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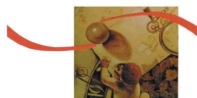

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Genetic admixture in species of *Conyza* (Asteraceae) as revealed by microsatellite markers

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ABSTRACT. The distinction among *Conyza canadensis*, *C. bonariensis*, and *C. sumatrensis* is a challenge for weed science. In the current study, primers for microsatellite (SSR) loci were used to investigate the molecular divergence among the three species, the genetic structure of the populations at the molecular level and the level of genetic admixture among *Conyza* plants from southern Brazil. Twelve primers amplified well-defined DNA segments for all 88 samples of the three *Conyza* species. The estimated proportion of SSR polymorphic loci, number of alleles, and mean expected heterozygosity were higher in samples of *C. bonariensis* than in samples of *C. sumatrensis* or *C. canadensis*. *Conyza canadensis* was the species with the lowest molecular diversity. High genetic divergence was observed among the three species. The well-defined ancestral groups for each species led to the identification of samples of *Conyza* with ancestral genomes from the three species. Hybridization events between pairs of these species may have occurred in crop fields from southern Brazil. The high molecular diversity in resistant biotypes of *C. sumatrensis* indicated that these biotypes have a high potential to colonize new areas, which increases its potential as a weed.

Keywords: horseweed, molecular divergence, SSR loci.

Mistura genética em espécies de *Conyza* (Asteraceae) reveladas por marcadores microsatélites

RESUMO. A identificação das espécies *Conyza canadensis*, *C. bonariensis* e *C. sumatrensis* tem sido desafiadora para a ciência das plantas daninhas. No presente estudo, *primers* para locos microssatélites (SSR) foram utilizados para investigar a divergência molecular entre as três espécies; mostrar como as populações estão geneticamente estruturadas em nível molecular e avaliar o nível de mistura genética entre as plantas de *Conyza* no Sul do Brazil. Doze *primers* amplificaram segmentos de DNA bem definidos em todas as 88 amostras das três espécies. A proporção estimada de locos SSR polimórficos, o número de alelos, e a heterozigosidade média esperada foram mais altos nas amostras de *C. banariensis* do que nas demais. *C. canadensis* foi a espécie com menor diversidade molecular. Divergência genética alta foi observada entre as três espécies. A formação de grupos ancestrais bem definidos para cada espécie levou à identificação de amostras de *Conyza* com genoma ancestral das três espécies. A ocorrência de hibridização entre as três espécies pode ter ocorrido nas lavouras do Sul do Brasil. A diversidade molecular alta em biótipos resistentes de *C. sumatrensis* indicou que estes biótipos têm um alto potencial para colonizar novas áreas, o que agrava seu potencial como planta daninha.

Palavras-chave: buva, divergência molecular, locos SSR.

Introduction

Some species of the genus *Conyza* are important weeds as they cause serious economic losses in agriculture (Thebaud & Abbott, 1995; Bossdorf et al., 2005). The species *C. canadensis* (L.) Cronquist (horseweed), *C. bonariensis* (L.) Cronquist (hairy fleabane) and *C. sumatrensis* (Retz.) E. Walker (Sumatran fleabane) are examples of weeds that occur in orchards; vineyards; corn, soybean, cotton, and forage crops; pastures, and fallow areas (Lazaroto, Fleck, & Vidal, 2008). *Conyza canadensis* is native to North America, whereas *C. bonariensis* and *C. sumatrensis* are native to South America. Among these three species, *C. canadensis* and *C. sumatrensis* are the most widespread worldwide (Thebaud & Abbott, 1995).

The increase in the economic relevance of these weeds is related to two main factors. One factor is the very efficient seed dispersal of these species and their prolific seed production, estimated at over 148,000 m^{-2} (Steckel & Gwathmey, 2009). The second factor is the intense use of chemical options for their control, which has led to the selection of biotypes that are resistant to several herbicide mechanisms of action. To date, resistance to EPSPS inhibitors, ALS inhibitors and Photosystem I (PSI) and II (PSII) inhibitors individually, and multiple resistance to PSI + EPSPS, ALS + EPSPS or ALS + PSII have been reported (Matzraf, Lazar, Sibony, & Rubin, 2015). Among all three species, cases of resistance to herbicides have been reported in 35 countries (Heap, 2016).

Conyza bonariensis and *C. sumatrensis* are common in Southern, Southeast and Midwestern of Brazil (Santos et al., 2014b). In contrast, *C. canadensis* occurs only in the Southern Region, particularly in Rio Grande do Sul State (Lazaroto et al., 2008). Identification keys (Pruski & Sancho, 2006) typically define characteristics that can be used to differentiate the three *Conyza* species. In *C. canadensis*, the leaves are yellowish green and glabrous, whereas in *C. bonariensis* and *C. sumatrensis*, they are greyish green and very hairy. The species vary in height and branching habit. *Conyza canadensis* branches from the middle of the main stem, *C. bonariensis* has branches that are taller than the main stem, and *C. sumatrensis* has branching towards the top of the main stem (Sansom, Saborido, & Dubois, 2013). However, in South America, definite identification of and distinction among the three species of important weeds is difficult due to morphological variability within species, the occurrence of varieties within some species, and hybridization between species (Thebaud & Abbott, 1995).

Herbicide susceptibility varies among these species (Gonzalez-Torralva et al., 2010; Zheng et al., 2011); however, due to the difficulty in their identification, reports of unsuccessful control might involve weed misidentification. Developing a deeper understanding of the genetic structure of these species may help scientists to develop more precise identification tools and effective strategies for control.

In contrast to the several studies on the characteristics of resistant biotypes in other weeds (Yamauti et al., 2010; Xiao-Ling et al., 2011; Santos et al., 2014a), few studies have addressed how the populations of *C. canadensis*, *C. bonariensis* and *C. sumatrensis* are genetically structured at the molecular level. Molecular analysis techniques allow us to estimate the genetic variability within and between species. They can also be used to search for specific unique molecular markers (alleles) of one or more species of *Conyza*; if found, such markers could be used to support the identification of and distinction among species. Differential allele frequencies could also be useful for species identification.

Simple sequences repeated of genomic DNA (SSR loci, also known as microsatellite loci) are efficient molecular markers to estimate genetic divergence and to reveal population structure in many plant species. In *Conyza* species, SSR loci have been used as molecular markers to investigate the evolution and spread of glyphosate resistance in *C. canadensis* in California (Yuan et al., 2010; Okada et al., 2013).

Invasive species that grow under strong selection pressure from herbicide application usually show significant genetic differentiation. Moreover, less genetic diversity is expected in samples of resistant biotypes that have been under selection due to the recurrent use of herbicides. In the current study, SSR loci were used to investigate the molecular divergence among the three species (*C. canadensis*, *C. bonariensis*, and *C. sumatrensis*) and to evaluate the level of genetic admixture among *Conyza* plants from southern Brazil.

Material and methods

The seeds of *C. sumatrensis* were collected from several plants in Campo Mourão, Floresta, Cascavel, Cafelândia, Peabiru, and Toledo in Paraná State, Brazil, in January 2011 (Figure 1; Table 1). Seeds were collected from plants in fields of glyphosate-resistant (GR) soybeans with a history of glyphosate applications of at least four years and for which farmers reported decreasing levels of control over repeated glyphosate applications. The seeds from each collection site were individually placed in paper bags to prevent the mixture of seeds from different collection sites.

Seeds from each site were randomly distributed for germination in separated 500-mL pots containing sterile soil. Plants obtained from germinated seeds were maintained at room temperature in the greenhouse at the State University of Maringá (latitude 23°23'44.91"S and longitude 51°57'3.13"W, altitude 510 m), irrigated daily, and used for the experiments. A sample consisting of young leaves collected from 43 plants of *C. sumatrensis* was used for DNA extraction. The young leaves were collected from plants 15-30 days after plant emergence. Samples of these plants in full bloom were then sent to the Institute of Biology

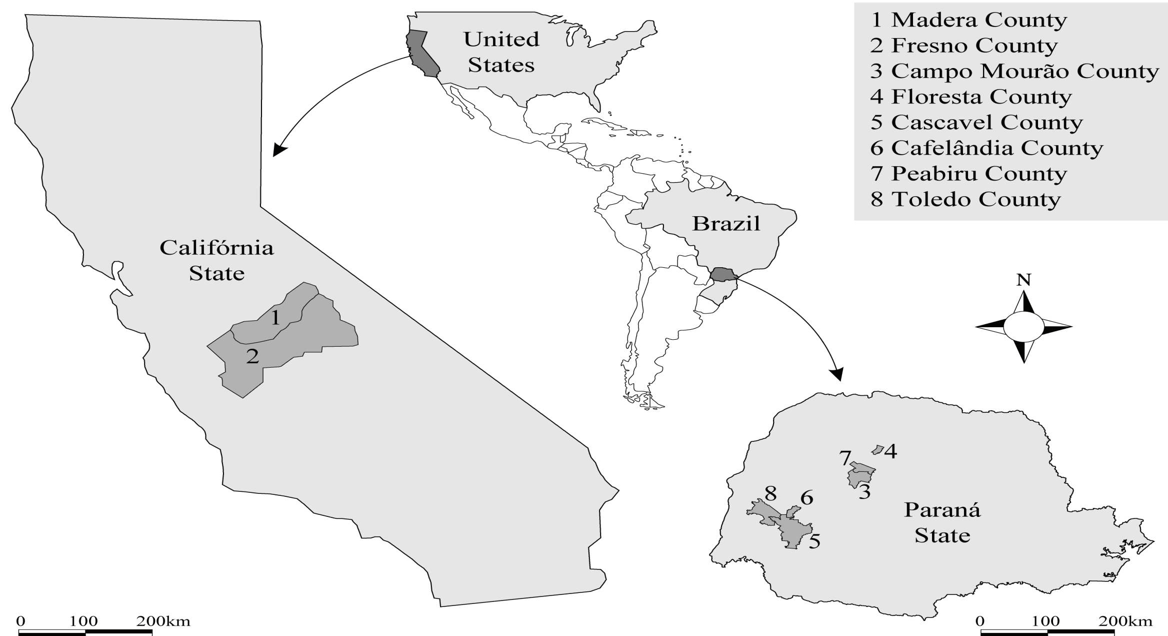

Figure 1. Distribution of the *Conyza* plants from which seeds were germinated to form samples representative of *C. sumatrensis* from Campo Mourão, Floresta, Cascavel, Cafelândia, Peabiru, and Toledo in Paraná State, Brazil, and of *C. canadensis* and *C. bonariensis* from the Central Valley of the State of California, United States.

(Uberlandense Herbarium) at the Federal University of Uberlândia (Minas Gerais State, Brazil), where they were cataloged and classified as *Conyza sumatrensis* (Retz.) E. Walker. The plants of *C. sumatrensis* were analyzed for possible resistance to glyphosate, which was applied at different stages of development according to procedures described by Santos et al. (2014b). The samples from Cascavel and Toledo were recorded as glyphosate resistant (SFR 6 and SFR 9, respectively; Table 1), whereas the remaining samples of *C. sumatrensis* (SFS) were recorded as sensitive to glyphosate.

The seeds of *C. canadensis* and *C. bonariensis* were collected in the Central Valley of the state of California, United States (Table 1; Figure 1). Since *C. canadensis* and *C. bonariensis* are well-differentiated species in North America, samples of the two species were collected in California and used as referential genomes to evaluate the level of genetic admixture among *Conyza* plants of southern Brazil. The broad morphological variability in *Conyza* plants from southern Brazil makes it difficult to obtain a secure identification of *C. canadensis* or *C. bonariensis* plants. Then, the seeds of resistant plants of *C. canadensis* (HWR) were collected in Madera County (Dinuba, CA), and the seeds of susceptible plants of *C. canadensis* (HWS 156) were collected in Fresno County. The seeds of *C. bonariensis* were collected from plants with multiple resistance (BH 51) to glyphosate and ALS in Fresno County (Parlier, CA) and from plants resistant to glyphosate (BH 53) in Parlier. Seeds were germinated in plastic trays (52 x 27 x 6 cm) in the greenhouse at the University of California (Davis, CA), using the substrate Sunshine Mix (Sungro Horticulture Canada, Ltd., Vancouver, British Columbia, Canada) for the establishment and growth of the plants. The plants were grown in greenhouse at the experimental field of the university (38°32'33.52"N and 121°45'47.92"W, elevation 17 meters). Samples consisting of young leaves collected from 45 plants of each species (*C. canadensis* and *C. bonariensis*) were used for DNA extraction.

Table 1. Samples of *Conyza sumatrensis* (SF) obtained from several sites (Campo Mourão, Floresta, Cascavel, Cafelândia, Peabiru, and Toledo) in Paraná State, Brazil, and samples of *Conyza canadensis* (HW) and *Conyza bonariensis* (BH) obtained from Dinuba, Fresno, and Parlier in the Central Valley of the State of California, USA.

	Site	Samples	Number of plants	Altitude (m)
Conyza sumatrensis	Campo Mourão	SFS 13	9	634
	Floresta	SFS 18	7	391
	Cascavel	SFR 6	9	625
	Cafelândia	SF 2	9	509
	Peabiru	SFS 17	4	501
	Toledo	SFR 9	5	531
Conyza canadensis	Dinuba	HWR	9	94
	Fresno	HWS 156	9	83
Conyza bonariensis	Parlier	BH51	4	103
	Parlier	BH53	23	106
	Total		88	

SFS: glyphosate-susceptible *C. sumatrensis*, HWR: glyphosate-resistant *C. canadensis*, HWS: glyphosate-susceptible *C. canadensis*, BH: *C. bonariensis*.

For DNA extraction, leaf pieces (300 mg) of each *C. sumatrensis* plant were separately ground in liquid nitrogen and homogenized with a glass rod in an Eppendorf microcentrifuge tube with the use of 800 μL extraction solution prepared with Tris-HCl 100 mM / EDTA 20 mM containing NaCl 1.4 M, CTAB (Cetyl Trimethyl Ammonium Bromide) 2%, PVP-40 (Polyvinylpyrrolidone-40) 2%, and β-mercaptoethanol 0.2% and maintained in an ice chamber. After homogenization, the microcentrifuge tubes were shaken gently and incubated at 65°C for 30 min. DNA was extracted according to the protocol by Doyle and Doyle (1990).

DNA extraction from plants of *C. canadensis* and *C. bonariensis* was performed using the Soltis Lab CTAB DNA Extraction Protocol (2002) developed from Doyle and Doyle (1990) and Cullings (1992) protocols (http://www.flmnh.ufl.edu/museum-voices/soltis-lab/files/2014/02/CTAB-DNA-Extraction.pdf). The young leaves were collected from plants with six weeks after plant emergence. For DNA extraction, leaf pieces (10–20 mg) were separately ground in liquid nitrogen and homogenized with a glass rod in an Eppendorf microcentrifuge tube with the use of 500 μL CTAB buffer extraction prepared according to the Soltis Lab CTAB DNA Extraction Protocol (2002).

After DNA extraction, DNA quantity and quality were determined by 0.8% agarose gel electrophoresis buffered with 1 x TAE (0.04 M Tris-Acetate and 0.001 M EDTA). A standard DNA (λ phage; 50, 100, and 150 ng) was used as a marker of concentration. The gel was stained with 0.5 μg/mL ethidium bromide, and the image was visualized with a Molecular Image Loccus L-PIX - HE (Loccus do Brasil Ltda., São Paulo, São Paulo State, Brazil) and the Picasa 3 program. The samples were also quantified using UV-visible Picodrop® spectrophotometer to verify the concentration of DNA per μL of each sample for dilution to be used in the PCR reactions.

Sixteen SSR primers previously developed for *C. canadensis*, i.e., HW01, HW02, HW06, HW07, HW14, HW27, HW29 (Abercrombie, Anderson, & Baldwin, 2009), HW17, HWSSR01, HWSSR03, HWSSR04, HWSSR06, HWSSR07, HWSSR09, HWSSR11, and HWSSR12 (Okada et al., 2013), were used with eight DNA samples to define the DNA quantity used for polymerase chain reaction (PCR). After initial screening, a 1.0 μL aliquot of DNA was defined for further analysis. PCR was performed using a PTC-200 Peltier thermal cycler. The reaction mixtures were prepared in microtubes (1.5 mL) and then loaded on a multiplex plate as described by Okada et al. (2013), with a final volume of 10 μL per reaction: dNTP 125 mM, 0.375 units of Taq polymerase (QIAGEN, Valencia, CA, USA), 1 x PCR buffer (QIAGEN), 10 ng DNA, 10 mM of the reverse and forward primers, and Milli-Q water to make up to 10 μL.

Microsatellite amplification was initially performed with initial denaturation at 94°C for 5 min. followed by 34 cycles at 94°C for 1 min.; annealing was carried out at 55 °C for 1 min, and extension was at 72°C for 1 min. and 30 seconds. Electrophoresis was performed in a 2% agarose gel using 0.5 x TBE buffer (44.5 $mmol \cdot L^{-1}$ Tris, 44.5 $mmol \cdot L^{-1}$ boric acid, and 1 $mmol \cdot L^{-1}$ EDTA) at 109 V for 60 min. Each gel was stained with ethidium bromide at 0.5 $\mu g \cdot mL^{-1}$, and the image was captured using a Kodak EDAS 290 digital camera. The sizes of the amplified DNA segments (alleles) were determined using a 100-bp DNA Ladder (Invitrogen).

The polymorphic SSR loci in the *C. sumatrensis*, *C. canadensis*, and *C. bonariensis* samples were used to estimate the average number of alleles per locus, the average observed heterozygosity (H_o), and the expected heterozygosity (H_e). The genetic diversity among the six samples of *C. sumatrensis* (F_{ST}) was estimated using the POPGENE 1.32 program (Yeh, Boyle, & Xiyan, 1999). The genetic distance matrix was calculated by the UPGMA clustering method (Unweighted Pair-Group Mean Average) using the values of Nei genetic distances (Nei, 1978).

Polymorphism in the SSR loci was also analyzed using the software Structure 2.0 (Pritchard, Wen, & Falush, 2010) and Instruct (Gao, Williamson, & Bustamante, 2007), which evaluate the level of genetic admixture among the three species of *Conyza*. The genotypes were clustered, with the number of clusters (K) ranging from 2 to 14, and were tested using the admixture model with a burn-in period of 200,000 repeats followed by 100,000 Markov Chain Monte Carlo (MCMC) repeats, considering the presence and absence of alleles across the sample. The true number of populations (K) is often identified using the maximal value of Δ (K) returned by the software. The most likely number (K) of subpopulations was identified as described by Evanno, Regnaut, and Goudet (2005). The graphical output display of the Structure results was taken as input data using the Structure Harvester, a website and software that are used to visualize Structure output and to implement the Evanno method (Earl & Von Holdt, 2012) to display a graphical representation. The graphical output display of the Instruct results was taken using the Distruct software (Rosenberg et al., 2002) to draw a bar plot of individual genome assignments. To explore the hierarchical partitioning of genetic variation within and among the samples of *Conyza*, we performed an Analysis of Molecular Variance (AMOVA, GenAlEx 6.2; Peakall & Smouse, 2006).

Results and discussion

Four primers (HW01, HW27, HWSSR06, and HWSSR12) did not produce well-defined amplified DNA segments for all 88 samples of *C. sumatrensis*, *C. canadensis*, and *C. bonariensis*, and only 12 (HW02, HW06, HW07, HW14, HW29, HW17, HWSSR01, HWSSR03, HWSSR04, HWSSR07, HWSSR09, and HWSSR11) amplified two or more alleles per polymorphic locus (Table 2). The estimated proportion of SSR polymorphic loci (%P) was higher in samples of *C. bonariensis* (91.67%) than in samples of *C. sumatrensis* (83.33%) or *C. canadensis* (75%). However, the number of alleles (N_a), effective number of alleles (N_e), and mean expected heterozygosity (H_e) were highest in samples of *C. sumatrensis*, indicating the highest molecular diversity. Samples of *C. canadensis* showed the lowest molecular diversity (Table 3).

The Nei identity (Nei, 1978) values calculated from analysis of the 12 microsatellite loci of the three species of *Conyza* showed that the highest value of genetic identity (I = 0.8293) was observed between *C. canadensis* and *C. sumatrensis* and that the most divergent species (I = 0.6303) were *C. canadensis* and *C. bonariensis*. The similarity between *C. canadensis* and *C. bonariensis* was lower than that between *C. canadensis* and *C. sumatrensis* and that between *C. bonariensis* and *C. sumatrensis* (0.7348) for number and frequencies of alleles at the 12 SSR loci. AMOVA showed higher genetic variation within (54%) than among (46%) the *C. canadensis, C. bonariensis,* and *C. sumatrensis* samples.

Table 2. Simple Sequence Repeat (microsatellite) primers used for analysis of the *Conyza sumatrensis*, *C. canadensis*, and *C. bonariensis* plants, and the number of alleles at each locus.

Primer	Sequence of nucleotides	Repeat type	Number of alleles
HW02	F: AGTATTTGGCAATCAAAATTCG R: TCACAATCACAAACAACACAAA	$(AC)_{17}$ $(AT)_8$	2
HW06	F: CTTGCATGGTAGTCAACGTCAT R: CAGAGGTGGTCATGTGATGTG	$(AT)_7$ $(GT)_6$ $(GT)_6$ $(CT)_{10}$	3
HW07	F: GTGTGGCGCTACTCATTTCC R: TGATCACACCTGCGATTTGT	$(AC)_7$ $(AT)_6$	2
HW14	F: AAACTAAGGGTGATTGGGGAAT R: TGGATAGCCAAAAAGCTACAAA	$(TG)_{10}$	2
HW17	F: ACATTTACTCCAAGCCCAAATG R: AACAAATCGGTCAAATGACAAG	$(CT)_{12}$	2
HW29	F: CTACTTGTTCAATTTATCCATAC R: AAACTGGTTACTTCTCTTCC	$(AC)_7$ $(ATAC)_{22}$	2
HWSSR01	F:TATGTTGTACGACTGACTGAGATC R: CCATTGACTGTAGACCAGTGTG	$(CTAT)_{21}$	4
HWSSR03	F: TTGACTCCAACTCGTAGTGTATG R: ACGTTAAATCTCTCGTGTCCTTC	$(TG)_7$ $(GTATAT)_7$	3
HWSSR04	F: GGAAAACTCCTGTCATAGTATTAGC R: ATTAAAATCTAGCAAGGCCGAAC	$(AAT)_{18}$	3
HWSSR07	F: AGGACTTAACCCAACACCTTAC R: CTAGATGAACGCAAAAAATGAC	$(GTAT)_{10}$	3
HWSSR09	F: CATGAGTTTGAGTTATCCCAGAT R: CGAATACTTTCAATGCTTACGAC	$(AATTT)_5$	2
HWSSR11	F: ATCGTTGACATCTGACTCTGC R: GATTCTTGCTCTGGTTCCTTG	$(GAT)_{15}$	2

Table 3. Number of alleles (Na) and effective number of alleles (Ne) per polymorphic SSR locus, mean observed heterozygosity (H_o), expected heterozygosity (H_e), and percentage of polymorphic loci (%P) in the samples from the three *Conyza* species (*C. canadensis*, *C. bonariensis*, and *C. sumatrensis*).

Species	N	N_a	N_e	H_o	H_e	%P
C. canadensis	18	1.7500	1.5092	0.0370	0.2775	75.00
C. bonariensis	27	2.0873	1.6481	0.1115	0.3500	91.67
C. sumatrensis	43	2.1667	1.9388	0.1053	0.4291	83.33
Total/Mean	88	2.5	2.0194	0.0899	0.4787	100

The clustering of the *C. canadensis*, *C. bonariensis*, and *C. sumatrensis* plants according to a model-based Bayesian algorithm is shown in Figure 2. Each bar in the graph represents a plant and its inferred proportion of genome admixture. The colors represent three different ancestral groups. The optimal K value determined by Bayesian analysis indicated that the plants were grouped into 3 clusters (ΔK2 = 0.00; ΔK3 = 91.5046; ΔK4 = 0.3635; ΔK5 = 1.3088; ΔK6 = 0.9398; ΔK7 = 1.2130; ΔK8 = 0.00; ΔK9 = 1.4745; ΔK10 = 8.9503; ΔK11 = 0.1505; ΔK12 = 3.0456; ΔK13 = 0.2263; ΔK14 = 0.00). The bar plot obtained for the K value (K = 3; ΔK = 91.5046), and the results showed that 96.9% of the *C. canadensis* samples were in the red group, 96.7% of the *C. bonariensis* samples were in the blue group, and 79.7% and 15.1% of the *C. sumatrensis* samples were in the green and red groups, respectively (Table 4).

The bar plot graphic (Figure 2) shows that several plants identified as *C. sumatrensis* in the ancestral group that were defined as prevalent in samples of *C. canadensis* and *C. bonariensis* from the Northern Hemisphere (Central Valley of California, USA). Unlike the analysis using the Structure software, the analysis using Instruct identified ten clusters for the 88 samples of the three *Conyza* species. Plants sharing alleles from the ten groups are evident in samples of the species *C. canadensis*, *C. bonariensis*, and *C. sumatrensis*. The selfing rate was high, ranging from 0.655 ± 0.028 (cluster 1) to 0.803 ± 0.013 (cluster 10). In the plants of *C. canadensis* from Dinuba (HWR), the Instruct analysis identified the predominance of the ancestral genome from the pink group, which suggests the selection of one of the ancestral groups in the resistant biotypes of *C. canadensis*.

The bar plot shows that the plants of Campo Mourão represent all three groups but predominantly represent the red group (*C. canadensis*). Plants from the blue group (*C. bonariensis*) are also evident in samples of Campo Mourão. In the samples from Floresta and Cafelândia, high proportions of plants in the red group

(*C. canadensis*) are evident. In the samples from Peabiru and Toledo, low proportions of plants of the red and blue groups are apparent.

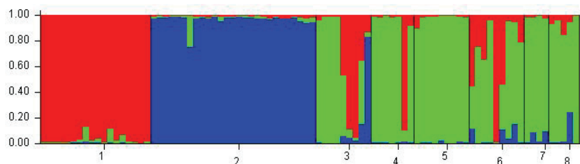

Figure 2. Bar plot of descendants from *Conyza canadensis* obtained from Dinuba (HWR) and Fresno (HWS 156), *C. bonariensis* obtained from Parlier, and *C. sumatrensis* obtained from Campo Mourão (3), Floresta (4), Cascavel (5), Cafelândia (6), Peabiru (7), and Toledo (8) in three inferred groups based on ΔK values. Each plantlet is represented by a single vertical line broken into K colored segments (K = 3), with lengths proportional to each of the K inferred clusters. Each color represents the proportion of membership of each individual, represented by a vertical line

Table 4. Proportion of *Conyza canadensis*, *C. bonariensis*, and *C. sumatrensis* samples in each group (K = 3) and the number of sampled plants.

Samples	Group			Number of plants
	Red	Green	Blue	
C. Canadensis	0.969	0.024	0.007	18
C. bonariensis	0.011	0.021	0.967	27
C. sumatrensis	0.151	0.797	0.051	43

A lower proportion of polymorphic loci was observed for *C. canadensis-R* plants (HWR: 41.67%) than for *C. canadensis-S* plants (HWS 156: 66.67%), suggesting that resistant plants show a reduction of polymorphism in microsatellite loci. Lower polymorphism in *C. bonariensis-2R* (BH51: 50%) plants than in *C. bonariensis-R* (BH53: 91.67%) plants was also observed but not considered of significance since a relatively low number of *C. bonariensis-2R* plants were analyzed.

In the samples of *C. sumatrensis* distributed among the six sites from southern Brazil (Paraná State), the highest proportion of polymorphic loci (83.3%) was observed in samples from Campo Mourão and Cafelândia. However, the highest number of alleles (Na = 2.0833) and the highest effective number of alleles (Ne = 1.8016), as well as the highest mean expected heterozygosity value (H_e = 0.3632), were observed in the samples from Toledo-*R* identified by Santos et al. (2014b) as resistant to glyphosate (Table 5). A high number of alleles (Na = 1.9167) and a high effective number of alleles (Ne = 1.6171) were also observed in samples of resistant biotypes from Cascavel-*R* (Table 5). Values of H_o were lower than H_e in the samples characterized as susceptive to glyphosate and those characterized as resistant, indicating a deficit of heterozygosity in all of the samples of *C. sumatrensis*. However, the selection for resistant biotypes due to glyphosate application has not reduced the number of alleles (N_a and N_e) in the resistant biotypes (from Toledo and Cascavel; Table 5).

Table 5. Number of alleles (Na) and effective number of alleles (Ne) per polymorphic SSR locus, mean observed heterozygosity (H_o), expected heterozygosity (H_e), and percentage of polymorphic loci (%P), in the samples of *Conyza sumatrensis* from six sites in southern Brazil.

Site	N	N_a	N_e	H_o	H_e	%P
Campo Mourão	9	2.0000	1.5915	0.1148	0.3344	83.3
Floresta	8	1.5455	1.3431	0.0779	0.2096	50.0
Cascavel	9	1.9167	1.6171	0.0556	0.3255	75.0
Cafelândia	9	2.0000	1.6750	0.1167	0.3517	83.3
Peabiru	4	1.5833	1.4175	0.1042	0.2370	58.3
Toledo	5	2.0833	1.8016	0.1708	0.3632	75.0
Total/Mean	43	2.1667	1.9388	0.1053	0.4291	83.3

The differential allele frequency in samples of *C. sumatrensis* among the six sites in southern Brazil is very high (F_{ST} = 0.31), indicating that the samples from the six sites are from structured sub-populations of *C. sumatrensis*. There are common alleles occurring at similar frequencies in all samples at the *HW29* (*HW29*[1]) and *HWSSR11* (*HWSSR11*[1]) loci, indicating an absence of genetic differentiation; however, the differential frequencies of alleles at other loci are sufficiently high to determine the genetic structure of the six samples of *C. sumatrensis*. The gene flow (Nm = 0.5535) was moderate among the samples of *C. sumatrensis* from the six sites, suggesting an exchange of alleles or dispersion of samples among sites.

The Nei identity (Nei, 1978) values calculated from the analysis of the 12 microsatellite loci of samples of *C. sumatrensis* from six sites in southern Brazil (Campo Mourão, Floresta, Cascavel, Cafelândia, Peabiru, and Toledo) showed that the highest value of genetic identity (I = 0.9275) was observed between samples from Cascavel and Peabiru and that the most divergent samples (I = 0.6931) were those from Floresta and Peabiru (Table 6). The high genetic similarity between the samples from Cascavel and Peabiru suggest that the implementation of similar control strategies could be effective in the two areas since plants with high genetic similarity can be expected to respond similarly to such strategies.

Table 6. Similarity matrix based on 12 SSR loci of the samples of *Conyza sumatrensis* from six sites (Campo Mourão, Floresta, Cascavel, Cafelândia, Peabiru, and Toledo) in southern Brazil.

	Campo Mourão	Floresta	Cascavel	Cafelândia	Peabiru	Toledo
Campo Mourão	-	0.7593	0.7782	0.9235	0.7122	0.7793
Floresta	0.2754	-	0.7872	0.7610	0.6931	0.7428
Cascavel	0.2508	0.2393	-	0.7978	0.9275	0.9119
Cafelândia	0.0795	0.2731	0.2258	-	0.7175	0.8196
Peabiru	0.3394	0.3666	0.0752	0.3319	-	0.8811
Toledo	0.2494	0.2973	0.0922	0.1990	0.1266	-

Nei's identity (1978) (above the diagonal) and genetic distance (below the diagonal).

Although the functional significance of the SSR loci (*HW02, HW06, HW07, HW14, HW29, HW17, HWSSR01, HWSSR03, HWSSR04, HWSSR07, HWSSR09, HWSSR11*) in *Conyza* species is unknown, the high number of alleles and the high levels of observed and expected heterozygosity in *C. sumatrensis* suggest that this species a higher potential than does *C. canadensis* or *C. bonariensis* to colonize new areas. High heterozygosity has been considered to indicate that the plant population has a substantial amount of adaptive genetic variation since there is a greater chance of finding plants that respond differently to changes or pressures exerted by the environment. SSR loci that occur in tandem are abundantly distributed in coding and non-coding regions of plant genomes (see review by Kalia, Rai, Kalia, Singh, & Dhawan, 2011) such that alterations in SSR loci that are located in a coding region or intron have the potential to regulate the differential expression of genes related to the environmental adaptive potential of the plant. The high molecular diversity estimated for *C. sumatrensis* appears to be consistent with the high morphological diversity that is reported by farmers and agronomists in the cropping fields in southern Brazil.

The molecular differentiation in SSR loci of the three *Conyza* species is consistent with the value of genetic identity found between the samples of *C. canadensis* and *C. bonariensis* (0.6303), *C. canadensis* and *C. sumatrensis* (0.8293), and *C. bonariensis* and *C. sumatrensis* (0.7348). Levels of genetic identity lower than 0.85 are frequently reported between populations from different species (Thorpe & Solé-Cava, 1994). The highest value of genetic identity reported in our study (I = 0.8293) was between *C. canadensis* and *C. sumatrensis.* The highest value of molecular similarity between *C. canadensis* and *C. sumatrensis* is in agreement with the highest similarity reported based on morphological parameters (Pruski & Sancho, 2006; Vladimirov, 2009).

AMOVA showed higher genetic variation within (54%) than among (46%) the samples of the three species, and the grouping of the *C. canadensis*, *C. bonariensis*, and *C. sumatrensis* plants according to Bayesian statistics revealed that the higher molecular diversity within the samples of the three species of *Conyza* is due to the high diversity within *C. sumatrensis*. The plants of Campo Mourão, Cafelândia, and Floresta represent all three ancestral groups (red, green, and blue), with genomes predominantly of the red group (*C. canadensis*) and the blue group (*C. bonariensis*), whereas in the samples from Peabiru and Toledo, low proportions of the genomes of the red and blue groups was observed.

The mixture of genomes, which were defined as ancestral groups prevalent in samples of *C. canadensis* and *C. bonariensis* from the Central Valley of California, in samples from sites in Paraná State suggest that all three species *Conyza* are present in these areas and that hybridization may be occurring. The presence of different *Conyza* species in an area can lead to difficulty in species identification. Seeds of a particular species can be carried to other areas by wind dispersal or via the movements of agricultural machinery. The potential exchange of alleles between *C. canadensis* and *C. bonariensis* has been noted in areas of São Paulo State, Brazil, based on analyses of loci for isozymes of esterase, malate dehydrogenase and acid phosphatase (Soares et al., 2015). Genomic admixture of *Conyza* species could explain why plants of one species (e.g., *C. sumatrensis*) can often exhibit the characteristics of another (e.g., *C. bonariensis* or *C. canadensis*). The hybridization between *C. canadensis* and *C. ramosissima* that was induced by Zelaya, Owen, and Vangessel (2007) produced interspecific hybrids of *Conyza* that exhibited intermediate phenotypes between the parents.

Despite the genomic admixture of the three ancestral groups in some plants in southern Brazil (evident in the bar plot graphic; Figure 2), the selfing rate revealed by the Instruct analysis for the three species of *Conyza* was high (0.655–0.803). Self-compatibility in *Conyza* species might explain the low values of H_o (Table 3) in the three *Conyza* species. *Conyza canadensi*s is described as a self-compatible species in which the pollen is released before the capitula have fully opened, suggesting that it is primarily self-pollinated (Weaver, 2001; Okada et al., 2013). High inbreeding coefficients (F_{IS} = 0.850 to 0.966) in a large sample of *C. canadensis* were recently reported by Okada et al. (2013) in a microsatellite loci analysis. Self-pollination has also been described in *C. bonariensis* (Ferrer & Good-Avila, 2007) but has not been described to date in *C. sumatrensis*. In *C. sumatrensis*, the analysis of SSR polymorphism indicated moderate gene flow (Nm = 0.5535) among the samples from different sites (Campo Mourão, Floresta, Cascavel, Cafelândia, Peabiru, and Toledo), suggesting out-crossing among plants from these sites. Values of Nm ranging from 0.25 to 0.99 indicate intermediate gene flow among populations (Govindaraju, 1989).

Despite the indication of moderate gene flow among the *C. sumatrensis* samples, the value of F_{ST} (0.31) was high, indicating that the frequency of alleles at the SSR loci varied among the six samples. Values of F_{ST} ranging from 0.01 to 0.05 indicate minimal divergence among populations; those from

0.05 to 0.15 indicate moderate divergence, whereas those ranging from 0.15 to 0.25 indicate high divergence. The observed F_{ST} was > 0.25, indicate very high divergence among the populations. *Conyza sumatrensis* species forms genetically well-structured populations according to both Wright's *F*-statistic (Wright, 1965) and Bayesian statistics. Highly differentiated populations with F_{ST} values ranging from 0.034 to 0.7530 have been reported by Okada et al. (2013) for 42 populations of *C. canadensis* from different geographical regions across the Central Valley of California. Most populations were highly differentiated, as expected for a highly selfing species. Since the establishment of a population of an invasive species often involves a small number of plants that contain only part of the variation present in the origin population, the "founder effect" in self-fertilization species can result in populations with different numbers and proportions of alleles. Moreover, invasive species that have evolved under strong selection pressure by herbicide application are expected to show high genetic differentiation.

The high proportion of polymorphic loci and the high values of observed and expected heterozygosity (Table 6) in samples of *C. sumatrensis* that were identified as resistant to glyphosate as well as those identified as susceptive to glyphosate conflicts with our hypothesis that lower genetic diversity is expected in samples of resistant biotypes that have been selected for by the use of herbicides. The molecular diversity estimated in the samples *C. sumatrensis* that were resistant to glyphosate indicates that the selection of resistant biotypes has not reduced the number of alleles or the observed and expected heterozygosity in the resistant biotypes.

Conclusion

The high genetic similarity at the molecular level observed between *C. canadensis* and *C. sumatrensis* is consistent with the high morphological similarity between the two species. The well-defined groups of ancestral genomes in the microsatellite loci for each species led to the identification of plants with the ancestral genome of *C. canadensis* in southern Brazil, where *C. sumatrensis* is the prevalent species, and to the identification of samples with admixture of ancestral genomes of two or three species. Moreover, the high molecular diversity in the resistant biotypes of *C. sumatrensis* indicated that these biotypes have a high potential to colonize new areas, which will facilitate their persistence and increase the difficulty of their control. The high molecular diversity of the resistant biotypes is consistent with the hypothesis of multiple mechanisms that are involved in herbicide resistance in *Conyza* species. The high and low values of genetic similarity between the *C. sumatrensis* samples may be used to investigate the efficiency of similar and different strategies, respectively, to control infestation in different areas.

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