Invasive Plant Science and Management

www.cambridge.org/inp

Research Article

Cite this article: Elam RJ and Culley TM (2023) Genetic analysis of invasive spread of wintercreeper (*Euonymus fortunei*), a popular ornamental groundcover. Invasive Plant Sci. Manag **16**: 207–214. doi: 10.1017/inp.2023.31

Received: 8 July 2023
Revised: 23 September 2023
Accepted: 25 October 2023
First published online: 6 November 2023

Associate Editor:
Marie Jasieniuk, University of California, Davis

Keywords:
'Coloratus'; cultivar; microsatellite

Corresponding author:
Theresa M. Culley;
Email: theresa.culley@uc.edu

Genetic analysis of invasive spread of wintercreeper (*Euonymus fortunei*), a popular ornamental groundcover

Robert J. Elam[1] and Theresa M. Culley[2]

[1]Adjunct Assistant Professor, Department of Biological Sciences, University of Cincinnati, Cincinnati, OH, USA and [2]Professor, Department of Biological Sciences, University of Cincinnati, Cincinnati, OH, USA

Abstract

An important route of introduction of some nonnative species that subsequently become invasive in the United States is through horticulture. One such plant is *Euonymus fortunei* (Turcz.) Hand.-Maz., commonly known as wintercreeper, an evergreen groundcover with more than 52 different horticultural varieties, which is still sold at many plant nurseries and garden centers in the midwestern United States. Although several states have recognized *E. fortunei* as an invasive species, it is unknown how its escape from cultivation has occurred and even the identity of spreading populations, including whether hybrids or cultivars are involved. Using codominant microsatellite markers, we sampled multiple invasive populations in Ohio, Kentucky, Kansas, and Minnesota and compared their genotypes with commercially available cultivars to determine how spread has occurred. All samples collected from invasive populations were genetically identical to one another and matched perfectly with the 'Coloratus' cultivar, the only cultivar to exhibit polyploidy. The data also suggest that *E. fortunei* may potentially reproduce via apomixis and/or clonally through propagule fragments, which can quickly fix favorable genotypes within a population. To curb continued invasive spread, we suggest that Coloratus be removed from commercial sale and distribution. We also propose that land managers, horticultural and landscaping businesses, and governmental agencies carefully monitor other *Euonymus* cultivars for invasive potential and spread.

Introduction

Invasive species reduce native biodiversity as well as threaten and endanger native species in the areas that they invade (Rozenberg 2017; Spatz et al. 2017; Walsh et al. 2016). Along with the ecological effects of biological invasions comes a substantial monetary cost, upward of $160 billion annually as of 2017 (Diagne et al. 2021). The difficulty in controlling invasive species comes from many factors that influence their rate of spread, such as the method of seed dispersal (especially bird or wind) and the invasibility of the habitats that are colonized (Alpert et al. 2000). Because no two invasive species are introduced or spread in the same exact way, creating a management plan to reduce the spread of a particular invasive species should take all aspects into account. This includes the history of the organism, country of origin, method of introduction, commercial uses (if any), mating and breeding systems, fecundity, and patterns or rate of spread (Bauer and Reynolds 2016; Courtois et al. 2018).

One route of introduction of nonnative species that may later become invasive in the United States is through horticulture, specifically through use in landscaping (Culley and Feldman 2023; Reichard and White 2001). Traits that make a species valuable as an ornamental may also aid in its spread and establishment into natural areas if it escapes cultivation (Lloret et al. 2005). For example, many gardeners desire plants that are hardy, resistant to disease and pests, grow well with minimal upkeep, and have good form and/or attractive flowers. A category of ornamental plants that are relatively overlooked as sources of invasion are evergreen groundcovers, a popular group of plants often installed by landscaping companies (Dirr 2011). Some of these groundcovers are nonnative lianas, or vines that undergo secondary growth, and are desired because of their attractive foliage. If regularly maintained, these plants will only spread vegetatively on the ground within a locally defined area and are not usually recognized as a problem plant. However, these plants can sometimes escape cultivation and spread through two ways. First, in gardens and landscaped areas that may go unattended, these plants can vegetatively establish themselves in a nearby forest understory (Gordon 1998). In the second method of spread, these same vines expand across the forest floor until they encounter a light gap (Gordon 1998). Under increased light levels, these lianas may find a vertical hold to climb, usually through adhesive, adventitious roots, before reaching their mature form; only then do they start to produce flowers and fruits (Conover et al. 2016; Leicht-Young 2014). The seed can then be dispersed to new areas via birds, further expanding the spread of the species.

Management Implications

Euonymus fortunei (wintercreeper) is an invasive vine in many areas of the Midwest, often forming thick mats that spread vegetatively from residential areas into adjoining natural areas, where the plants can smother natural vegetation. *Euonymus fortunei* may also appear in the middle of forested areas, originating from seeds carried by birds that eat fruit produced by flowering vines under high light (usually after climbing up trees). Control of the species as a groundcover is often difficult and time-intensive, as the vines intertwine and can root repeatedly along their extent.

Despite its invasive behavior, *E. fortunei* is still a popular and economically important ornamental plant used in landscaping as a groundcover. The vine is still commonly found in a variety of commercial plant nurseries and big box stores throughout the United States. To reduce the continued spread of *E. fortunei* in the United States, it is important to curtail the commercial distribution of any genotype(s) escaping into natural areas. This information is currently unknown. Are escapees the straight species itself that was initially introduced, an artificially selected cultivar of *E. fortunei* or other *Euonymus* species, a hybrid between cultivars, or perhaps a hybrid between the *E. fortunei* species and another invasive *Euonymus* species such as winged burning bush (*Euonymus alatus (Thunb.) Siebold*)? The answer to this question informs not only land managers trying to control this species on their properties but also state regulators who need to know what taxon should be included on regulatory invasive plant lists. Currently, some state-based regulations ban the sale of all cultivars of a species assessed as invasive, even if some economically important cultivars are shown to be sterile. This makes it very challenging to list any species with ornamental or horticultural use because of projected economic market losses to the nursery industry. This results in many species not being included at all on state regulatory lists. An alternate approach is to list those genotypes or cultivars shown to be invasive, which will ensure that any problematic genotypes are much more quickly removed from commercial production. In the case of *E. fortunei*, this information would also allow the nursery industry to stop production of problematic genotype(s), focusing its efforts instead on alternative groundcovers that do not exhibit invasive behavior. These alternative groundcovers could include nonspreading *Euonymus* cultivars or, ideally, native species with similar characteristics, such as wild ginger (*Asarum canadense* L.). Taken together, the knowledge of what genotype or genotypes are actually invasive is extremely helpful in limiting the further spread of *E. fortunei* in the United States.

Some of the most popular groundcovers sold by big box stores, plant nurseries, or otherwise installed by landscapers in the midwestern United States are English ivy (*Hedera helix* L.), lesser periwinkle (*Vinca minor* L.), and wintercreeper [*Euonymus fortunei* (Turcz.) Hand.-Maz., also known as climbing euonymus] (Dirr 2011; Okerman 2000). In the eastern United States, there are more than 20 states where *E. fortunei* has reportedly escaped (EDDMapS 2022; Remaley 2005; Schwegman 1996). Although states such as Indiana (IDNR 2022) and Maine (MDACF 2022) have banned the commercial sale or import of *E. fortunei*, there have been relatively few legislative efforts regarding the introduction or sale of this species elsewhere—largely due to its economic importance and unique characteristics for the trade as an effective groundcover.

A member of the Celastraceae (spindle-tree family), *E. fortunei* is native to China, Korea, and northern Japan (Rounsaville 2017). Dirr (1998) described three different species varieties and 52 cultivars that vary largely in terms of leaf morphology and size (including variegation). But since then, 105 cultivars and varieties have been listed by the National Gardening Association (2023), including White Album® and 'Wolong Ghost'. The species itself is naturally phenotypically plastic, easily cloned via cuttings, and amendable to mutation, creating natural mutated ramets ("sports") that can be readily developed as new cultivars (Graves 1940). For example, White Album® is a sport of 'Emerald Gaiety', which itself was selected as a mutant from 'Emerald Pride'. Some cultivars are also developed from morphologically similar taxa (spreading euonymus *Euonymus kiautschovicus* (Turcz.) Hand.-Maz. and evergreen spindle *Euonymus japonicus* Thunb.) but sold together with *E. fortunei* as "wintercreeper," so gardeners often assume they are the same species. *Euonymus fortunei* is highly desirable as an ornamental groundcover, in part because it has a rapid growth rate, is evergreen throughout winter, and persists in a variety of different habitats where other species may be reluctant to grow. For example, *E. fortunei* prefers moist, loamy soils derived from limestone bedrock, but is tolerant of a wide range of soil, water, and light conditions (Nordman 2004). It is hardy in colder climates and grows easily from cuttings (Weeks and Weeks 2012).

Euonymus fortunei also exhibits broad morphological plasticity (Graves 1940). The plant has oppositely branched evergreen leaves, which are ovate-elliptic with finely toothed margins, but leaf shape can vary greatly depending on environmental conditions and resource availability (Dirr 1998; Figure 1A). In its juvenile stage, *E. fortunei* is procumbent, forming dense groundcover mats, which in natural areas can deplete soil moisture and nutrients, creating a positive soil feedback loop and altering soil chemistry and microbiota (Smith and Reynolds 2015). Once mats form, they prevent sunlight from reaching the forest floor and reduce the germination of native herbaceous and woody species (Bauer and Reynolds 2016). The chamaephytic juvenile transitions to the phanerophytic adult form after the liana attaches itself to a vertical structural host, usually a tree, where it can ultimately reach a height of more than 20 m (Dirr 1998; Weeks and Weeks 2012; Figure 2D–F). Being able to quickly grow to this height, it can overtop trees, preventing their photosynthesis and eventually leading to the death of the trees (Weeks and Weeks 2012).

Once the phanerophytic form of the plant reaches a stem diameter of ~1 cm, it can produce clusters of flowers (Zouhar 2009). Flowering begins in late June/early July and consists of clusters of axillary compound cymes with abundant flowers. The flowers are 6.5 mm in diameter, perfect, 4-merous, greenish white (Dirr 1998; Figure 1B and 1C). These flowers produce capsules that start as pale green but turn a pinkish white after ripening in late October and early November (Figure 2A and 2B). The capsule dehisces to reveal between one and four seeds covered in an orange aril (Figure 2C). Seeds are facultatively dormant, being able to germinate with or without cold stratification, and can have a germination rate of up to 98% (Dirr 1998; Rounsaville et al. 2018). The plant is also able to reproduce vegetatively through fragment propagules that are broken off during high winds or storms. These clonal propagules can wash away to colonize a site away from the maternal individual, possibly rooting in the place they come to rest (Merritt et al. 2010).

Euonymus fortunei had been reported as "escaped" in Ohio as early as 1961 (Braun 1961), but it did not become a regional problem until sometime in the 1980s (Liang 2010). Recent studies of *E. fortunei* have focused on the 'Coloratus' cultivar, which was once one of the most common varieties sold at garden centers and

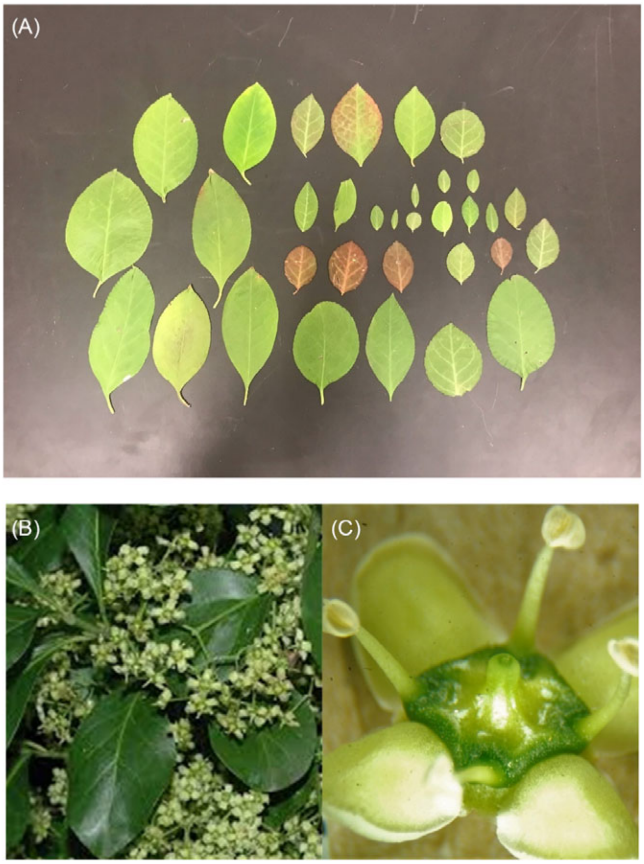

Figure 1. Morphology of *Euonymus fortunei* showing (A) variation in leaf shape and size, (B) clusters of flowers on a mature *E. fortunei*, and (C) close-up of *E. fortunei* flower.

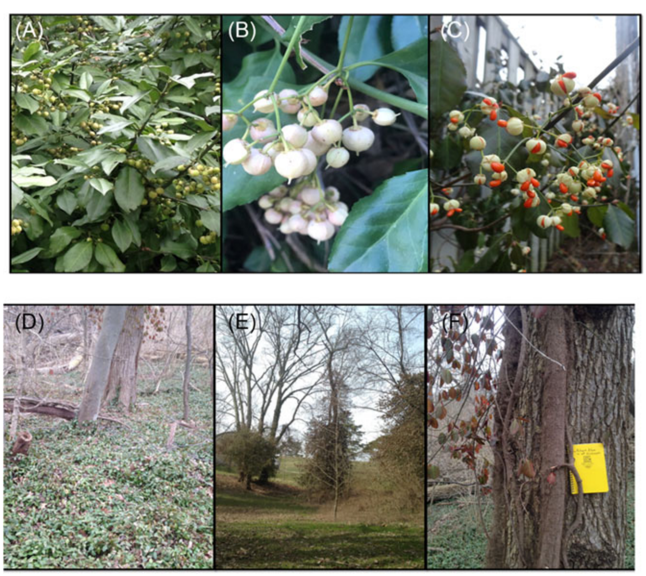

Figure 2. Fruit development and growth of *Euonymus fortunei*. Shown are (A) newly formed, pale green fruits; (B) ripening fruits, turning a pinkish white; (C) capsules dehiscing, exposing the bright red arils covering the seeds; (D) chamaephytic juvenile of *E. fortunei* creating thick mats across a forest floor; (E) phanerophytic mature *E. fortunei* growing up and overtopping trees; and (F) well-established phanerophyte (10 × 16 cm field notebook for scale).

nurseries (Mattingly 2016; Rounsaville 2017; Tanner et al. 2017). This cultivar is noted for its distinct purple-colored foliage and rapid growth; it also most closely resembles the morphology and purple/reddish coloration of *Euonymus* that has invaded wooded areas (Rounsaville 2017; Sink et al. 2000).

The goal of this study was to identify the origin of invasive populations of *E. fortunei* to better understand the spread of this species and provide information for potential commercial regulation of the species. To do so, we compared genetic samples taken from invasive populations across several states with genetic samples from cultivated varieties (cultivars) of *E. fortunei* available at local nurseries. We also aimed to determine whether there was any hybridization occurring among cultivars or with a closely related invasive species, winged burning bush [*Euonymus alatus* (Thunb.) Siebold].

Materials and Methods

A sample size of 259 individuals of cultivated *E. fortunei* and *E. japonicus*, *E. alatus*, and *E. kiautschovicus* were taken from local plant nurseries and big box stores in Cincinnati, OH, or residential and university landscaping, and from invasive individuals growing in local parks and preserves. The latter included Avon Woods, Burnet Woods, California Woods, LaBoiteaux Woods, Mt Airy Forest, Rawson Woods, a residential area (Clifton), as well as Spring Grove Cemetery and Arboretum, all of which are in the Cincinnati, OH, greater metropolitan area (Figure 3). Branches or leaf samples were also collected from Devou Park and Boone County in northern Kentucky; additional samples were collected from the downtown area of Rochester, MN. Two samples were also collected by Denis Conover from Fort Hill in Highland County, OH, and 20 samples were obtained from Mead Island, KS, sent by James Beck of Wichita State University (Table 1). A sample of a herbarium specimen collected in 1978 from *E. fortunei*'s native range in northern Japan was obtained from Miami University (MU), Miami, OH.

From each small branch cutting, approximately 150 mg of leaf tissue was removed from the area of newest growth and subsequently extracted or frozen at −20 C until extraction. All the remaining tissue collected in the field was pressed and prepared for deposition to the Margaret H. Fulford Herbarium at the University of Cincinnati (CINC), with vouchers representing each population (Elam0010 to Elam0018).

DNA was extracted from plant tissue using a modified version of the CTAB method (Doyle and Doyle 1987). DNA was then amplified through PCR and genotyped at eight different microsatellite loci (Table 2) using markers obtained from Mori et al. (2017), combined in a multiplexed reaction. Each multiplex PCR reaction consisted of 10 µl reaction volumes as follows: 5 µl of GoTaq Master Mix (Promega, Madison, WI), 1 µl of Primer Mix (consisting of 2 µM reverse primer and 2 µM forward fluorescently labeled primer for each marker), 3.8 µl of H_2O, and 0.4 µl of DNA. Samples were then amplified on an Applied Biosystems SimpliAmp thermal cycler (Applied Biosystems, Fortune City, CA) with the following conditions: initial denaturation of 95 C for 15 min, followed by 35 cycles each of 94 C for 30 s, 57 C for 90 s, and 72 C for 60 s; with a final extension of 60 C for 30 min. PCR products were then sent to Cornell University's Life Sciences Core Laboratory Center (Ithaca, NY) for fragment analysis on a 3730 × l DNA Analyzer (Applied Biosystems) using a LIZ 500 internal size

https://doi.org/10.1017/inp.2023.31 Published online by Cambridge University Press

Figure 3. Collection sites of invasive *Euonymus fortunei* around the Greater Cincinnati area.

standard. The resulting fragment analysis data were examined using GeneMarker v. 1.85 (SoftGenetics, State College, PA) to identify alleles. The data were analyzed using GenAlEx v. 6.503 (Peakall and Smouse 2012) to match across multilocus genotypes and then using STRUCTURE v. 2.3.4 (Pritchard et al. 2000) to genetically group the samples, using a model of no ADMIXTURE and specified LOCPRIOR, and independent loci with 10,000 burn-in iterations and 100,000 iterations for the test. K was run from 2 to 20 with 5 iterations at each K value (Evanno et al. 2005). A K = 20 was chosen, because although there were originally 27 populations sampled, many cultivars were a genetic match to one another as revealed by an initial multilocus match comparison in GenAlEx.

Results and Discussion

Comparison of the multilocus genotypes based on the eight microsatellite loci confirmed that multiple individuals of the same cultivar were genetically identical to one another, as expected, because cultivars are propagated through cloning. Furthermore, some cultivars were also genetically identical to one another ('Gold Spot' and Silver Princess™—both *E. japonicus*) or nearly so with a different allele at one to two loci (White Album® and Emerald Gaiety—*E. fortunei*). In all other cases, cultivars were differentiated from one another, exhibiting different multilocus genotypes, even with the limited number of eight loci. As determined from the microsatellite data, all *Euonymus* cultivars are diploid, with a maximum of two alleles per locus, except for *E. fortunei* Coloratus and *E. alatus*, which both appear to be a tetraploid with four alleles for many of the loci (see Supplementary Material).

The multilocus genotypes of nearly all of the 195 samples from invasive *E. fortunei* populations sampled across different states were identical to one another and completely matched the Coloratus tetraploid genotype. There were four invasive individuals that exhibited allele dropout for one to two loci, but otherwise matched to Coloratus (Figure 4). Two other individuals that did not match Coloratus were provided to us from Kansas; upon further inspection of the leaves, these were determined not to be *E. fortunei* and were likely misidentified in the field. The STRUCTURE analysis indicated that these two outlying samples were the *E. kiautschovicus* cultivar 'Manhattan'; these samples were the last two collected at this site, and presumably may have been planted. Interestingly, one sample collected in Fort Hill was not tetraploid, matching instead to the Japanese herbarium sample, and may be an escaped cultivar not yet sampled. A closer examination of the sampled leaves confirmed their leaf shape was different from that of Coloratus. Finally, all seven samples of *E. alatus* were identical to one another with a tetraploid multilocus genotype, and hybrids between *E. fortunei* and *E. alatus* were never detected in the sampled invasive populations.

The STRUCTURE analysis (Fig. 4) indicated that a K of 8 best matched the data, using the highest model log likelihood (Evanno et al. 2005). As with the GenAlEx analysis, all individuals within each cultivar were genetically identical to one another, and several cultivars of *E. fortunei* grouped together in this analysis. For example, White Album®, Gold Splash®, Green Lane™, 'Moon Shadow', Emerald Gaiety, and 'Emerald & Gold' formed one group (although with some slight differences in multilocus genotypes; see Supplementary Material); exceptions were 'Kewensis', Wolong Ghost, and the tetraploid Coloratus. As the only dwarf *E. fortunei* variety, Kewensis was introduced from Japan to Kew Gardens in 1893 (International Dendrology Society 2023), an origin that was consistent with its genetic separation from other cultivars. Although Wolong Ghost appears to be of hybrid origin (potentially of Japanese ancestry with Kewensis), it is a Chinese genotype originally collected in the Wolong Nature Reserve in China (Leighty, 2018). The grouping of many other *E. fortunei* cultivars is consistent with their origins, according to U.S. patent records. Gold Splash® is a sport of Emerald & Gold, which itself was derived from 'Emerald Cushion', an older cultivar that also gave rise to 'Sunspot', which was used to develop Moon Shadow and also likely

https://doi.org/10.1017/inp.2023.31 Published online by Cambridge University Press

Table 1. List of species, cultivar or invasive population, and number of samples taken from each.

Species	Cultivar[a]	Invasive population	*N*
Euonymus fortunei	White Album® ('Alban')	—	5
	'Kewensis'	—	3
	Gold Splash® ('Roemertwo')	—	5
	Green Lane™ ('Grezam')	—	2
	'Moon Shadow'	—	3
	'Emerald Gaiety'	—	4
	'Wolong Ghost'	—	1
	'Emerald & Gold'	—	4
	'Coloratus'	—	12
Euonymus kiautschovicus	'Manhattan'	—	3
Euonymus japonicus	'Gold Spot' ('Auero-Variegata'[b])	—	6
	Silver Princess™ ('Moness')	—	4
	'Chollipo'	—	7
	'Aueromarginatus'[b] ('Gold Edge')	—	6
Euonymus fortunei	—	Avon Woods, OH	23
	—	California Woods, OH	17
	—	Clifton area of Cincinnati, OH	19
	—	Boone County, KY	18
	—	Rawson Woods, OH	13
	—	Mt Airy, OH	21
	—	Spring Grove, OH	20
	—	DeVou Park, KY	20
	—	Rochester, MN	12
	—	Mead Island, KS	18
	—	LaBoiteaux, OH	14
	—	Japan (i.e., herbarium)	1
	—	Highland County, OH	2
Euonymus alatus	'Compactus'	Clifton area of Cincinnati, OH	7

[a]In cases where a cultivar is also known by a different trade name, both are provided.
[b]Plants with this name are commercially sold under the Latin female (-*a*) or male (-*us*) format. The cultivar name may also be separated by a hyphen or run entirely together. For example, 'Auero-Variegata' is also sold as 'Auerovariegatus'.

had the same source as Emerald Gaiety, which gave rise to White Album®. Green Lane™ is another cultivar that grouped together with these others; although its parentage is unknown, the genetic data here indicate it may also descend from the 'Emerald' group, selected by Clifford Corliss in the 1950s in Massachusetts. In *E. japonicus*, cultivars also tended to cluster together, albeit in two groups: one group consisting of Gold Spot and Silver Princess™ and another group of 'Chollipo' and 'Aueromarginatus'. One sample from the Fort Hill population and the herbarium sample of *E. fortunei* from Japan also grouped together. Finally, *E. alatus* was distinct from all other samples, exhibiting tetraploidy with a unique allele for marker ef05.

This genetic analysis indicates that the source of escaped and invasive populations of *E. fortunei* in several different states is the Coloratus cultivar, which is consistent with the reddish-purple coloration often observed in invasive populations. Interestingly, Graves (1940) mentioned that Coloratus was not known in the wild at that time. A single genotype (cultivar) spreading so extensively, at least within the locations sampled here, is relatively unusual, although it has been seen in species like common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) (Saltonstall 2002). For *E. fortunei*, spread of a single genotype could be due to several non–mutually exclusive reasons. First, spread of Coloratus may simply reflect propagule pressure, with the popularity of this ornamental groundcover leading to increased chance of escape from ornamental plantings into surrounding natural areas. Second, Coloratus could be a general-purpose genotype that does well in a variety of habitats. In this case, a clone with the largest breadth of tolerance to environmental conditions and phenotypic plasticity will be selected within natural areas, which over time could lead to eventual fixation, as other less-adaptable genotypes do not survive and persist (Parker 1979). Third, polyploidy (i.e., tetraploidy) in Coloratus may provide a selective advantage by amplifying adaptive traits, which can allow species potentially to expand to new habitats (Levin 1983) and enhance phenotypic plasticity, adaptability to new environments, and vigor (Schinkel et al. 2016). In fact, traits such as larger, thicker leaves (Hannweg et al. 2016) and increased flower production and color (Pyšek and Richardson 2007; Sajjad et al. 2013) may naturally occur in some species because of polyploidy (Mo et al. 2020). Future investigations should compare the morphology of Coloratus with that of other *Euonymus* cultivars, especially given that polyploidy may be selected for and even induced during horticultural development.

The genetic homogeneity found in the invasive samples of *E. fortunei* is also suggestive of possible apomixis (Jank et al. 2011), especially if populations are founded by a single apomictic propagule (Vrijenhoek and Parker 2009). Apomixis can also be an important driver in invasiveness of introduced plants (Kumar et al. 2019). In the introduced and invasive apomictic plant purple pampas grass (*Cortaderia jubata* (Lem.) Stapf), Okada et al. (2009) showed that the introduction of a single clonal genotype had a significant founder effect, resulting in a single general-purpose genotype adapted to the introduced environment. The possibility of apomixis in *E. fortunei* is currently being examined in a companion genetic study of parentage of *E. fortunei* seeds.

The invasion of *E. fortunei* into wooded areas is of concern for many private landowners, land managers, and governmental agencies (Bray et al. 2017). The removal and control of a well-established invasive species can be prohibitively expensive (Jardine and Sanchirico 2018), and many parks and private landowners have neither the resources nor the workforce needed to manage a large-scale infestation (Courtois et al. 2018). This limitation can

https://doi.org/10.1017/inp.2023.31 Published online by Cambridge University Press

Table 2. Microsatellite forward and reverse sequences (from Mori et al. 2017) and motif of the simple sequence repeat (SSR) used in this study of *Euonymus fortunei.*

Locus ID	Forward primer sequence	Reverse primer sequence	SSR motif
ef01	CGGAGAGCCGAGAGGTGTCCTGTTCAAATCACATCCTCCG	GTTTCTTCCGAGCGAATGTAAGGACACGC	$(AG)_{13}$
ef02	GCCTCCCTCGCGCCATGTCGATCAACCCACCGGAACAG	GTTTCTTTCAACGAGCTCAGGATGTTCC	$(TTA)_{14}$
ef03	CGGAGAGCCGAGAGGTGCGATGGCGAAATCGGAAGAAGCAG	GTTTCTTATCATCTGCAGTGTGTCGGGTGC	$(TC)_{11}$
ef04	CGGAGAGCCGAGAGGTGCATAATTTGCCTAGGTCCTTTCTTG	GTTTCTTATTCAGTCTCAGCGTTCCGGCTC	$(CT)_{16}$
ef05	CAGGACCAGGCTACCGTGACACCAAGTGATCAACCTGCATTC	GTTTCTTGGAGCCTTTCCACTTCTGCTCTC	$(AT)_{14}$
ef06	CAGGACCAGGCTACCGTGAGGTCAAACCATGCCAGAACTTGC	GTTTCTTGCCGCTTCTTTGTCCTGAACTCG	$(TC)_{14}$
ef07	CAGGACCAGGCTACCGTGACCACTCAATACCCTCCAAGCCC	GTTTCTTCGATTTCCCAACTCCAGAGTCTCC	$(GA)_{11}$
ef08	CAGGACCAGGCTACCGTGACAGAGCTGCAAACATATTTGGGAGC	GTTTCTTGTGAAATGG CAGTGGTATGGATGC	$(TG)_{14}$
ef09	CGGAGAGCCGAGAGGTGTCGCATCCTCATCACAACTCCCAC	GTTTCTTAGGCGGATCAGTGTAGTCCTTGG	$(CT)_{12}$
ef10	CGGAGAGCCGAGAGGTGACCTAGGCAGACCTCGAGAACTC	GTTTCTTAGCCTCAAATCTCCAAGAATCTCCC	$(CT)_{12}$

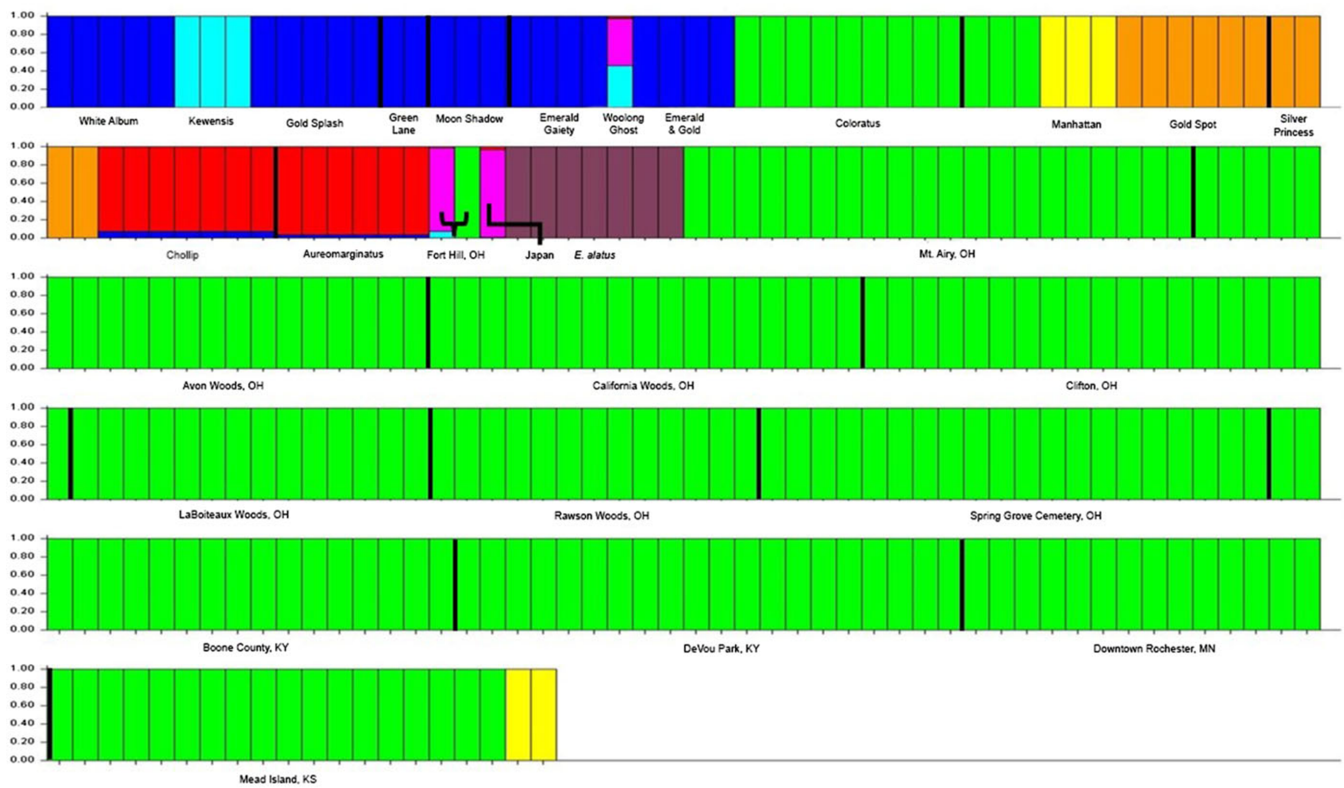

Figure 4. STRUCTURE output comparing samples of *Euonymus* species collected from invasive populations with horticultural varieties. Each individual sample is indicated by a column, and samples from different cultivars and collection sites that share similar or identical multilocus genotypes are grouped together. See Table 1 for sample sizes.

lead to the unchecked growth of invasive plant populations, further accelerating their spread and establishment into new areas. It is especially imperative for land managers to work as diligently as possible to prevent *E. fortunei* invasions, which have been documented to alter biogeochemical cycles, reduce plant diversity (Mattingly 2016), and impact land and vegetation (Bray et al. 2017; Smith and Reynolds 2015).

Given the evidence provided here, we suggest that action be taken to end the introduction and commercial sale of *E. fortunei* Coloratus, especially in geographic locations in which the plant has not yet spread. Plant nurseries, landscaping companies, big box stores, and even online distributors that sell this cultivar are exacerbating spread of this invasive species (Beaury et al. 2021). State-based invasive plant regulation should also take immediate note and include *E. fortunei* Coloratus on lists of regulated plant species. As other cultivars are not yet evident in escaped populations, it is possible that they do not have the invasive ability of Coloratus nor can they currently persist in the forest understory environments, at least not yet at the locations sampled in this study. Further investigation should explore additional locations and populations of *E. fortunei* to determine whether other cultivars might also be contributing to invasive populations. We suggest caution and thorough testing of any existing or new *Euonymus* cultivars, especially those that might be polyploid in origin. In this way, continued expansion of *E. fortunei* populations can be limited and ideally prevented in areas where spread has not yet occurred.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/inp.2023.31

Data Availability. The microsatellite data in Microsoft Excel format will be made available at Scholar@UC (https://doi.org/10.7945/qg5w-ny75) or in the Supplementary Material.

Acknowledgments. The authors thank James Beck of Wichita State University for providing samples from Kansas, and Denis Conover for his help in collecting samples of the Fort Hill, OH, population. Eric Tepe, Denis Conover, and George Uetz also provided helpful comments on the article. Funding was provided by the University of Cincinnati Department of Biological Sciences Wendel-Weiman-Benedict Scholarship to RJE.

No conflicts of interest have been declared.

References

Alpert P, Bone E, Holzapfel C (2000) Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. Perspect Plant Ecol Evol Syst 3:52–66

Bauer JT, Reynolds HL (2016) Restoring native understory to a woodland invaded by *Euonymus* multiple factors affect success. Restor Ecol 24:45–52

Beaury EM, Patrick M, Bradley BA (2021) Invaders for sale: the ongoing spread of invasive species by the plant trade industry. Front Ecol Environ 19:550–556

Braun EL (1961) The woody plants of Ohio. Ohio State University Press: Columbus, OH. 362 p

Bray SR, Hoyt AM, Yang Z, Arthur MA (2017) Non-native liana, *Euonymus fortunei*, associated with increased soil nutrients, unique bacterial communities, and faster decomposition rate. Plant Ecol 218:329–343

Conover D, Geiger D, Sisson T (2016) Dormant season foliar spraying slows the spread of wintercreeper, English ivy, and lesser periwinkle in wooded natural areas. Ecol Restor 34:19–21

Courtois P, Figuieres C, Mulier C, Weill J (2018) A cost–benefit approach for prioritizing invasive species. Ecol Econ 146:607–620

Culley TM, Feldman TH (2023) The role of horticulture in plant invasions in the midwestern United States. Int J Plant Sci 184, 10.1086/724662

Diagne C, Leroy B, Vaissière AC, Gozlan RE, Roiz D, Jarić, Salles J-M, Bradshaw CJA, Courchamp F (2021) High and rising economic costs of biological invasions worldwide. Nature 592:571–576

Dirr MA (1998) Manual of Woody Landscape Plants: Their Identification, Ornamental Characteristics, Culture, Propagation and Uses. Stipes Publishing LLC, Champaign, IL; 1187 pp. [5th edition]

Dirr M (2011) Dirr's Encyclopedia of Trees and Shrubs. Portland, OR: Timber Press. 952 p

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytol Bull 19:11–15

EDDMapS (2022) Early Detection & Distribution Mapping System. University of Georgia Center for Invasive Species and Ecosystem Health. https://www.eddmaps.org. Accessed: October 4, 2022

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Gordon DR (1998) Effects of invasive, non-indigenous plant species on ecosystem processes: lessons from Florida. Ecol Appl 8:975–989

Graves G (1940) Fruiting forms and variegations in the wintercreeper. J NY Bot Gard 41:285–287

Hannweg K, Visser G, De Jager K, Bertling I (2016) In vitro-induced polyploidy and its effect on horticultural characteristics, essential oil composition and bioactivity of *Tetradenia riparia*. S Afr J Bot 106:186–191

[IDNR] Indiana Department of Natural Resources (2022) Indiana Terrestrial Plant Rule. https://www.in.gov/dnr/files/ep-terrestrial_plant_rule.pdf. Accessed: October 4, 2022

International Dendrology Society (2023) *Euonymus fortunei* (Turcz.) Hand.-Mazz. Trees and Shrubs Online. https://www.treesandshrubsonline.org/articles/euonymus/euonymus-fortunei. Accessed: September 5, 2023

Jank L, Valle CD, Resende RMS (2011) Breeding tropical forages. Crop Breed Appl Biotechnol 11:27–34

Jardine SL, Sanchirico JN (2018) Estimating the cost of invasive species control. J Environ Econ Manag 87:242–257

Kumar S, Saxena S, Rai A, Radhakrishna A, Kaushal P (2019) Ecological, genetic, and reproductive features of *Cenchrus* species indicate evolutionary superiority of apomixis under environmental stresses. Ecol Indicators 105:126–136

Leicht-Young SA (2014) Seeing the lianas in the trees: woody vines of the temperate zone. Arnoldia 72(1)

Leighty M (2018) *Euonymus fortunei* 'Wolong Ghost'. Nursery Management. https://www.nurserymag.com/article/euonymus-fortunei-wolong-ghost. Accessed: September 5, 2023

Levin DA (1983) Polyploidy and novelty in flowering plants. Am Nat 122:1–25

Liang Y (2010) Exotic Invasive Plants in Kentucky. Master's thesis. University of Kentucky, Lexington, KY. 94 pp

Lloret F, Medail F, Brundu G, Camarda I, Moragues E, Rita J, Lambdon P, Hulme PE (2005) Species attributes and invasion success by alien plants on Mediterranean islands. J Ecol 93:512–520

[MDACF] Maine Department of Agriculture, Conservation, and Forestry (2022) Criteria for Listing Invasive Terrestrial Plants. https://www.maine.gov/dacf/php/horticulture/invasiveplants.shtml. Accessed: October 4, 2022

Mattingly KZ (2016) Recovery of forest floor diversity after removal of the nonnative, invasive plant *Euonymus fortunei*. J Torrey Bot Soc 143:103–116

Merritt DM, Nilsson C, Jansson R (2010) Consequences of propagule dispersal and river fragmentation for riparian plant community diversity and turnover. Ecol Monogr 80:609–626

Mo L, Chen J, Lou X, Xu Q, Dong R, Tong Z, Huang H, Lin E (2020) Colchicine-induced polyploidy in *Rhododendron fortunei* Lindl. Plants 9:424

Mori H, Ueno S, Matsumoto A, Uchiyama K, Kamijo T, Masaki T, Tsumura Y (2017) Isolation and characterization of microsatellite markers from the RAD sequence of two temperate liana species: *Euonymus fortunei* (Celastraceae) and *Schizophragma hydrangeoides* (Hydrangeaceae). Silvae Gen 66:40–42

National Gardening Association (2023) Plants Database. https://garden.org/plants. Accessed: September 5, 2023

Nordman C (2004) Vascular Plant Community Classification for Stones River National Battlefield. Durham, NC: NatureServe. 157 p

Okada M, Lyle M, Jasieniuk M (2009) Inferring the introduction history of the invasive apomictic grass *Cortaderia jubata* using microsatellite markers. Divers Distrib 15:148–157

Okerman A (2000) Combating the "ivy desert": the invasion of *Hedera helix* (English ivy) in the Pacific Northwest United States. Restor Reclam Rev 6(4)

Parker ED (1979) Ecological implications of clonal diversity in parthenogenetic morphpspecies. Am Zool 19:753–762

Peakall R, Smouse PE (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Pyšek P, Richardson DM (2007) Traits associated with invasiveness in alien plants: where do we stand? Biological Invasions Pages 97–125 *in* Nentwig W, ed. Biological Invasions. Ecological Studies 193. Berlin: Springer

Reichard SH, White P (2001) Horticulture as a pathway of invasive plant introductions in the United States: most invasive plants have been introduced for horticultural use by nurseries, botanical gardens, and individuals. BioScience 51:103–113

Remaley T (2005) Fact sheet: climbing euonymus—*Euonymus fortunei* (Turcs.) Hand.-Mazz. In: Weeds Gone Wild: Alien Plant Invaders of Natural Areas. Plant Conservation Alliance's Alien Plant Working Group. https://www.invasive.org/weedcd/pdfs/wgw/climbingeuonymus.pdf. Accessed November 23, 2023

Rounsaville TJ (2017) Invasion Dynamics of the Exotic Liana *Euonymus fortunei* (Turcz.) Hand.-Mazz. (Wintercreeper). PhD Dissertation. University of Kentucky, Lexington, KY. 133 pp

Rounsaville TJ, Baskin CC, Roemmele E, Arthur MA (2018) Seed dispersal and site characteristics influence germination and seedling survival of the invasive liana *Euonymus fortunei* (wintercreeper) in a rural woodland. Can J For Res 48:1343–1350

Rozenberg AG (2017) Assessment of economic and environmental impact of invasive plant species. Biol Bull Rev 7:273–278

Sajjad A, Jaskani MJ, Mehmood A, Ahmad I, Abbas H (2013) Effect of colchicine on in vitro polyploidy induction in African marigold (*Tagetes erecta*). Pak J Bot 45:1255–1258

Saltonstall K (2002) Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. Proc Natl Acad Sci USA 99:2445–2449

Schinkel CCF, Kirchheimer B, Dellinger AS, Klatt S, Winkler M, Dullinger S, Horandl E (2016) Correlations of polyploidy and apomixis with elevation and associated environmental gradients in an alpine plant. AoB Plants 8: plw064

Schwegman JE (1996) *Euonymus fortunei*—wintercreeper. Page 56 *in* Randall JM, Marinelli J, eds. Invasive Plants: Weeds of the Global Garden. Handbook 149. Brooklyn, NY: Brooklyn Botanic Garden

Sink RA, Einert AE, Klingaman GL, McNew RW (2000) 226 juvenile and adult growth characteristics of newly planted *Euonymus fortunei* 'Coloratus'. HortSci 35:430–430

Smith LM, Reynolds HL (2015) *Euonymus fortunei* dominance over native species may be facilitated by plant–soil feedback. Plant Ecol 216:1401–1406

Spatz DR, Zilliacus KM, Holmes ND, Butchart SH, Genovesi P, Ceballos G, Tershy BR, Croll DA (2017) Globally threatened vertebrates on islands with invasive species. Sci Adv 3:e1603080

Tanner R, Branquart E, Brundu G, Buholzer S, Chapman D, Ehret P, Fried G, Starfinger U, van Valkenburg J (2017) The prioritisation of a short list of alien plants for risk analysis within the framework of the Regulation (EU) No. 1143/2014. NeoBiota 35:87–118

Vrijenhoek RC, Parker ED (2009) Geographical parthenogenesis: general purpose genotypes and frozen niche variation. Pages 99–131 *in* Schön I, Martens K, Dijk P, eds. Lost Sex. Dordrecht, Netherlands: Springer

Walsh JR, Carpenter SR, Vander Zanden MJ (2016) Invasive species triggers a massive loss of ecosystem services through a trophic cascade. PNAS 113:4081–4085

Weeks SS, Weeks HP (2012) Shrubs and Woody Vines of Indiana and the Midwest: Identification, Wildlife Values, and Landscaping Use. West Lafayette, IN: Purdue University Press. 475 p

Zouhar K (2009) *Euonymus fortunei*. *In* Fire Effects Information System. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. https://www.fs.usda.gov/database/feis/plants/vine/euofor/all.html. Accessed November 23, 2023