A look at ploidy levels of *Vincetoxicum nigrum* (L.) Moench and *V. rossicum* (Kleopow) Barbar. (Apocynaceae) from the perspective of a study of their invasion success

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Vincetoxicum sp. seed pods

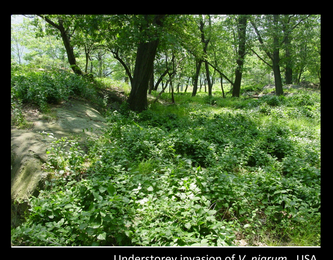

V. rossicum , USA


V. rossicum, Sweden


V. nigrum, France


V. nigrum, Spain


V. nigrum, USA


Understorey invasion of *V. nigrum* , USA


Isolated plant of *V. nigrum* , Mallorca


V. hirundinaria , France

INTRODUCTION

Many polyploid plants are well-adapted, successful weedy species, which reinforces the idea that polyploidy may have predisposed species to become invaders. Interestingly, newly formed polyploids are frequently invasive species, which suggests that polyploidy confers an immediate ecological aptitude to invade new habitats. Invasive weedy species of recent origin are excellent model systems to investigate the early evolutionary mechanisms associated with invasiveness. *Vincetoxicum nigrum* (L.) Moench [Black swallow-wort, BSW] is native to southwestern Europe and *V. rossicum* (Kleopow) Barbar. [Pale swallow-wort, PSW] (Apocynaceae) originates from Ukraine and southwestern Russia (Figure 1). Both species are invasive in natural areas, abandoned pastures and rural sites in the U.S. and Canada (Figure 1). The most likely source of introduction of both species was importation as specimens for botanical gardens in the late 19th Century though this remains uncertain. From a review of the literature, data for the ploidy level and chromosome numbers for swallow-wort species in the introduced and native ranges are rare and moreover ambiguous. One single chromosome count of $2n$ =22 was reported for PSW in Canada, whereas chromosome counts for BSW vary from $2n$=22 to $2n$=44 for two populations in Italy.

The present study aimed to document precisely the patterns of chromosome counts and/or ploidy level variation of both species in both native and introduced ranges and to address the hypothesis that species invasiveness had been induced by a switch in ploidy level.

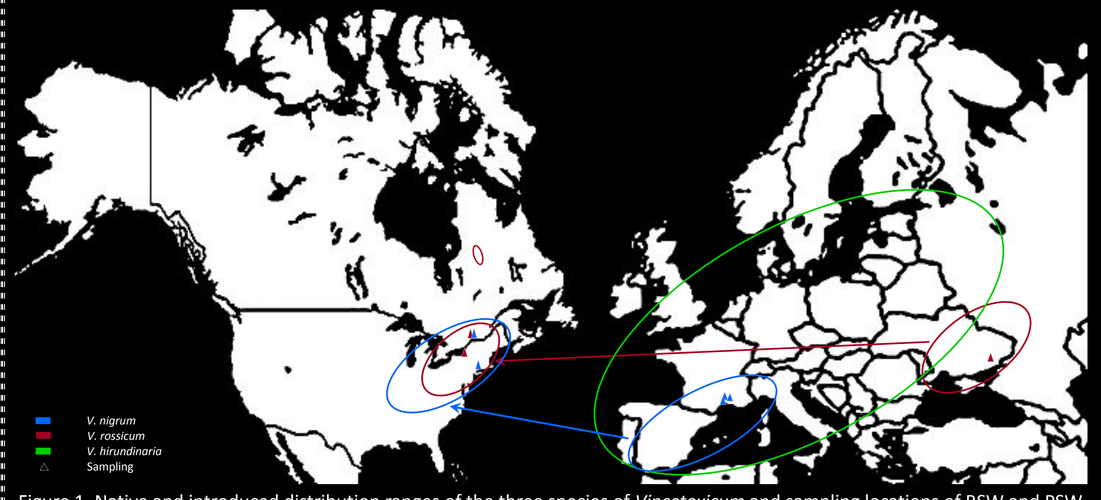


Figure 1. Native and introduced distribution ranges of the three species of *Vincetoxicum* and sampling locations of BSW and PSW.

MATERIELS & METHODS

Ploidy levels of both species were determined by i) chromosome counts in root tips of 4-day-old seedlings that germinated in moistened Petri dishes and ii) flow cytometry from fresh foliar tissues in adult leaves of plants growing in a Phytotron.

Chromosome counting was conducted in collaboration with Marguerite Goud at the CIRAD Molecular Cytogenetics platform (UMR- DAP). The chromosome preparation from root tips was done according to D'Hont et al. (2000) and the protoplast preparations were performed as described in D'Hont et al. (1996). The metaphase chromosomes were counterstained with (2.5µg/ml) DAPI (4'.6-diamino-2-phenylindole) in vectashield antifade mounting solution (Vector Laboratories) and observed with a microscope (Leica DMRAX2) under a wavelength of 456 nm. Data acquisition was done with the software Volocity (Perkin Elmer).

Flow cytometry analyses were carried out in two phases. In the first phase, the nuclear DNA content was estimated in plants at different developmental stages and growing in a Phytotron in order to determine, besides other things, the most appropriate choice of leaf explant for the second phase. Plants originated from four populations of BSW in France (Saint Clément, Vidauque, Lecques, Clapiers; Figure 1). Preliminary flow cytometry analyses were conducted by EBCL under the supervision of Frédéric Bakry at the CIRAD Flow Cytometry platform (Multiplication Végétative-UR75).

In the second phase, the nuclear DNA content was estimated in plants of all populations of BSW and PSW from both ranges (Figure 1). This comparative study was conducted by Spencer Brown at the Cytometry Service, Institut des Sciences du Végétal, CNRS UPR2355.

At CIRAD, approximately 0.5 cm² of healthy leaf of the sample was shopped together with the internal standard in 1,500 µl of modified LB01 nuclei extraction buffer (Dolezel *et al* 1989). The solution obtained was filtered with a 30 µm nylon mesh to eliminate cell debris and treated with 0.03% RNAse II A. After filtration, 50 µl of propidium iodide (0.2mg/ml) was added for DNA staining. After 30 min of incubation, the samples were analyzed using a Partec PAII laser Flow Cytometer (Partec GmbH, Münster, Germany) equipped with a Blue Solid State laser (488 nm). A minimum of 5000 nuclei per sample and a minimum of 5 independent replications were analyzed. Histograms were analyzed using the Partec FloMax software, which determines peak position, coefficient of variation (CV), and the relative ploidy index of the samples. Reference standards were *Oryza sativa* cv "NipponBar" (2C=2x=0.91pg) at CIRAD and *Lycopersicum esculentum* cv

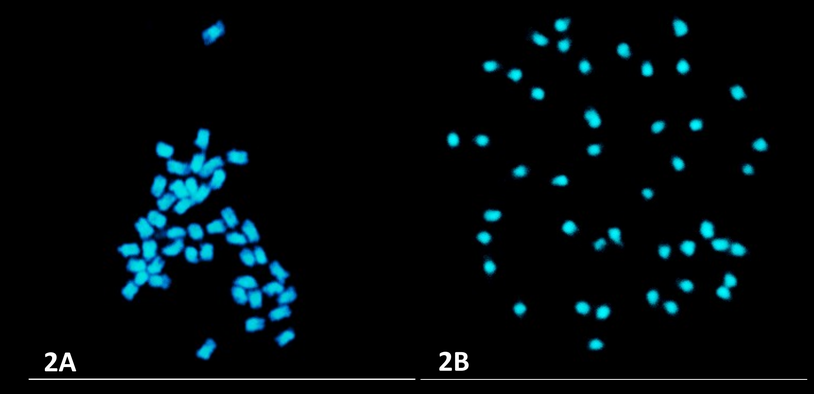


Figure 2. Metaphase chromosome spreads of *V. nigrum* from USA, Bear Mountain (2A) and Antwerp (2B), from France, Saint Clément (2C) and Baillarguet (2D). Magnification x630.

RESULTS (1)

Chromosome constitution in all four BSW populations considered (Bear Mountain and Antwerp, New York State, USA; Baillarguet and Saint Clément, Hérault, France) was $2n$=44; (Figures 2A, 2B, 2C and 2D). Chromosome constitution in two PSW populations (Jamesville, New York State USA; Donetsk, Ukraine) was $2n$=22 (Figures 3A and 3B). Given that the basic chromosome number in the genus *Vincetoxicum* is x=11, our results confirmed the diploidy and tetraploidy of PSW and BSW respectively.


V.rossicum seeds


V.rossicum seedlings for chromosome counting

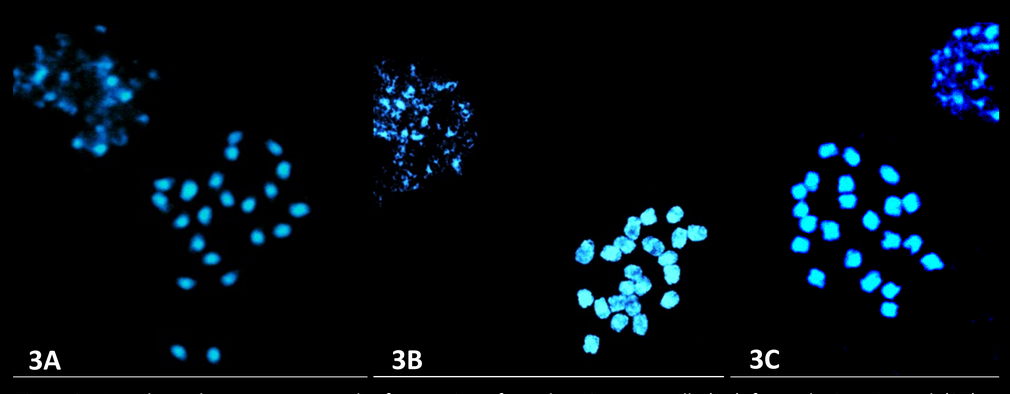


Figure 3. Metaphase chromosome spreads of *V. rossicum* from the USA, Jamesville (3A), from Ukraine, Donetsk (3B) and *V. hirundinaria* from Austria, Mösern (3C). Magnification x630.

RESULTS (2)

Flow cytometric analysis of isolated nuclei resulted in histograms of their DNA content compared to that of standard references and represented one peak corresponding to the 2C level (G_0/G_1 phase) (Figure 4).

In the preliminary study, the 2C DNA content of leaf nuclei was assessed in BSW plants from four populations in the native range (Table 1). One way ANOVA indicated that there was no significant difference in the DNA content among populations of BSW (P=0.101, α=0.05). From a qualitative standpoint, the most appropriate choice of explant was a leaf of the second or the third last leaf pairs (Figure 5).

In the final study, the 2C DNA content of leaf nuclei was compared between populations in native and introduced ranges for each species (Table 2), CV varied from 0.59 to 3.38%. For both species, no significant difference in the DNA content and ploidy level was observed between native and introduced ranges (after t-test , α=0.05).

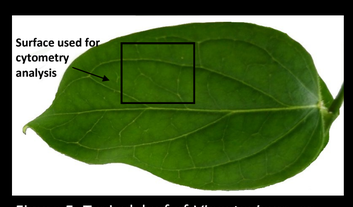


Figure 5. Typical leaf of *Vincetoxicum sp.* used for flow cytometry

Table 1. Preliminary study of nuclear DNA content in BSW from the native range.

French population	Nb. of Individuals	DNA content [pg/2C ± S.D.]
Saint-Clément, Hérault	5	1.29 ± 0.01
Vidauque, Vaucluse	5	1.27 ± 0.02
Lecques, Gard	8	1.24± 0.03
Clapiers, Hérault	5	1.28 ± 0.02

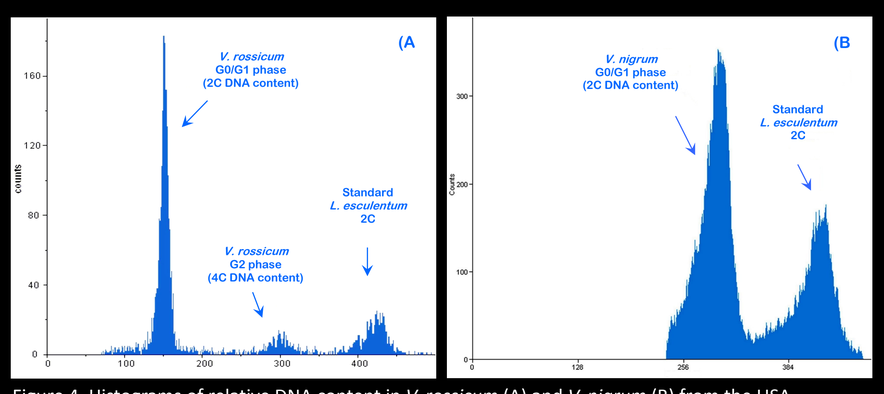


Figure 4. Histograms of relative DNA content in *V. rossicum* (A) and *V. nigrum* (B) from the USA

Table 2. Nuclear DNA content (mean ± standard deviation), ploidy level, and mean genome size of BSW and PSW

Species	Range	Nb of measures	DNA content [pg/2C ± S.D.]	Ploidy level	Genome size [Mbp/1C] [b]
BSW	Native (Baillarguet, France) [a]	11	1.47 ± 0.03	4	723± 23
	Introduced (Bear Mountain, USA [a]& Ottawa, Canada)	13	1.45 ± 0.04	4	715± 19
PSW	Native (Donetsk, Ukraine) [a]	3	0.70 ± 0.00	2	343± 2
	Introduced (Jefferson Co., USA & Ottawa, Canada)	12	0.72 ± 0.02	2	352± 8

a: Ploidy level is also assumed based on samples on which chromosomes were counted.
b: One copy of nuclear genome. 1pg = 978 Mbp (Dolezel et al., 2003)

MAIN CONCLUSIONS

- The present study confirmed the tetraploidy of BSW and the diploidy of PSW. Moreover, PSW is diploid like one of its phylogenetically closely related species i.e. *V. hirundinaria* Medik (White swallow-wort, WSW), (Figure 3C) that is sympatric to BSW and PSW in its native range but is not known to be naturalized in the North America. (Figure 1). Discrepancies found in the literature per the chromosome counts in BSW within its native range are most likely due to misidentification between BSW and WSW taxa.
- This is the first report of DNA content values in any species of the genus.
- The present study gave evidence that neither change in ploidy level, nor change in DNA content has occurred during the introduction of these two weeds in North America. Given that the invasive spread of BSW and PSW was not triggered by differences in ploidy level, alternative explanations should be sought including neutral genetic and or phenotypic variations.

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