

# EPPO Datasheet: *Solanum carolinense*

Last updated: 2023-10-23

## IDENTITY

**Preferred name:** *Solanum carolinense*

**Authority:** Linnaeus

**Taxonomic position:** Plantae: Magnoliophyta: Angiospermae:  
Lamiids: Solanales: Solanaceae

**Other scientific names:** *Solanum floridanum* Raf., *Solanum obliquatum* Raf.

**Common names:** Carolina horse nettle, bull nettle, horse nettle (US), sand brier

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**EPPO Categorization:** A2 list, Alert list (formerly)

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**EPPO Code:** SOLCA



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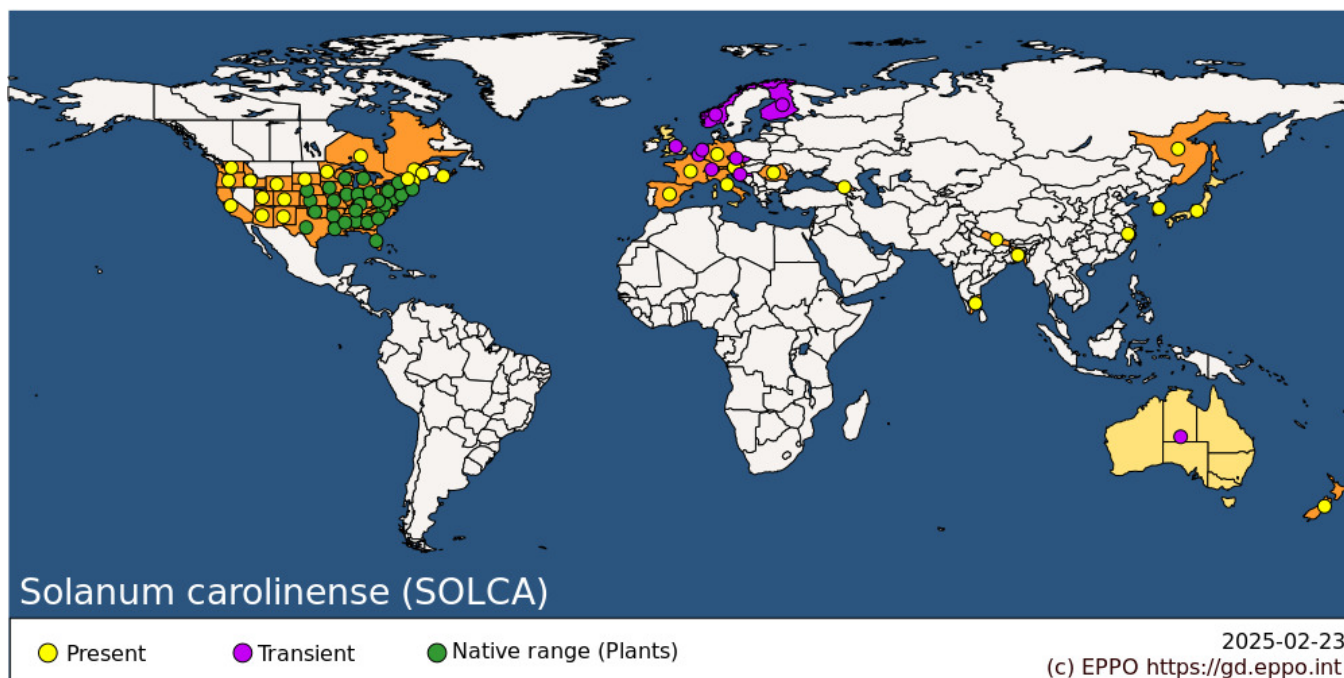
## GEOGRAPHICAL DISTRIBUTION

*Solanum carolinense* is native to North America (USDA, NRCS, 2021). The species occurs in all states of the USA except Nevada, Montana, North Dakota, Hawaii and Alaska (USDA, NRCS, 2021; Wahlert *et al.*, 2015). It also can be found in the southernmost parts of Canada (Quebec, Ontario) as well as in Nova Scotia (Bassett & Munro, 1986; VASCAN, 2021).

The database USDA, NRCS (2021) indicates ‘native’ for all states, although it is clear that the species has spread and has now invaded other parts of the USA (Wahlert *et al.*, 2015). USDA GRIN (2021), for example, recognizes 32 states including 1 province in Canada (Ontario) and 1 state in Mexico (Sonora) as native. Wahlert *et al.* (2015) pointed out that the native range prior to European settlement could not be determined with full certainty. In their study, they interfered its native distribution based on herbarium specimens and stated that its distribution ‘... extends from central Florida north to New York and Massachusetts and west to Texas, Oklahoma, Kansas, and Nebraska to about the 97th meridian west’.

The occurrence of the species in Mexico, Haiti and in South America is not entirely conclusive. Websites such as [inaturalist.org](http://inaturalist.org) and databases (CABI, 2021; GBIF, 2021; USDA GRIN, 2021) list findings of *S. carolinense*. However, Martínez *et al.* (2017) do not list the species in Mexico. Wahlert *et al.* (2015) stated that there is no evidence that *S. carolinense* ‘... has been collected in Brazil since the time of Pohl's collections [from 1852] (Stehmann *et al.*, 2013)’. The authors also do not recognize any further occurrences in Mexico, Central or South America.

*Solanum carolinense* was first introduced into the EPPO region presumably in the second half of the 20th century. Early records were of small, transient populations scattered across the EPPO region (e.g. in Belgium, Croatia, Georgia, the Netherlands, and Norway).



**EPPO Region:** Austria, Belgium, Croatia, Czech Republic, Finland, France (mainland), Georgia, Germany, Italy (mainland), Netherlands, Norway, Romania, Russia (Far East), Spain (mainland), Switzerland, United Kingdom

**Asia:** Bangladesh, China (Zhejiang), India (Tamil Nadu), Japan, Korea, Republic, Nepal

**North America:** Canada (Nova Scotia, Ontario, Québec), United States of America (Alabama, Arizona, Arkansas, California, Colorado, Connecticut, Delaware, District of Columbia, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New Hampshire, New Jersey, New Mexico, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Utah, Vermont, Virginia, Washington, West Virginia, Wisconsin, Wyoming)

**Oceania:** Australia, New Zealand

## MORPHOLOGY

### Plant type

Perennial herb.

### Description

The following description is primarily based on Bassett and Munro (1986) and Wahlert *et al.* (2015): *S. carolinense* is a perennial herb, up to 1.2 m tall, unbranched or branched near the base, with both vertical and horizontal roots, the latter spreading horizontally up to 5 m. Stems are armed with slender yellowish spines (prickles) up to 6 mm long. Leaves are also sparsely to moderately armed with prickles up to 6.5 mm long on the major veins abaxially and adaxially. Leaf blades 2–15 × 2–10 cm in size, margins lobed with 1–4 lobes per side, sometimes very deeply lobed almost to the midrib, apex is acute to obtuse, and the petioles are 0.4–4 cm in size. Inflorescences consist of 1–20 flowers. They are white, lilac, or purple and star-shaped with five yellow poricidal anthers. Fruits are 1–2 × 1–1.8 cm in size, light green with darker green mottling or pale greenish-white when immature, bright yellow at maturity and glabrous. Seeds are 1.7–2.4 × 1.6–1.8 mm in size, flattened-reniform, lenticular, yellow, and the surface is finely foveolate.

## BIOLOGY AND ECOLOGY

### General

In the north-east of the USA, the growing season (i.e. seed emergence) of *S. carolinense* begins in the middle of May (Ilnicki & Fertig, 1962). In Northern Florida, the growing season typically begins in April and ends in October (Hakes *et al.*, 2018). Bassett and Munro (1986) stated that the species reaches anthesis by early July, while fruits begin to mature by mid-September in Canada. In Japan, the period of shoot emergence is from late April to early June (Miyazaki *et al.*, 2005; Miyazaki & Ito, 2004).

*Solanum carolinense* propagates by creeping roots and seeds. The extensive root system consists of a taproot and horizontally growing roots (Ilnicki & Fertig, 1962; Miyazaki, 2008). The taproot can reach a depth of 240 cm and the roots grow horizontally in soil depths up to 45 cm and become several metres long (Ilnicki & Fertig, 1962). Miyazaki (2008) demonstrated that different sections of the root system had different functions: the bending part forms new shoots; the horizontal part extends into the surrounding area; and the vertical part is used for storage. Shoots are produced from adventitious root buds. In this way, the species can form large clusters (up to 10 m from the parent plant) covering large areas within a few years. Belowground parts over-winter, and new shoots (= ramets) emerge in the spring. Root fragments form buds within a few weeks and thus new plants. Ilnicki & Fertig (1962) demonstrated that fragments 2 cm in length and 3.5 mm in diameter show a 100% regeneration success. Root fragments grown in a greenhouse at 23–32°C showed regeneration at a level of 63% for 1 cm length and 94% for 2 cm length (Wehtje *et al.*, 1987).

*Solanum carolinense* is a prolific seed producer. It can produce ca. 40–170 seeds per fruit, with a single plant producing up to ca. 5000 seeds (Bassett & Munro, 1986; Ilnicki & Fertig, 1962). Seeds can germinate and plants emerge from depths of 10 cm (Ilnicki & Fertig, 1962). Seeds retain viability for at least 3 years when buried at depths of 8–12 cm according to Brown and Porter (1942). Solomon (1983) remarked that seeds remained viable for at least 7 years when stored under laboratory conditions.

*Solanum carolinense* is pollinated by a variety of generalist insects. In North America, non-specialist bees (*Lasioglossum* spp., *Bombus* spp., *Xylocopa* spp.) are described as the main pollinators of this species (Quesada-Aguilar *et al.*, 2008; Wahlert *et al.*, 2015). The poricidal anthers must be vibrated by pollinators to release pollen (i.e., buzz pollination; Hardin *et al.*, 1972). *S. carolinense* is an andromonoecious species (i.e. plants bear either hermaphrodite flowers or male flowers or both) with a system of gametophytic self-incompatibility (GSI; Travers *et al.*, 2004), which is quite uncommon among other weed species. Travers *et al.* (2004) showed that there is some plasticity in the strength of GSI in *S. carolinense*: flowers become more self-compatible as they age and self-fertility increases on plants when cross pollen is scarce. Moreover, genotypes differ in their degree of self-fertility indicating ‘... that there is broad sense heritability for plasticity in the strength of self-incompatibility’ (Travers *et al.*, 2004). See Kariyat *et al.* (2011) for further details.

## Habitats

In North America, *S. carolinense* grows in various habitats, such as prairies, deciduous woodlands, swamps, and pine forests, and in disturbed areas such as road-sides, grazed and mowed pastures, ditches, cultivated fields, urban waste areas, and utility and railroad embankments (Wahlert *et al.*, 2015). The species is a weed in many crops (Table 1).

Table 1. Main crops which *Solanum carolinense* is associated with.

Crop	Country (ISO code)	References
<i>Arachis hypogaea</i>	US	Hackett <i>et al.</i> (1987)
<i>Beta vulgaris</i>	IT	Vidotto and Selvaggi (2028)
<i>Cucurbita pepo</i>	AT	Follak (2020)
<i>Glycine max</i>	AT, IT, RU, US	Follak (2020); Hackett <i>et al.</i> (1987); Van Wychen (2015)
<i>Gossypium hirsutum</i>	US	Hackett <i>et al.</i> (1987); Van Wychen (2015)

<i>Medicago sativa</i>	US	Van Wychen (2015); Van Wychen (2020)
<i>Phaseolus vulgaris</i>	US	Frank (1990)
<i>Solanum tuberosum</i>	US	Hackett et al. (1987); Van Wychen (2015)
<i>Triticum aestivum</i>	US	Hackett et al. (1987); Van Wychen (2015)
<i>Zea mays</i>	AT, FR, IT, US	Whaley and Vangessel (2002); Vidotto and Selvaggi (2018)

Within the EPPO region, the species is recorded as growing in different habitats including banks of major rivers (e.g. Waal; Dirkse *et al.*, 2007), ruderal habitats (e.g. roadsides, port areas; Junghans, 2013; Pérez *et al.*, 2020), pastures and crop fields (Follak, 2020; Hohla & Zahlheimer, 2018; Klingenhagen *et al.*, 2012; Selvaggi *et al.*, 2018). In Austria, it invades roadsides and crop fields, such as maize, oil-pumpkin and soybean (Follak, 2020). In Italy, the species has been recorded in crops, such as sugar beet, beans and soybean, mainly in Northern Italy (Saglia *et al.*, 2006; Selvaggi *et al.*, 2018). Additionally, the species has also been reported from disturbed sites such as roadsides and ditches (Barberis *et al.*, 2014; Zanotti, 1993). In the Netherlands, since 2004, *S. carolinense* has been found in several localities on sandy riverbanks particularly along the River Waal (Dirkse *et al.*, 2007; FLORON Verspreidingsatlas Vaatplanten, 2021).

Apart from its presence on banks of rivers, *S. carolinense* is not recorded in natural habitats in the EPPO region.

### Environmental requirements

*Solanum carolinense* occurs over a wide climatic range. In North America, *S. carolinense* occurs predominantly between northern latitudes of 28° to 45° and western longitudes of 70° to 98° (GBIF, 2021; Wahlert *et al.*, 2015).

The distribution of *S. carolinense* is limited in cool environments by intense frost, and the length of the growing season (Bassett & Munro, 1986). Stems are frost sensitive and tops usually die following frost in autumn. Roots of *S. carolinense* can tolerate low temperatures of 3°C (in 6 cm soil depth), but were killed at temperatures between ?2 and ?4°C (Bassett & Munro, 1986; Wehtje *et al.*, 1987). Nishida *et al.* (2004) reported that roots (0.5 mm in diameter and 35 cm in length) from seedlings were not killed at ?4°C for 12 h.

*Solanum carolinense* needs warm temperatures for germination, sprouting and growth. The plant grows rapidly during hot weather (Ilnicki & Fertig, 1962). Miyazaki *et al.* (2005) demonstrated that under controlled conditions, sprouting of detached roots was highest at temperatures between 15 and 30°C. This temperature range for optimal growth is in accordance with results of Onen *et al.* (2006) under western Japanese conditions (Osaka Prefecture). Nishida *et al.* (2000) pointed out that germination of *S. carolinense* does not occur at temperatures below 14°C under field conditions. Seeds were killed by exposure to heat at 55°C for 72 h and at 60°C for 24 h (Nishida *et al.*, 1999b).

Seedlings of *S. carolinense* are resistant to shading. Urakawa & Koide (2004b) reported that the growth of shoots and roots of *S. carolinense* did not decrease by shading (50% of sunlight), while it sharply declined under shading of ?75% of sunlight.

Experimental data indicated that *S. carolinense* can tolerate a broad range of soil types and textures, but thrives on light textured, well-drained soils (Ilnicki & Fertig, 1962). It can also grow under high moisture conditions, as it can persist on riverbanks, along field margins of paddy rice fields, in ditches and other moist to periodically saturated locations (e.g. Imaizumi *et al.*, 2006). Moreover, the species was found to be drought resistant, which was attributed to its deeply penetrating roots (Bassett & Munro, 1986; Ilnicki & Fertig, 1962).

### Natural enemies

Within the EPPO region, there are no host specific natural enemies of *S. carolinense*. Generalist natural enemies will

potentially attack the plant, but these are unlikely to cause enough damage to influence establishment.

## Uses and benefits

*Solanum carolinense* is not used for anything in particular.

## PATHWAYS FOR MOVEMENT

Seeds of *S. carolinense* may be a contaminant in grain imported for (1) animal feed mixtures and (2) human consumption, including for processing. The grain imported for human consumption is likely to be less contaminated than that for animal consumption as regulations are stricter. In EPPO countries, it is assumed that the species was introduced from North America together with soybean.

In Norway, the species was introduced as a contaminant of imported soybean most likely from the USA (Ouren, 1987). For Germany, Jehlik (1989) and Junghans (2013) noted that contaminated soybean originating from America was the probable source of transient populations of the species. In Romania it is reported by Costea (1996) as probably originated from shipping, especially from trade ('soya-bean waste'). Kurokawa (2001) assumed that it has further been most likely introduced to Japan from the USA via contamination of grain.

*Solanum carolinense* can infest many crops, in particular maize and soybean (Prostko *et al.*, 1994; Van Wychen, 2015; Wiles *et al.*, 1992), and these crops are harvested at a period when seeds of *S. carolinense* are present. Seed lots can therefore be infested by seeds of *S. carolinense*. Seed lots of soybean and maize are most at risk of being contaminated. Seed of wheat was not included in pathways for movement as wheat would be harvested too early in the season for seed of *S. carolinense* to be present. Klingenhagen *et al.* (2012) and Zanotti (1993) assumed that the occurrence of *S. carolinense* in Germany and Italy was due to the cultivation of contaminated maize varieties (*Zea mays*) from abroad. In Austria, *S. carolinense* first appeared in a maize field following soybean, where the seeds were thought to have been obtained from Canada (Follak, pers. communication 2021).

A recent survey (2020) revealed that *S. carolinense* is the fourth most common and the second most troublesome weed in pastures, rangeland, or other hay fields in the USA (Van Wychen, 2020). Indeed, it is considered that the spread of *S. carolinense* (both berries and seed) in the USA has occurred through the movement of hay (Robbins *et al.*, 1952). Imported hay from the USA may be contaminated with fruits or seeds (Anonymous, 1896). Kurokawa (2001) checked samples of imported hay into Japan. Although many seeds were recognized in each sample, they were not those of the recently observed non-native noxious weed species (incl. *S. carolinense*). Likewise, Asai *et al.* (2007) did not detect seeds of *S. carolinense* in imported hay including *Phleum pratense*, alfalfa, Sudan grass) from the USA and Canada.

In North America, and in Japan, *S. carolinense* has shown to have spread over long distances presumably by both natural and human assisted mechanisms. In the USA, long distance spread has been speculated to be due to the interstate movement of hay (Robbins *et al.*, 1952).

In Japan, Imaizumi *et al.* (2006) reported that *S. carolinense* has been recorded in pastures and orchards from the 1970s onwards. The species was limited to a small area in 1981 and from the 1990s onwards, it has become more widespread and infested areas have increased rapidly. In 1994, *S. carolinense* was reported on approximately 25% of all surveyed pastures (Nishida *et al.*, 1999a). In 2013, a survey recorded that *S. carolinense* had infested 76.3% of the total surveyed area of forage crop fields (11 200 ha; cited in Tominaga & Kurokawab, 2020).

In the EPPO region, in Austria, Follak (2020) noted that the movement of root fragments via agricultural machinery was strongly suspected to be the main dispersal vector from field-to-field. This was underlined by the fact that most observed populations of *S. carolinense* occurred at field margins and headlands along farm tracks and roads (southern Styria, Austria). It was observed that the species has spread at least 2 km within 10 years.

## IMPACTS

### Effects on plants

*Solanum carolinense* infests many crops, in particular spring crops such as peanuts, maize, cotton, potato, alfalfa, green beans, tomato, vegetables, and soybeans (e.g. Van Wychen, 2015, 2020; Webster, 2008). Unfortunately, competition of *S. carolinense* with crops is not well documented. Only a few studies focused on the impact of the species on crop yield. The extent of yield loss depends largely on the density of *S. carolinense* but also on the crop type, and low-growing crops seem to be more affected.

Some authors have documented effects of various densities of *S. carolinense* on yield of peanut (*Arachis hypogaea* L.) and snap bean (*Phaseolus vulgaris* L.). The impacts are expressed as crop yield reductions. The study of Hackett *et al.* (1987) seemed to indicate that *S. carolinense* is not a major problem in peanuts. They showed that in 1 year 32 specimens in a 10 m of row (the highest density) reduced yield, but in the second year the same density did not have any effect on the yield of the peanuts. In contrast, Frank (1990) demonstrated that the yield of snap bean was greatly reduced due to the presence of *S. carolinense*. Eight *S. carolinense* specimens planted in a 4.6-m row the first year and 16 specimens per row for the second year reduced snap bean yield 36% and 55%, respectively.

Whaley and Vangessel (2002) noted that *S. carolinense* was not a strong competitor with maize. At all sites (Delaware, USA), no significant differences or trends in maize yield were observed in field trials (untreated control vs. different herbicide control options). Prostko *et al.* (1994) demonstrated similar findings, although a trend of higher yields was observed in plots with herbicide treatments.

A recent survey (2020) revealed that the species is the fourth most common and the second most troublesome weed (just after *Cirsium arvense*) in pastures, rangeland, or other hay production in the USA (Van Wychen, 2020). In pastures, *S. carolinense* is considered as a drought-resistant competitor and is presumed to reduce the yield and quality of forage crops (Beeler *et al.*, 1994). For example, population density of *S. carolinense* on an experimental site averaged 86 stems in 10 m<sup>2</sup> in a tall fescue dominated pasture (Richmond/USA; Tolson *et al.*, 2012). Pasture weeds, such as *S. carolinense*, reduce desirable forage biomass through direct competition for resources or displacement of valuable forage species. However, specific data on forage yield losses are not available.

The potential economic impact of *S. carolinense* in the EPPO region for farmers could be significant if the species spreads and establishes in further areas. The studies conducted in North America indicate the degree to which *S. carolinense* may impact crop and forage yield. Thus, effective weed control is essential in *S. carolinense* infested crops and pastures.

*Solanum carolinense* occurs already locally in crop (maize, soybean, oil-pumpkin) fields in the EPPO region (Austria: Follak, 2020; Italy: Selvaggi *et al.*, 2018; Germany: Klingenhagen *et al.*, 2012), though extensive data on the area of distribution and infestation levels are not available.

Specific studies on yield loss or additional operating costs are not accessible, with the exception of Todua (1975), who showed in Georgia (Abkhazia) that the yield of essential oil crops (*Pelargonium roseum* Wild) was decreased with the presence of *S. carolinense*. In addition, the yield of tea (*Camellia sinensis*) in plantations was shown to decrease with the presence of *S. carolinense* and the quality of tea deteriorated.

In general, *S. carolinense* can be managed in crops and pastures in the same way as other weeds by herbicide use or mechanical measures. However, the control of the species by ploughing, cultivation and mowing is considered difficult because of its extensive root system and high capacity of regeneration. Moreover, *S. carolinense* is only moderately susceptible to several herbicides and multiple applications are required for adequate control (e.g. Armel *et al.*, 2003; Beeler *et al.*, 1994). The species will most likely show the same behaviour in the EPPO region. Therefore, additional weed management actions, such as specific herbicide programs (e.g. Whaley & Vangessel, 2002) or multiple tillage passes, may be required and this could raise control costs.

*Solanum carolinense* is a host to many pests that can cause damage to a variety of crops. The species is a reservoir for pathogens, such as *Alternaria solani* Sorauer, *Septoria lycopersici* Speg., tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV). Some of the important phytophagous insect pests include *Leptinotarsa decemlineata* (Say), *Leptinotarsa juncta* (Germar), *Gargaphia solani* (Heidemann), *Trichobaris trinotata* (Say), *Epitrix fuscula* (Crotch), *Epitrix cucumeris* (Harris), *Manduca sexta* (Haworth), *Zonosemata electa* (Say), and *Phthorimaea operculella* (Bassett & Munro, 1986; Nichols *et al.*, 1992; Wahlert *et al.*, 2015; Wise, 2018; Wise & Sacchi, 1996).

## Environmental and social impact

There are currently no studies available on the potential negative impact of *S. carolinense* on biodiversity. In general, for *S. carolinense* most natural habitats of high conservation value have a low potential to be invaded, thus negative effects of this plant on biodiversity are considered of low importance. However, in South Korea, *S. carolinense* has invaded island ecosystems and the interior of natural forests (Kim *et al.*, 2017).

*Solanum carolinense* is considered toxic to live- stock (Bassett & Munro, 1986). Fortunately, the species is not palatable and is not readily grazed unless animals are confined in overgrazed fields. The species contains glycoalkaloids, primarily asolasonine and asolamargine (Cipollini & Levey, 1997). Glycoalkaloids may induce gastrointestinal and systemic effects, with potential neurotoxicity. Bassett and Munro (1986) presented a small amount of anecdotal data on intoxication of livestock.

## CONTROL

A pro-active and integrated weed management strategy is required to effectively manage *S. carolinense*. It should be noted that in natural environments, management practices should be tailored to the habitat invaded. NPPOs should provide land managers, farmers and stakeholders with identification guides including information on preventive measures and control techniques.

Control of the species is difficult, because of its extensive root system, its ability to grow from small root fragments and the number of seeds produced. It is most successful when multiple tactics are employed, such as the combination of preventive methods, chemical, mechanical, and cultural control techniques.

### Mechanical control

Frequent mowing is ineffective in the control of *S. carolinense* (Gorrell *et al.*, 1981). In this respect, Ilnicki and Fertig (1962) demonstrated that *S. carolinense* likely forms a rosette growth pattern and thus keeps the root system sufficiently supplied with carbohydrates when mowed frequently at very low heights.

Deep tillage practices (ploughing) normally reduce perennial weed populations, because the underground root system (i.e. the development of below-ground storage organs) is disturbed. Tillage by ploughs, disks, or cultivators may increase *S. carolinense* infestations by relocating root fragments to new areas of the crop field or by breaking the dormancy of adventitious buds, resulting in new shoot growth (Wehtje *et al.*, 1987).

### Chemical control

Herbicides are the most common method of controlling the species in fields and pastures. Applications with certain mixtures and treatments of glyphosate, auxintype herbicides (e.g. dicamba, picloram, aminopyralid), sulfonylureas (primisulfuron, nicosulfuron) and triketones (mesotrione) are somewhat effective (e.g. Armel *et al.*, 2003; Beeler *et al.*, 2004; Klingenhagen *et al.*, 2012; Prostko *et al.*, 1994); Whaley & Vangessel, 2002.

## REGULATORY STATUS

In the EPPO region, *S. carolinense* is included on the EPPO A2 list of pests recommended for regulation as a quarantine pest. In addition, *S. carolinense* is 'black- listed' (banned from sale) in the Italian region of Piemonte (Piedmont) according to D.G.R. no. 46-5100 of 18 December 2012 and under monitoring in the network of protected areas (Natura, 2000).

In Canada, *S. carolinense* is listed under 'Primary Noxious Weed Seeds' under the Weed Seeds Order of the Seeds Act (<http://www.gazette.gc.ca/rp-pr/p2/2016/2016-05-18/html/sor-dors93-eng.html>).

In the USA, *S. carolinense* is declared as a 'noxious weed' in Alaska, Michigan, Maryland, Iowa and Nevada (<https://>



[www.invasive.org/browse/subinfo.cfm?sub=6440](http://www.invasive.org/browse/subinfo.cfm?sub=6440)).

In New Zealand, *S. carolinense* has the status of a 'Quarantine pest' (Official New Zealand Pest Register: <https://pierpestregister.mpi.govt.nz/PestsRegister/ImportCommodity/>).

## PHYTOSANITARY MEASURES

EPPO (2022) recommends phytosanitary measures for grains of *Glycine max*, *Zea mays* and *Triticum aestivum*. Grains should be produced in a pest-free area (PFA) for *S. carolinense* or produced in a Pest free production site (PFPS) or Pest free place of production (PFPP) for *S. carolinense* coupled with sorting of the consignment, or grain should be sampled according to ISPM 31 and inspected, and the grain lot found free from *S. carolinense*, or the grain should be devitalized according to an appropriate method. Certified seed of *Glycine max* and *Zea mays* should be used.

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