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REVIEW SCIENTIFIC ARTICLE

Aleksandra M. SAVIĆ[*]

Institute for Plant Protection and Environment, Department of Herbology
Teodora Drajzera 9, Belgrade 11000, Serbia

Ambrosia trifida L. (GIANT RAGWEED)

ABSTRACT: *Ambrosia trifida* L. belongs to the group of invasive weeds, which in colonized areas cause great damage to the biodiversity of autochthonous flora and vegetation as well as in plant production. It originates from North America wherefrom it has spread to many parts of Europe. In Serbia it is currently locally present in the area of Bačka (the northern region of the country). Early and rapid growth rate, vegetative and generative production, high degree of morphological and reproductive plasticity have given it a competitive advantage over many other weeds, hence in many countries it is considered one of the most problematic weeds in agricultural production. *A. trifida* could cause great damage in root crops, vegetable gardens and orchards and its harmfulness is measured by the negative impact on biodiversity by suppressing indigenous and other non-indigenous species. With its allergens, *A. trifida* negatively affects human health. Observing its vegetative and generative potential and climate change on the other hand, recent research indicates the potential for the spread of *A. trifida* in our country and in Europe, which could be a serious risk for agrophytocenoses and the ecosystem as a whole. In 2019, it was added to the EPPO A2 List of quarantine pests recommended. It can be controlled with the use of mechanical, biological and chemical measures.

KEYWORDS: *Ambrosia trifida* L., Ambrosia spp., allergenic species, competition, invasive weeds, secondary metabolites, weed control, yield losses

INTRODUCTION

Invasive plant species may be a serious threat to crop fields and natural habitats (Pyšek et al., 2009; Vilà et al., 2011; Essl et al., 2009; 2015). Since they do not have their particular natural agent of control in the new area, they can grow fast and compete for resources. If establishment is effectual, invasive species generate noticeably (two to five times) more biomass than indigenous species and better exploit the available resources (Szymura et al., 2018). Considering their conspicuous competitive potential, they suppress indigenous populations and gravely disturb biodiversity (Gioria and Osborne, 2014).

[*] Corresponding author. E-mail: aleksandra.m.savic@gmail.com

The species of genus Ambrosia (family Asteraceae) are widespread and heavily deleterious invasive plants. The genus has around 40 species, originating mostly from North America and they have been introduced into new countries in the 19th century (Makra et al., 2005; Gerber et al., 2011; Smith et al., 2013). In North America and Europe, it started causing grave trouble in the past decades, contributing to an evident increase in respiratory allergic reactions (D'Amato and Spieksma, 1992; D'Amato, 2007). Pronounced adaptability to new conditions makes them able of surviving and generating colony of an invasive species. Beside *Ambrosia artemisiifolia* L., which is the most damaging species of this genus (Kazinczki et al., 2008), *Ambrosia trifida* L. also causes considerable damage in agricultural production (Weaver, 2001; Harrison et al., 2001). It has been present in numerous countries in Europe (Follak et al., 2013, www.cabi.org), and its harmfulness in crop has been remarked in Serbia (Malidža and Vrbničanin, 2006). In recent years, *A. trifida* has been a species that has attracted the attention of many researchers (Harrison et al., 2001; Follak et al., 2013; Page and Nurse, 2015; Savić et al., 2019a, b; 2020a, b; 2021).

TAXONOMIC HIERARCHY

Domain: Eukaryota
 Kingdom: Plantae
  Phylum: Spermatophyta
   Subphylum: Angiospermae
    Class: Dicotyledonae
     Order: Asterales
      Family: Asteraceae
       Genus: Ambrosia
        Species: *Ambrosia trifida*
         Sub-species: *Ambrosia trifida* var. *texana* and *Ambrosia trifida* var. *trifida*
          EPPO code: AMBTR

DISTRIBUTION

Ambrosia trifida originates from North America and it is distributed from Western to Central Europe. Currently, it inhabits all continents except Africa and Oceania. It is assumed that it was introduced by imports of commercial grain and oilseed and during World War II, when military movements were also vectors of introduction for this species in Europe (Lawalree, 1947; Follak et al., 2013; Ardenghi and Polani, 2016). At this time, it is present in many parts of Europe (Slovak, Serbia, Germany, Switzerland, Italy, Slovenia, Bulgaria, Austria, Romania, Czech Republic, etc.). Furthermore, this species it has been introduced in Asia, Japan, China, Mongolia and South Korea, Georgia, Israel, etc.

(Follak et al., 2013; www.cabi.org). In 2019, *A. trifida* was added to the EPPO A2 List of quarantine pests.

Follak et al. (2013) researched the rate of spread of *A. trifida* in Central and Eastern Europe. The number of reports peaked in the periods 1951–1990 and thereafter reduced suddenly. For a relatively long time, the percentage of established populations was rare. It is supposed that the decrease in the spread rate was impacted by the fact that *A. trifida* mostly inhabits ruderal habitats and thus its distribution is less intense than invasive species present in rural and urban areas (Follak et al., 2013; Chauvel et al., 2015). Seeds are rich in fat and protein and Harrison et al. (2003) found that predators (moths, mice and birds) feed on seeds of *A. trifida* and in that way they reduce the number and limit its spread to some extent. In addition, it is determined that seeds of *A. trifida* are a food for species from the order *Diptera*, *Lepidoptera* and *Coleoptera* (Harrison et al., 2001). The percentage of identified populations did not change for a relatively long period. However, it is recently reported that the population of *A. trifida* is permanently increasing and this fact could be connected to climate changes which are affecting almost all European countries (Johnson et al., 2005; Follak et al., 2013; Mora et al., 2018). Since it is well adjusted to warm habitats with more light and nutrient-rich soils (Dinelli et al., 2013), it is assumed that climate change has affected its re-spread.

On the Balkan Peninsula, it was first found in the 1980s in Banat (Serbia) (Koljadžinski and Šajinović, 1982). A few years later, it was recorded in Slovenia (Vasić, 1990). After that, Malidža and Vrbničanin (2006) recorded a large population of *A. trifida* in Central Bačka (Despotovo, Kucura, Savino Selo and Ravno Selo). Thereafter, reports on its incidence in the crop fields have arisen in Serbia, specifically in Pannonia Plain region (along the roads in villages, between settlements, and on field edges, as well as in sunflower, maize, soybean, and sugar beet crops) (Malidža and Vrbničanin, 2006; Vrbničanin et al., 2012; 2015).

BIOLOGY AND ECOLOGY

Ambrosia trifida is a broadleaf annual plant (therophyte, T4) which is only propagated from seeds. Drier, warm habitats, with more light, and the nutrient-rich soils suit this species (Vrbničanin et al., 2015). Taking into account different growth conditions, it can display substantial plasticity in height, degree of branching, number and size of leaves, amount of reproduction (Abul-Fatih and Bazzaz, 1980). It forms an upright, strong and branched stem, covered in short, white hairs in its upper part, while in the lower part the stem is bare. Root is very strong, with a dense system of lateral roots. *A. trifida* is also characterized by leaf variability, where individuals often form a leaf plate with three to five lobes, or without lobes, and sometimes the lobes absence is a result of varied environmental conditions during the plants development. *A. trifida* is a diploid species (2n = 24; Payne, 1964). In nature, there has been observed the formation of hybrid forms between *A. artemisiifolia* and *A. trifida* (a new taxon, *A*. x *helenae*) which affects the creation of morphological and genetic diversity (Wagner, 1958; Vincent et al., 1988; Strother, 2006).

The final plant height may reach 6 m. Their leaves (20–30 cm in length) are mainly placed in the terminal part of the plant and depending on their size and position; the available light is notably reduced to the other species (Basset and Crompton, 1982). This species has high photosynthetic (C3 photosynthesis system) and net assimilation rates (Abul-Fatih and Bazzaz, 1979). It is a monoecious plant which flowers and produces seeds from July to September. An allometric fruit distribution is typical for this species (Harrison et al., 2001). The flowers are unisexual, with males flowers producing pollen and females flowers producing seeds. Anthers in male flowers form clusters, located on the terminal part of the plant and contain 10–15 flowers grouped in inflorescences up to 30 cm long (Bassett and Crompton, 1982). Female heads are 6–10 mm in size and are located in the axils of the upper leaves. The pistils are located in groups at the leaf base below the anthers. One plant can produce about 10 million pollen grains a day. Pollen grains (tricolpate, three-celled pollen) (16–27 µm in diameter) are spherical with spines and spinules (Basset and Terasmae, 1962; Curtis and Lersten 1995; Liu et al., 2012). The pollen grain is round with spikes and contains about 50 proteins (antigens) that act as allergens. During the day, the concentration of pollen is highest from five to ten o'clock in the morning. Pollen stays in the air for more than 100 days, with the highest concentrations in August and September (Johnson et al., 2007). Pollination in this species is anemophilous and is more successful between than within individual plants (Bassett and Crompton, 1982). The fruit is achenia (seed) and characterized by 6–8 blunt teeth at the top, without papules, grey-green to light dark in colour. The achenes are 6–11 mm wide and 7–14 mm long (Bassett and Crompton, 1982). *A. trifida* produces up to 5,000 seeds/plant (Abul-Fath and Bazzas, 1979). Seeds of this species can germinate at a depth of 0.5 cm, but active soil seed bank is typically up to 5 cm of soil depth, however, large seed size makes it able to germinate from deep soil of 16 cm (Abul-Fath and Bazzas, 1979). Depending on the depth of seed burial, it can establish a seed bank of soil for up to 21 years (Toole and Brown, 1946; Stoller and Wack, 1974; Harrison et al., 2007).

Seeds are polymorphic (of different dimensions and colours). Larger seeds have a higher ability to germinate. The seeds fall from the plant in the period of full physical maturity (autumn). The incorporation of seeds into the soil takes place with the help of precipitation, the activity of earthworms and other predators or during tillage (Harrison et al., 2003). In addition, the germination rate of this species could also be affected by other weed species which, if their competitive characteristics enable it, hinder germination, growth and development (Savić, 2019a, b; 2020a, b; Savić et al, 2021). However, *A. trifida* germinates in early spring, so it has a potentially much better chance than other species to occupy a given area and achieve its vegetative and generative production (Savić, 2020b). *A. trifida* germinates and emerges from early spring (March/April). It germinates at wide range of temperatures (from 4 to 41 °C), with an optimum between 10 and 24 °C. It is preferable if soil moisture conditions are suitable (17% to 55% soil moisture, with an optimum at 20% to 30%)

(Ballard et al., 1996). Opposite that, Schutte et al. (2008) found that emergence occurs during relatively dry periods. Additionally, Cui et al. (2007) showed that *A. trifida* is a good accumulator of heavy metals (Pb, Zn, Cu, Cd) at the root level and it can grow successfully in the soil where the concentration of metals is quite high.

COMPETITIVE ABILITY

Many scientists have paid special attention to studying the competitive abilities of *A. trifida* (Abul-Fatih and Bazzaz, 1979; Webster, 1994; Williams and Masiunas, 2006). It is a highly competitive species that can dominate the annual plant community due to a rather early germination and emergence and high rates of growth (Webster, 1994; Malidža and Vrbničanin, 2006; Follak et al., 2013; Harrison et al. 2001; Page and Nurse, 2015). As a strong competitor, it efficiently draws water and nutrients from the soil and thus reduces the natural resources needed for the growth and development of other plants, which can lead to significant losses in crop yields. When it occurs on agricultural land, it quickly conquers space and thus hinders the growth and development of crops, especially wheat, corn, soybeans, sunflowers, beans and other crops (Weaver, 2001; Williams and Masiunas, 2006; Vrbničanin et al., 2012). *A. trifida* is more competitive and causes greater yield losses in soybeans than in maize. Some authors have reported yield losses of 13% in maize and 50% in soybean with as few as one plant of *A. trifida*/m^2 (Baysinger and Sims, 1991; Harrison et al., 2001). Additionally, Harrison et al. (2001) found yield loss in maize of 60% with 14 plants of *A. trifida*/10 m^2. According to these results, Harrison et al. (2001) predicted yield losses of maize as high as 90% if *A. trifida* density was 14 plants/m^2. Similar study determines that 1.7 *A. trifida* plant/10 m^2 reduces yield of maize by 18% (Webster et al., 1994). If we compare the influence of two different species of ragweed on maize yield, one plant/m^2 of *A. artemisiifolia* and *A. trifida* reduced maize yield by 6% and 14% (Weaver, 2001; Harrison et al., 2001). Unlike *A. artemisiifolia*, *A. trifida* reduced twice the maize yield, so compared to *A. artemisiifolia* it can be characterized as more harmful to crops (Weaver, 2001; Harrison et al., 2001). According to Vrbničanin et al. (2012) 2 plants of *A. trifida*/m^2 reduced dry mass of sunflower by 25.3%. Webster et al. (1994) found that one plant of *A. trifida*/m^2, can reduce soybean yield by 77%, unlike other species at the same conditions (*Abutilon theophrasti*, *Amarantus retroflexus*, *A. artemisiifolia*, *Chenopodium album* and *Datura stramonium*) that reduce the yield soybeans by 9, 18, 12, 14 and 15%, respectively (Rathmann and Miller, 1981; Kirkpatrick et al., 1983; Weaver, 2001; Bensch et al., 2003). According to other authors, only a few plants of *A. trífida*/m^2 reduced soybean yield by 70%, while by the similar density *Xanthium strumarium* and *A. artemisiifolia* can reduce soybean yield by 30% and 15% (Coble et al., 1981; Bloomberg et al.,1982). *A. trifida* has relative strong interspecific competitive ability (Montagnani et al., 2017). Liebman and Nichols (2020) modelled *A. trifida* population dynamics in different crop rotation, where rotations of crops (two-year corn-soybean; corn-soybean-rye-alfalfa system) have a higher probability of controlling *A. trifida* populations.

Although it can cause grave damages to the crops, it can also negatively affect biodiversity by suppressing other species (Abul-fatih and Bazzaz, 1979; Follak et al., 2013). There are not much data on studying the competition of weeds. However, some literature sources cite the results of inter/intraspecies competition between *A. trifida* and other weeds species. Savić et al. (2019a; 2020a, b; 2021) revealed that the different ratio of *A. trifida* and *A. artemisiifolia*/m^2 was reflected in all vegetative parameters. With an increase in the number of *A. trifida*/m^2 in different treatments (20/80%, 40/60%, 60/40%, 80/20%, 100/0% *A. trifida/A. artemisiifolia* /m^2) where the total numbers were 10 and 100 plant/m^2, values of vegetative parameters (height, plant width, number of leaves and dry mass) of *A. trifida* decreased. Its highest dry weight was recorded in treatments with its lowest abundance *A. trifida* (20%/m^2) compared to *A. artemisiifolia* (80%/m^2) ,while its lowest dry weight was recorded in monoculture treatment of *A. trifida* (100%/m^2), which indicates a more pronounced intraspecific competition. Taking into account the vegetative production of *A. trifida*, especially at a lower number of plants/m^2 compared to *A. artemisiifolia*, its competitive strength is more pronounced. Moreover, the large vegetative production of *A. trifida* enabled greater dominance in relation to other weed species *Chenopodium album*, *Polygonum aviculare*, *Setaria viridis*, *Bilderdykia convolvulus*, *Echinochloa crus-galli*, *Sorghum halepense* (Savić, 2020; Savić et al., 2021). Savić et al. (2021) found that due to more pronounced intraspecific competition, with a high population *A. trifida* suppressing itself, harmful effects on other species and ecosystem occurs only with a smaller number of plants per unit area (up to 40, maximum 50 plants/m^2). At high numbers, intraspecific competition occurs; the number of *A. trifida*/m^2 decreases, and this favours the growth and development of other plant species.

IMPACT OF SECONDARY METABOLITES OF *A. trifida* ON OTHER ORGANISMS

Many weed species have been characterized as highly recognizable for their secondary metabolites that influence the germination of other plants in nature (Todaria et al., 2005). In species of the genus Ambrosia, allelopathic influence on neighbouring plants has been confirmed. Several studies have shown that these species can synthesize various secondary metabolites including flavonoids, sesquiterpenes, lactones, phenolic acids, ambrosine, isabelin, psilostachine, and others. These compounds have been found to have a broad spectrum of biological activity, primarily inhibiting or stimulating the growth of other species (Beres et al., 2002; Wang et al., 2005; Kong et al., 2010).

The allelopathic effects of *A. trifida* on other plants are also well documented. Root exudates, leaf leachate, and decaying leaves produce allochemical compounds that inhibit germination and growth of other species, both in natural and agricultural environments (Kong et al., 2007). Secondary metabolites (carotenesquiterpenes, thiarubrins and thioephenes, etc.) isolated from this species show biological activities on microorganisms and nematodes and some of them inhibit the growth of other plants (Wang et al., 2006; Kong et al., 2007). Sarić-Krsmanović et al. (2020) determined components of an essential oil produced from leaves of *A. trifida* (monoterpene hydrocarbons 25%; oxygenated monoterpenes 35%; sesquiterpene hydrocarbons 22%; oxygenated sesquiterpenes 13%; phenylpropanoids 0.8%). According to these authors, an increase in essential oil concentration leads to decrease in seed germination of watermelon, lettuce, tomato and cucumber. Wang et al. (2005) revealed that the volatile oil of *A. trifida* significantly inhibited the seed germination and seedling growth of maize and wheat. Kong et al. (2007) confirmed that the high invasiveness of *A. trifida* resulted in side effects on wheat growth and yield. *A. trifida* produces sesquiterpenes of the carotene type (1 α-angelo-yloxy-carotol and 1 α-2-methyl-butyro-yloxy-carotol) and their low concentrations (20 mg^{-1}) can inhibit wheat growth by more than 10% (Williams and Masiunas, 2006; Kong et al., 2007). Wang et al. (2006) confirmed the main essential oil components of *A. trifida* (bornyl acetate, borneol, caryophyllene oxide, α-pinene, germacrene D, β-caryophyllene, trans-carveol β-myrcene, camphor, limonene) and determined strong bactericidal and fungicidal activity against *Staphylococcus aureus*, *Candida albicans*, *Klebsiella pneumoniae*, while *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Asperigillus niger* were less sensitive. In addition, *A. trifida* was recorded as a host of the *Xylella fastidiosa* (Black et al., 2004), aster yellows, tobacco mosaic, tobacco ring spot and tobacco streak viruses (Royer and Dickinson, 1999).

Interestingly, the presence of *A. trifida* may contribute to a lower number of parasitic nematodes. The study revealed a tendency that the number of nematodes in the soybean rhizosphere with the presence of *A. trifida* was lower than without *A. trifida*. Populations of several parasitic nematodes in soybean rhizospheres were suppressed by the presence of *A. trifida* and in particular, the number of *Aphelenchoides*, *Filenchus* and *Tylenchus* nematodes was significantly reduced. Additional experiments showed that the root of *A. trifida* secreted allelochemicals, such as acetylenes and their sulfur derivatives, which show high biological activity against parasitic nematodes in neighbouring soybean rhizospheres (Wang et al., 1998).

Contrary to the negative influence of this species on the ecosystem, Ahmad et al. (2013) state the positive characteristics of this species and possibilities converting of *A. trifida* biomass to biochar and its use as an adsorbent for the depuration of trichloroethylene contaminated water. Furthermore, Yakkala et al. (2013) confirmed that biochar derived from vegetative tissues of this species can be used to remove heavy metal ions from aqueous solutions.

PREVENTION AND CONTROL OF *A. trifida*

As one of the plants with strong allergenic properties, which grow in our climate, it must be systematically controlled in order to protect and improve human health and the environment, which includes the application of various measures: preventive, agrotechnical, mechanical, chemical and administrative measures. In order to control the spread of *A. trifida,* it is necessary to carry out its constant destruction. The main goal of the control programme of *A. trifida* should be based on the attempt to reduce seed dispersal and the formation of new populations in new habitats, as well as to reduce their numbers below the harmfulness threshold. The choice of measures depends on the crop in which this species is located, the method of cultivation as well as the presence of other weed species. The application of integral control measures of *A. trifida*, as in the case of other weeds, implies the application of all measures (crop rotation, tillage, planting of quality planting material, crop care, proper fertilization, application of biological control measures and herbicide application). Mowing will effectively reduce generative production. Morover, control of *A. trifida* using electrical discharges proved to be good to excellent (Rasmusson et al., 1980). In general, in the conditions of intensive agricultural production, agrotechnical measures and herbicides are mostly used. There is a little research on the control of newer weed species such as *A. trifida*.

Many publications have reported results of potential biocontrol agents of *A. trifida* (insects, fungi, bacteria). They have been identified as biological agents for suppressing ambrosia species: *Stobaera concinna*, *Trigonorhinus tomentosus*, *Tarachidia condefacta*, *Euaresta bella* and *E. festiva* (Sheppard et al., 2006), *Zygogramma suturalis*, *Epiblema strenuana* (Zhou et al., 2014). Additionally, some microorganisams can be used for biocontrol of *A. trifida*. For example, *Puccinia xanthii* forma *specialis ambrosid-trifidae* (Batra, 1981) and the bacterial pathogen *Pseudomonas syringae* pv. *tagetis* are the causal agents of a disease characterized by apical chlorosis on several members of Asteraceae. In many countries, introductions of biological control agents have been made against Ambrosia spp. in China, Australia, Russia, Georgia (Julien and Griffiths, 1998). The most of biocontrol agents are specific only to the genus of ragweed, and no doubt some of the successfully introduced agents also attacked *A. trifida* if it was present.

Registered active substances that can be used to control of *A. trifida* are: 2, 4-D, bentazone, glyphosate, chlorimuron, dicamba, diflufenzopyr, glyphosate imazaquin, acifluorfen, imazethapyr, isoxaflutole, mesotrione, prosulfuron, rimsulfuron (Weed Science Society of America, 2003). In addition to growth traits, the species propensity to develop resistance contributes to the successful survival of *A. trifida* populations. As one of the most competitive weeds in row crops, it has evolved resistance to multiple herbicide biochemical sites of action within the plant, necessitating the development of new and integrated methods of weed control. Glyphosate-resistant of *A. trifida* was first reported in 2004 (Stachler, 2008) and as of 2016 has been confirmed in 12 US states (Heap, 2016; Vink et al., 2012). Moreover, populations of this species have been reported to be resistant to (ALS) – inhibitors acetolactate synthase, which raises concerns about future chemical control choices (Patzoldt and Tranel, 2002).

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ПРЕГЛЕДНИ НАУЧНИ РАД

Ambrosia trifida L. (АМБРОЗИЈА ТРОЛИСНА)

АЛЕКСАНДРА М. САВИЋ

Институт за заштиту биља и екологију
Одсек за хербологију
Теодора Драјзера 9, Београд 11000, Србија

РЕЗИМЕ: *Ambrosia trifida* L. припада групи инвазивних корова који у колонизованим подручјима наносе велику штету биодиверзитету аутохтоне флоре и вегетације, као и биљној производњи. Пореклом је из Северне Америке, одакле се раширила у многе делове Европе. У Србији је тренутно локално присутна на подручју централне Бачке. Рана и брза стопа раста, вегетативна и генеративна производња, висок степен морфолошке и репродуктивне способности, омогућили су јој конкурентску предност у односу на многе друге корове, па се у многим земљама сматра једним од најпроблематичнијих корова у пољопривредној производњи. *A. trifida* би могла проузроковати велике штете у окопавинама, повртњацима и вочњацима, а њена штетност се мери и негативним утицајем на биодиверзитет потискујући алохтоне и аутохтоне врсте. Својим алергенима негативно утиче на здравље људи. Имајући у виду њен вегетативни и генеративни потенцијал и узимајући у обзир климатске промене (које су захватиле многе земље), новија истраживања указују могућност ширења ове врсте у нашој земљи и Европи, што би могло представљати озбиљан ризик за агрофитоценозе и екосистем у целини. Од 2019. године додат је на EPPO A2 листу препоручених карантинских штеточина. Може се сузбијати механичким, биолошким и хемијским мерама.

КЉУЧНЕ РЕЧИ: *Ambrosia trifida* L., алергене врсте, губици приноса, инвазивни корови, компетиција, секундарни метаболити, сузбијање корова