Distinguishing an alien invasive vine from the native congener: morphology, genetics, and hybridization

Report to the Center for Invasive Species Management
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Investigators: Dr. Noel B. Pavlovic
Dr. Stacey Leicht Young
Great Lakes Science Center, U.S. Geological Survey
Lake Michigan Ecological Research Station
1100 N. Mineral Springs Rd.
Porter, IN 46304

Proposal: We proposed to use genetic analysis of the two bittersweet species (American bittersweet, *Celastrus scandens* and oriental bittersweet, *C. orbiculatus*) on material of both known and unknown plants to aid identification in the field. We have already taken morphological data on 50 fruiting individuals of each species to determine if they can be identified using various characters. Fruit characters were the most reliable, although there was a slight difference in the leaf length-to-width ratio: 1.6 for *C. scandens* and 1.4 for *C. orbiculatus* (Leicht-Young et al. 2007). However, this subtle difference could be difficult to differentiate in the field and is not near to the ratio of 2 in the literature (Swink and Wilhelm 1994). In addition, specific leaf area (cm^2/g) was also found to be different for the two species. Using the genetic data, we could ascertain if the leaf and other habit characters are "good" characters for the identification of the two species. This information could then be relayed to the land managers so that they can target only the invasive bittersweet. In addition, we want to create and study more extensively the hybrid bittersweet. In this way, we can gather basic morphological data for this plant so that it can be watched for in the field. Finally, using the hybrid bittersweet as well as the two parent species, we can compare their chromosome numbers to determine if the ploidy level of the hybrid is different than that of the parent plants (n = 23). If it is, this may be a simple way to establish if an unknown plant is a hybrid.

Methods: *Hybrid creation*

Before the flowers opened in mid-May 2006 we bagged them using veil material to prevent pollination. Flowering of both species overlapped each other, so we conducted hand pollinations on five females of each species from May 22 to May 30, 2006. Each female was pollinated using five interspecific males (five flowers per male) and one intraspecific male (five flowers per male). Five flowers were not pollinated as a control. Flowers were marked using colored pieces of floss and kept bagged until flowering was complete. Pollination success was scored on June 8, 2006 and fruits collected in October 2006. Seeds were extracted from the fruit and were cold-stratified for three months from February to May 2007. Pollination success between the two crosses was analyzed using a t-test.

cpDNA Analysis

We collected at least 10 young leaves from 50 individuals of each species of *Celastrus* in September – October 2006 that had been positively identified as either *C. orbiculatus* or *C. scandens* using fruit position the previous year. In addition, we collected leaves from 50 "unknown" *Celastrus* that were not fruiting. All of these plants were located several meters apart to avoid collecting leaves from the same clone. We put the leaves in zip-top bags with silica gel and put them in a cool dark place until we returned from the field. Leaves were then stored in these bags at -20ºC.

We aligned available sequences for *rbc*L cDNA genes (GenBank Accession No. AY788194 and AY788195) using the ClusterIW program on the EBI website (Chenna et al. 2003). We found 10 single-base sites that differed between the two species. We then used NEBCutter V2.0 to identify unique restriction fragment length polymorphism (RFLP) sites (Vincze et al. 2003).

For DNA extraction, we removed a small sample of leaf and extracted total DNA using the Sigma® RED-Extract-N-Amp™ Plant PCR kit. Following extraction, we amplified a portion of the cpDNA *rbc*L gene using the custom-made primer pairs *rbc*L (5' CTGGCGTTAAAGATTATAAATTGAC) and *rbc*LR (5' CCTCCACCGAATTGTAGTACG). The PCR reactions contained 25μL of the RED-Extract-N-Amp™ PCR ReadyMix (buffer, salts, dNTPs, Taq polymerase and JumpStart Taq antibody), 1 μL each of *rbc*L and *rbc*LR, 4μL chloroplast extract (0.5 – 1 μg DNA) in a total volume of 50 μL. The thermal cycling profile, using a Thermo Electron Corporation PxE 0.2 thermal cycler, was 2 min. at 94° followed by 35 cycles of 94° for 45 s, 59° for 45 s and 72° for 90 s, followed by a final extension of 72° for 2 min. Ten mL of the PCR product was digested using *Nla*IV and *Pvu*II according to the manufacturer's protocol. The restriction fragments were electrophoresed in ethidum bromide stained 1.75% TBE agarose gels and visualized with UV light.

Results:

Hybrids

The *C. scandens* × *C. orbiculatus* cross was significantly more successful than the *C. orbiculatus* × *C. scandens* cross ($t_{0.05,\,8} = -2.1$, $P = 0.005$), and the intraspecific crosses were equally successful for both species ($t_{0.05,\,7} = 5.096$, $P = 0.075$, Table 1).

cpDNA Analysis

The amplified *rbc*L region was 1171 bp long. The *Nla*IV restriction endonuclease cut *C. orbiculatus* cDNA once, to give bands at 944 bp and 227 bp in length. *Nla*IV cut *C. scandens* twice, to give products of lengths 844 bp, 227 bp and 100 bp in length (Figure 1). *Pvu*II did not cut *C. orbiculatus*, but cut *C. scandens* once, to give bands of 1012 bp and 159 bp in length (Figure 1).

Table 1. Pollination results for hybrid study. CO is *C. orbiculatus* and CS is *C. scandens*. Bold numbers identify the five male and female individuals of each species. Numbers are successful pollinations out of five. "–" indicates that data were not obtained.

CO Female ×	CS Males					CO Male	Control
	1	**2**	**3**	**4**	**5**		
1	0	1	0	0	0	5	2
2	0	0	0	0	0	4	0
3	0	0	0	0	0	5	3
4	0	0	0	1	1	5	-
5	0	0	0	0	0	-	1
CS Female ×	CO Males					CS Male	Control
	1	**2**	**3**	**4**	**5**		
1	0	0	0	0	0	0	0
2	0	0	1	0	0	0	0
3	4	4	5	5	5	3	0
4	5	4	2	3	4	2	0
5	4	0	1	3	1	1	1

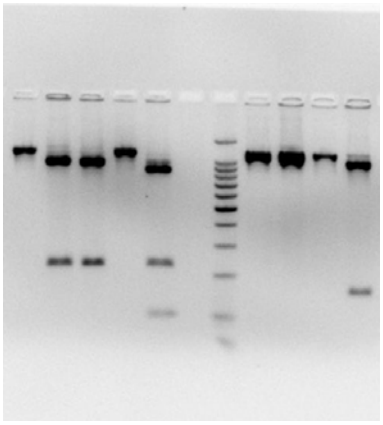

Figure 1: Gel showing differing banding patterns for *C. orbiculatus* and *C. scandens* using RFLPs. From left to right, lanes are: 1. *C. orbiculatus*, uncut *rbc*L gene, 2-3. *C. orbiculatus* cut with *Nla*IV, 4. uncut *C. scandens*, 5. *C. scandens* cut with *Nla*IV, 6. negative control, 7. 1500bp ladder, 8. uncut *C. orbiculatus*, 9. *C. orbiculatus* uncut by *Pvu*II, 10. uncut *C. scandens*, 11. *C. scandens* cut with *Pvu*II

Discussion: We successfully created hybrids of *C. orbiculatus* and *C. scandens* using hand pollinations in the field. Interestingly, the *C. scandens* × *C. orbiculatus* cross was much more successful than the reverse. Previous studies have also shown this result, with White and Bowden (1947) finding that the *C. orbiculatus* × *C. scandens* cross only produced four fruit, which contained abnormal seed, none of which germinated. While the *C. scandens* × *C. orbiculatus* cross produced 12 fruit with normal seeds, of which

some germinated. In addition, Pooler et al. (2002) were unsuccessful with the *C. orbiculatus* × *C. scandens* cross as well, although they attributed this to poor fruit set overall for that *C. orbiculatus*. These results imply that *C. orbiculatus* would indeed be capable of swamping out *C. scandens* genetically if populations of *C. orbiculatus* were large enough. This would make conservation of the native species even more challenging, as it would be a concern if the plant that is being conserved is "pure" *C. scandens* versus a hybrid or a backcrossed individual.

RFLP analysis of the two species provided a means of distinguishing between the native and invasive species using the *rbc*L chloroplast gene. We were also able to identify the 50 unknown species successfully using this technique. Having this tool available would be useful if one had vegetative material and needed a definitive identification. The only drawback to this technique is that since chloroplasts are maternally inherited, we were really only able to identify the maternal parent species of the individual. Given what we have discovered about the possibility of hybrid plants in places where the two species overlap, this technique would be unable to distinguish between a hybrid and its maternal parent plant. Further research would be necessary to be able to make this distinction using molecular methods.

Publications:

Leicht-Young, S. A., N. B. Pavlovic, R. Grundel, and K. J. Frohnapple. 2007. Distinguishing native (*C. scandens* L.) and invasive (*C. orbiculatus* Thunb.) bittersweet species using morphological characteristics. Journal of the Torrey Botanical Society (in press).

Pavlovic, N. B., S. A. Leicht-Young, R. Grundel and K. J. Frohnapple. 2007. American and oriental bittersweet identification. Fact sheet for U.S. Geological Survey Great Lakes Science Center.

Literature Cited:

Chenna, R., H. Sugawara, T. Koike, R. G. Lopez, T. J. Gibson, D. G. Higgins, and J. D. Thompson. 2003. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Research 31:3497-3500.

Leicht-Young, S. A., N. B. Pavlovic, R. Grundel, and K. J. Frohnapple. 2007. Distinguishing native (*C. scandens* L.) and invasive (*C. orbiculatus* Thunb.) bittersweet species using morphological characteristics. Journal of the Torrey Botanical Society (in press).

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Vincze, T., J. Posfai, and R. J. Roberts. 2003. NEBcutter: a program to cleave DNA with restriction enzymes. Nucleic Acids Research 31:3688-3691.

White, O. E., and W. M. Bowden. 1947. Oriental and American bittersweet hybrids. Journal of Heredity 38:125-128.

Products: Dr. Mary Ashley, University of Illinois at Chicago (UIC) used the chloroplast DNA method with middle school teachers participating in the Nature, Math, and Science Partnership in a program called "Change Over Time." This program was funded by the state of Illinois and is a joint project between UIC and The Peggy Notebaert Nature Museum of the Chicago Academy of Science. About 20 teachers participated.

Long-Term Goal/s and Continued Progress of Research: Our long-term goals are to determine in a more definitive manner the genetic status of bittersweet plants throughout the Great Lakes region and the rest of the country. We are now collaborating with the University of Illinois at Chicago and The Field Museum's Pritzker Molecular Lab to develop DNA microsatellites to aid in determining if there are indeed hybrid/backcrossed bittersweet individuals in the field. In addition, we have solicited leaf specimens from National Parks across the country that have one or both of the species present so that we can determine the distribution of potential hybrids across the country. The results of this research would be significant if plants that appear morphologically to be the native *C. scandens* are in fact hybrid or backcrossed individuals. This would imply that pure *C. scandens* is more rare than previously realized and that more steps would need to be taken not only to protect populations of *C. scandens* from harm but to make sure that these populations are actually the true species. In addition, *C. scandens* is often marketed as a native plant appropriate for restoration and horticultural purposes. Care would need to be taken to determine if the stock being used was truly *C. scandens* or a hybrid/backcrossed individual.

Benefits of Seed Money: As we wrote in the previous paragraph, the benefits of this seed grant were enormous in terms of obtaining additional collaborators to accomplish the long-term goals of this project. Without the seed money, we would not have been able to conduct the initial genetic work to obtain the preliminary data necessary to inform the next part of our project. We were able to use a little to go a very long way. In addition, we obtained additional USGS funds this summer to further the DNA microstellites genetic work because our Center Director saw the importance of the work we were doing.

Advancing This Research: To advance this research further, we would need to form additional partnerships with those on the ground doing restoration work that involves *C. scandens* so that, if many of the plants are not of pure lineage, we can work to make sure that the stock being planted is pure stock. In addition, further work would need to be conducted on the ecological implications of a potential hybrid swarm of *C. orbiculatus*, *C. scandens* and their hybrids and backcrosses.

Budget

See next page.

Center for Invasive Plant Management

Distinguishing an Alien Invasive Vine from the Native Congener: Morphology, Genetics and Hybridization SM06/10

Outflows BP64C **05/01/2006 through 9/30/2007**

Class Description	***Deposit***	***Local Travel***	***Contracts***	***Fees & Permits***	***Office***	***Lab***	***Field Supplies***	***Chemicals***	***OVERALL TOTAL***
Deposit	5,000.00	0	0	0	0	0	0	0	5,000.00
Travel	0	-3.60	0	0	0	0	0	0	-3.6
Contracts & Maintenance	0	0	-2,790.61	-29.00	0	0	0	0	-2,819.61
Supplies & Materials	0	0	0	0	-53.49	-1,925.28	-110.60	-87.42	-2,176.79
OVERALL TOTAL	5,000.00	-3.60	-2,790.61	-29.00	-53.49	1,925.28	-110.600	-87.42	**0**

Payee	
Carlin Hort.	46.48
Chesterton Feed & Garden	16.99
Close MSU	9.81
Erika Beals	2,790.61
Fisher	87.42
Fisher Scientific	386.58
Hamilton	562.90
Hobby Lobby	12.99
Integrated DNA Tech	57.40
Meijers	33.21
Menards	43.07
Michaels	31.98
New England BioLabs	64.80
Pavlovic	3.60
Sigma-Aldrich	777.14
Walmart	46.02
Warren Dunes State Park	29.00
OVERALL TOTAL	5,000.00

Category Description	
Local Travel	3.60
Contracts	2,790.61
Fees/Permits	29.00
Office	53.49
Lab	1,925.28
Field supplies	110.60
Chemicals	87.42
TOTAL OUTFLOWS	5,000.00
OVERALL TOTAL	-5,000.00

Acknowledgments:
We thank Claudia Wing for helping with protocol development and determining the cpDNA seguences to analyze. We thank Richard Whitman and Murulee Byapanahalli for the use of the genetics lab and for Kasia Przybyla-Kelly for assisting Stacey with the genetic analysis procedures.

Appendices (attached separately as files)

1. Paper accepted by the Journal of the Torrey Botanical Club.
2. Protocol for chloroplast DNA analysis.
3. Draft USGS fact sheet: will soon be posted on USGS website.

Distinguishing native (*Celastrus scandens* L.) and invasive (*C. orbiculatus* Thunb.) bittersweet species using morphological characteristics[1]

Stacey A. Leicht-Young[2], Noel B. Pavlovic, Ralph Grundel, and Krystalynn J. Frohnapple

U. S. Geological Survey, Great Lakes Science Center, 1100 N. Mineral Springs Rd., Porter, IN 46304

*Address for correspondence same as above, e-mail: sleichtyoung@usgs.gov

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[2] We thank David Zaya, Peter Carlson and Erika Beals for their help with field work and Jean Adams for statistical assistance. Douglas Wilcox and Cynthia Jones provided helpful comments on previous versions of this manuscript. We also thank the following agencies for permission to conduct this research at their properties: National Park Service at Indiana Dunes National Lakeshore, Michigan Natural Features Inventory at Warren Dunes State Park, Lake Country Forest Preserve District at Lyons Woods and Illinois State Parks at Illinois Beach State Park. Author for correspondence; e-mail: sleichtyoung@usgs.gov.

LEICHT-YOUNG, S. A., N. B. PAVLOVIC, R. GRUNDEL, AND K. J. FROHNAPPLE (U. S. Geological Survey, Great Lakes Science Center, 1100 N. Mineral Springs Rd., Porter, IN 46304). J. Torrey Bot. Soc. XXX: 000 000. 20XX.—*Celastrus orbiculatus* is an invasive liana in the Eastern United States. Its native congener, *C. scandens*, is less common and declining in the Northeast. The correct identification of these two species is often difficult because of their similar vegetative characteristics. Using morphological characteristics of both species growing naturally along a sand dune/forest ecotone, we built models for use in discriminating between the species, given a suite of leaf and fruit traits. We confirmed that the two species can be discriminated effectively using fruit characters, notably fruit volume and seed number. Several leaf traits, such as length-to-width ratio and leaf apex length can also discriminate between the species, but without the same predictive reliability of fruit traits. In addition, we determined that at leaf out in the spring the leaves of the two species were folded differently in the bud allowing them to be successfully discriminated in the early spring. Land managers could use this information to differentiate between the two species in the field and thereby control for the invasive *C. orbiculatus*, while preserving remaining populations of *C. scandens*.

Key words: Congeners, hierarchical partitioning, invasive species, lianas, morphology, morphometric analysis, native species

One of the first steps in managing invasive species is proper species identification. This is often a simple task, as many invasive species can be readily differentiated from their native counterparts. There are cases, however, in which identification of the species of interest can be ambiguous (Mehrhoff et al. 2003), leading to misidentification of native or invasive species. One such example is the invasive *Celastrus orbiculatus* Thunb. (Oriental bittersweet) and its native congener *C. scandens* L. (American bittersweet). *Celastrus scandens* appears to be on the decline in many natural areas. This is especially true in the Eastern United States, where *C. scandens* has become difficult to find in many historic habitats (Fike and Niering 1999, Steward et al. 2003, Leicht 2005, Stoos 2006). Both *Celastrus* species are lianas (woody vines) and climb up adjacent vegetation. The native species grows slower than the invasive (Dreyer et al. 1987, Leicht 2005, Leicht and Silander Jr. 2006), while the invasive can blanket the neighboring vegetation, adding extra weight that can lead to breakage of host plants during high winds or ice storms (Siccama et al. 1976), can girdle trees (Lutz 1943, McNab and Meeker 1987), and can shade out native seedlings and saplings (McNab and Meeker 1987). *Celastrus orbiculatus* is spreading westward from the Eastern United States but has not yet completely covered the historic range of *C. scandens* (USDA NRCS 2006).

The two species occur in the same habitats, often adjacent to each other (S. Leicht-Young and N. Pavlovic, pers. obs.), especially in the Midwestern United States, where the native species is still common and *C. orbiculatus* has more recently invaded. Given the similar appearance of the two species, this can make effective management of the two species problematic. This is especially true when not all plants are reproductive,

since reproductive traits are often the most useful traits for discriminating between the species. So far, the only definitive way of distinguishing between the two species is by the location of inflorescences and infructescences. Both species are dioecious or polygamo-dioecious, having both male and female plants (Gleason and Cronquist 1991). *Celastrus orbiculatus* has flowers and fruits located in multiple leaf axils along the stems of the plants while *C. scandens* has fruits and flowers in a terminal panicle (Hou 1955, Radford et al. 1968, Voss 1985, Dreyer et al. 1987, Gleason and Cronquist 1991). Male *C. orbiculatus* plants can sometimes have terminal flowers, while *C. scandens* occasionally has flowers/fruit in the upper axils (Hou 1955, Dreyer et al. 1987). Both situations can lead to confusion in identification. *Celastrus scandens* in these cases will lack a vegetative bud adjacent to the fruit or flower (Hou 1955, Dreyer et al. 1987). However, male *C. orbiculatus* plants rarely only have the terminal flowers, in most cases having the accompanying diagnostic axillary flowers (S. Leicht-Young, pers. obs.), while *C. scandens* males lack these axillary flowers on the lower parts of the stems. Another difference between the male flowers of the two species is pollen color. *Celastrus orbiculatus* pollen is white, *C. scandens* pollen is yellow (Pooler 2002, Leicht-Young pers. obs.). The color of valves of the capsule covering the crimson aril also varies between species when fruits have ripened in the fall. In *C. orbiculatus*, valves are yellow, while in *C. scandens*, valves are orange (Dreyer 1994, S. Leicht-Young, pers. obs.). To further complicate matters of identification, the two species are known to hybridize (White and Bowden 1947, Wyman 1950, Pooler et al. 2002, Mehrhoff et al. 2003), although the extent of this hybridization in natural settings is unknown.

A drawback with using fruit characteristics to distinguish these species is that fruits are only present on mature female plants in the summer or fall. Flower characteristics are available in the spring on male and female plants, but again the plants have to be mature. However, if one wants to identify vegetative plants, comparing differences in leaf characteristics between species might be useful (Dreyer et al. 1987). According to published floras, *C. scandens* has a leaf that is about twice as long as wide (Swink and Wilhelm 1994) and elliptic to oblong or ovate and acuminate in shape (Radford et al. 1968, Gleason and Cronquist 1991). *Celastrus orbiculatus*, however, has leaves "scarcely longer than wide" (Swink and Wilhelm 1994), and they are suborbicular to broadly oblong-ovate in shape (Radford et al. 1968, Gleason and Cronquist 1991). Thus, it would appear that the leaves of the two species are rather different. Often, land managers and others cite this leaf difference as a way to tell them apart (Leicht-Young, pers. obs.). However, others have stated that this is an unreliable method (Dreyer et al. 1987).

We set out to determine systematically the validity of using leaves for species differentiation by measuring both leaf and fruit characters on wild *Celastrus* plants and using these measures to build predictive models. This type of systematic, morphometric approach has only occasionally been used for identification of invasives (Baret et al. 2003a, Baret et al. 2003b, Rogers et al. 2006). We predicted the leaf characteristics would be less effective than fruit characteristics in differentiating the two species. However, it was our goal to examine how leaf and fruit characteristics performed in discriminating between the invasive *C. orbiculatus* and the native species in the field.

Methods. *Celastrus* plants used in this study were located along foredune/forest ecotones at Indiana Dunes National Lakeshore (INDU: 41º37´N, 87º05´W) in Porter, IN, located at the southern tip of Lake Michigan, Illinois Beach State Park (IBSP: 42º25´N, 87º48´W) in Zion, IL and Lyons Woods (LW: 42º24´N, 87º49´W) in Waukegan, IL, located on the western shore of Lake Michigan, and Warren Dunes State Park (WD: 41º54´N, 86º35´W) in Sawyer, MI, located on the southeastern shore of Lake Michigan.

LEAF AND FRUIT MORPHOLOGY OF MATURE PLANTS. In late September and October of both 2005 and 2006, we measured morphological characteristics of 50 fruiting female individuals of each *Celastrus* species at INDU. To locate individuals in the field, we walked along the interface of dune and forested habitats. We selected adult fruiting plants because these were the most easily identified to species. From each individual, we collected three randomly selected mature leaves and three randomly selected fruits.

We took several measurements to characterize leaf blade shape and size. Using the fall 2006 leaves from INDU, we scanned the fresh leaves to make measurements of leaf length and width with Adobe Photoshop® and perimeter using Scion Image (Scion Corporation 2000). Using the perimeter measurements, we calculated both the shape factor and feret-diameter ratio (Huff et al. 2003):

$$\text{shape factor} = 4\pi \frac{\text{leaf area}}{\text{leaf perimeter}^2} \qquad \text{feret - diameter ratio} = \frac{\text{feret diameter}}{\text{major axis length}}$$

The more dissected or toothed a leaf is, the lower its shape factor. The more oblong a leaf, the lower the feret-diameter ratio. The feret-diameter is the diameter of a

126 circle having the same area as the leaf. The major axis length is the longest dimension of
127 a leaf (Huff et al. 2003). For both shape factor and feret-diameter ratio, a circle has a
128 value of one and a line a value of zero. In addition to differences in shape of the main
129 part of the leaf, the two *Celastrus* species appeared to have different lengths of leaf
130 apices. To quantify apex length, we used the scanned images in Adobe Photoshop® and
131 drew a straight line between the two inflection points at the leaf apex. The inflection
132 point was where the curvature of the body of the leaf changed direction and began the
133 apex. From this straight line, we measured to the end of the leaf to estimate apex length.
134 It was possible to have "negative lengths" when the end of the leaf actually went inwards.
135 We also calculated a leaf apex ratio, which was the ratio of the length of the apex to the
136 length of the body of the leaf without the apex. These same leaf data were collected in
137 early summer of 2007 from approximately 25 plants of each species at IBSP, LW and
138 WD to compare and validate data collected from INDU. These additional plants were
139 identified to species using position at leaf out (see below) and included both male and
140 female plants of each species.

141 On the fruits collected in fall 2005, we measured both longitudinal and latitudinal
142 diameter with calipers. Using these diameters, and converting them to semi major and
143 minor axis lengths, we calculated fruit volume using the formula for the volume of a
144 spheroid, with an oblate spheroid being a fruit that has a larger latitudinal size and a
145 prolate spheroid being a fruit with a larger longitudinal size.

146 $$\text{Oblate spheroid} = \frac{4}{3}\pi a^2 b \qquad \text{Prolate spheroid} = \frac{4}{3}\pi a b^2$$

147 We also calculated a fruit ratio, which was the ratio of the longitudinal and latitudinal
148 diameters, to determine if there were differences in the overall shape (*i.e.*, roundness) of

149 the fruit. Finally, we dried the fruit so that seeds could be extracted and number of seeds
150 per fruit determined.
151
152 COMPARISON OF LEAF OUT FOLDING. We observed in late April 2006 that the
153 leaves of two species of *Celastrus* appeared to have different leaf folding upon leaf out.
154 The leaves were either conduplicate (two sides of the leaf folded against each other) or
155 involute (leaf margins rolled in like a scroll). To investigate the consistency of this
156 observation, we examined our previously marked individuals at INDU and recorded leaf
157 display for each of these plants. As with the morphological leaf measurements, we took
158 additional observations in early spring 2007 at IBSP, LW and WD sites to compare these
159 to our observations from INDU.
160
161 COMPARISON OF DEVELOPMENTAL SEQUENCE OF LEAVES. In late May 2006, we
162 selected ten different mature individuals of each species, using the position of the
163 inflorescence to identify the species positively. From each of these plants, we took an
164 actively growing leader and harvested the entire developing series of leaves, from the
165 most recently mature leaf to the newest leaf whose edges had fully unrolled. We
166 recorded the same measurements (length:width, feret-diameter ratio, shape factor, apex
167 length, apex ratio) on these leaves as we did on the leaves collected in the fall.
168
169 ANALYSES. To examine whether mean values of each of the different leaf
170 measures and fruit traits were statistically different between species, we first used a
171 mixed-model ANOVA, with species as a fixed factor and individual plant as a random

172 factor. In this way, we could determine if the variability within a given plant was
173 significant compared to the variability within a species. These were followed by paired *t*-
174 tests

175 We then built predictive models using logistic regression to determine if the two
176 species could be discriminated based on the leaf morphological traits from the fall 2006
177 data and from fruit traits from the fall 2005 data collected at INDU. The leaf traits that
178 we considered for the model were length:width, shape factor, feret-diameter ratio,
179 $\log_{10}$apex length, and apex ratio. Fruit traits were fruit volume, fruit ratio, and seed
180 number. In addition, we constructed a combined model that incorporated both the leaf
181 and fruit traits listed above. For all of these models, a response of one identified the plant
182 as *C. orbiculatus*, while *C. scandens* was coded as zero.

183 For the leaf traits, after doing a preliminary correlation analysis, we determined
184 that all of the leaf measurement variables were significantly correlated (e.g., $r^2 > 0.5$) and
185 thus could not be used simultaneously in logistic regression without first determining the
186 factors that most contributed to the model. Since we had three leaves (or fruits) from
187 each plant, we trained the logistic regression on a randomly selected set of two leaves
188 from INDU ($N = 98$ for *C. orbiculatus* and $N = 96$ for *C. scandens*) from each plant. We
189 then tested this model using the remaining set of leaves from INDU ($N = 49$ for *C.*
190 *orbiculatus* and $N = 48$ for *C. scandens*) and then the leaves collected from IBSP and LW
191 ($N = 75$ for *C. orbiculatus* and $N = 72$ for *C. scandens*) and WD ($N = 87$ for *C.*
192 *orbiculatus* and $N = 84$ for *C. scandens*). The default cutoff probability for identification
193 as *C. orbiculatus* was 0.5 and above.

194 To determine how much variation in the response was explained by each
195 parameter, we used hierarchical partitioning (Walsh and Mac Nally 2005) followed by a
196 randomization test for hierarchical partitioning to assess which of the factors'
197 contributions to the model were significant (Mac Nally 2002). In addition, we calculated
198 a logistic regression incorporating only the length:width, as this is the most easily
199 measurable characteristic in the field and the vegetative characteristic most commonly
200 pointed to as being diagnostic for the species. We followed the same protocol for the
201 predictive model of the fruit data as the leaf data, except that only data from INDU were
202 used to train and test these models. However, since fruit traits were not highly correlated
203 with each other, all fruit traits were entered together into the logistic regression and
204 significant predictors were selected for the final model. Finally, we developed a model
205 that combined fruit and leaf characteristics together and used hierarchical partitioning to
206 determine which characteristics might explain the most variation in the logistic regression
207 response.
208 To compare developmental sequences across plants, we created a Leaf
209 Developmental Index, using leaf length as a proxy for developmental age:

210 $$\text{Leaf Developmental Index} = \frac{\text{leaf length} - \min(\text{leaf length})}{\text{range}}$$

211 where leaf length is length of an individual leaf on a leader, min (leaf length) is
212 minimum leaf length within that leader, and range is the range of leaf length values in the
213 leader. This results in an index between zero and one. We divided the Leaf
214 Developmental Indices into five age classes so that changes in leaf traits with age could
215 be analyzed using *t*-tests Age class 1 was leaves with an index between 0 – 0.2, class 2
216 was 0.21 – 0.4, class 3 was 0.41 – 0.6, class 4 was 0.61 – 0.8 and class 5 was 0.81 – 1.

We used a sequential Bonferroni adjustment to adjust α-value when conducting the five separate t-tests within a leaf trait (Quinn and Keough 2002). All analyses were conducted using SPSS (SPSS Inc. 2003).

Results. LEAVES AS PREDICTORS OF SPECIES IDENTITY. All leaf characteristics differed significantly between the two *Celastrus* species at all of our sites (Table 1). The individual plant the leaf was collected from was not a significant random factor, indicating that there was not a trend present based on the plant as an individual. *Celastrus orbiculatus* had a lower length:width, apex length, and apex ratio and greater feret-diameter ratio and shape factor than *C. scandens*. This was true across all three of our sites. Despite these statistically significant results, there was substantial overlap in the data. Using INDU as an example, ranges of the leaf shape predictors for the two *Celastrus* species exhibited between 91% to 99% overlap for the five predictors (Fig. 1).

Hierarchical partitioning of the leaf data indicated that length:width, feret-diameter ratio, and $\log_{10}$apex length contributed significantly to the predictive model. The logistic regression model incorporating these predictors had a McFadden's ρ^2 of 0.206 (Table 2). Percentage of correct prediction by the model for *C. orbiculatus* was 72% and 70% for *C. scandens* for the training data. Using the remaining set of leaves from INDU to test the model resulted in similar predictive rates of 76% for *C. orbiculatus* and 76% for *C. scandens*. Using the IBSP and LW data to test the model resulted in 91% correct for *C. orbiculatus* and 67% correct for *C. scandens*. Finally, the WD data had 54% correct for *C. orbiculatus* and 86% correct for *C. scandens*.

When we only used length:width in the model as a predictor, ρ^2 decreased to 0.14 (Table 2). The ability of the model to predict *C. orbiculatus* and *C. scandens* successfully, however, was similar to the previous model with the other leaf traits, with *C. orbiculatus* correctly predicted 73% of the time and *C. scandens* 69% for the training data and 65% of *C. orbiculatus* and 67% of *C. scandens* correctly predicted for the test data from INDU. Using leaves from IBSP and LW, we had correct predictive rates of 91% for *C. orbiculatus* and 73% for *C. scandens*, and from WD, rates were 77% and 86%.

FRUIT CHARACTERISTICS AS PREDICTORS OF SPECIES IDENTIFICATION. *Celastrus scandens* had significantly greater fruit volume and lesser seed number than *C. orbiculatus* (Table 1, Indiana). Fruit shape ratio, however, did not differ between the two species, indicating that there is no difference in the overall shape of the fruit (Table 1). Peak frequency in fruit volume and seed number for the species differed distinctively (Figures 2A, C). The frequency distributions for fruit ratio, however, were nearly identical for the two species (Figure 2B).

In logistic regression, fruit volume and seed number were significant predictors, while fruit ratio was not (Table 2). Fruit characteristics as expected, were better predictors of the two species than leaf data alone. $\rho^2 = 0.74$ for fruit characteristics alone. The percent correct species identification using fruit characteristics was 92% for *C. orbiculatus* and 91% for *C. scandens* using the training data and a 0.5 cutoff. Using the testing data, we obtained correct predictions in 92% of cases for *C. orbiculatus* and 86% for *C. scandens*.

LEAVES AND FRUIT AS PREDICTORS OF SPECIES IDENTIFICATION. The hierarchical partitioning randomization test using leaf and fruit data indicated that length:width, $\log_{10}$apex length, fruit volume, and seed number were significant contributors to the model. Using this combination of leaf and fruit traits, we obtained a model with ρ^2 = 0.70. In this model, $\log_{10}$apex length was not significant, while the other factors remained significant (Table 2). The predictive rates for this model with the 0.5 cutoff was 92% for *C. orbiculatus* and 92% for *C. scandens* in the training dataset and 94% and 92% for the testing dataset.

LEAF OUT FOLDING. Of the plants identified in fall 2005 at INDU as *C. orbiculatus*, 100% had leaves folded in a conduplicate manner while 100% of the *C. scandens* were involute in spring 2006 (Figure 3). We had the same results when observing the leaves at IBSP, LW and WD in early spring 2007.

DEVELOPMENTAL MORPHOLOGY. In general, younger leaves (*i.e.*, categories 1 and 2) had fewer significant morphological differences between species than did more mature leaves (categories 4 and 5, Table 3). However, length:width and shape factor differed in all leaf age categories except category 1. Apex length and feret-diameter ratio did not differ in any of the categories except for category 5 (Table 3).

Discussion. We determined that leaf data, in the absence of fruits, provided a moderate level of discrimination between the two *Celastrus* species. Unexpectedly,

285 folding of the leaves at leaf out proved to be perhaps the most certain means of
286 discrimination, and does not rely on the presence of fruit or flowers to make the
287 determination. The more complicated measures of leaf shape (shape factor and feret-
288 diameter ratio) did not provide any greater level of discrimination than the basic
289 length:width. In fact, in the logistic regression, feret-diameter ratio came out as not
290 significant. Although the leaf apices of *C. scandens* were generally longer than for *C.*
291 *orbiculatus*, we found through our validation data that this is the trait that causes differing
292 rates of success (91-54% correct). For instance, on average, *C. scandens* from IBSP had
293 shorter tips than those from INDU and WD, while *C. orbiculatus* from WD, on average,
294 had longer tips (Table 1). In addition, using our data from the developing leaves, we
295 found that younger, less developed leaves of both species had longer leaf apices, making
296 them more indistinguishable between species at that stage. Thus, in the broad sense, the
297 mature leaves of the species tend to follow the descriptions in the published floras
298 (Gleason and Cronquist 1991, Swink and Wilhelm 1994), and making determinations
299 based on younger leaves would be inadvisable.

300 Despite the ability to distinguish the species statistically on leaf characteristics,
301 there is the question of the practicality of using this method. It must be noted that the
302 differences between species were based on mean values. The ranges in values for the two
303 species, however, could be rather large. For instance, in length:width at INDU, the mean
304 value for *C. orbiculatus* was 1.49 and for *C. scandens* was 1.77. However, the
305 length:width range for *C. orbiculatus* was 0.9 – 2.6, and for *C. scandens* was 1.2 – 2.8
306 (Figure 1A) resulting in a 91% overlap in values. Not only is there a significant amount
307 of variation, but also, measurements often only differ by a few millimeters. Thus, since

using leaf data alone does not have great (*i.e.*, > 90%) reliability in the logistic regression for both species, decisions of identification should be made with caution, and should be treated as suggestive of which species is present.

Fruit data, however, have proven much more reliable in identification of the species (Dreyer et al. 1987). Not only fruit location, but fruit volume and seed number can assist in identification – the latter two traits probably being more useful for fruits separated from the plant. The combined model of leaf and fruit data, however, did not prove to be any more successful than fruit alone, showing how strong the fruit traits were in their predictive power.

The folding of the leaves at leaf out in the spring, however, was one of the most interesting and perhaps most useful differences between the two species. These differences allow for differentiation of the two species regardless of their maturity or sex. It is especially useful in places where the two species could be present. At INDU and WD in particular, although the two species are often growing in close proximity, the difference in leaf folding is very clear. However, it is important to make these observations just after the leaves have broken bud and are expanding, because as the leaves become more mature, the differences are not as apparent. In addition, leaves that emerge later in the season do not present the discriminating features. Thus, there is a narrow window of opportunity to use this feature to discriminate between the species, which at INDU in 2006 and WD in 2007 was the last days of April and early May. IBSP was later in its phenology and early May was the best time. Obviously, the time of leaf out is highly variable depending on region and spring weather temperatures, and careful observation is necessary to catch the plants at the proper time. We have provided

pictures to assist with this determination (Figure 3). If land managers want to use this feature for delineating areas and individual plants for control, they need to be proactive, marking the plants in the spring for control later in the summer or fall. Although this takes some planning, it could be the most effective way to identify the native and exotic species where they both occur.

One final issue is that of the potential hybridization of these two species in the field. Based on the literature in which crosses were created of these two species, the hybrid plants would be intermediate in appearance of the two species, especially in terms of fruit position (White and Bowden 1947, Wyman 1950, Pooler et al. 2002). Since these plants do grow in such proximity, there is a very real possibility that there are hybrid plants in these areas and that some of our specimens could have been hybrid or back-crossed individuals. Morphology, especially vegetative morphology, would probably not prove useful in discriminating hybrids and the only way to truly discern how these two species are interacting would be to use molecular methods.

In summary, we confirmed that the two *Celastrus* species are best discriminated using either fruit position or fruit volume. In addition, we observed a highly consistent difference in the folding of the leaves at leaf out for the two species, allowing for differentiation of all individuals, even if they are not mature or fruiting females. There are statistical differences in the leaf morphology of the two species, but caution must be exercised when using these traits, as they can be variable. Using this basic identification information for the two *Celastrus* species, it could be possible to target areas for management where the invasive *C. orbiculatus* is present and more effectively preserve the native *C. scandens* in areas where both species can co-occur. To assist in the

discrimination of these two species, we have developed a diagrammatic key which summarizes useful characters for identifying these two species (Figure 3).

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Table 1. Mean (± SE) for leaf and fruit characteristics for mature plants of *Celastrus* spp. in three states. Different letters within a state indicate significant differences between species for the leaf or fruit measurement at the $\alpha = 0.05$ level.

	Indiana		Illinois		Michigan	
	C. orbiculatus	*C. scandens*	*C. orbiculatus*	*C. scandens*	*C. orbiculatus*	*C. scandens*
Feret-diameter ratio	0.77 ± 0.01^a	0.69 ± 0.01^b	0.82 ± 0.01^a	0.71 ± 0.01^b	0.77 ± 0.01^a	0.68 ± 0.01^b
Length:width	1.49 ± 0.03^a	1.77 ± 0.03^b	1.36 ± 0.02^a	1.82 ± 0.04^b	1.49 ± 0.02^a	1.82 ± 0.02^b
Apex Length (cm)	0.53 ± 0.05^a	0.99 ± 0.05^b	0.45 ± 0.05^a	0.68 ± 0.05^b	0.79 ± 0.06^a	1.00 ± 0.05^b
Apex Ratio	0.11 ± 0.01^a	0.20 ± 0.01^b	0.10 ± 0.01^a	0.11 ± 0.01^b	0.11 ± 0.01^a	0.14 ± 0.01^b
Shape Factor	0.64 ± 0.01^a	0.58 ± 0.01^b	0.56 ± 0.01^a	0.52 ± 0.01^b	0.51 ± 0.01^a	0.48 ± 0.01^b
Fruit volume (mm^3)	166.69 ± 5.57^a	290.69 ± 10.02^b	-	-	-	-
Fruit ratio	0.96 ± 0.01^a	0.97 ± 0.01^a	-	-	-	-
Seeds/fruit	4.10 ± 0.10^a	2.80 ± 0.10^b	-	-	-	-

429

Table 2. Results of logistic regression models. The McFadden's ρ^2 is the model fit value for the whole model. All models were built using data from INDU. The leaves only model and the length:width only models were tested using data from INDU, IBSP, LW and WD, and the fruit only and combined models were tested using data from INDU.

	Coefficient	Standard error	Wald statistic	*P*-value	ρ^2
Leaves Only					
Length:width	-3.85	1.73	4.94	0.03	0.20
Feret-diameter ratio	-7.76	6.31	1.51	0.22	
$\log_{10}$apex length	-4.58	1.76	6.78	0.01	
Constant	12.87	7.47	2.96	0.09	
Length:Width Only					
Length:width	-3.40	0.62	30.38	<0.001	0.14
Constant	5.47	1.00	29.78	<0.001	
Fruit Only					
Fruit volume	-0.05	0.01	35.95	<0.001	0.74
Fruit ratio	-1.39	3.15	0.19	0.66	
Seed number	2.30	0.39	34.95	<0.001	
Constant	2.87	3.31	0.75	0.39	
Combined Model					
Fruit volume	-0.04	0.01	27.63	<0.001	0.70
Seed number	2.36	0.45	28.22	<0.001	

Length:width	-2.73	1.38	3.93	0.05
$\log_{10}$apex length	-3.37	2.42	1.93	0.16
Constant	6.27	2.28	7.57	0.01

431

Table 3. Means (± SE) for developmental leaf sequences for *Celastrus* spp. Leaves are ordered from youngest (1) to oldest (5). Different letters within a leaf category indicate significant differences using t-tests at the sequential Bonferroni-adjusted α-level (see methods for description).

	Leaf Category	*C. orbiculatus*	*C. scandens*
Length:width	1	2.32 ± 0.05[a]	2.48 ± 0.12[a]
	2	2.01 ± 0.05[a]	2.50 ± 0.14[b]
	3	1.88 ± 0.04[a]	2.17 ± 0.11[b]
	4	1.72 ± 0.04[a]	1.96 ± 0.08[b]
	5	1.61 ± 0.03[a]	1.91 ± 0.04[b]
Feret-diameter ratio	1	0.52 ± 0.00[a]	0.51 ± 0.02[a]
	2	0.57 ± 0.01[a]	0.52 ± 0.02[a]
	3	0.61 ± 0.01[a]	0.56 ± 0.02[a]
	4	0.64 ± 0.01[a]	0.60 ± 0.01[a]
	5	0.71 ± 0.01[a]	0.63 ± 0.01[b]
Shape factor	1	0.45 ± 0.01[a]	0.42 ± 0.02[a]
	2	0.51 ± 0.01[a]	0.44 ± 0.01[b]
	3	0.56 ± 0.01[a]	0.48 ± 0.01[b]
	4	0.59 ± 0.01[a]	0.51 ± 0.02[b]
	5	0.64 ± 0.01[a]	0.56 ± 0.01[b]
Apex length (cm)	1	1.13 ± 0.03[a]	1.13 ± 0.09[a]

	2	1.19 ± 0.04^{a}	1.29 ± 0.10^{a}
	3	1.08 ± 0.06^{a}	1.25 ± 0.12^{a}
	4	1.09 ± 0.06^{a}	1.09 ± 0.08^{a}
	5	0.74 ± 0.04^{a}	0.97 ± 0.04^{b}
Apex Ratio	1	0.54 ± 0.02^{a}	0.49 ± 0.03^{a}
	2	0.37 ± 0.01^{a}	0.40 ± 0.03^{a}
	3	0.29 ± 0.02^{a}	0.31 ± 0.03^{a}
	4	0.26 ± 0.02^{a}	0.22 ± 0.02^{a}
	5	0.13 ± 0.01^{a}	0.16 ± 0.01^{b}

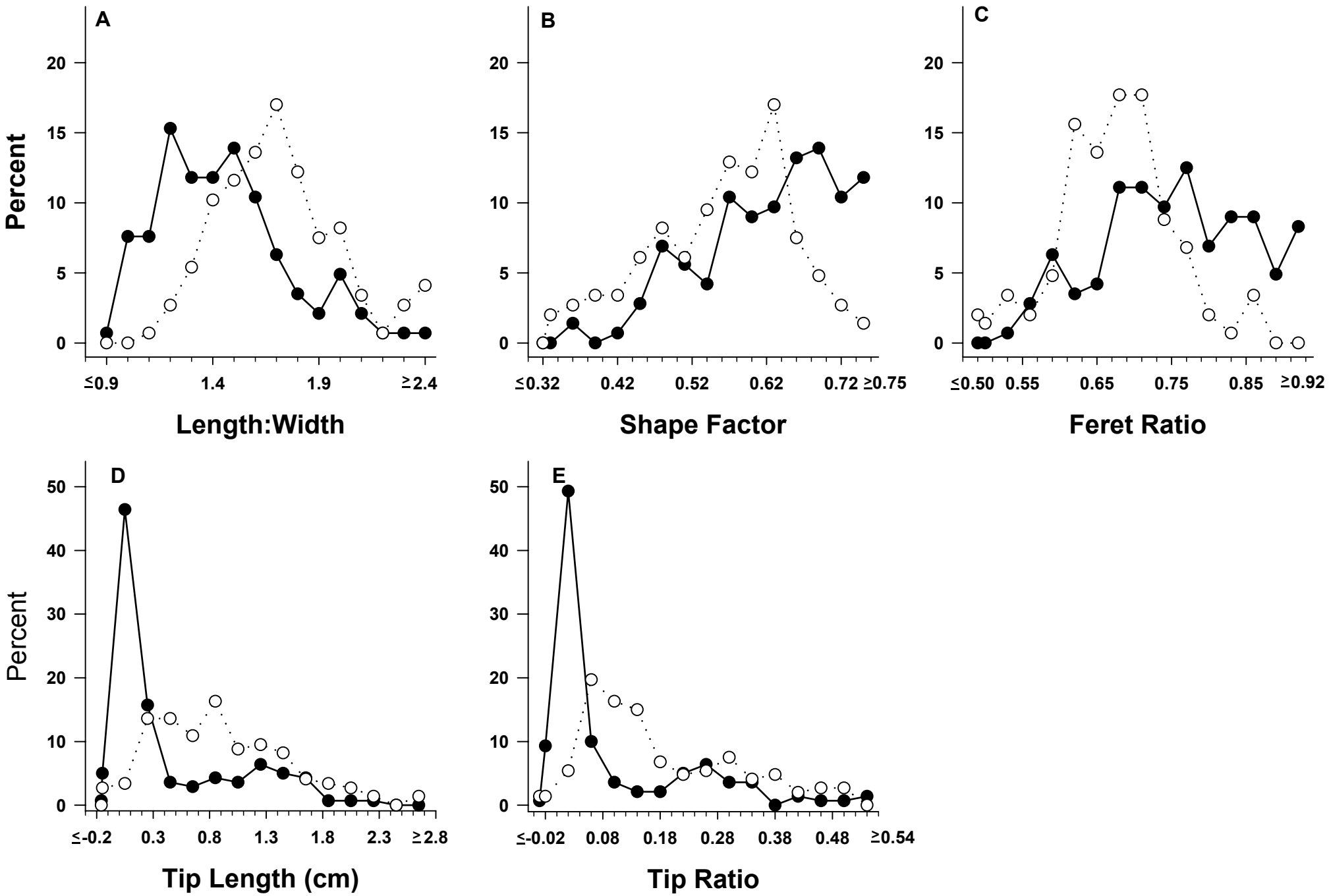
432

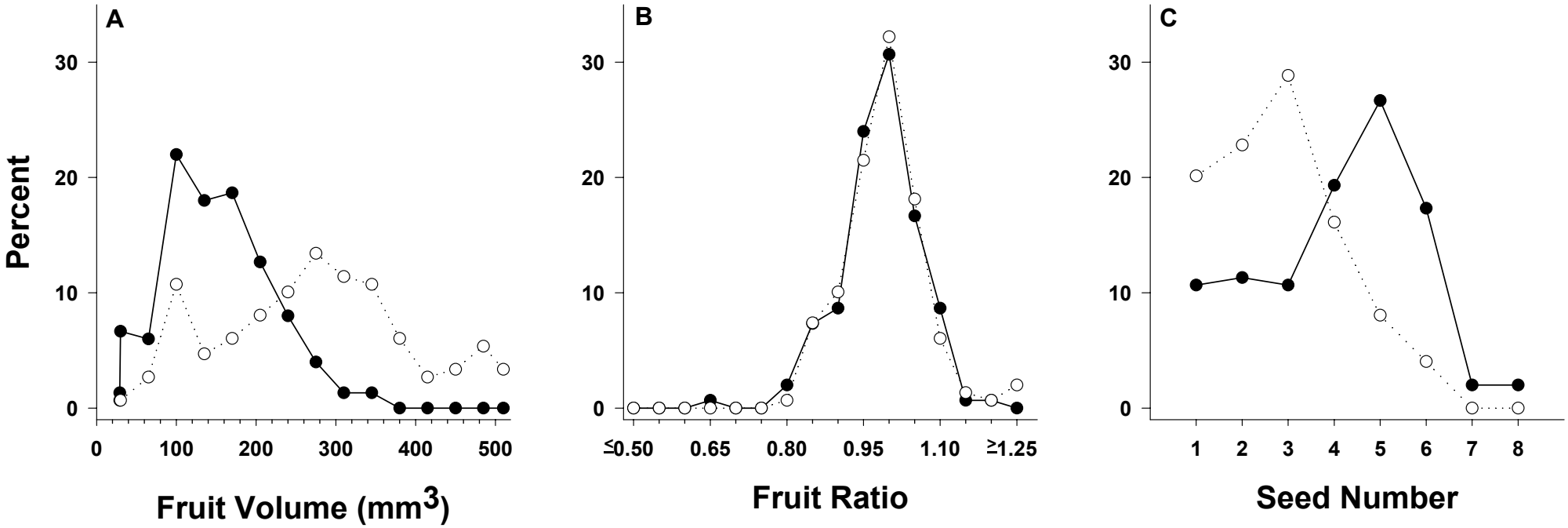
Figure Legends

Figure 1. Distributions of *Celastrus orbiculatus* and *C. scandens* for leaf measurements. Black circles and solid lines are *C. orbiculatus* and white circles and dashed lines are *C. scandens*. Data are broken up into equal intervals. Untransformed data are shown for ease of interpretation. (A) length:width, (B) shape factor, (C) feret-diameter ratio, (D) apex length, and (E) apex ratio.

Figure 2. Distributions of *Celastrus orbiculatus* and *C. scandens* for fruit measurements. Black circles and solid lines are *C. orbiculatus* and white circles and dashed lines are *C. scandens*. Data are broken up into equal intervals. (A) Fruit volume, (B) fruit ratio, and (C) number of seeds.

Figure 3. Diagrammatic key for identification of *C. orbiculatus* and *C. scandens* using traits from the fruit and leaf models, as well as other field-observed characteristics. An asterisk indicates a 90% probability of correct identification based on the data collected for this study. Traits without an asterisk are qualitative and therefore reliable distinguishing characteristics. Questions followed by a dagger indicate that the trait needs to be measured/calculated in the lab. It is important to note that the characteristics with asterisks are based on data that has shown overlap for the two species and are only suggestive of which species is present.


A
B
C
D
E
Percent
Percent
0
5
10
15
20
30
40
50
≤0.9
1.4
1.9
≥2.4
Length:Width
≤0.32
0.42
0.52
0.62
0.72
≥0.75
Shape Factor
≤0.50
0.55
0.65
0.75
0.85
≥0.92
Feret Ratio
≤-0.2
0.3
0.8
1.3
1.8
2.3
≥2.8
Tip Length (cm)
≤-0.02
0.08
0.18
0.28
0.38
0.48
≥0.54
Tip Ratio

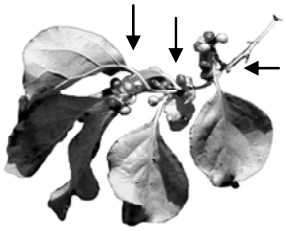

A
B
C
Percent
30
20
10
0
0
100
200
300
400
500
Fruit Volume (mm3)
≤0.50
0.65
0.80
0.95
1.10
≥1.25
Fruit Ratio
1
2
3
4
5
6
7
8
Seed Number

Is plant in fruit? **Yes** → Are ripe fruit capsules orange? → *C. scandens*

yellow? → *C. orbiculatus*

Are fruit borne in terminal panicles? / axillary cymes?

C. scandens



C. orbiculatus

Do fruits have ≤ one seed?* → *C. scandens*

≥ five seeds?* → *C. orbiculatus*

Is fruit volume ≥ 250 mm^3?*† → *C. scandens*

≤ 115 mm^3?*† → *C. orbiculatus*

No

Is plant in bloom? **Yes** → Are flowers borne in terminal panicles? → *C. scandens*

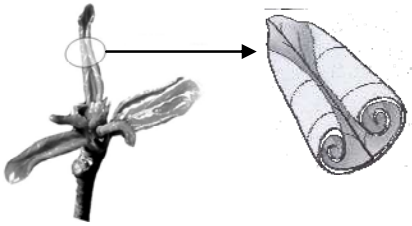
axillary cymes? → *C. orbiculatus*

Are flowers staminate? **Yes** → Pollen yellow → *C. scandens*

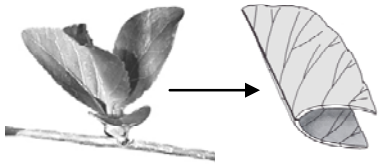
Pollen white → *C. orbiculatus*

No

Is plant leafing out? **Yes** → Are leaves involute? / conduplicate?



C. scandens



C. orbiculatus

No

Are mature leaves present? **Yes** → Is length:width ≥ 2.0?* → *C. scandens*

Is length:width ≤ 1.4?* → *C. orbiculatus*

Is leaf tip > 1.5 cm?* → *C. scandens*

Is leaf tip < 0.3 cm?* → *C. orbiculatus*

Is feret-diameter ratio < 0.60?*† → *C. scandens*

Is feret-diameter ratio < 0.77?*† → *C. orbiculatus*

No

If dormant season, only fruit characters applicable

Celastrus DNA protocol

I. Isolation of the chloroplast DNA for analysis:

1. Set water bath to 95°C…it takes a long time to heat up.

2. Once the water bath has hit 95°C, begin leaf punch collection. Using forceps that were sprayed down with ethanol and dried, pull a leaf out of the bag. Holding the leaf with forceps, use metal hole punch sprayed with ethanol and dried with paper towel to gently punch a hole in the leaf, allowing the punch to fall into a labeled watch glass.

3. Repeat this procedure for all leaves, being careful to spray down forceps and hole punch between each leaf. Each punch should be in its own glass.

4. In the hood, add one hole punch to each labeled 1.5mL tube.

5. Add 100µL extraction buffer (XNAP kit) to each tube and vortex briefly. When adding extraction buffer, use the tip of the pipette to push down the leaf punch so that it is completely covered in solution.

6. Place tubes in float and put in water bath for 10 minutes.

7. Add 100µL of dilution solution (XNAP kit) and vortex (at least 30s) to mix.

8. Store the extract in freezer, or fridge if being used soon.

II. PCR amplification:

1. Thaw components of the PCR on ice.

For each 0.2mL PCR tube, volumes below are what is needed, scale up for making Master Mix and add negative control:

Reagent	**Volume**
PCR H_2O	19µL
REDExtract-N-Amp PCR ReadyMix	25µL
Forward primer *rbcL* (10 pmol)	1µL
Reverse primer *rbcLR*	1µL
Leaf disk extract	4µL
Total Volume	50µL

2. Centrifuge if necessary to bring all components to the bottom.
3. Put tubes in thermocycler, making sure all lids are tight. Use program number 3.

Cycling parameters

94°	2 min	denaturation
94°	45 sec	
59°	45 sec	35 cycles amplification
72°	90 sec	
72°	2 min	final extension
4°	soak	

Place sample in freezer or fridge until ready for PCR gel and RFLP analysis.

III. PCR product gel

1. Set water bath to 55°C.
2. To make 0.5X TBE, add 50mL 10X TBE to 950mL distilled water. Mix in large container.
3. To make the gel, put 100mL of the 0.5X TBE in a 250mL orange-capped bottle with 1.25g of the SeaKem agarose.
4. Swirl contents gently to begin dissolving of agarose.
5. Put solution in the microwave with cap on loosely, and set for 3 minutes on high (you'll be stopping it intermittently during this time). Microwave until mixture starts to boil, stop microwaving and swirl. Put bottle back and microwave again until boiling, stop, swirl. Continue until all little pieces of agarose are completely dissolved.
6. Put bottle in water bath for at least 15-20 min so that the solution cools to 55°C.
7. Add 3µL ethidium bromide to solution…gently swirl until there is no color left.
7. Cast the gel using the 27 well comb.
8. Run 5µL of the PCR products at 100V for about 1 hour to visualize product.

IV. Restriction enzyme digestion

1. Heat water bath to 37°C.

Digest using the following conditions per tube, scale up for Master Mix and remember negative control:

NlaIV		***PvuII***	
PCR product	10µL	PCR product	10µL
Digestion buffer 4	1.5µL	Digestion buffer 2	1.5µL
NlaIV	2.5µL	*PvuII*	0.25µL
BSA	1µL	PCR H_2O	3.25µL
Total Volume	15µL	Total Volume	15µL

Place tubes in float and place in water bath for 2 hours.

2. Take DNA ladder out to thaw about 20 min before 2 hours are up. Mix 5µL DNA with 1µL dye and mix with pipette…place in fridge if digestion not complete.

3. Make TBE and gel. To make TBE, use 100mL 10X TBE and 900mL distilled water. Use 1.75g of agarose in 100mL of 1X TBE. Follow same protocol as above, using the 27 well comb.

4. Run the whole 15µL digestion on a 1.75% agarose gel using 1X TBE at 77V for about 1-1hr 15min. Also run negative controls and undigested product.

USGS
science for a changing world


American bittersweet

American and Oriental Bittersweet Identification


Oriental bittersweet

Invasive species are one of the greatest threats to native ecosystems. They can crowd out native species and change the natural nutrient cycling processes that take place in ecosystems. One of the best ways to combat invasive species is by identifying small infestations and removing them.

One invader threatening Midwestern ecosystems is oriental bittersweet (*Celastrus orbiculatus*). This woody vine was introduced to the eastern United States in the mid-1800s. It has spread from the east to the south and west and is now moving into Midwestern natural areas. Oriental bittersweet can be found in a variety of habitats, from roadsides to interior forests and sand dunes. It has the ability to girdle and overtop adjacent vegetation – often to the detriment of native species. To halt the spread of oriental bittersweet, significant control measures are needed.

However, a native bittersweet species, American bittersweet (*Celastrus scandens*), can be mistaken for oriental bittersweet. Although American bittersweet is also a vine and climbs on nearby vegetation, it does not appear to grow as rapidly or as large as oriental bittersweet. In the northeastern United States, American bittersweet is declining because of habitat change and possible hybridization, while in the Midwest, it is still common.

Because the two bittersweet species look so similar, there can be difficulty knowing which plants to target for control. Using fruit and leaf characters, the two species can be discriminated from each other. However, certain traits are more reliable for correct identification than others. Classically, the position of the fruit and flowers on the stems has been cited as the most definitive means of discriminating between the species.

Oriental bittersweet has fruit and flowers located in the leaf axils along the length of the stem. American bittersweet, however, only has fruit and flowers in terminal clusters. There is also a difference in the color of the capsules surrounding the ripened fruit in the fall. Oriental bittersweet has yellow capsules, while those of American bittersweet are orange. Another difference in color is the pollen color of the male flowers. The pollen of oriental bittersweet is white while that of American bittersweet is yellow.

Some less definitive fruit traits for discrimination are size of the fruits and number of seeds per fruit. American bittersweet has generally larger fruit than oriental bittersweet. If fruits have a volume of greater than 250 mm^3, there is a 90% probability of a plant being American bittersweet, while if the fruit has a volume of 115 mm^3 or less; it has a 90% chance of being oriental bittersweet. Values in between these numbers overlap to some extent between the species. Similarly, if the fruit has one or fewer seeds, it is 90% likely to be American bittersweet, while five or more seeds have a 90% chance of being oriental bittersweet. The greater number of seeds of oriental bittersweet gives it a reproductive advantage over the native species.

The problem with using fruit and flower traits for discriminating between the two species is that, for fruits, only mature female plants have this character available for identification. In terms of flowers, only mature male and female plants have these present, and only for a brief time of the year during the spring.

Vegetative traits apply to plants regardless of their sex or maturity. The most definitive vegetative trait is the posture of the leaves at leaf out of the first buds in the spring. The leaves of oriental bittersweet are conduplicate (two sides of the leaf folded against each other) and tightly packed in the bud when they emerge in the spring. The leaves of American bittersweet are involute (leaf margins rolled in like a scroll) and not as tightly packed in the bud.

Other leaf traits are not as reliable as the leaf-out posture. Although the ratio of length-to-width (length:width) of the leaves is generally greater for American bittersweet, this trait is quite variable. If the length:width of the leaf is greater than or equal to 2, there is a 90% chance of the plant being American bittersweet, while if the ratio is less than or equal to 1.4, there is a 90% chance of it being oriental bittersweet. The tips of the leaves of American bittersweet are also generally longer than those of oriental bittersweet. Plants with leaf tips of 1.5 cm or greater have a 90% chance of being American bittersweet, while plants with leaf tips of 0.3 cm or less have a 90% chance of being oriental bittersweet.

By using these traits, plants could be marked at the appropriate time of year (spring or fall) for control at a later point. In this manner the invasive species can be targeted without harming the native. The key on the next page summarizes the key traits for discrimination of these two species in the field.

U.S. Department of the Interior
U.S. Geological Survey

For more information, contact:
Plant Ecologist, Lake Michigan Ecological Research Station
1100 N. Mineral Springs Rd; Porter, IN 46304
(219) 926-8336 ext. 428

Fact Sheet 07 - #
October 2007

Is plant in fruit? **Yes** → Are ripe fruit capsules orange? → *C. scandens*

yellow? → *C. orbiculatus*

No ↓

Are fruit borne in terminal panicles? / axillary cymes?

C. scandens

C. orbiculatus

Do fruits have ≤ one seed?* → *C. scandens*

≥ five seeds?* → *C. orbiculatus*

Is plant in bloom? **Yes** → Are flowers borne in terminal panicles? → *C. scandens*

No ↓ axillary cymes? → *C. orbiculatus*

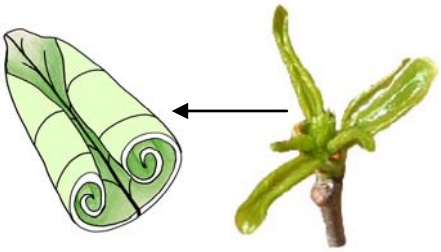
Are flowers staminate? **Yes** → Is pollen yellow? / white?

C. scandens

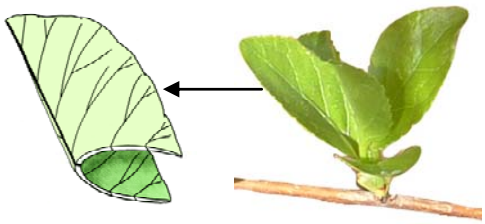
C. orbiculatus

Is plant leafing out? **Yes** → Are leaves involute? / conduplicate?

No ↓



C. scandens



C. orbiculatus

Are mature leaves present? **Yes** → Is length:width ≥ 2.0?* → *C. scandens*

Is length:width ≤ 1.4?* → *C. orbiculatus*

No ↓ Is leaf tip > 1.5 cm?* → *C. scandens*

Is leaf tip < 0.3 cm?* → *C. orbiculatus*

If dormant season, only fruit characters applicable

* Indicates a 90% probability of correct identification based on the data collected for this study.
Colors in text boxes are to be used as a guide only, actual colors seen in the field may differ.

Collaborators: Noel B. Pavlovic, Stacey Leicht Young, Ralph Grundel, and Krystalynn J. Frohnapple