extracted from green and ripe *P. americana* fruit differ, and investigated their substrate specificity and their inactivating factors.

Yamagami et al. (1998) examined a chitinase from *P. americana* leaves, sequenced it and compared it with other chitinases.

Shimoda et al. (2012) showed that *P. americana* cell cultures do not produce curcumin-reducing enzyme.

**Pigments.** Betacyanins are water-soluble betalain pigments. The plant accumulates them in vacuoles. They occur in some members of the order Caryophyllales, including *Phytolacca americana* (Takahashi et al., 2015a)

Table 1. List of compounds subject to glycosylation using *P. americana* enzymes.

Glycosylated compound	Source of enzyme	Literature source
(+)-e -viniferin	E.coli	Hamada et al., 2015
8-nordihydrocapsaicin	cell cultures	Hamada et al., 2003
Artepillin C	cell cultures	Shimoda et al., 2014
Chrysin	cell cultures	Fujitaka et al., 2020
Daidzein	cell cultures	Fujitaka et al., 2017a, 2020
Daidzein	cell cultures Bacillus subtilis	Hamada et al., 2021
Dihydrocapsaicin	cell cultures	Shimoda et al., 2021
Gnetol	cell cultures	Shimoda et al., 2020
Hesperetin	cell cultures	Fujitaka et al., 2020
Hydroxyflavones	cell cultures	Shimoda et al., 2015b
6- and 7-hydroxyflavone	cell cultures, E. coli	Iwakiri et al., 2013a
Capsaicin	cell cultures	Hamada et al., 2003 ; Homma et al., 2008 ; Shimoda et al., 2021
Capsaicin	E.coli	Noguchi et al., 2009 ; Maharjan et al., 2022
Kaempferol	E. coli	Maharjan et al., 2020 ; Maharjan et al., 2022
Cinnamic acid	cell cultures	Shimoda et al., 2016
Ferulic acid	cell cultures	Shimoda et al., 2016, 2018
Hydroxybenzoic acid	E.coli	Hamada et al., 2016
p-coumaric acid	cell cultures	Shimoda et al., 2016
Quercetin	cell cultures, E. coli	Yasukawa et al., 2015
Quercetin	cell cultures	Fujitaka et al., 2017b
Myricetin	cell cultures	Fujitaka et al., 2017b
Naringenin	cell cultures	Fujitaka et al., 2020
Oxyresveratrol	cell cultures	Shimoda et al., 2020
Piceatannol	E.coli	Iwakiri et al., 2013b ; Maharjan et al., 2020
Piceatannol	cell cultures	Sato et al., 2014 ; Shimoda et al., 2019b
Piceid	E.coli	Shimoda et al., 2019a
Pinostilbene	E.coli	Uesugi et al., 2017
Pterostilbene	cell cultures	Sato et al., 2014 ; Hamada et al., 2017 ; Shimoda et al., 2019b
Pterostilbene	E.coli, pure enzyme	Uesugi et al., 2017
Resveratrol	enzyme isolated from <i>P. americana</i>	Shimoda et al., 2015a
Resveratrol	E.coli	Uesugi et al., 2017
Resveratrol	cell cultures	Shimoda et al., 2019b ; Fujitaka et al., 2020 ; Sato et al., 2014
Resveratrol	B. subtilis, cell cultures	Hamada et al., 2021
Resveratrolside	E.coli	Shimoda et al., 2019a
Trans-resveratrol	E. coli	Ozaki et al., 2012
$\alpha$ -tocopherol	cell cultures	Fujitaka et al., 2020
$\alpha$ -tocopherol derivatives	B. subtilis, cell cultures	Hamada et al., 2021

E.coli – enzyme extracted from cultures of transformed *Escherichia coli* cellsB.subtilis – enzyme extracted from cultures of transformed *Bacillus subtilis* cellsCell cultures – cell cultures of *Phytolacca americana*

imparting a characteristic reddish-purple colour to various parts of the plant. They are not found in plants that produce anthocyanins (Shimada et al., 2004).

Shimada et al. (2004) and Takahashi et al. (2009) studied the links between the anthocyanin and betacyanin biosynthetic pathways, also their genetic determinants and regulatory factors. Shimada et al. (2005) explained that the production of betacyanin instead of anthocyanin is due to the absence or inhibition of 2 enzymes and tried to determine the timing of the separation of these metabolic pathways in phylogeny.

Takahashi et al. (2015 a,b) investigated the biosynthesis of betacyanin. Tyrosinase catalyses the hydroxylation of tyrosine to DOPA, and dioxygenases catalyse the conversion of DOPA to betalamic acid, which is the main component of betacyanin. They compared the DOPA dioxygenase activity of *P. americana* from stained leaves and peel of green, ripening and mature fruit, determined their biochemical properties and proposed a three-dimensional model of the enzyme structure. Joy et al. (1995) studied genes encoding polyphenol oxidase, an enzyme involved in the production of the betalain.

Hirano et al. (1992) found that one of the cytokinins limits pigment production by reducing the amount of tyrosine incorporated into betacyanin, and Hirano et al. (1996) investigated the mechanism by which ABA (abscisic acid) inhibits betacyanin production in *P. americana* cell cultures.

### Other compounds

Yamaguchi et al. (1996) studied lectins isolated from young roots of *P. americana* and determined the amino acid composition of one showing the strongest haemagglutinating and mitogenic activity. The properties of lectins were also studied by Reisfeld et al. (1967) and Minic et al. (2000). Barbieri et al. (1979) dealt with the immunostimulatory effect of synthetic lectin and explained the reason for its lower activity. Yang et al. (2006) found that the activation of macrophages leading to the production of pro-inflammatory cytokinins and NO attributed to commercial lectins is rather the result of their contamination with toxins. Yamaguchi et al. (1997) analysed

the amino acid sequence in lectin B and, on this basis, tried to explain its very high haemagglutinating and mitogenic activity. Yamaguchi et al. (2004) studied the structure and properties of two isomers of lectin D, with weaker mitogenic properties, and compared them with the other lectins of *P. americana*.

Yu et al. (2016) found that esculentosides were responsible for the toxicity of *P. americana* roots. They isolated four such compounds. Since *P. americana* roots are similar to *Panax ginseng* roots but poisonous, Osathanunkul and Madesis (2019) developed a barcode-based method to distinguish between these materials.

Suga et al. (1978) isolated 3 major saponins from the roots of *P. americana* and studied their structure. Kanzaki et al. (1999) used the inhibitory effect of a triterpene glucoside isolated from *P. americana* to study the mechanism of plant transformation by *Agrobacterium tumefaciens*.

Burke and Le Quesne (1971) isolated acetyloleanolic acid from *P. americana* seeds and oleanolic acid from the roots. Minami et al. (1998) isolated antimicrobial and antifungal peptides from *P. americana* seeds, and Shahin-Kaleybar et al. (2020) compared them with other high-cysteine peptides.

Callus of *P. americana* is a good source of active substances that can be produced in *in vitro* culture. Trunjaruen et al. (2022, 2023) determined the optimal medium composition for organogenesis, the possibility of inducing callus from cotyledons, leaves, nodes and internodes. They also investigated the total content of saponins, phenols, flavonoids and antioxidant activity of the resulting material.

#### THE POTENTIAL MEDICINAL USE OF *PHYTOLACCA AMERICANA*

In areas where *P. americana* has long been found, it is widely used in folk medicine. However, care must be taken in analysing these reports, as they sometimes refer to other species of the genus. A number of articles present the properties of preparations (extracts) from various parts of the plant. The active substances responsible for the medicinal and toxic properties of *P. americana* have been the subject of numerous studies.

#### Use of *P. americana* in folk medicine

Bellavite et al. (2006) report the use of this plant in a homeopathic mixture used in tonsillitis. It has immunomodulatory, analgesic and anti-inflammatory effects. No serious adverse effects have been reported. Fenner et al. (2006) confirmed the antifungal properties of *P. americana* used in folk medicine in Brazil. Gras et al. (2017) found information on the analgesic and anticancer properties of *P. americana* in a herbarium from the 18th/19th century.

Li et al. (2024) found, based on surveys, that *P. americana* frequently appears in relation to folk medicine in southern China, and Hu et al. (2022) reported that it is used

there for baths and in the form of decoctions with detoxifying and diuretic effects.

#### Properties of *P. americana* extracts

Tomlinson et al. (1974) demonstrated antiviral activity of *P. americana* leaf extract against two strains of influenza virus. Patra et al. (2014) found the selective effect of extracts from the aboveground parts of *P. americana* with different solvents against the bacteria that cause periodontal inflammatory diseases and dental caries: *Porphyromonas gingivalis* and *Streptococcus mutans*. Marinas et al. (2021) investigated the chemical composition (phenolic content, flavonoids, carotenoids, betalains, saponins) and effects of an alcoholic extract of *P. americana* berries and leaves. They found its antioxidant effects, as well as those directed against microorganisms and biofilm: *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and the yeasts *Candida famata*, *C. utilis*, *C. albicans*. Demirkan et al. (2022) tested *P. americana* seed extract for total phenolic content, antioxidant, antimicrobial and biofilm effects against *Escherichia coli*, *Enterococcus faecalis*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Yersinia enterocolitica*, and anti-cancer against breast cancer. They also identified the mechanism of toxic effects on cancer cells.

Zheleva-Dimitrova (2013) found, used in the prevention and treatment of neurodegenerative diseases, the antioxidant and acetylcholinesterase-inhibiting properties of a methanolic extract from the fruit and leaves of *P. americana*.

Marinas et al. (2023) proposed the use of *P. americana* fruit extract as a component of a novel dressing material made from the pods of *Gleditsia triacanthos*.

*Phytolacca americana* can be poisonous, so the side effects of its use have been studied. Han et al. (2020) found no toxic effects on rats after long-term, 13-week orally administration of *P. americana* extract, while Karami et al., 2010 found histopathological changes in studies on isolated rat liver.

#### Active compounds of *P. americana* used in medicine

Tanaka et al. (1987) isolated various lignans from *P. americana* seeds, including americanin A, which has a protective effect on the liver, and studied the structure of this compound. Takahashi et al. (2001) isolated from cell cultures and studied the structure of a triterpene glucoside with neurotrophic properties. Takahashi et al. (2003) investigated the structure of 3 lignan derivatives isolated from a methanolic extract of *P. americana* and showed a positive effect of one of them on neuritogenesis of cortical neurons.

*P. americana* lectins have been used in modelling studies of reactions to histamine release (Matsuda et al., 1994), as well as biomarkers of glycans (Itakura et al., 2017) and for the detection of cervical (Clark et al., 2014) and



colorectal cancers (Faragó et al., 2024). Pérez-Campos-Mayoral et al. (2008) showed that *P. americana* lectins are not suitable for distinguishing between leukaemia types. Saleri et al. (2017) studied saponins from *P. americana* and their antiproliferative properties on gastrointestinal cancer cell lines. Bekeredjian-Ding et al. (2012) investigated the mechanism of lectin action on immunoglobulin-secreting B cells of the immune system. They concluded that this action was due to the synergistic effect of lectin and other substances present in the preparations.

George Thompson et al. (2015) showed that astragal-glucoside derived from *P. americana* inhibits excessive carbohydrate transport across the cell membrane through inhibition of the proteins involved.

Yu et al. (2016) found that esculentosides isolated from *P. americana* induced gastrointestinal irritation and the release of inflammatory mediators.

D’Cruz et al. (2004) showed that the use of an antiviral protein gel from *P. americana* does not cause vaginal irritation and can be used for HIV prophylaxis. D’Cruz and Uckun (2001a, b) found that the use of antiviral proteins from *P. americana* does not have a negative effect on fertility.

#### **Use of lectins from *P. americana* in veterinary medicine and zootechnics**

Castagnaro et al. (1991) used *P. americana* lectins in the diagnosis of kidney disease in rainbow trout.

Jones et al. (2009) used them as biomarkers in a histochemical study of the placenta of the Hottentot Golden Mole, and Jones et al. (2017) used them in a study of the origin of pronghorn.

Pandey et al. (2009) found that the introduction of lectin from *P. americana* into the solution in which *in vitro* matured oocytes of *Bubalus bubalis* cattle improved their quality and ability to produce an embryo.

#### **PHYSIOLOGY OF PHYTOLACCA AMERICANA**

The publications on physiology mainly focus on traits related to the competitiveness of the *P. americana* plant - speed of germination and growth, ability to adapt to different conditions. The chapter also includes papers on metal hyperaccumulation by *P. americana*. There are also articles in which *P. americana* chloroplasts provided material for the study of photosynthesis.

#### **Competitiveness, adaptation to stressful conditions**

He and Bazzaz (2003) and He et al. (2005) studied the effects of elevated CO<sub>2</sub> concentration on *P. americana* reproduction under conditions of different population densities and proximity to other plants. Kim and You (2010) determined the effects of elevated CO<sub>2</sub> concentration and temperature on photosynthesis and water utilisation by *P. americana*. Pepe et al. (2022b) found that *P. americana* responded strongly to prolonged water stress, but leaf mor-

phology was not affected. Dougherty et al. (2024) and Neller et al. (2016, 2019) studied gene expression of *P. americana* under stress conditions simulated by the presence of jasmonic acid. Neller et al. (2018) identified miRNAs mediating hormone biosynthesis, information signalling and defence against pathogens. Cahill and Casper (1999) studied the response of a plant to nutrient concentration/dispersal under conditions of competition with other species.

Liu et al. (2024) showed that *P. americana* germinated faster than, native to the study area, the *P. acinosa* species due to earlier activation of genes responsible for amylases regulating the supply of free sugars from stored starch in the seed. Panero et al. (2024) found that *P. americana* seeds germinated better at higher temperatures and after cold stratification, but at the same time highlighted the high plasticity of the plant in this respect in adapting to habitat conditions. Pepe et al. (2022a) also confirmed that *P. americana* germinated faster at higher temperatures, and had a rapid height gain. Orrock and Christopher (2010) speculate that rapid germination is one of the evolutionary adaptations of the plant to grow in dense patches. They also found that the seeds are largely resistant to pathogens. However, Orrock et al. (2006) showed that *P. americana* seeds are often eaten by animals from different systematic groups. Guzzetti et al. (2017) assessed the physiological value of *P. americana* seeds depending on the degree of fruit maturity, and Strgulc Krajšek et al. (2023) studied the germination of *P. americana* seeds from fruit with different degrees of seed maturity without and with pericarp, and fruit ripening on cut plants.

#### **Metal hyperaccumulation**

Chen et al. (2017) compared the gene expression of control and Cd excess stressed plants. From this, they identified genes responsible for cadmium uptake, transport, accumulation and tolerance. They also noted that the response varied depending on the plant organ. Zhao et al. (2019) found that different genes are activated in the defence response. The end result is the activation of the defence system further stimulated by the formation of reactive oxygen species. Zhao et al. (2021) included metal binding in cell wall lignin, chelation, isolation in the vacuole, and an increase in thiol compounds among the main mechanisms activated during detoxification. Dou and Qi (2023) noted that in hydroponic culture under high Mn concentration conditions, *P. americana* produces manganese phosphate crystals on trichomes and roots. They assumed that this is a new mechanism in the plant response to excess Mn, under phosphorus-abundant conditions. *P. americana* can also deposit Mn in the leaves, but this is not sufficient in situations of heavy manganese contamination. Zhou et al., 2023 found that, induced by exposure to high concentrations of Mn, the production of secondary metabolites – tannins, phenols and flavonoids – leads to a reduction in the plant’s susceptibility to pest attack, tested using the example of *Spodoptera litura* larvae.

Wang et al. (2021) evaluated, under laboratory conditions, the feasibility of using phytoremediated plants for biogas production. In the case of *P. americana*, manganese was found to ultimately reduce the amount of biogas produced relative to control plants, although the process parameters change over time.

Grosjean et al. (2019) studied the ability of *P. americana* to uptake and accumulate rare earth metals, under hydroponic culture conditions. They described the plant response – changes in root morphology, chlorophyll content and other pigments in the leaves – to different concentrations of metals. According to Guo et al. (2023), rare earth metals are bound in the cell wall mainly in pectin. The plant can control the transport and binding of different fractions, accumulating more ‘heavy’ metals in the roots and ‘light’ metals in the leaves.

### Photosynthesis

Milner et al. (1958) investigated how to restore the photochemical activity of chloroplasts after extraction with petroleum ether, which removes fatty substances. Habermann (1960) determined the effect of Mn ions on the course of the Mahler reaction, and Homann (1988) investigated the stabilising function of Cl<sup>-</sup> ions on photosystem II. Hiyama et al. (2000) checked which photosystem specific polypeptides isolated from chloroplasts are associated with.

Iida et al. (1983) attempted to use the chloroplasts of the *P. americana* plant to produce hydrogen. They constructed a suitable cell and determined the conditions for its operation and durability.

### ECOLOGY OF THE *PHYTOLACCA AMERICANA*

The works included in the ‘ecology’ chapter mainly deal with the development of *P. americana* in different ecosystems, habitat requirements and expansiveness. In many countries, *P. americana* is treated as a plant that threatens biodiversity, but it has not yet been included in the European Union’s list of invasive plants (Implementing Regulation..., 2016). Articles on pathogens of *P. americana* and insects that feed on it are also included in the chapter.

### Relationships between *P. americana* and other plants

Chen et al. (2019) tested whether a stable natural forest ecosystem is more resistant to *P. americana* invasion than industrial robinia plantations. They found that in forests, allelopathic activity and nutrient deficiencies, mainly phosphorus, affect reduced seed germination and seedling growth. Wang et al. (2024b) found that in China, *P. americana* grows well in robinia and pine forests because it has good light conditions there, nutrient availability and soil microbial properties that favour its competitive advantage over other plants in the undergrowth. Liu et al., 2022 found that the advantage of *P. americana* over *P. acinosa* under shade conditions is due to, among other things, the secre-

tion of active volatile organic compounds (VOCs) and greater plant height. Riveiro et al. (2024) demonstrated that aqueous leaf extracts of the invasive trees *Acacia melanoxylon* and *Eucalyptus globulus* inhibit the germination of the *P. americana* seeds. Liu et al. (2021) confirmed that *P. americana* retains the same seed dispersal mode by birds as for a similar native species in terms of fruiting characteristics, and that passage through the digestive tract of animals improves seed germination.

### Occurrence and invasiveness

De Groot et al. (2024) collected information on the habitat preferences of *P. americana* and plotted threat maps. They found that the plant grows near water and appears frequently near railway lines. In forests, it most often inhabits areas of forest gaps and clearings. Lee et al. (2018) estimated that forest edges are most susceptible to invasion. De Groot et al. (2024) located *P. americana* sites mainly in lower-lying areas, but Zima and Stefanic (2021) from Croatia noted its preference for hilly areas, and Do Kim et al. (2013) reported its occurrence in mountains. Xu et al. (2023) and Nan et al. (2024) distinguished climatic factors affecting the spread of *P. americana* in China and, based on this, created expansion models with climate scenarios for 2050 and 2070. Obrock et al. (2006) investigated the role of different limitation factors in dissemination of *P. americana* in large-area experiment. Yazlık and Ambarlı (2022) considered *P. americana* as a high-risk plant on the basis of biological traits, environmental impacts and control options. Bentley et al. (2015) investigated the possibility of using microsatellite markers to track the spread of this species in native and new habitats.

Essert et al. (2023) report it as an invasive species in Croatia. Verloove (2013, 2017) reports the emergence of the *P. americana* in the Canary Islands. Mosyakin and Mosyakin (2021) noted that in and around Kyiv, *P. americana* is often cultivated and goes wild. However, they also reported the presence of an established plant colony in a firebreak ditch in the forest, far from the cultivation sites. Strgulc Krajšek et al. (2023) reported that in Ljubljana *P. americana* is used as an ornamental plant. In addition to the work of Strgulc Krajšek et al. (2023) are maps showing how the range and number of *P. americana* listing sites in Slovenia changed in the years up to 2000, 2000–2010 and after 2010. Follak et al. (2022) described the occurrence of *P. americana* in agricultural areas in the Czech Republic, Germany, Hungary, Italy, Slovakia, Slovenia and Switzerland on the basis of literature and expert consultation. It usually occurred on forest edges, less frequently in ruderal habitats (e.g. rubbish dumps), in the *Panicum-Setarion* association, together with *Chenopodium polyspermum* L., *Chenopodium album* agg., *Setaria pumila*, *Amaranthus powellii*, *Calystegia sepium* L. According to Strgulc Krajšek et al. (2023), in Slovenia *P. americana* does not form dense patches, growing in communities with other plants.



De Groot et al. (2020) analysed the challenges to EU legislation posed by invasive organisms at different trophic levels. *Phytolacca americana* was chosen as an example of a plant with invasive potential.

### Pathogens and phytophages

Baskett et al. (2020), on the occasion of a study on the influence of latitude on the relationship between plants and entomofauna, determined the intensity of insect visitation on flowers, the nectar supply and self-pollination rate of *P. americana*, and the intensity of insect feeding on leaves. Baskett and Schemske (2018) included the caterpillars *Disclisioprocta stellata* Guenée (the most common species, Geometridae), *Psara obscuralis* Lederer (Crambidae), *Asciodes gordialis* Guenée (Crambidae), and *Argyrotaenia velutinana* Walker (Tortricidae) among the caterpillars of *P. americana*. Zhou et al. (2023) used larvae of the polyphagous moth *Spodoptera litura* in their study of *P. americana*.

Dong et al. (2023) found that *P. americana* is a new host of the fungus *Torula phytolaccae*.

Parrella et al. (2021) confirmed by molecular studies and serological methods that *P. americana* is a host plant of *Parietaria mottle virus* (PMoV). Kwak et al. (2024) isolated *Turnip yellows virus* and additionally one of the viroids (*Citrus exocortis viroid*) from a *P. americana* plant showing infestation symptoms. Suzuki et al. (2021) isolated from *P. americana* and reported the complete genome sequence of MiCMV virus, which causes leaf mosaic of *Mirabilis jalapa*. Di (2016) studied the virus that causes *mosaic* in *P. americana*, which breaks the plant resistance associated with the production of antiviral proteins. He highlighted the genetic diversity of isolates from different locations

### IMPORTANCE OF *PHYTOLACCA AMERICANA* IN AGRICULTURE

Throughout the resource, there are few works relating directly to agriculture. This is understandable, as the plant is not commonly found in agricultural fields. Included in the chapter is an extensive paper by Follak et al. (2022) reporting on a case of weed infestation of pumpkin by *P. americana*, as well as papers on the contribution of *P. americana* to pathogen and pest transmission, its potential negative effects on crop plants, and its antiviral and antimicrobial activities. Only one article deals with an attempt to use *P. americana* in animal nutrition.

### Occurrence of *P. americana* in field crops

Follak et al. (2022) described a case of weed infestation in pumpkin crops in Austria. They do not predict that the problem of *P. americana* in fields will increase in the near future, but warn that in a further phase of invasion *P. americana* may start to colonise crops. Based on literature and

surveys, they gave examples of such weeding in vineyards, abandoned orchards, peppers, sunflowers, soybeans, corn, cereals. They warn that, when present in large numbers, it can cause a decrease in yield, contaminate the raw material with seeds and make harvesting difficult. Problems can arise especially on sandy loam soils and with no-till practices. The authors also gave ways to control *P. americana* in crops. Also Strgulc Krajšek et al. (2023), based on their experiments on plant germination, proposed methods to control the occurrence of *P. americana* in fields.

### Pest and pathogen transmission

Kenis et al. (2016) studied the host plants of the East Asian fly *Drosophila suzukii*, which has reached Europe in recent years. The pest attacks soft fruits. They found that *P. americana* is heavily colonised by this insect and may be a reservoir for the pest. Diepenbrock et al. (2016) confirmed the feeding of *D. suzukii* on *P. americana* and concluded that the fly can survive on it during periods of the year when there are no other fruits. Lee et al. (2015) also found the possibility of *D. suzukii* feeding on *P. americana*, but believe that the desirability of removing wild hosts from the field environment needs to be investigated as they may provide an alternative to crops.

Parrella et al. (2021) confirmed that *P. americana* may be a reservoir of *Parietaria mottle virus* PMoV, which can attack tomato and pepper crops (Panno et al., 2021). Kwak et al. (2024) found that *P. americana* can host *Turnip yellow mosaic virus* (TuYV), which can attack oilseed rape (Jajor, Mrówczyński, 2016) and *Citrus exocortis viroid*, a potential threat to tomato crops (Mishra et al., 1991)

### Effects of *P. americana* extracts on crop plants

Mikulic-Petkovsek et al. (2022) investigated the effect of different *P. americana* extracts on germination and growth of stems and roots of the *perennial* ryegrass *Lolium perenne*. The preparation caused a strong reduction in germination and a significant reduction in grass root length. Wang et al. (2024a) found that *P. americana* root extract harvested at the vegetative stage inhibited the growth of wheat and rice seedlings wild and modified, but to varying degrees. Crops reduced growth and produced defensive substances. The authors anticipate that the cereals tested may adapt to the active substances produced by *P. americana*.

### Impact on crop pathogens and pests

Tomlinson et al. (1974) showed that *P. americana* leaf extract has antiviral activity against *Cucumber mosaic virus* (CMV). Sipahioğlu et al. (2017) investigated the possibility of using *P. americana* proteins against Zucchini yellow mosaic virus on pumpkin. They found a protective effect against mechanical infection and a slightly negative effect on the crop. Picard et al. (2005) found that *P. americana* proteins can inactivate *Brome mosaic virus in vitro*

(in barley protoplasts) and reduce its accumulation *in vivo*. They considered that another mechanism besides ribosome depurination was active.

Kobayashi et al. (1995) demonstrated that incubation of P.a. kallus with the fungus *Botrytis fabae* results in the production of a fungicide substance by the plant and identified this compound. Bajpai et al. (2012) studied the effect of extract from the aboveground part of *P. americana* on the pathogenic fungi *Magnaporthe oryzae*, *Rhizoctonia solani*, *Botrytis cinerea*, *Phytophthora infestans*, *Puccinia recondita*, *Blumeria graminis* f. sp. *hordei*, *Colletotrichum coccodes* in pot experiments. The best results were obtained for *Magnaporthe*, *Botrytis* and *Phytophthora*, the extract had no effect on *Blumeria graminis*.

Lopes et al. (2020) tested the insecticidal effect of an extract of, among others, lyophilised leaves and fruits of *P. americana* in dichloromethane and ethanol water. The test subjects were cell cultures of the *Spodoptera frugiperda* butterfly. The best effects, similar to the synthetic insecticide chlorpyrifos, were found with *P. americana* leaves and dichloromethane, but *P. americana* was not qualified for further testing.

#### Use of *P. americana* in animal nutrition

Barnett (1975) showed that *P. americana* fruits were toxic to turkey poult. Their addition to the diet resulted in reduced growth and increased mortality, and in larger quantities led to clumsiness of movement, ankle joint damage, ascites and altered bile composition.

#### OTHER TOPICS

Jasińska et al. (2024) proposed the use of methanolic extract from *P. americana* fruit as a component for the production of a natural, biodegradable, non-toxic material for furcellan and gelatine packaging.

Iglesias et al. (2023) investigated the possibility of using environmentally removed invasive plants in the paper industry. Paper made from *P. americana* had properties comparable to those produced from wood, and interesting surface structural effects predisposed it to artistic applications.

Xue et al. (2016) studied the effect of the addition of *P. americana* powder to a sulphuric acid solution in the leaching of manganese from its ore. They found that the addition of plant material in the range studied increased its efficiency.

Wang et al. (2018) tested the potential of *P. americana* biomass to capture Pb ions from aqueous solutions (potentially wastewater). They found that the material exhibited both physical and chemical sorption, and it was also easy to recover.

Dogan et al. (2015) described, known from Macedonia, the use of *P. americana* leaves as a component of sarma, to wrap rice or meat.

Omokawa et al. (1988) used *P. americana* leaves in a study of the efficacy of triazine herbicides.

#### SUMMARY

The overview of research topics related to the *P. americana* plant presented in this paper is based on two selected bibliographic databases, and only to these can the final conclusions be referred. It is important to be aware that the world literature is probably much richer, covers a wider time range, and contains more papers from the plant native range.

On the basis of the overview presented, it can be concluded that the research topics related to the *P. americana* are extensive and concern both basic and applied sciences. The largest part of the selected material consists of articles related to the biochemical properties of the plant. For many compounds, the papers include not only a detailed description of the properties and chemical structure, but also the genetic basis of their production by the plant. However, only a few articles date from recent years, and the rapid development of analytical methods makes it possible to deepen study and verify previous hypotheses. Both research into the influence of various factors on the production of active substances and results enabling the efficient and safe production of compounds that have already found industrial application are still needed.

Many articles refer to the possibility of using the active substances of *P. americana* in medicine, and there are many new reports published after 2020. This is due to the high demand for effective yet safe medicines and natural methods of maintaining health, which also explains the drawing on the knowledge of folk medicine. Changing risk factors are prompting the search for new remedies and therapeutic methods, also through new applications of already described compounds. However, publication possibilities in this area are sometimes restricted by patent requirements or trade secrets.

The publications on the physiology of *P. americana* are mainly concerned with the initial developmental phases and the characteristics that facilitate its adaptation to new conditions, including those predicted for the future. However, the information presented in the articles analysed is fragmentary and needs to be supplemented from other sources, for example appendix bibliography, or new research conducted. Several papers refer to the already known ability of *P. americana* to hyperaccumulate heavy metals, but new reports also speak of its potential in the phytoremediation of soils contaminated with rare earth metals.

The ecological research discussed in the resource under review focuses primarily on the competitiveness of *P. americana* in relation to other, including invasive, plants. Much space is devoted to the topic of habitat requirements, which are related to the ability and rate of colonisation of new areas. Recent articles in this area are often the result of collaboration between specialists from different countries. *P. americana* has been present in Europe or Asia for a short time and strategies to predict and reduce its range and potentially detrimental effects on biodiversity are still

relevant. For obvious reasons, there is a lack of information on the relationship of *P. americana* with other organisms (plants, animals, microorganisms) and the environment in new areas. This is an interesting field for original research, as such data should relate to local conditions. The research on the ecology of *P. americana* as a potentially invasive species is also needed to provide a scientific basis for policy decisions and legislation.

In the resource analysed, there are few reports on the impact of *P. americana* on field crops. At present, there is not much threat to crops in Europe from this species, but, according to the authors quoted, it may appear in later stages of invasion. It is important to carry out research in this area, taking into account local production conditions: soil characteristics, tillage methods, crop rotation, field size and location in the agricultural landscape.

Previous experience with other plants shows that it is not possible to effectively eradicate from the environment a plant species that has found good conditions for growth in a new area. Besides, changes in the species composition of both the flora and representatives of other systematic groups are only a visible manifestation of uncontrollable changes in the environment. Hence, it is likely that *P. americana* will become increasingly common, also in Poland, and information about it will therefore become more important. Its increasing availability is an opportunity not only for, previously impossible, environmental research, but also for innovative uses, examples of which are presented in the literature resource under discussion.

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