Zeitschrift: Bollettino della Società ticinese di scienze naturali

Herausgeber: Società ticinese di scienze naturali

Band: 109 (2021)

Artikel: Juglans ailantifolia : a new alien walnut tree species naturalized in Switzerland and Italy

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DOI: https://doi.org/10.5169/seals-1003000

Juglans ailantifolia
A new alien walnut tree species naturalized in Switzerland and Italy

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Abstract: We present a new species of *Juglans* (walnuts, Juglandanceae), *J. ailantifolia* Carrière, a tree native to Japan and Sakhalin and naturalized in southern Switzerland and northern Italy. First found in Canton Ticino in 2019, its identification has been difficult, due to the (unresolved) taxonomic complexity of this genus. The species was documented and morphologically characterized using field surveys, photographs, and by taking voucher herbarium specimens. A dichotomous key to identify *Juglans* species in Switzerland and Italy is presented, including similar species. To assess vitality and potential for resprouting, a three-ring girdling experiment was carried out on 30 trees. *Juglans ailantifolia* is characterized by compound leaves up to 90 cm long with 11-19 densely hairy and only weakly asymmetric leaflets, erect red-pink female inflorescences, and up to 20 fruits. In Switzerland, the species was found in the areas of Bellinzona and Mendrisio, in moist lowland environments. It went unnoticed for decades, as the largest stands derive from abandoned private plantings dating back several decades. In Italy, it occurs in Lombardy, with young individuals also found along rivers, but it might be present also in Piedmont and Veneto. Its biological characteristics (relatively few fruits dispersed by gravity, but nuts can float, no vegetative reproduction) and its relatively limited spread in Canton Ticino and northern Italy (despite the age of the stands) do not indicate an invasive behavior in the Insubric area. Furthermore, its regenerative capacity after girdling is weak: trees die within a year and resprouting is low. Nevertheless, we recommend to control the species especially along waterways, avoid uncontrolled cutting and/or girdling, and to prefer *J. regia* for timber plantations.

Keywords: alien species, biological recognition, invasive potential, *Juglans mandshurica*, neophyte

Juglans ailantifolia **– Una nuova specie di noce naturalizzata in Svizzera e Italia**

Riassunto esteso
Introduzione: In questo studio presentiamo *Juglans ailantifolia* Carrière (Juglandaceae), una nuova specie alloctona di noce naturalizzata in Svizzera meridionale e Italia settentrionale, originaria di Giappone e Sakhalin. È stata trovata prima in Cantone Ticino nel 2019 e in seguito in Italia. Poiché la sua identificazione è stata complessa a causa di una tassonomia ancora irrisolta, proponiamo una chiave dicotomica per l'identificazione delle specie di *Juglans* presenti in Svizzera e Italia, incluse le specie simili *J. cinerea* (America settentrionale) e *J. mandshurica* (Asia orientale). Viene valutata inoltre la sua vitalità e la sua capacità di formare ricacci e discusso l'eventuale potenziale invasivo in base alle sue caratteristiche biologiche e alla sua diffusione geografica.
Materiale e metodi: La specie è stata documentata e caratterizzata mediante rilievi in campo durante l'intera stagione vegetativa, fotografie e campioni d'erbario. Per l'identificazione sono state consultate le opere tassonomiche e le flore di riferimento. Per la distribuzione in altri paesi europei sono state consultate banche dati online e sono stati contattati esperti locali. Per valutarne la vitalità e il potenziale di formazione di ricacci, su 30 alberi è stato applicato il metodo di cercinatura con tre anelli, seguito da rilievi di controllo.
Risultati: *Juglans ailantifolia* presenta foglie composte lunghe fino 90 cm, con 11-19 foglioline densamente pelose e con base debolmente asimmetrica, infiorescenze maschili verdi e pendule, femminili erette e di colore rosso-rosa, fino a 20 frutti indeiscenti, con esocarpo tomentoso-ghiandoloso e noce legnosa relativamente spessa. Fiorisce in aprile e fruttifica a fine estate. Viene discusso il problema tassonomico con *J. mandshurica*. In Svizzera, *J. ailantifolia* è stata trovata unicamente nel Bellinzonese e nel Mendrisiotto, in ambienti freschi. I due popolamenti più estesi sembrano derivare da impianti privati risalenti a vari decenni fa. In Italia, è presente in Lombardia, con individui giovani in diffusione lungo i fiumi, e potrebbe essere naturalizzata anche in Piemonte e Veneto. Altrove, in Europa, sembra trovarsi allo stato selvatico unicamente in un paio di località della Germania e del Belgio. La cercinatura ha mostrato una debole capacità rigenerativa: gli alberi muoiono entro un anno e la formazione dei ricacci è bassa. Inoltre, molti degli alberi indeboliti sono stati attaccati dal bostrico esotico *Xylosandrus germanus*.
Conclusioni: Il processo di naturalizzazione di *J. ailantifolia* in Svizzera e Italia è in corso già da alcuni decenni, ma questo albero è rimasto inosservato fino ad ora. Le sue caratteristiche biologiche (pochi frutti, dispersi soprattutto per gravità a ridosso

degli alberi maturi, nessuna riproduzione vegetativa) e la sua diffusione geografica limitata (nonostante l'età degli alberi più grandi) non indicano attualmente un comportamento invasivo nella regione insubrica. Tuttavia, per la capacità delle noci di galleggiare, si raccomanda di sorvegliare la specie soprattutto lungo i corsi d'acqua, di evitare il taglio e la cercinatura incontrollata e di preferire *J. regia* per gli impianti da legno.

Parole chiave: *Juglans mandshurica*, neofite, potenziale invasivo, riconoscimento biologico, specie alloctone

INTRODUCTION

Every year new alien plant species naturalize in Switzerland and northern Italy becoming part of the wild flora. Particularly, the Insubric region is well known for its rich exotic flora (Banfi & Galasso 2010; Schoenenberger et al. 2014), where new naturalized species are constantly discovered (e.g., Galasso & Banfi 2020; Mangili et al. 2020). This region encompasses the area of the great lowland pre-alpine lakes on the southern side of the Alps in Canton Ticino (Switzerland) and Piedmont and Lombardy (Italy), and is characterized by a nearly subtropical climate (MeteoSvizzera 2012).
In this study, we present a new naturalized alien walnut species, *Juglans ailantifolia* Carrière (Juglandaceae), discovered in 2019 in Canton Ticino, which adds to the two already recognized *Juglans* species of the Swiss flora: the archaeophyte *J. regia* L. and the casual alien *J. nigra* L. from North America (Juillerat et al. 2017). This new species was then discovered also in northern Italy, initially misidentified as the North American *J. cinerea* L. (Federici et al. 2013). The latter species and *J. mandshurica* Maxim., a close relative of *J. ailantifolia*, are occasionally cultivated as ornamentals in Central Europe (Roloff & Bärtels 2018).
Juglans is a rather small genus of deciduous trees in the family Juglandaceae, including 20-21 extant species (Whittemore & Stone 1997; Lu et al. 1999; Grimshaw 2003). There is no monography of this genus, despites Manning's life dedication to it (e.g., Manning 1957, 1960, 1978). The closely related East-Asian *J. ailantifolia, J. mandshurica, J. cathayensis* Dode, forming *J.* sect. *Cardiocaryon* Dode, and the North American *J. cinerea,* forming *J.* sect. *Trachycaryon* Dode ex W.E.Manning are very closely related, but there is incongruence in molecular data. A sister relationship between *J. cinerea* and taxa of the eastern Asian *J.* sect. *Cardiocaryon* was supported by plastid DNA data, while nuclear data reveal *J. cinerea* nested in the northern American *J.* sect. *Rhysocaryon* Dode near *J. nigra* (Stanford et al. 2000; Aradhya et al. 2005, 2007; Dong et al. 2017; Zhang et al. 2019; Mu et al. 2020). So, *J. cinerea* likely originated from hybridization and massive introgression from an immigrating Asian butternut into the genome of an American black walnut (Zhang et al. 2019). The taxonomy of the East-Asian species of this clade (known as the Oriental butternuts or Asian heartnuts) is complicated, although more recent studies (Bai et al. 2016) unravel their biogeographic history, in which the formation of the Japanese archipelago may have contributed to the separation of *J. ailantifolia* from *J. mandshurica* as a distinct lineage about 3 to 5 Ma ago (Pliocene split). Other molecular phylogenetic studies (Mu et al. 2017), however, suggested that the three East-Asian taxa should be interpreted as a single species. As there is no international consensus, we prefer to treat *J. ailantifolia* and *J. mandshurica* as preliminarily accepted species, though as part of an aggregate *J. mandshurica* s.l.
We here propose a dichotomous key to identify *J. ailantifolia* from the other wild and cultivated *Juglans* species of Switzerland and Italy, provide a morphological description and discuss the taxonomic debate. We also describe its known range of distribution, degree of naturalization, habitat and ecology, and possible origin of the populations. Finally, we test its regenerative capacity and vitality to provide best management practices and explore whether the species has any potential to become invasive.

MATERIALS AND METHODS

To identify *J. ailantifolia* and infer an identification key, several references were used in the absence of a monography of *Juglans* worldwide: the original description by Carrière (1878), Flora of North America (Whittemore & Stone 1997), Flora of China (Lu et al. 1999), Grimshaw (2003), Flora of Japan (Ohba 2006), a US field guide (Farlee et al. 2010), Flora of Woody Plants (Roloff & Bärtels 2018), and the identification key by De Langhe (2006-2012). For the wood anatomy we used Miller (1976). All found occurrences of *J. ailantifolia* in Switzerland were reported to the national database (Info Flora) using their application FlorApp, and data from any other reports of this species were requested. Both a flowering and a fruiting Swiss voucher specimen are deposited at LUG (herbarium acronyms according to Thiers 2021+), the herbarium of the Natural History Museum of Canton Ticino (Lugano, Switzerland); Italian specimens are hosted at MSNM (Museo di Storia Naturale di Milano). The Swiss vouchers were the first specimens at LUG.
Juglans ailantifolia was not present in the Swiss (Juillerat et al. 2017) or Italian (Galasso et al. 2018) Checklist of vascular plants nor was there any data of *J. ailantifolia* or *J. mandshurica* species in the Info Flora database at the moment of discovery of the plant in 2019, in the Portal to the Flora of Italy (Martellos et al. 2020; Galasso et al. 2020) or in the available online databases of major Swiss or Italian herbaria (FI, G, Z-ZT; both accessed 26 May 2021).
To have an idea of the alien range of distribution of the species, especially any occurrences in the wild in Europe, the international online databases Euro+Med Plantbase (Uotila 2011), the Global Biodiversity Information Facility (www.GBIF.org; accessed 1 February

2021) and the French National Inventory of Natural Heritage (INPN, https://inpn.mnhn.fr; accessed 18 February 2021; with the respective current taxonomic reference TAXREF 14.0, https://inpn.mnhn.fr/telechargement/referentielEspece/taxref/14.0/menu) were consulted. In addition, experts of the alien flora from neighboring countries (Austria, France, Germany) and Belgium were contacted by email to inquire about any naturalization in their countries. It was beyond the scope of this study to investigate in detail the history of introduction and distribution of cultivated trees in Italy, Switzerland or elsewhere in Europe.

To document the species biology and ecology, the trunk diameter of several individuals at the site of first discovery of the species (in the locality of Gnosca, Switzerland) was measured to estimate age, and their phenology (from leaf, flower and fruit development to fruit dispersal and leaf senescence) was observed during one year starting late Summer 2019 until early Summer 2020. Other plant species occurring at the site were also identified (and reported to the national database using FlorApp) to infer the type of vegetation and habitat preferences of *J. ailantifolia*. In Spring 2021, the number of flowers in 36 female inflorescences from 6 individuals at this site and one individual from Italy were counted, and damage of Spring frost (MeteoSvizzera 2021) was documented with photographs.

To test the capacity for vegetative regeneration of *J. ailantifolia*, such as resprouting and suckers, a girdling experiment was carried out at the site in Gnosca, using the three-ring method with a chainsaw (on the 3 of June 2019) and subsequent regular resprouting controls (until September 2020). Among the measured trees at the site, 30 trees with a diameter of 6-12 cm were selected and treated at a height of 1.0 m and a depth of 1-2 cm. After the treatment, resprouting was controlled every month for four months, the vitality status of the tree was evaluated by observing the foliage and documented with photographs; any basal and radical sprouts were first counted and measured in height, and then eliminated with a sickle.

To assess whether the species has any potential to become invasive its biological features and its spread potential were analyzed by comparing them with the criteria listed in the catalogue of criteria by Info Flora (2014, used to compile the lists of invasive and potentially invasive alien species of Switzerland). The criteria to estimate the spread potential of a species in Switzerland consider aspects of the sexual and vegetative reproduction (including the number of seeds produced, presence of a seed bank in the soil, and presence of organs for clonal growth like stolons), dispersal modes (by natural vectors and also human activities), and the spread dynamic and speed of populations across the country. The criteria to estimate its impact potential examine health impacts on humans and animals, environmental impacts on native species and habitat structure, and economic impacts (such as damages to buildings, agricultural losses, etc.). Data relevant for these criteria was gathered by searching the literature on *J. ailantifolia* in Google and Google Scholar, using the species name alone and also combined with 'weed', 'invasive' and 'noxious'.

RESULTS AND DISCUSSION

Key to the species of *Juglans* wild in Italy and Switzerland, and their allies. (Figs. 1, 2)

1a. Leaves with 5-7(-11) leaflets (fig. 1A); leaflets ovate, with entire or almost entire margin (sometimes serrate in young plants and young leaves), glabrous except for the domatia in the axils of the veins on the abaxial side of lamina ....***J. regia***

1b. Leaves with >9 leaflets (fig. 1A); leaflets ovate-lanceolate, with serrate or toothed margin, with glandular, simple and/or stellate hairs, domatia absent...**2**

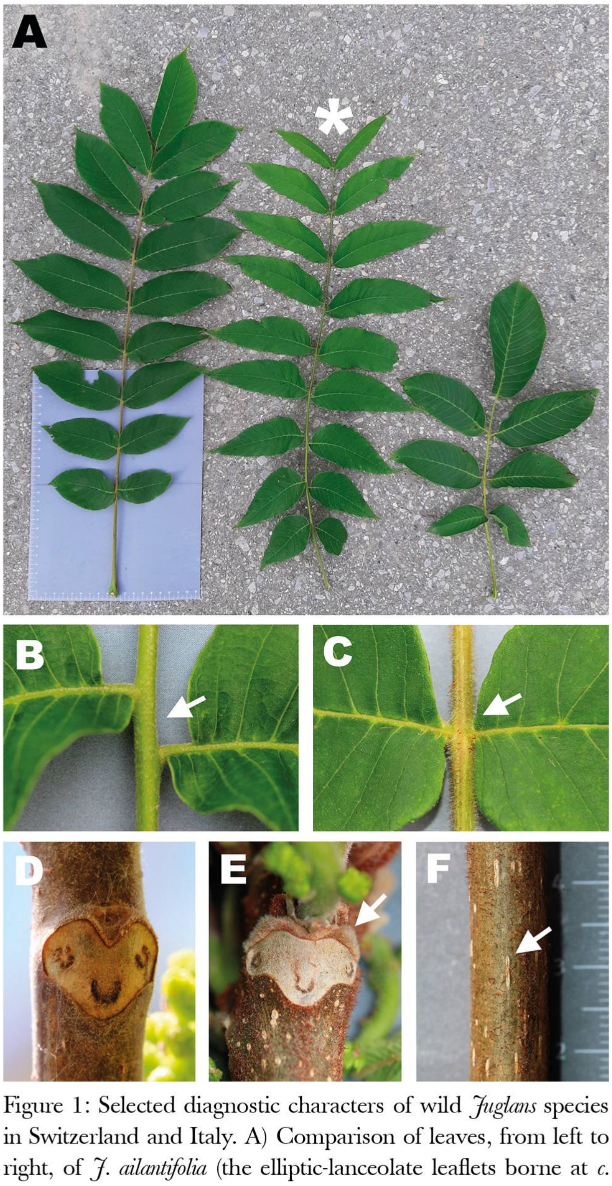

Figure 1: Selected diagnostic characters of wild *Juglans* species in Switzerland and Italy. A) Comparison of leaves, from left to right, of *J. ailantifolia* (the elliptic-lanceolate leaflets borne at *c.* 45 ° to rachis are clearly visible), *J. nigra* (arrow indicates lacking terminal leaflet), and *J. regia*, respectively. B, D) *J. nigra*. C, E-F) *J. ailantifolia*; B) Base of leaflets asymmetric. C) Base of leaflets only very weakly asymmetric. D) Leaf scar lacking "moustache" of dense hairs on its upper margin. E) "Moustache" of dense hairs on upper margin of leaf scar (arrow; young bud has been removed left of the arrow). F) Elongated lenticels on twig.

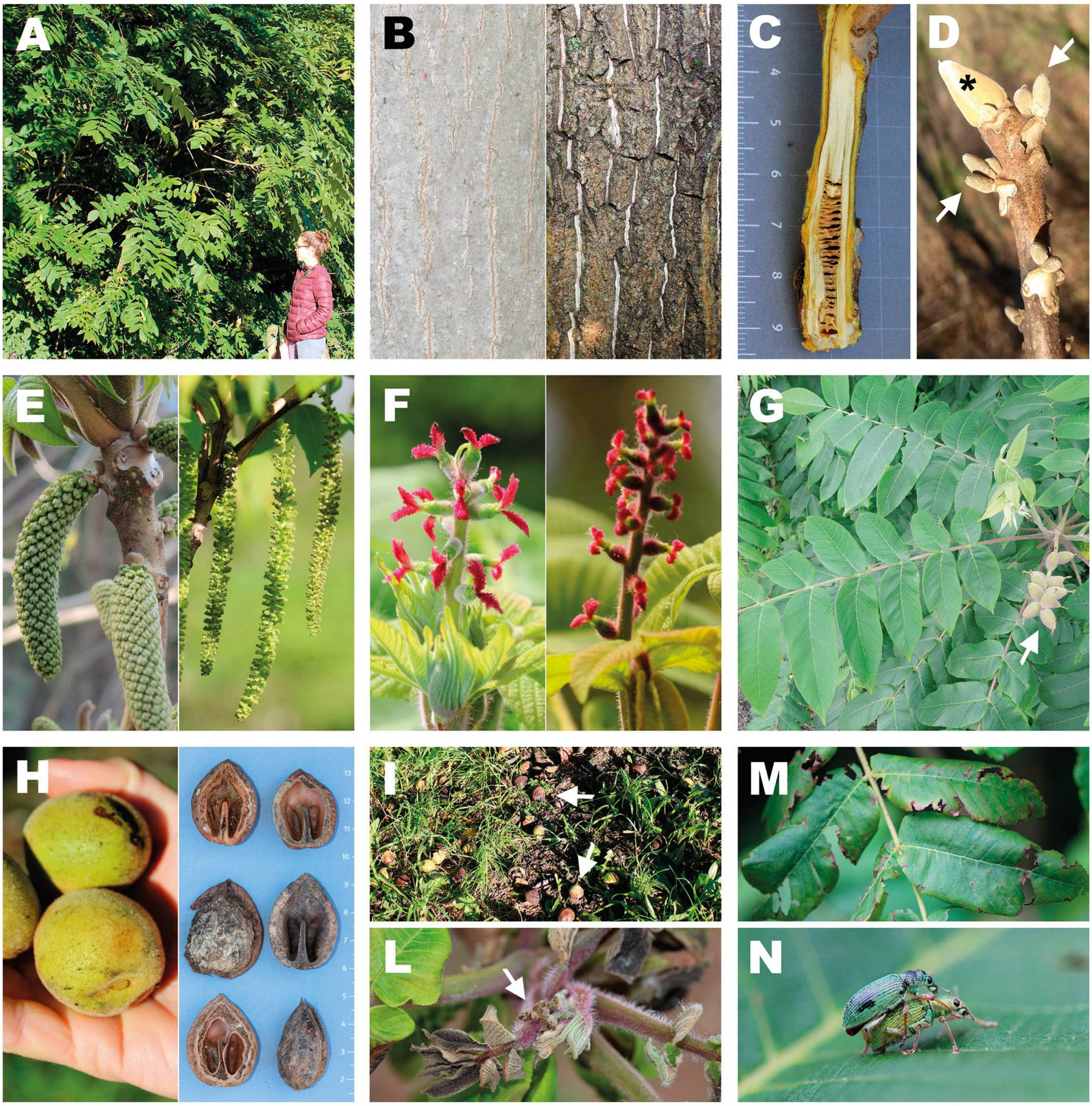

Figure 2: Morphology and biology of *Juglans ailantifolia*. Images from individuals of the site in Gnosca (southern Switzerland) from early Fall 2019 to late Spring 2020 (A-I, M-N), and Spring 2021 (F, L). A) Foliage of young trees on the margin of the stand. B) Trunk of a young tree (left, diameter *c.* 20 cm) and of an old tree (right, diameter = *c.* 40 cm). C) Longitudinal section of a young shoot showing the light-brown pith. D) Pale brown apical leaf bud (asterisk), and young male inflorescences (arrows)(11 March). E) Leaf emergence and young developing male inflorescences (left, 2 April) and flowering male inflorescences (right, 17 April). F) Flowering female inflorescences (17 and 21 April, respectively left and right). G) Young infructescence (5 June). H) Older mature fruits with tomentose-glandulose exocarp = husk (left, 16 October), and variability in the woody mesocarp = "nut" (right). I) Fruits (some are indicated by arrows) on the ground on the margin of the stand (16 October). L) Frost damages on emerging leaves and early developing inflorescences (21 April). M) Damaged leaves (10 June); the elliptic-lanceolate leaflets that borne at *c.* 45 ° to rachis are clearly visible. N) Copulating individuals of species native weevil species *Polydrusus formosus*.

2a. Terminal leaflet absent or reduced in size (fig. 1A); leaves never sticky to the touch; base of leaflets distinctly asymmetrical (fig. 1B); upper margin of leaf scars lacking a "moustache" of dense velvety hairs (fig. 1D); female flowers (and fruits) 1-2(-5); husks globose, rarely ellipsoid, nuts with numerous warty ridges; trees up to 40(-50) m .... ***J. nigra***

2b. Terminal leaflet present (fig. 1A); leaves sometimes sticky to the touch (glandular hairs); base of leaflets not or only weakly asymmetrical (fig. 1C); upper margin of leaf scars with a "moustache" of dense velvety hairs (fig. 1E); female flowers (and fruits) 3-10(-20) (fig. 2F); husks ellipsoid to ovoid, nuts smooth or with up to 8 +/– sharp longitudinal ridges; trees up to 30 m **3**

3a. Fruits 3-5, nuts with about 8 high, narrow longitudinal main razor-sharp ridges interspersed with smaller, interrupted ridges or lamellae, suture inconspicuous resembling the longitudinal ridges; lenticels small and round or slightly elongating horizontally across the branch; pith very dark brown; leaf bud +/– cylindrical, white to greyish; upper margin of leaf scars flat .... ***J. cinerea***

3b. Fruits 5-10(-20) (fig. 2F,G), nuts smooth or ridged, suture prominent and easily distinguished; lenticels lengthwise elongated (fig. 1F); pith light to medium brown (fig. 2C); leaf bud +/– pyramidal, beige to pale brown (fig. 2D) or blackish; upper margin of leaf scars slightly emarginated (fig. 1E) .... **4.** (*J. mandshurica* aggregate)

4a. Leaflets ovate-elliptic, shortly acuminate, borne at right angles to rachis; leaf bud blackish; nuts ridged... ***J. mandshurica***

4b. Leaflets elliptic-lanceolate, acuminate, borne at *c.* 45° to rachis (figs. 1A, 2L); leaf bud beige to pale brown; nuts ridged or smooth (fig. 2H) ....***J. ailantifolia***
– Nuts smooth, heart shaped, rather compressed (depressed) .... *J. ailantifolia* 'Cordiformis'

Juglans ailantifolia Carrière, Rev. Hort. (Paris) 50(21): 414(-415, figs. 85-86). 1878 [1 Nov 1878].
Juglans sachalinensis Komatsu; *Juglans sieboldiana* Maxim., non Göpp., nom. illeg.

Specimina visa

SWITZERLAND: Gnosca, bosco al lato della strada cantonale, 100-200 ind., E 2'721'499, N 1'122'640, 259 m, 16 October 2019, *A. Rosselli & B. Marazzi* (LUG; Info Flora Obs_ID No. 8470767); *ibidem*, 17 April 2020, *A. Rosselli & B. Marazzi* (LUG; Info Flora Obs_ID No. 8824481).
ITALY: Lombardia, Zogno (Bergamo), fraz. Ambria, sponda destra del Torrente Ambria, alla base del parapetto/discesa al torrente della SP27 (Via Piave), di fronte allo stabilimento dell'Acqua Bracca (WGS84: 45.808406°N 9.685911°E), scarpata fluviale, 339 m, S, 25 June 2012, *G. Federici* (APP, MSNM sub *J. cinerea*); *ibidem*, 14 October 2020, *G. Galasso* (MSNM); Lombardia, Piario (Bergamo), sentiero in fondo alla Via A. De Gasperi di Villa d'Ogna (WGS84: 45.897209°N 9.924416°E), bosco ripariale, 506 m, NW, 14 October 2020, *G. Galasso* (MSNM).

Description

Wide-crown trees up to 20 m (fig. 2A), deciduous, monoecious. Bark of young trees light gray with orange narrow longitudinal furrows, of older trees dark-brown-greyish with white furrows (fig. 2B); sapwood whitish to light brown, heartwood light brown or chestnut and barely distinguishable from the sapwood. Twigs with lenticels lengthwise elongated (fig. 1F) and pith light to medium brown (fig. 2C); upper margin of leaf scars with a "moustache" of dense velvety hairs (conspicuous or almost invisible, but always detectable) slightly emarginated (fig. 1E); leaf buds +/– pyramidal, beige to pale brown (fig. 2D). Leaves compound, petiolate and odd-pinnate, up to 70(-90) cm long, rachis and abaxial side of leaf lamina densely hairy, with simple eglandular and glandular, and some stellate hairs; leaflets 11-19 (fig. 1A), base only weakly asymmetric, margin serrate (fig. 1C). Twigs and leaves are +/– sticky to the touch. Male inflorescences pendent, green, almost to 20 cm long (fig. 2E); female inflorescences erect, flowers 7-20+, apetalous, carpels with bright pink-reddish papillose stigmas (fig. 2F). Fruits ellipsoid to ovoid, drupe-like nuts with a densely glandular pubescent exocarp (husk) and a relatively thick woody nut, spherical to ovoid (or compressed and hearth-shaped in the cultivar Cordiformis) with a prominent and easily distinguished suture; nut surface with rather superficial longitudinal ridges, which tend to become smooth once dropped to the ground and exposed to the environment (fig. 2G-H) (smooth in the cultivar Cordiformis). Flowering: Spring (April-early May). Altitude: 210-510 m a.s.l. (lowland layer).

Taxonomy

There is currently no international consensus on the taxonomy of East-Asian *Juglans* species of section *Cardiocaryon.* Two recent molecular phylogenies come to opposite conclusions. Bai et al. (2016) who focus on relationships among *J. ailantifolia*, *J. cathayensis* and *J. mandshurica* find that they form distinct lineages. Their analyses can be considered as robust, as they are based on a large sample size (1,460 adult individuals of 70 natural populations) and on one nuclear and eight chloroplast DNA markers. In contrast, Mu et al. (2017) who focus on more Asian *Juglans* species, using RAD-seq and a sampling of only 42 samples, suggest that the three East-Asian taxa are to be considered as a single species. They base their conclusion essentially on a lack of resolution in their phylogenetic tree, in which *J. ailantifolia* samples resulted to be nested in the cluster formed by *J. cathayensis* and *J. mandshurica* (Mu et al. 2017). Therefore, while Bai et al. (2016) appear to support the traditional taxonomic view in which *J. ailantifolia* is a separate entity from *J. mandshurica* (e.g., Aradhya et al. 2007), Mu et al. (2017) would be more in line with Flora of Japan (Ohba 2006) that considers *J. ailantifolia* a variety of *J. mandshurica* (*J. mandshurica* var. *sachalinensis* (Komatsu) Kitam.). There is still no consensus: for instance, the synonymy has been adopted by the International Dendrological Society (Sutton 2019), but Tropicos (Tropicos.org; accessed 18 February 2021) and other nomenclatural databases still list them as separate species.

The fundamental problem is the morphological circumscription and differentiation of the three East-Asian *Juglans* species. Bai et al. (2016) also admit that "The three Asian butternuts are closely related, with minute morphological differences […]". We have noticed that several keys (e.g., Huxley et al. 1992; Roloff & Bärtels 2018) differentiate *J. ailantifolia* by the number of female flowers (and fruits) up to 20, but both the Flora of Japan (Ohba 2006) and the plate contained in the Protologue (Carrière 1878) indicate a smaller number of fruits (7-10 flowers), a number similar to *J. mandshurica* (and *J. cathayensis*). However, in their study on flower phenology of *J. mandshurica* in China, Bai et al. (2006) describe female inflorescences as typically consisting of 10–18 flowers, while in Flora of China (Lu et al. 1999) describe *J. mandshurica* (incl. *J. cathayensis*) with 5-10(-13) nuts. In our study, examined inflorescences where bearing from 8 to over 20 flowers, with a mean of 15 flowers (Tab. 1; see fig. 2F and the infructescence illustrated in fig. 2G). Therefore, there is definitely a need to clarify the morphological circumscription of these taxa across their whole distribution range, identify diagnostic characters to distinguish them, and provide a lectotypification of the names. Only a comprehensive study combining morphometrics, molecular phylogenetics and biogeography could show that the geographic separation that lead to the *J. ailantifolia* lineage (Bai et al. 2016) also lead to a morphologically distinct taxon. Finally, considering also the other *Juglans* species, there is definitely a need of a worldwide revision of this genus.

Table 1: Number of flowers in female inflorescences in Italian and Swiss naturalized *Juglans ailantifolia*. Counted on 21 and 26 April 2021 (Swiss, CH, and Italian, IT, individuals, respectively). Asterisks indicate counts of flowers at the bud stage.

Individual	Nr. flowers/inflorescence					
Gnosca 1 (CH)	10	11	12			
Gnosca 2	15	16	18			
Gnosca 3	14	22*	25*	26*		
Gnosca 4	9	10	11	11		
Gnosca 5	14	17	17			
Sementina (CH)	8					
Zogno (IT)	14	15	15	17	18	18
Mean	*15*					
StDev	*5*					

Alien range of distribution and naturalization

In Switzerland, *J. ailantifolia* occurs in Canton Ticino, in at least six lowland localities (fig. 3). At least three of them consist of few individuals of unknown origin, far from the other sites known to derive from cultivated trees. The two largest sites are located in Gnosca and Novazzano. The first has an extension of about 1 ha, and appears to be an abandoned plantation for which there is apparently no archival documentation. There are adult trees over 20 m tall with a trunk diameter of up to 45-50 cm, suggesting that they are 60-70 years old. The second site is more extended, as mature individuals are sparse along a small valley for at least 3 km. These two stands seem to derive from private plantings dating back several decades ago, but the current owners have no other information. Seedling recruitment and a rich renewal are observed, in the understory with young mature individuals of at least the third generation. While in southern Switzerland the species can be considered subspontaneous and possibly even established (at least in Novazzano), in the rest of Switzerland it is so far only known as a rarely cultivated tree, as for example in the Arboretum of the University of Fribourg (Walker 2013).

The species is naturalized also in northern Italy, in Lombardy, where it went unnoticed, as it was misidentified as another casual alien, *J. cinerea* (Federici et al. 2013; Galasso et al. 2018). In 2020, the discovery of a rich stand of *J. ailantifolia* in Val Seriana (Piario) prompted the check of the previous report of *J. cinerea* in Ambria (Val Brembana, municipality of Zogno), which turned out to be *J. ailantifolia*. In Ambria there is only one individual, which does not bear fruits yet. On the other hand, in Piario along the Serio river there is a grove of numerous trees that bear fruit and reproduce regularly. The size of this stand will need to be checked. A brief investigation made it possible to discover its origin. Around 1946, a man planted three "Canadian walnut" seedlings (probably *J. nigra*, to which plants of *J. ailantifolia* must have been added) obtained from the forest nursery of Curno (today of the ERSAF Lombardia – Ente Regionale per i Servizi all'Agricultura e alle Foreste –, at the time of the CFS – Corpo Forestale dello Stato, Ispettorato Ripartimentale delle Foreste di Bergamo, today Carabinieri Forestali –). Likely Curno nursery produced the seedlings starting from seeds distributed by the Vallombrosa forest nursery (Tuscany), at the time having both "*J. sieboldiana*" and "*J. mandshurica*" in its catalog. If the documentation is still available, archival research will be able to establish the exact origin of the material planted in the postwar. The same person planted three other seedlings in a neighboring place, which will need to be checked. In Lombardy, *J. cinerea* was also reported in the Cremona administrative province (Bonali 2020), but a check of the related herbarium samples made it possible to ascertain that they belong to *J. nigra*.

The species is likely present also in other Italian administrative regions. According to the GBIF database, an individual was reported on 26 September 2019 in the locality of Fondotoce (municipality of Verbania), Piedmont, very close to the localities in Ticino. Images of its leaves are consistent with those of *J. ailantifolia*. However, almost certainly the posted individuals correspond to cultivated trees (Alberto Selvaggi, Instituto per le Piante da Legno e l'Ambiente, pers. comm.). A field check is necessary to confirm its identification and status. Lastly, in Piedmont the casual alien *J. cinerea* is reported (Siniscalco et al. 2009; Galasso et al. 2018). It is certainly necessary to check this data, because it

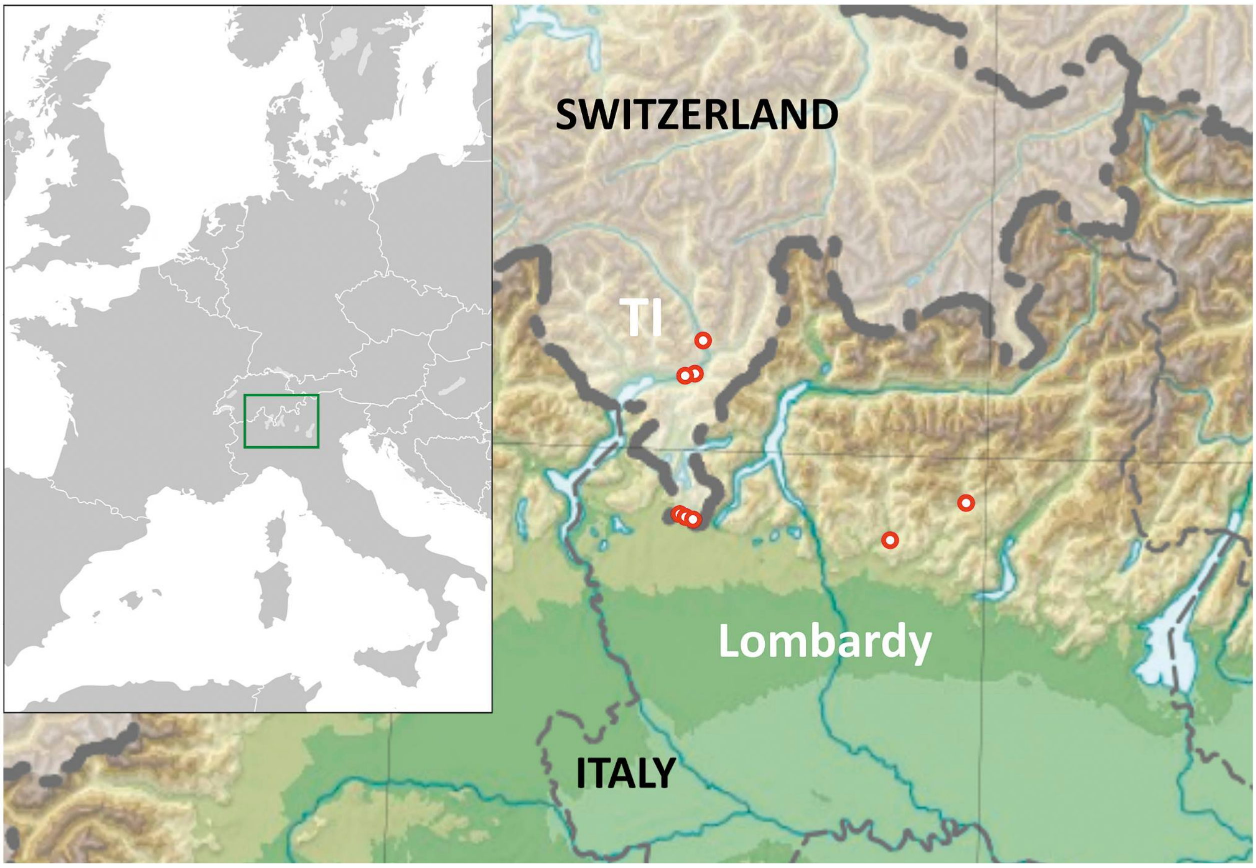

Figure 3: Distribution of *J. ailantifolia* in Switzerland and northern Italy. In Canton Ticino (TI), *J. ailantifolia* sites were found in three central (Gnosca, Gudo, and Sementina) and three southern localities (Castel San Pietro, Coldrerio, and Novazzano). In Lombardy, two main localities are confirmed so far. Map of Europe showing the close-up map with the Insubric Region that encompasses the area of the great lakes illustrated here in light blue. (Close-up map with localities of *J. ailantifolia* based on 'Italy_topographic_map-blank.svg' by Eric Gaba, CC BY-SA 3.0, Commons.wikimedia.org. Map of Europe based on 'Blank map of Europe (with disputed regions).svg' by Maix, CC BY-SA 3.0, Commons.wikimedia.org.).

could be *J. ailantifolia*. In Veneto casual young trees of *J. cinerea* were reported near Melara (Rovigo) along the Po river (Masin 2014; Galasso et al. 2018; Argenti et al. 2019). The photographs published by Argenti et al. (2019) could match *J. ailantifolia*, but a check is also required in this case. If confirmed, the presence of the Japanese walnut would be compatible with a float of nuts from trees certainly present further upstream.
Elsewhere in Central Europe, the species is reported to occasionally escape in Belgium, southern Germany, and perhaps in western France. In Belgium it is known for only one station, referred as *J. mandshurica* s.l. (Filip Verloove, Meise Botanic Garden, pers. comm.). In Germany, escaped individuals of *J. ailantifolia* are reported from a couple localities in North Rhine-Westphalia (Gausmann et al. 2017). In France, the species is not considered as established, but occasional and not naturalized (INPN https://inpn.mnhn.fr/; accessed 18 February 2021). It includes reports from individuals that were planted in Alsace in 1959 under the synonym *J. sieboldiana* (Geissert 2002). To date, however, the taxon is considered to be absent from the Alsatian territory, and more broadly from the Grand Est region, former regions of Alsace, Lorraine and Champagne-Ardenne (Jérôme Hog, Conservatoire Botanique d'Alsace, pers. comm.; Amblard et al., in press), and from the whole France (Tison & de Foucault 2014). The species has not been reported in the wild in Austria (Christian Gilli, University of Vienna, pers. comm.). According to the distribution map displayed on Euro+Med plant database (Uotila 2011), *J. ailantifolia* is a 'casual alien' in Poland, an alien with 'status unknown' in Latvia, and 'in large-scale cultivation' in Belarus, Lithuania and Ukraine, while *J. mandshurica* is an alien with 'status unknown' in Estonia and Central European Russia, and in large-scale cultivation' in Lithuania and Ukraine.

Biological and ecological aspects

Habitat ecology – The trees in Gnosca, Novazzano, and Piario area grow in cool mesophilic environments. In Gnosca, the soils are acidic and the understory is covered with *Equisetum hyemale* L. typical of floodplain forests in general, and clear forests on soils with variable humidity in particular (Delarze et al. 2015). Trees found in Sementina appear as an exception as they occurred on the mountain side together with abandoned open woods with *Castanea sativa* Mill.; but, did not appear to form a rich renewal in the understory. The stand in Piario grows in an ash wood of *Fraxinus excelsior* L. subsp. *excelsior*, while the plant found in Ambria developed on an artificial river bank. *Juglans* trees are well known for their allelopathic effects (reviewed in Willis 2000). In its natural range on Japan and Sakhalin, the tree is widely distributed. It grows in different types of mixed riparian forests (Tamura & Hayashi 2008).

Phenology – In 2020, leaf emergence occurred early in April together with development of the inflorescences (fig. 2E). Leaves were fully expanded by mid-June (fig. 2G). Flowering occurred in April (figs. 2E-F). While *J. mandshurica* is heterodichogamous, with some individuals flowering first male then female and *viceversa* in other individuals (Bai et al. 2006), it was beyond the goal of our study to verify this in *J. ailantifolia.* Fruits develop in summer (fig. 2G) and become mature and ready to fall by October (fig. 2H). On the ground, the exocarp (husk) rots exposing the woody mesocarp, i.e., the nut, which remains in the ground for longer time as an "empty shell" (fig. 2I). Germination tests show that nuts germinate after about 40 days (Bonner 2008a). In 2021, frost in Spring affected some trees by damaging ("burning") young leaves and young female inflorescences, in some cases, completely compromising flower development (fig. 2L).

Interactions with animals – Flowers of *Juglans* are wind pollinated, but fruits could be dispersed by mammals. In its natural range, fruits of *J. ailantifolia* are a major food source for both Japanese squirrel (*Sciurus lis* Temminck, 1844) and Japanese field mouse (*Apodemus speciosus* Temminck, 1844). Both animals contribute to the species' dispersal through scatter-hoarding. Tamura & Hayashi (2008) showed that squirrels preferred larger fruits than mice, triggering evolutionary processes through this plant-animal interaction. Japanese areas outside the range of the Japanese squirrel showed significant smaller fruits, since they are harvested and thus dispersed by field mice. In Ticino, several nuts found on the ground appeared to have been gnawed by rodents, perhaps squirrels (B.M. and A.R., personal observation).

On leaves, we observed no herbivory damages, except in a young tree in early summer 2020, with damages probably caused by phytophagous insects (fig. 2M). Indeed, on these leaves we found several adult individuals of the native and polyphagous weevil species *Polydrusus formosus* (Mayer, 1797) (Coleoptera: Curculionidae) that was using the leaves as a mating site (fig. 2N). This weevil species is abundant in Spring and early Summer, but it is a little less abundant in Ticino than in the North of the Alps (Christophe Germann, pers. comm.).

During the gridling experiment (see below), dying trees with characteristic external leftovers of the alien xylophagous black stem borer *Xylosandrus germanus* (Blandford, 1894) were discovered on almost all treated trees (fig. 4G). It was, however, not possible to capture a voucher specimen. In a first phase, holes were found in the inner part of the rings; in a second phase, also in the lower part of the trunk. This is a species of ambrosia beetle in the family Curculionidae native to eastern Asia, but is an invasive alien in Europe and North America (CABI 2019). Although not reported to occur in Canton Ticino, two other alien curculionid bark beetles are noteworthy to mention here in relation to *Juglans* species. The first one is the Asian *Dryocoetes himalayensis* Strohmeyer, 1908 that has so far been found only North of the Swiss Alps (Sanchez et al. 2020). Although the host in Switzerland is unknown, elsewhere in its alien range in Europe it was found on *J. nigra* (Foit et al. 2017). The other species is the walnut twig beetle, *Pityophthorus juglandis* Blackman, 1928 from northern Mexico and southern US that is specific to Juglandaceae trees. It has been recently reported to occur in northern Italy (Piedmont) as the first place in Europe and outside the rest of its alien range in the US (Montecchio & Faccoli 2014) and it is currently still absent from Canton Ticino (Lucia Pollini, Natural History Museum of Canton Ticino, pers. comm.). This beetle forms an insect-pathogen complex responsible for the thousand cankers disease, as it inoculates the phloem with a fungal pathogen, *Geosmithia morbida* M. Kolařík, Freeland, C. Utley & Tisserat (Kolařík et al. 2011). In its alien range of distribution, it seems to prefer *J. nigra*, while *J. ailantifolia*, *J. cinerea*, *J. mandshurica*, and our arquaeophyte *J. regia* are less suitable hosts (Hefty et al. 2018).

Vegetative regeneration test

Vitality – Only five weeks after the treatment with the three-ring gridling method, five trees (17%) were already dead; after three months 11 trees (37%) had died, and all others displayed a low vitality. One year after (June 2020) all but one treated tree had died (fig. 4A, C-D). The exception was a tree to which the girdling had not been done properly (rings were not closed completely) and displayed a normal vitality. *Juglans ailantifolia* displayed a relatively low regenerative capacity, as their vitality declined fast after the three-ring gridling method was applied, and trees died within one year (fig. 4D). Other alien tree species like *Ailanthus altissima* (Mill.) Swingle take much more time to die after the application of the three-ring gridling method (Wunder et al. 2019), although it has to be mentioned that the tested trees of *J. ailantifolia* were of small dimensions. In September 2020, a dozen trees had fallen. Dying trees were attacked by xylophagous insects, such as *Xylosandrus germanus* (fig. 4G, found in 20 trees) and xylophagous fungi (fig. 4H).

Resprouting capacity – Resprouting capacity of *J. ailantifolia* in reaction to girdling was weak (fig. 4B). The number of basally resprouting shoots or basal sprouts (fig. 4E) fell continuously from 12-18 (average 8) in July 2019 to no such shoots in September 2020. As for the maximum height of these basal shoots, there was a decrease in the first control year from 25-75 cm (mean of 56 cm) in July to 5-65 cm (mean of 19 cm) in September 2019, then an increase to 40-120 cm (mean of 57 cm, but only 13 of 30 trees) in June and a decrease to no shoots in September 2020, i.e., ending the second year of control (fig. 4B). It must be noted, that suckers can develop, if trees are cut at the foot (data not shown). Two months after the three-ring treatment, half of the trees began to close the top ring in order to heal the wound (fig. 4E). This behavior is also known in other alien species. For example, *A. altissima* closes the wound from top to bottom with bark (Forest Service of Canton Ticino, unpublished data), whereas *J. ailantifolia* develops a new woody structure made of a spongy tissue (fig. 4F).

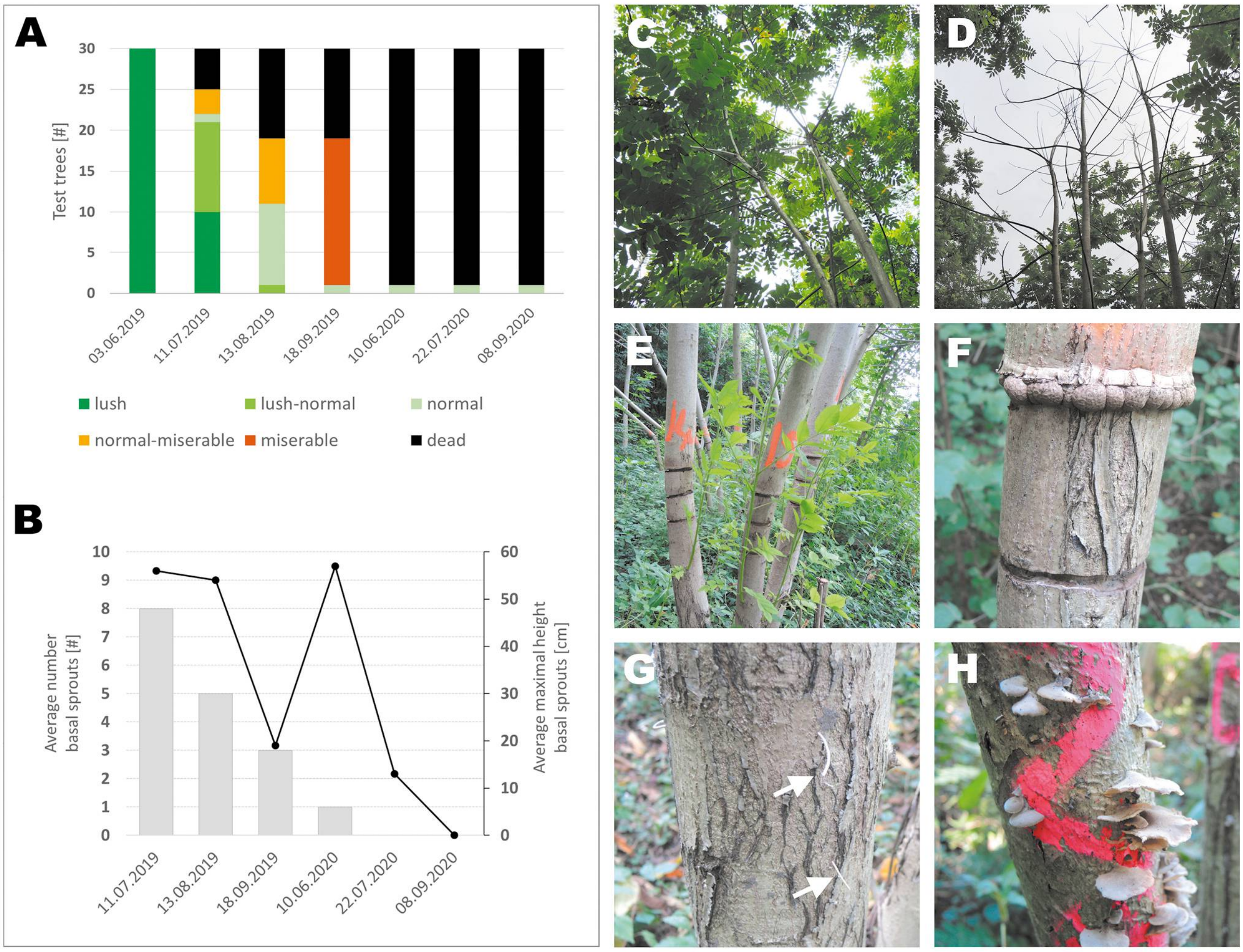

Figure 4: Three-ring girdling experiment on *Juglans ailantifolia.* A-B. Results of the experimental plot at the site in Gnosca (Switzerland). A) Vitality after girdling, described in categories by the aspect of the foliage (from lush to miserable) and tree death. B) Resprouting capacity in time, as indicated by the mean number and the mean maximum height of basal sprouts. C) Individuals with intact foliage at the beginning of the experiment (3 June 2019). D) The same individuals, dead and lacking foliage (8 September 2020). E) Basal resprouting as a reaction to the girdling (11 July 2019). F) Spongy bark tissue closing the top ring of the girdling experiment (observed in ca. 50% of treated individuals). G) Dying tree with typical signs by the xylophagous alien beetle *Xylosandrus germanus.* H) Dying individual attacked by xylophagous fungi.

Invasion potential

When such a conspicuous leafy tree is found in a site in which it formed a rich renewal in the understory, it is understandable that the question raises as whether this species can become invasive. In the following, we discuss the spread potential and impact potential, consisting of features about reproduction, dispersal and recent geographic spread according to the criteria catalogue of Info Flora (2014). The species reproduces only sexually, and sets the first seeds at a minimum of 10 years (Bonner 2008a). Individuals in the wild produce most likely less than 1'000 nuts/tree (each nut contains one seed) and should not be compared with *Juglans* trees farmed for nut production. It is unclear whether a seed bank is formed. We mainly found old "empty" nut halves, and studies on seed storage and seed longevity suggest that *Juglans* nuts would rarely retain viability for more than 10 years with current storage technology (Bonner 2008b). Furthermore, Spring frost may affect the early flowering of *J. ailantifolia* and cause flowers to abort (fig. 2L).

Dispersal by natural vectors is most likely by gravity, hence nearby the mother plant (fig. 2I). Even if nuts are dispersed by squirrels, their spread would be close. The rich renewal observed in the understory of the site in Gnosca is not to be compared with dense monoclonal stands of invasive alien plants, like *Reynoutria japonica* Houtt. or *Prunus serotina* Ehrh. This situation is somewhat artificial, as it was possible due to the high density of cultivated trees as a private (then abandoned) plantation. However, nuts can float, meaning that nuts from trees close to streams, rivers, lakes, or in floodplains could be dispersed by water, which is considered a dispersal vector for long distances (over 1 km) (Info Flora 2014). This is the case in New Zealand, where the species has spread in the Waikato Region, for example along Mangapiko and Mangaohoi streams on public and private land, and has been targeted by local groups and also reported on local media (Cursey 2010; Te Awamutu Courier 2020). The Waikato Region is the only place known to us where the species has been reported as an unwanted weed. It is banned from propagation or sale and is listed in the governmental management category of 'site-led pest plants', i.e., *where the outcome for the programme is to exclude, eradicate, contain, reduce or control the pest that is capable of* *causing damage to a place and its values* (Waikato Regional Council 2019, p. 25). Although New Zealand adopts a hard line against alien species to protect its unique biota, we should keep their case in mind, as there is some degree of climatic similarity between the lowlands of Canton Ticino and Lombard Prealps and the Waikato Region, characterized by relatively warm temperatures in the summer (20-25°C mean daily maximum temperature), relatively cold temperatures during the winter (0-8°C mean daily minimum temperature), and relatively abundant rainfall throughout the year (Chappell 2014).

Dispersal of *J. ailantifolia* by human activities is most likely very low, as it is not a garden ornamental in Switzerland and Italy. The plant is unable to set roots and resprout from pruned foliage and shoots and to give rise to a new stand in this way. The fact that the species occurs in the wild in few places where it has been overlooked for many decades, and that few adult trees of second and third generation exist far from cultivated mother plants, indicates the geographic spread of *J. ailantifolia* in southern Switzerland and northern Italy has been slow in the last decades. This could also be explained by the fact that the species does best in a relatively narrow range of environmental conditions, that are rather uncommon in the Insubric area.

As it was not possible for us to quantitatively assess the impact of *J. ailantifolia* on native species, we discuss potential impacts based on the current vegetation observed at Gnosca, probably the site with the most favorable conditions to the species. However, we were unable to infer any relevant impact on native species, because we must bear in mind that this site was an artificially created and then abandoned private plantation. Actually, the dominant presence of *Equisetum hyemale* in the undergrowth rather suggests that the native understory alluvial vegetation was able to colonize the site after the plantation was abandoned, hence despite the presence of the alien species and its seedling recruitment. While further studies are needed to understand any potential impacts of *J. ailantifolia* from wild stands, such as in Piario, it is unlikely to expect hybridization with the archaeophyte *J. regia*. This was not the case in the United States: as *J. ailantifolia* and its cultivar Cordiformis were introduced already in the mid-19th century and became widely cultivated for the heart-shaped nuts, they have hybridized with the native *J. cinerea* (Hoban et al. 2009) originating *J. ×bixbyi* Rehder to such an extent to raise concern for its conservation (Farlee et al. 2010; Pike et al. 2020). No hybrids seem to occur with *J. regia* (Farlee et al. 2010), although *J. hopeiensis* Hu seems to have originated as a hybrid between *J. regia* and *J. mandshurica* (Mu et al. 2017).

In sum, the biological characteristics, spread history, and current observable impacts indicate that the potential spread and impact of *J. ailantifolia* in Switzerland and Italy is low and do not support its classification as an invasive species. Nevertheless, because nuts can be dispersed along waterways, we recommend to control its spread close to alluvial habitats, streams, rivers or lakes, as well as avoid any cultivation of this tree in or nearby these habitats. Given its ability to resprout, if trees are cut to the foot or girdled, it is necessary to control for suckers and resprouting of basal shoots. We recommend to prefer *J. regia* for timber plantings and native tree species for reforestation.

ACKNOWLEDGMENTS

We are grateful to Nicola Bomio-Pacciorini, Giovanni Galli, and Gisella Novi for help with the girdling experiment; to Lucia Pollini and Christophe Germann for identification of *Polydrusus formosus*; to Arnaud Albert, Michael Hassler, Jérôme Hog, Christian Gilli, Emmanuelle Sarat, and Filip Verloove for information on *J. ailantifolia* in their countries; to Pierino Bigoni, Germano Federici, Rizzieri Masin, and Alberto Selvaggi for information on alien *Juglans* in Lombardy, Piedmont and Veneto, and for the Lombard map; to Giovanni Galipò and Duccio Baldassini for information from Vallombrosa; and to David Frey for valuable comments that improved this manuscript.

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