

ABSTRACT

POST, ANGELA R. A Systematic Study of *Cardamine* Weed Species in United States Container Nurseries. (Under the direction of Dr. Joseph C. Neal).

Cardamine species present in the United States nursery trade were identified and characterized based on morphological and molecular evidence. The genus *Cardamine* L., or the bittercresses, contains some of the most prolific weed species in the container nursery industry. Though most bittercress in the U.S. nursery industry was thought to be hairy bittercress (*Cardamine hirsuta*), we suspected other species to be present due to the global movement of nursery stock. We surveyed 21 nurseries in California, Mississippi, Missouri, New York, North Carolina, and Oregon to collect a representative sample of bittercress species occurring in the U.S. nursery industry. Type material and other herbarium samples were examined for all species encountered in order to identify each accession and determine which characters were most important for identification. From these nursery surveys four *Cardamine* species were collected: *Cardamine corymbosa*, *C. hirsuta*, *C. flexuosa* and *C. oligosperma*, each representing 90%, 6%, 2%, and 2% of collections respectively. *C. flexuosa* was originally described in Europe but also has distribution throughout Asia. European and Asian populations may represent separate taxa based on molecular evidence. Here we treat them as the same taxon. Type material no longer exists for *C. flexuosa* as it was described in Europe. A lectotype is designated here. *Cardamine corymbosa*, native to New Zealand, is a newly reported species for the United States. *Cardamine hirsuta* has worldwide distribution and *C. oligosperma* is native to the United States. Representatives of each species collected, as well as select herbarium specimens, were chosen for a molecular study to examine the relationships

between these species. Sequence was generated for the nrDNA Internal Transcribed Spacer region (*ITS*) and for the single copy nuclear COP1-Interacting Protein 7 region (*CIP7*). Sequence data was aligned and subjected to maximum parsimony and bayesian inference. When comparing the phylogenies created by *ITS* and *CIP7* datasets, there were areas of congruence and incongruence. The *ITS* data resolved *C. corymbosa* as sister to *C. flexuosa* whereas the *CIP7* data resolved *C. corymbosa* sister to *C. hirsuta*. The combined alignment resolved the *Cardamine* accessions with higher bootstrap (BS) support and posterior probability (PP) than either of the separate matrices alone. In the combined matrix *C. hirsuta* and *C. corymbosa* resolve as sister taxa. However, no analysis fully resolved the relationships within this closely related species complex.

A Systematic Study of *Cardamine* Weed Species in United States Container Nurseries

by
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BIOGRAPHY

Angela R. Post was born Angela Rose Richardson in Asheboro North Carolina and grew up in the nearby rural town of Franklinville. From childhood Angela had a love of plants and always enjoyed flower and vegetable gardening with her grandmother. Her high school horticulture program set her on the path to become a plant scientist and in 2001 she began an undergraduate program in Horticultural Science at North Carolina State University. One year later Angela changed her major to Botany for a more rigorous math and science base. She completed her B. S. Botany in 2003 followed by a B. S. in Biological Sciences in 2004 and a B.A. in Multidisciplinary Studies with a concentration in Medicinal Ethnobotany in 2005. Angela returned to North Carolina State as a teaching assistant and research technician in Fall 2006 before starting her M. S. program in Horticultural Science. Under the direction of Dr. Joseph Neal Angela studied the systematics and taxonomy of weedy *Cardamine* species in United States container nurseries. During her time as a master's student Angela traveled to five major herbaria to study her group and presented research at nine local, regional, and national meetings. Just before her program began she was married to John Richard Post and a year and a half later they welcomed their first child Liana Rose Post to the family. Upon completion, Angela will move on to Cornell University to pursue Ph.D. in Plant Biology with a focus on systematics. In the future Angela hopes to become a university professor with a teaching and research appointment.

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ABBREVIATIONS

Herbaria

BM = Natural History Museum of London Herbarium
CAS = California Academy of Sciences
GH = Gray Herbarium
K = Kew Gardens
LINN = Linnean Herbarium
MO = Missouri Botanical Garden Herbarium
NCSC = North Carolina State University Herbarium
NY = New York Botanical Garden Herbarium
US = United States National Herbarium
WTU = Washington University

Collection Site Acronyms

BGS = Bayer's Garden Shop St. Louis Co. MO
BLM = Bloom's UK
BNB = Beds and Borders NY
BNT = Bentley Plants UK
CHIR = Dr. Robert Bulcke's garden UK
HFL = Horticulture Field Labs Wake Co. NC.
HKR = Hawksridge Nursery Wilkes Co. NC
HNR = Hefner's Nursery Wilkes Co. NC
ICR = Icard's Nursery Wilkes Co. NC
MCL = McLamb's Nursery NC
MBG = Missouri Botanical Garden St. Louis Co. MO
NYB = New York Botanical Garden Bronx Co. NY
OMS = Othikkeba Co. MS
ORC = Clackamas Co. OR
ORW = Washington Co. OR
ORY = Yamhill Co. OR
PPG = Pinewood Perennial Garden NY
RTC = Robbin Tacchi's Nursery UK
SMT = Smith's Nursery Johnston Co. NC
STN = Stephenson's Nursery Johnston Co. NC
STZ = Setzer's Nursery Wilkes Co. NC
TCN = Trimble's of Corchaug Nursery NY
VCA = Valley Center CA
WMS = Washington Co. MS
ZKA = Zelenka Nursery NC

ABBREVIATIONS (continued)

Herbarium Samples

COR = *Cardamine corymbosa*

HIR = *Cardamine hirsuta*

OLK = *Cardamine oligosperma*

IMP = *Cardamine impatiens*

NOF = *Nasturtium officinale*

PVA = *Cardamine parviflora*

RPA = *Rorripa palustris*

SCOPE AND JUSTIFICATION

Bittercress (*Cardamine* L., Brassicaceae) is one of the most common and costly weeds in container nurseries (Mathers 1996; Bond and Turner 2006) despite the availability of numerous herbicides labeled for its control. In the 2007, USDA IR-4 Ornamental Horticulture annual survey bittercress was reported to be the most prominent weed in United States greenhouse and nursery production, and the third most prominent weed in landscapes. Bittercress is particularly problematic for herbaceous annual and perennial nursery crops, as few broad spectrum herbicides are labeled for these crops (SCNLA 2005). A single plant is capable of producing thousands of seeds and projecting them several feet. Seeds also become quite sticky when wet which aids in their dispersal (Salisbury 1961). It can complete a life cycle in about 5 weeks resulting in multiple generations per year, and has been reported to fruit between 8 and 12 months out of the year in irrigated environments (Bachman and Whitwell 1995; Bond and Turner 2006).

The efficacy of herbicides applied for bittercress control in nurseries is often unsatisfactory (Altland et al. 1998; *personal communication*). Smith et al. (1997) reported 85% control of bittercress with Gallery WG in gravel beds surrounding production areas 12 weeks after application. Similarly, Altland et al. (1999) observed 92% control of small bittercress plants in containerized 'Nachez' crepe myrtle and 'Carousel' barberry. However, only 71% control was observed for intermediate sized bittercress and 48% for large bittercress plants over the same time period. The reasons for variable or unsatisfactory control have not been resolved. However, resistance to isoxaben (Gallery) has been reported for certain populations of *C. flexuosa* in Europe (Eelen & Bulcke 1997).

Most bittercress in U.S. container nurseries is thought to be hairy bittercress or *C. hirsuta* L. However, due to the morphological diversity and variable responses to herbicides we and other researchers have questioned this assumption. Fain et al. (2005) reported three other species to occur in the trade including *C. flexuosa* With. native to Asia, *C. oligosperma* Nutt. native to the United States, and *C. scutata* Thunb. native to Japan. However, Fain et al.'s research included only 12 populations of *Cardamine*, depended upon genetic data from unconfirmed sources for identification, and did not relate genetic data to morphological evidence. Therefore, it is important to extend this research to include more populations from throughout the US and to pair good morphological evidence with the molecular data to accurately identify the *Cardamine* species in U.S. nurseries.

Cardamine is a member of the mustard family (Brassicaceae, Cruciferae) with worldwide distribution. There are about 3700 species in 338 genera in Brassicaceae (Appel & Al-Shehbaz 2003; Al-Shehbaz et al. 2006). Members of this family are characterized by having four petals, which may be reduced or absent in some cases, alternating with four sepals. Flowers typically have six stamens, the outside two being shorter than the inside four. Fruit is most often a two-valved capsule with a false septum commonly known as a silique or silicle depending on length to width ratio. Fruits dehisce longitudinally releasing 1-300 seeds (Appel & Al-Shehbaz 2003). The most commonly used characters of fruiting morphology and arrangement of the cotyledons are widely convergent traits and should not be used alone to distinguish taxa within Brassicaceae (Koch et al. 2003).

Within *Cardamine* morphological characters are so variable that it is often difficult to distinguish between species, especially those weedy members thought to occur in U.S.

nurseries and landscapes. Many authors have cited phenotypic plasticity and morphological overlap among weedy *Cardamine* species around the world (Eelen & Bulcke 1997; Fain et al. 2005; Bond & Turner 2006; Lihova et al. 2006). *Cardamine* species may be annual, biennial, or perennial herbs. Basal leaves vary and may be pinnately compound, trifoliate, pinnatifid, or entire. Cauline leaves are typically petiolate or sessile and can be simple or compound. Plants may be glabrous or pubescent with simple trichomes. Petals are white to purple in color with flowers having six stamens or rarely, four. Fruits are a linear silique that dehisces explosively to release seeds projecting them one meter or more from the parent plant (Bachman & Whitwell 1995; Appel & Al-Shehbaz 2003; Bond & Turner 2006). Current keys to *Cardamine* in North America do not include all species present, resulting in frequent misidentification (Detling 1937; Hitchcock et al. 1925; Hickman; Radford 1968; Rollins 1993; Weakley 2007). Consequently, confusion remains regarding the correct identification of weedy *Cardamine* in nursery crops.

Several molecular taxonomic studies have attempted to resolve phylogenetic relationships in the Brassicaceae and *Cardamine*. Molecular studies of the Brassicaceae have demonstrated homoplasy for every morphological character used in the taxonomy of the family (Price et al. 1994; Zunk et al. 1996; Mummenhoff et al. 1997a; Koch & Mummenhoff 2001; Mummenhoff et al. 2001a, 2001b) and especially for the genus *Cardamine* (Franzke et al. 1999b; Sweeney & Price 2000). The most commonly used system for dividing the family into tribal groups is Schulz's treatment of the family (1936) recognizing 19 tribes and 30 subtribes, though there have been criticisms of the artificial nature of this system (Janchen 1942; Lihova et al. 2006). The newest classification system (Al-Shehbaz 2006) recognizes 25

tribes and moves *Cardamine* out of the tribe Arabideae as described by Schulz (1936), placing it in its own tribe Cardamineae with ten other genera. This Cardaminine alliance (c. 340 species) includes *Armoracia* G.Gaertn., B.Mey. & Scherb., *Barbarea* R. Br., *Cardamine* L., *Dentaria* L., *Iti* Garn.-Jones, *Iodanthus* (Torr. & A. Gray) Steud., *Leavenworthia* Torr., *Nasturtium* R.Br., *Planodes* Greene, *Rorripa* Scop., and *Selenia* Nutt. Despite extensive work on certain members of Brassicaceae, such as *Arabidopsis thaliana* and *Brassica oleracea*, studies are lacking on several important alliances within the family including the Cardaminine alliance (Koch et al. 2003). Among these genera *Cardamine* is an especially difficult genus containing many weedy members which are self-compatible (Kimata 1983; Preston 1986; Appel and Al-Shehbaz 2003) but may also hybridize readily with one another (Urbanska et al. 1997).

Cardamine is a cosmopolitan genus with ca. 220 species worldwide occurring on every continent except Antarctica (Al-Shehbaz 1988). The genus was first described by Linnaeus (1753) but the earliest complete treatment of the genus was O.E. Schulz's monograph in 1936. The most recent treatments of the genus have been in New Zealand (Pritchard 1957), Central and South America (Sjostedt 1975), Carpathians and Pannonia (Marhold 1994, 1995a, 1995b), the Iberian Peninsula (Lihova et al. 2000), and China (Al-Shehbaz et al. 2001). The taxonomy of several complex groups within *Cardamine* have been addressed separately including the *C. pratensis* complex (Marhold 1994; Marhold 1996; Franzke & Hurka 2000), and the *C. amara* complex, both of Eastern Europe (Marhold 1995; Lihova et al. 2000; Marhold 2002a), the *C. concatenata* alliance of Eastern North America (Sweeney & Price 2001), and the *C. flexuosa* complex (Lihova et al. 2006). Most important to note is

there have been no comprehensive taxonomic studies of the genus for North America in the recent past. Currently there is widely followed sectional classification within the genus. Although Schulz (1936) divided the group into 13 sections, six of them monotypic, this sectional classification has not been widely accepted or upheld by phylogenetic studies (Lihova and Marhold 2003).

Other studies on *Cardamine* include a phylogenetic study for the high-mountain *Cardamine* species of the Southern Hemisphere (Bleeker et al. 2002). Taxonomic studies have also focused on particular species such as *C. penzesii* (Marhold & Anceev 1999), *C. dentata* (Wojcicki & Marhold 2000), and *C. flexuosa* (Kudoh et al. 2002), and comparative reproductive studies have been performed on *C. flexuosa*, *C. impatiens*, *C. scutata*, and *C. lyrata* (Kimata 1983). Multivariate morphological analyses have also been conducted on the *C. concatenata* alliance (Sweeney & Price 2001) and the *C. pratensis* group (Marhold 1996). The sheer number of taxonomic works and unresolved phylogenetic relationships on this genus illustrates the poor understanding and the need of further work on this group. There have been no studies focusing specifically on the weedy species occurring in container nurseries; however, Lihova et al. (2006) did include these weedy species in a worldwide phylogeny for the *C. flexuosa* complex.

Hybridization and polyploidy have been reported extensively in *Cardamine* (Neiffer & Jahnke 1997; Urbanska et al. 1997; Franzke et al. 1998; Franzke & Mummenhoff 1999; Franzke & Hurka 2000; Lihova et al. 2000; Bleeker et al. 2002a; Marhold et al. 2002a, 2002b, 2004) and several natural hybrids exist. The genus has chromosome numbers ranging from $n=8$ to $n=128$ (*C. diphylla* and *C. concatenata*). The latter is the highest chromosome

number known for the family (Appel and Al-Shehbaz 2003). In North America approximately 70 taxa from this genus are represented including accepted subspecies (USDA, NRCS 2008). Morphologically, the genus *Cardamine* has several complexes that are difficult to distinguish and at least one of these complexes exists in North America, the *C. concatenata* alliance (Sweeney & Price 2001). It is probable, based on hybridization and polyploidy among *Cardamine* that other complexes exist on this continent. Polyploid complexes are well documented within *Cardamine* including *C. pratensis* (Lovkvist 1957), *C. amara* (Lovkvist 1957), and *C. concatenata* (Easterly 1963) groups. One study has reported that up to 58% of all known *Cardamine* are exclusively polyploid and that 8% of taxa include both diploid and polyploid populations (Lihova and Marhold 2003; Marhold et al. 2004). In cases where multiple cytotypes have been reported for a single species, it is possible that taxonomy is unresolved or that these complexes represent dynamic systems that may eventually produce stable hybrids (Lihova and Marhold 2003).

The basic characters of weedy *Cardamine* include a basal rosette of pinnately compound leaves which vary in size and shape, inflorescences in a raceme with flowers having four white petals and typically six stamens. Fruits are a forcefully dehiscent silique with yellowish to brown seeds less than 1 mm. The vegetative characters among *Cardamine* species are especially plastic and vary according to their environment, making on-sight identification of these members difficult. Reproductive characters are more reliable for identification; however, these parts are so small that identification remains difficult without the use of a hand lens.

In phylogenetic studies within Brassicaceae, the nuclear ribosomal ITS sequences have been the most commonly used marker (Bleeker et al. 2002; Fain et al. 2005; Al-Shehbaz et al. 2006). It is important to note, however, that *ITS* sequences frequently have paralogous copies and may require cloning to obtain reliable data sets (Al-Shehbaz et al. 2006). Chloroplast DNA markers that have been commonly used for phylogenetic studies in Brassicaceae include the non-coding regions of *trnT*, *trnL*, *trnF*, and intergeneric spacers *trnT-trnL*, *trnL-trnF*, and *psbA-trnH* (Al-Shehbaz et al. 2006). A few studies used other markers, such as the nuclear-encoded chalcone synthase gene (*Chs*) and the chloroplast gene *matK* (Koch et al. 2001). These markers helped to partially resolve the relationships in the genus (e.g. *trnL* intron and the *ndhF* gene in Sweeney & Price 2001 and Bleeker et al. 2002; combined data of *ITS* and chloroplast *trnT-L* and *trnL* intron sequences in Franzke et al. 1998). Franzke and Hurka (2000) also used a combined approach with allozymes, *ITS* sequencing, non-coding cpDNA, and RAPD's to resolve relationships in the *C. pratensis* complex. Treatment of the *C. amara* complex was also supported using RAPD's (Lihova et al. 2000). Most recently Marhold used *ITS* and AFLP analysis to elucidate the relationships between groups using diploid taxa from polyploidy complexes (Marhold et al. 2004). In all previous phylogenetic analyses, the weedy species of nurseries were either not all included, or their relationships remained incompletely resolved due to insufficient variation of the molecular markers, leaving their molecular identity in question.

With the recent development of several nuclear gene regions variable within species in Brassicaceae (Schranz et al. 2007), it may be possible to further resolve phylogenetic relationships of the closely related weedy species of *Cardamine* occurring in United States

nursery crops. Paired with detailed morphological examinations, these nuclear DNA regions allow us to better characterize the identity of the weedy species at both molecular and morphological levels and to develop keys for the species.

OBJECTIVES

Our main objectives were to: (1) to conduct a molecular and morphological analysis on *Cardamine* populations with thorough sampling from nurseries throughout the major production zones in the United States, (2) determine what species and evolutionary lineages occur in US nurseries, and (3) develop keys and provide descriptions, and illustrations to facilitate their identification. We addressed these objectives by first surveying the container nursery industry of the United States and collecting representative vouchers of morphologically variable bittercress species. We examined over 4000 herbarium specimens in order to morphologically characterize each species encountered in the nursery trade. Molecular evidence was then generated by sequencing the nrDNA Internal Transcribed Spacer region (*ITS*) and the COP-1 Interacting protein 7 region. We performed phylogenetic analysis of the molecular data to examine the relationships and evolutionary divergence between these weedy species.

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Chapter 1

On the identity of the weedy *Cardamine* (Brassicaceae) species in United States nurseries:
Evidence from molecules and morphology¹

(In the format appropriate for submission to Systematic Botany)

On the identity of the weedy *Cardamine* (Brassicaceae) species in United States nurseries:
Evidence from molecules and morphology¹

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ABSTRACT

Cardamine (Brassicaceae) is one of the most prolific and costly weeds of the container nursery industry. *Cardamine* accessions from container nurseries throughout the major production zones in the United States were examined and compared to herbarium specimens. The identity of these weedy *Cardamine* species were further explored using sequences of the nrDNA internal transcribed spacer region (*ITS*) and the nrDNA region for the COP1-interacting protein 7 (*CIP7*). We identified four species of *Cardamine* occurring in the nursery industry of the United States including *Cardamine corymbosa*, *Cardamine hirsuta*, *Cardamine flexuosa*, and *Cardamine oligosperma*. The taxon referred to here as *Cardamine flexuosa* likely contains both genotypes previously reported by Lihova et al. (2006): European *C. flexuosa* and Asian '*C. flexuosa*'. Phylogenetic relationships between the four species we examined, particularly how each is related to *C. flexuosa*, were not fully resolved by the molecular evidence generated for this study. *Cardamine corymbosa* is alien and does not appear in current keys to the species for the United States. *Cardamine flexuosa* is also an alien species which appears in some U.S. keys but not all. A key to the weedy *Cardamine* species of US container nurseries is provided as well as detailed descriptions and illustrations to aid nurserymen and botanists in the identification of these four closely related species.

INTRODUCTION

Cardamine L. (Brassicaceae) is a cosmopolitan genus with ca. 220 species worldwide occurring on every continent except Antarctica (Al-Shehbaz 1988). In North America, approximately 70 taxa from this genus are represented including accepted subspecies (USDA, NRCS 2008). The genus was first described by Linnaeus (1753) but the earliest complete treatment of the genus was O.E. Schulz's monograph in 1903. Schulz (1903)

placed *Cardamine* in the tribe Arabideae but there have been criticisms of the artificial nature of this placement and his system of classification in general (Janchen 1942; Lihova et al. 2006). Al-Shehbaz (2006) recognized 25 tribes within Brassicaceae and moved *Cardamine* out of the tribe Arabideae, placing it in the tribe Cardamineae with ten other genera. This Cardaminine alliance (c. 340 species) includes *Armoracia* G.Gaertn., B.Mey. & Scherb., *Barbarea* R. Br., *Cardamine* L., *Dentaria* L., *Iti* Garn.-Jones, *Iodanthus* (Torr. & A. Gray) Steud., *Leavenworthia* Torr., *Nasturtium* R.Br., *Planodes* Greene, *Rorripa* Scop., and *Selenia* Nutt. Among these genera *Cardamine* is a morphologically variable genus containing many weedy members which are self-compatible (Kimata 1983; Preston 1986; Appel and Al-Shehbaz 2003) but may also hybridize readily with one another (Urbanska et al. 1997).

Hybridization and polyploidy have been reported extensively in *Cardamine* and several natural hybrids exist (Neiffer & Jahnke 1997; Urbanska et al. 1997; Franzke et al. 1998; Franzke & Mummenhoff 1999; Franzke & Hurka 2000; Lihova et al. 2000; Bleeker et al. 2002a; Marhold et al. 2002a, 2002b, 2004). The genus has chromosome numbers ranging from $n=8$ to $n=128$ (*C. diphylla* Wood and *C. concatenata* (Michx.) O. Schwarz). The latter is the highest chromosome number known for the family (Appel and Al-Shehbaz 2003). Morphologically, the genus *Cardamine* has several complexes that are difficult to distinguish and at least one of these complexes, the *C. concatenata* alliance, exists in North America (Sweeney & Price 2001). Well documented polyploid complexes include *C. pratensis* L. (Lovkvist 1957), *C. amara* L. (Lovkvist 1957), and *C. concatenata* (Easterly 1963) groups. It was reported that up to 58% of all known *Cardamine* are exclusively polyploid and that 8% of taxa include both diploid and polyploid populations (Lihova and Marhold 2003; Marhold et al. 2004).

The vegetative characters among *Cardamine* species are extremely plastic and vary according to their environment, making identification of these members difficult. The basic characters of weedy *Cardamine* include a basal rosette of pinnately compound leaves which vary in size and shape, inflorescences in a raceme with flowers having four white petals and typically six stamens. Fruits are a forcefully dehiscent silique with yellowish to brown seeds less than 1 mm in diameter. Currently there is no widely followed sectional classification within the genus. Though Schulz divided the group into 13 sections, six of them monotypic, this sectional classification so far has not been upheld by phylogenetic studies (Lihova and Marhold 2003).

In *Cardamine*, several molecular approaches have been utilized to distinguish taxa at the species level. Nuclear ribosomal *ITS* sequences have been used most commonly across the family (Al-Shehbaz et al. 2006) including studies on *Cardamine* (Bleeker et al. 2002; Fain et al. 2005). It is important to note that *ITS* sequences frequently have multiple motifs within the same individual and require cloning to obtain homogeneous data sets (Al-Shehbaz et al. 2006). Non-coding regions of chloroplast DNA including *trnT*, *trnL*, *trnF*, the *trnL* intron, and intergeneric spacers: *trnT-trnL*, *trnL-trnF*, and *psbA-trnH* are also commonly used markers for phylogenetic studies of Brassicaceae (Al-Shehbaz et al. 2006). Other studies have used the nuclear-encoded chalcone synthase gene (*Chs*) and the chloroplast gene *matK* (Koch et al. 2001). Relationships in the genus have been partially resolved using chloroplast DNA sequence data from the *trnL* intron and the *ndhF* gene (Sweeney & Price 2001; Bleeker et al. 2002). Franzke et al. (1998) partially resolved relationships among ca. 20 taxa within *Cardamine* using *ITS* sequences, chloroplast *trnT-L* gene and the *trnL* intron. Franzke and Hurka (2000) also used a combined approach with allozymes, *ITS* sequencing,

non-coding cpDNA, and RAPD's to resolve relationships in the *C. pratensis* complex. Treatment of the *C. amara* complex was also supported using RAPD's (Lihova et al. 2000). Most recently Marhold used *ITS* and AFLP analysis to elucidate the relationships between groups using diploid taxa from polyploidy complexes (Marhold et al. 2004).

The most recent treatments of the genus have been in New Zealand (Pritchard 1957), Central and South America (Sjostedt 1975), Carpathians and Pannonia (Marhold 1994, 1995a, 1995b), the Iberian Peninsula (Lihova et al. 2000), and China (Al-Shehbaz et al. 2001). Several complex groups within *Cardamine* have been addressed separately including the *C. pratensis* complex (Marhold 1994; Marhold 1996; Franzke & Hurka 2000), and the *C. amara* complex, both of Eastern Europe (Marhold 1995; Lihova et al. 2000; Marhold 2002a), the *C. concatenata* alliance of Eastern North America (Sweeney & Price 2001), and the *C. flexuosa* With. complex (Lihova et al. 2006). Most important to note is there have been no comprehensive taxonomic studies of the genus, for North America in the recent past. For this study we focus on the identity of weedy *Cardamine* present in the United States container nursery trade.

Container nurseries have often been a foothold for introduced weed species and *Cardamine* (bittercress) includes some of the most prolific weed species in the container nursery industry. Despite the availability of numerous herbicides labeled for bittercress control, it remains one of the most common and costly weeds in container nurseries (Mathers 1996; Bond and Turner 2006). In 2007, the USDA IR-4 Ornamental Horticulture survey reported bittercress to be the most prominent weed across the United States in greenhouse and nursery production, and the third most prominent weed in landscapes. A single plant is capable of producing thousands of seeds and projecting them several feet. Seeds also

become quite sticky when wet which aids in their dispersal (Salisbury 1961). *Cardamine* is particularly problematic for herbaceous annual and perennial nursery crops, as it can complete a life cycle in about 5 weeks and has been reported to fruit between 8 and 12 months out of the year in irrigated environments (Bachman and Whitwell 1995; Bond and Turner 2006).

The efficacy of herbicides applied for bittercress control in nurseries is often unsatisfactory (Altland et al. 1998; Altland & Neal *pers. comm.*). Smith et al. (1997) reported 85% control of bittercress with Gallery WG in gravel beds surrounding production areas up to 12 weeks after application. Similarly, Altland et al. (1999) observed 92% control of small bittercress plants in containerized ‘Nachez’ crepe myrtle and ‘Carousel’ barberry. However, only 71% control was observed for intermediate sized bittercress and 48% for large bittercress plants over the same time period. The reasons for variable or unsatisfactory control have not been resolved. However, resistance to isoxaben (Gallery) has been reported for certain populations of *C. flexuosa* in Europe (Eelen & Bulcke 1997).

Most bittercress in U.S. container nurseries is thought to be hairy bittercress or *C. hirsuta* L. However, due to the morphological diversity and variable responses to herbicides we and other researchers have questioned this assumption. Fain et al. (2005) reported three other species to occur in the trade including *C. flexuosa* native to Asia, *C. oligosperma* Nutt. native to the United States, and *C. scutata* Thunb. native to Japan. However, Fain et al.’s (2005) research included only twelve populations of *Cardamine*, depended upon genetic data from unconfirmed sources for identification, and did not relate genetic data to morphological evidence. Therefore, it is important to extend this research to include more populations from throughout the US and to correlate morphological evidence with molecular data to accurately

identify the *Cardamine* species in U.S. nurseries. Many authors have cited phenotypic plasticity and morphological overlap among weedy *Cardamine* species around the world (Eelen & Bulcke 1997; Fain et al. 2005; Bond & Turner 2006; Lihova et al. 2006) and a previous study has shown many of these species to be very closely related (Lihova et al. 2006). To date there have been no studies focusing specifically on the weedy species occurring in United States container nurseries; although, Lihova et al. (2006) included these weedy species in a worldwide study focusing on relationships within the *C. flexuosa* complex. As a result, current keys to *Cardamine* in North America do not include all species present, which leads to difficulty in correct identification and may result in frequent misidentification (Detling 1937; Hickman 1925; Hitchcock et al. 1955-1969; Radford et al. 1968; Rollins 1993; Weakley 2007).

In phylogenetic studies within Brassicaceae, the nuclear ribosomal ITS sequences have been the most commonly used marker (Bleeker et al. 2002; Fain et al. 2005; Al-Shehbaz et al. 2006). It is important to note, however, that *ITS* sequences frequently have paralogous copies and may require cloning to obtain reliable data sets (Al-Shehbaz et al. 2006). Chloroplast DNA markers that have been commonly used for phylogenetic studies in Brassicaceae include the non-coding regions of *trnT*, *trnL*, *trnF*, and intergeneric spacers *trnT-trnL*, *trnL-trnF*, and *psbA-trnH* (Al-Shehbaz et al. 2006). A few studies used other markers, such as the nuclear-encoded chalcone synthase gene (*Chs*) and the chloroplast gene *matK* (Koch et al. 2001). These markers helped to partially resolve the relationships in the genus (e.g. *trnL* intron and the *ndhF* gene in Sweeney & Price 2001 and Bleeker et al. 2002; combined data of *ITS* and chloroplast *trnT-L* and *trnL* intron sequences in Franzke et al. 1998). Franzke and Hurka (2000) also used a combined approach with allozymes, *ITS*

sequencing, non-coding cpDNA, and RAPD's to resolve relationships in the *C. pratensis* complex. Treatment of the *C. amara* complex was also supported using RAPD's (Lihova et al. 2000). Most recently Marhold used *ITS* and AFLP analysis to elucidate the relationships between groups using diploid taxa from polyploidy complexes (Marhold et al. 2004). In all previous phylogenetic analyses, the weedy species of nurseries were either not all included, or their relationships remained incompletely resolved due to insufficient variation of the molecular markers, leaving their molecular identity in question.

To aid in species identification and nursery weed management, we conducted a detailed molecular and morphological analysis to characterize the weedy species from major nursery production areas across the United States. Although a number of phylogenetic studies were performed at different taxonomic levels for various groups of Brassicaceae, most of these studies did not include the weedy species of *Cardamine* with the exception of Lihova et al. (2006). However, the molecular markers used in Lihova et al (2006) focused on one species complex (*C. flexuosa*) and did not fully resolve the relationships among these closely related species. With the recent development of several nuclear gene regions variable between species that are suitable for the study of speciation in Brassicaceae (Schranz et al. 2007), it may be possible to further resolve phylogenetic relationships among the closely related species of *Cardamine* occurring in United States nursery crops. Our main objectives were to: (1) increase sampling of *Cardamine* populations from nurseries throughout the major production zones in the United States, (2) employ molecular and morphological data to accurately identify the encountered species, and (3) provide appropriate keys, descriptions, and illustrations to facilitate their identification.

MATERIALS AND METHODS

Taxa Sampling—*Cardamine* accessions were collected from 21 nurseries in six major nursery stock producing states including California, Missouri, Mississippi, North Carolina, New York, and Oregon. At each nursery, collections were made of the most common bittercress phenotype present as well as any phenotypes which differed from the most common one. This sampling methodology allowed us to capture a representative sample of bittercress species occurring in United States nurseries.

Morphological Analysis—In addition to investigation of fresh materials collected from US nurseries, on-site examinations of *Cardamine* herbarium specimens at two major US herbaria (NY, MO) and three European herbaria (K, LINN, and BM) were undertaken to ensure a thorough survey of *Cardamine* species and to investigate morphological variation. Additional specimens were examined through loans from five other institutions: CAS, GH, TEX, WTU, and US. Type specimens were examined for weedy taxa already reported for the United States, and other closely related taxa. For all herbarium specimens, we examined leaf, stem and root morphology, surface hairs, floral parts and fruiting characters. Suitable specimens were also selected for molecular analysis.

Molecular Analysis—A total of 37 accessions were included in the analysis, including specimens from 21 container nurseries in six states (CA, MO, MS, NC, NY, OR) and from selected herbarium material (Appendix A). *Rorippa* Scop. and *Nasturtium* R.Br. were selected as outgroups following Franzke et al. (1998). Total genomic DNA was extracted from freshly collected and herbarium material using the DNeasy Plant Mini Kit (Qiagen, Valencia, California). Sequence data were obtained from nuclear gene region *CIP7* using primers designed for *Arabidopsis* (Lawton-Rauh *unpublished*). Primers ITS4 and ITS5

(White et al. 1990) were used to amplify *ITS* in a single piece. PCR reactions (50 μ L) consisted of the following components: 2 μ L template DNA, 10X PCR buffer (Promega), 2.5 mM each dNTP, 25 mM MgCl₂, 1 μ L each primer (Table 1.1), and 1 unit of *Taq* polymerase (+ 1.5 μ L 10mg/mL DMSO for amplification of *ITS*). PCR cycling parameters for *CIP7*: initial denaturation step at 95° C for 5 minutes, then 35 cycles of denaturation at 95° C for 1 minute, primer annealing at 50° C for 30 seconds, and extension at 72° C for 2 minutes with a final extension cycle at 72° C for 6 minutes. PCR reactions for *CIP7* were performed in a Biorad icycler thermal cycler. PCR reactions for *ITS* were run on a Stratagene Robocycler gradient 96. Prior to sequencing, PCR reactions for *CIP7* were run on a 1% TAE agarose gel and bands extracted using 15 minute UltraClean DNA purification kit (MoBio Laboratories) following the manufacturer's protocol. PCR reactions for *ITS* were cleaned up using an enzymatic cleanup reaction of Exonuclease I and Antarctic Phosphatase (MCLAB) to purify them for cloning. The enzymes were added to the PCR product and run on the Biorad icycler thermal cycler at 37° C for 15 minutes followed by 80° C for 15 minutes. *ITS* was cloned using the TOPO TA cloning kit (Invitrogen) and 5 clones from each of 6 samples were sequenced. DNA sequencing was performed using the ABI prism BigDye Terminator cycle sequencing ready reaction kit v.3.1 (Applied Biosystems) and sequencing products were detected on a 3730xl Sequencer (Applied Biosystems; North Carolina State University Genome Sciences Laboratory). DNA sequences generated from this study have been deposited in GenBank (Appendix A). After examining the sequences we did not observe any paralogous copies and all clones of the same individual grouped together.

Phylogenetic Analysis—Three matrices were analyzed. The first matrix contains the *CIP7* sequences from 33 *Cardamine* accessions and includes the outgroups *Nasturtium* and *Rorripa*. Due to the fact that a gap of ~40bp was detected when trying to align these sequences in Vector NTI (Invitrogen) only 186 bp of the forward sequence was used to construct the matrix. The length of the gap was determined based on comparison with previously published *Arabidopsis CIP7* sequence in GenBank (NM_118877.3). The second matrix consists of newly generated sequence of seven *Cardamine* accessions from container nurseries and herbarium specimens, as well as previously published sequence for *Rorippa* (X98638) and comparison sequences from Lihova et al. (2006) (Appendix A). There were two to four clones for each of four *C. flexuosa* accessions, three clones of one *C. corymbosa* accession, two clones of one *C. hirsuta* accession, and three clones of one *C. oligosperma* var. *kamtschatica*. The selected *Cardamine* accessions from Lihova et al. (2006) included three of Asian *C. flexuosa*, three of European *C. flexuosa*, two of *C. hirsuta*, two of *C. oligosperma*, two of *C. scutata*, and one of *C. corymbosa* (Appendix A). The previous accessions of *C. flexuosa*, *C. hirsuta*, and *C. oligosperma* were chosen because they were collected in the United States and are appropriate to the scope of this research. There were no U.S. accessions of *C. scutata* or *C. corymbosa* available. Japanese *C. scutata* accessions were included because this species has been reported for the United States by previous authors and a Canadian accession of *C. corymbosa* was included because we identified *C. corymbosa* in the United States during the course of morphological studies. The *ITS* matrix contains 631 bp. The third matrix consists of the combined sequences of *ITS* and *CIP7* and is 817 bp long. For each matrix sequences were aligned using ClustalW and adjusted by eye in Vector NTI 10.0 (Invitrogen).

Each matrix was subjected to phylogenetic analysis using maximum parsimony in PAUP* v. 4.0b10 (Swofford 2002) and Bayesian inference in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). For the parsimony analysis of *ITS* and combined matrices we used a heuristic search with 1000 random-addition-sequence replicates, tree-bisection-reconnection (TBR) branch swapping, and MULtrees option in effect, characters equally weighted, and character state unordered (Default). For the parsimony analysis of *CIP7*, phylogenetic trees were generated first with the MULtrees option off. The resulting most parsimonious trees were then used as the starting trees for a second round of analysis with MULtrees option on and the same settings as the *ITS* and combined data (Liede-Schumann et al. 2005; Krings et al. 2008). The second search hit the memory limit of PAUP* with 700,000 equally most parsimonious and was stopped due to these memory limitations. For all matrices, support for each clade was estimated using bootstrap analysis in PAUP* using fast heuristic search with 10000 replicates (Felsenstein 1985).

Phylogenetic analyses of each dataset using Bayesian inference were performed with MrBayes v. 3.1.2. Akaike Information Criterion (AIC) was used to determine DNA substitution models in ModelTest v. 3.7. Four independent Bayesian analyses were run for 1 million generations with sampling of trees every 100 generations. The 1 million generations well passed the stationary point. Burn-in was estimated by plotting likelihood scores using Tracer (Rambaut and Drummond 2004). After excluding 1100 trees from the burn-in phase a majority rule consensus tree was generated to estimate the posterior probabilities (PP) of clades.

RESULTS

The *CIP7* alignment included 37 accessions and 186 characters of which 45 were variable (23 autapomorphic; 22 parsimony informative) (Figure 1.1). The *ITS* matrix included 8 accessions from our collections, 14 accessions from Lihova et al. (2006), and one out group. There were 631 characters of which 68 were variable (39 autapomorphic; 24 parsimony informative). The combined alignment includes 29 taxa and 817 characters (*ITS*: 631 bp; *CIP7*: 186 bp) of which 70 were variable (2 autapomorphic; 68 parsimony-informative). The strict consensus of most parsimonious trees from *CIP7* data resolved four major clades corresponding to three species: *C. hirsuta*, *C. parviflora*, and *C. corymbosa* and a subgroup of *C. flexuosa* with moderate support (Figure 1.1). The majority of *C. flexuosa* accessions each form a distinct lineage not allied with any of these four clades. *Cardamine hirsuta* was grouped with *C. parviflora*, which in turn are sister to *C. corymbosa* (Figure 1.1). The 50% majority rule tree from Bayesian inference is consistent with the parsimony tree as shown in Figure 1.1. The trees resulting from parsimony and Bayesian analysis of *ITS* sequences similarly resolved the clades of *C. hirsuta*, *C. corymbosa*, and accessions of *C. flexuosa* in several distinct lineages, including one subclade containing accessions from NY and UK, a subclade of Asian origin, a subclade from NC and OR, and four other distinct lineages with three consisting of a single accession of European origin and one from UK (Figure 1.2). Relationships among the species appeared to be different than those in *CIP7* trees. The *ITS* trees showed that *Cardamine scutata* is nested within a paraphyletic *C. flexuosa*, and *C. corymbosa* is grouped with *C. oligosperma* var. *kamschatica* (a species not sampled in the *CIP7* analysis) (Figure 1.2). The trees resulting from analysis of combined *ITS-CIP7* data is congruent with the *CIP7* trees, having a number of *C. flexuosa* accessions

that do not ally with any other accessions. *Cardamine hirsuta* and *C. corymbosa* were grouped as sisters nested within the *C. flexuosa* complex (Figure 1.3). The single difference between the trees from the combined data and *CIP7* data is the recognition of a second subclade of *C. flexuosa* consisting of six accessions in the combined data trees but not present in *CIP7* trees (Figure 1.3).

DISCUSSION

Based on molecular evidence alone, we identified at least three *Cardamine* species occurring in the United States container nursery industry including *Cardamine corymbosa*, *C. flexuosa* and *C. hirsuta*. A fourth species *Cardamine oligosperma*, native to the United States, also occurs; however sequence data for this species was incomplete and not useful for analysis. The previously reported *Cardamine scutata* (Fain et al. 2005) was not encountered in this study. This is either due to incomplete sampling in our study or the previous report was inaccurate in identification. We sampled *Cardamine* from six states important to US nursery production including California, Mississippi, Missouri, New York, North Carolina and Oregon. Two major nursery producing states, Florida and Texas, are not represented in our molecular data. Two Texas nurseries were surveyed late in this study and therefore were not included in the molecular analysis, but morphologically, the only species we encountered in Texas was *C. flexuosa*. Although Florida is missing completely from our sampling of major production areas, anecdotally, it is unlikely to harbor any species not encountered elsewhere in the country, as many Florida container nurseries ship their stock, and likewise their weeds, nationwide.

Cardamine hirsuta and *Cardamine corymbosa* resolve sister to one another based on molecular data which is congruent with morphological evidence. Both these species exhibit at least partial loss of two stamens. *Cardamine hirsuta* exhibits complete loss of the two outside stamens and *C. corymbosa* exhibits six stamens in early petaliferous flowers and only four later in the season, when it begins to produce cleistogamous flowers. Along with the loss of stamens *C. corymbosa* also exhibits an apparent loss of petals in flowers with only four stamens. Even in early flowers producing six stamens, petals are sometimes fewer than four either due to fusion or loss. *C. corymbosa* also has the ability to form stolons and a corymbose inflorescence which is atypical of the family (Figure 1.3).

Based on molecular data, only six accessions of *C. flexuosa* form a well defined group sister to the previous taxa (*C. corymbosa* and *C. hirsuta*). Morphology supports *C. flexuosa* as a distinct species exhibiting six stamens and stems which flex at the nodes (See below).

Our morphological analysis indicated that the majority of accessions collected from United States container nurseries belong to *Cardamine flexuosa* (90%), which is very likely to consist of at least two evolutionary lineages based on the molecular phylogeny (Figure 1.3). The earliest report of *C. flexuosa* in the United States is from the 1933 (Fernald, Long & Fogg 1721 NY) and its current widespread distribution in the U.S., suggests that it has likely been frequently misidentified as *C. hirsuta*, which has been considered the most common. The two species are actually quite different in androecium, with *C. flexuosa* having tetradynamous stamens (4 long and 2 short) and *C. hirsuta* having only four long stamens; although, vegetatively, they are not so easily distinguished. However, a common mistake can occur in differentiating between the species when examining floral characters,

counting only the four long stamens, and assigning an identification of *C. hirsuta* not taking into account that two stamens are shorter and not easily seen without dissecting the flowers. The molecular study focusing on *C. flexuosa* (Lihova et al. 2006) similarly indicates *C. flexuosa* has at least two distinctive genotypes in the United States, one of Asian origin and one of European origin, that ought to be considered as distinct taxa. Based on our molecular evidence, additional evolutionary lineages may exist (Figure 1.3). Our molecular evidence shows that *C. flexuosa* in United States nurseries consists of populations of Asian origin, and probably also European origin. However, morphologically, there is no discontinuity between these subgroups, thus it remains unclear whether it is better to consider the two genotypes as distinct taxa. We placed these two genotypes in the same category under the description of *C. flexuosa*.

Our morphological survey indicated that *Cardamine hirsuta* comprised 6% of our accessions, and *C. corymbosa* and *C. oligosperma* comprised only 2% each. The previously reported *Cardamine scutata* (Fain et al. 2005) was not encountered in this study. If the species occurs it is only present in very low abundance, as the nature of our sampling method was biased towards encountering species that occurred at lower densities (see above).

Our data demonstrate that, in contrast to prior assumptions, *C. flexuosa* is the most common bittercress encountered in container nursery crop production. Furthermore, there is significant morphological and genetic diversity within the species *C. flexuosa*.

Phylogenetic Utility of CIP7—The gene regions explored in this study included the COP-1 Interactive Protein 7 which is a single copy nuclear gene believed to positively regulate light-regulated genes in *Arabidopsis* (Yamamoto et al 1998). Low copy and single copy nuclear regions such as this are often highly variable between species and have demonstrated utility

on species level phylogeny reconstruction. However, many times these regions exhibit fewer parsimony informative characters than the more commonly used internal transcribed spacer region (Hughes et al. 2006), as is the case here. Data for *CIP7* generated for this study showed variation among the closely related species that are phylogenetically informative, although, not completely resolving the relationships among the *C. flexuosa* accessions. One possible reason for the lack of monophyly of *C. flexuosa* is polyploidy. Lihova et al. (2006) reported both tetraploid populations and putatively octaploid populations within *C. flexuosa*.

TAXONOMIC TREATMENT

Cardamine L. Sp. Pl. 2: 654. 1753.—Type: “*Habitat in Europae pascuis aquosis*”.

[lectotype: no. 835.15 LINN *non. vidi.* designated by Britton and Brown 1914].

Annual, biennial or perennial often rosetted. *Basal leaves* pinnately compound, trifoliate or pinnatifid. *Cauline leaves* petiolate or sessile, simple or compound. *Petals* four, white, pink, or purple obovate to spatulate. *Stamens* 6, rarely 4. *Stigma* capitate, entire, or two lobed. *Ovules* few to many. *Siliques* linear, valves elastically dehiscent. *Seeds* uniseriate.

Each of the four *Cardamine* species encountered in United States nurseries have odd pinnately compound leaves which can be extremely variable in size and shape. Basal leaves typically have 3–7 pairs of lobed leaflets, the terminal leaflet being up to 2 times larger than lateral ones. Lateral leaflets get progressively smaller closer to the stem. Cauline leaves are also odd pinnately compound with fewer leaflet pairs than basal leaves. Inflorescences are mostly ebracteate. Petals are white and 2–4 mm long. All four species arise from fibrous root systems or a weak taproot and all have forcefully dehiscent siliques. We provide a key,

and detailed illustrations for each species known or reported to occur in the nursery industry (Figures 1.4–1.8), designed to aid in the identification of these species in several stages of growth:

Key to Cardamine in United States container nurseries

1. Basal leaves many forming a tight persistent rosette; stems 1–many, erect.
 2. Cauline leaves nearly ovate to obovate.....***C. oligosperma***
 2. Cauline leaves lanceolate to oblanceolate with or without slight lobing.
 3. Stamens 4; siliques < 1 mm wide upright and parallel to rachis, stem straight < 20 cm tall.....***C. hirsuta***
 3. Stamens 6; siliques > 1 mm wide upright but held at an angle to rachis; stem straight to flexuous > 20 cm tall.....***C. flexuosa***
1. Basal leaves rosetted upon germination then deciduous and few, or open wiry rosette; stems 1–many, erect or trailing.
 4. Plants forming stolons rooting at nodes; wiry decumbent habit; fibrous root system.....***C. corymbosa***
 4. Plants without stolons; erect habit; 1–many stems arising from fibrous roots or weak taproot.
 5. Sepals present; petals present; stamens four or six
 6. Petals four, 3–4 mm long.
 7. Petals cuneate-obovate to obovate, held open; stamens six.
 8. Cauline leaves linear to nearly oblanceolate***C. flexuosa***
 8. Cauline leaves oblanceolate to oblong.....***C. scutata***
 7. Petals spatulate held upright to slightly open; stamens four.....***C. hirsuta***

6. Petals 2–4, or four with one or more appearing fused, 3–4 mm long.....*C. corymbosa*
5. Sepals present; petals absent; stamens four.....*C. corymbosa*

Descriptions

Cardamine corymbosa Hook. f., Ic. Pl. t. 686. 1844.—Type: “Campbell’s Island” (fl & fr).

[holotype: *Hook f. s.n.*, K!]

Decumbent annual. *Stems* wiry, unbranched, creeping and rooting at the nodes to produce daughter plants. *Basal leaves* alternate, compound, leaflets 3–5, sessile or nearly so, and ovate to orbicular terminal leaflet up to 2 times larger than lateral ones. *Flowers* dimorphic (see notes below) four sepals alternating with four white petals, one or more petals fused. *Stamens* six, four long and two short. *Inflorescence* indeterminate corymb rather than the typical cruciferous raceme, pedicels 0.8–1.5 cm long. *Siliques* 1.8–2.7 cm 6–7 mature seed in each valve (or 12–14 per fruit) (Figure 1.4).

Notes: Later in the life cycle, flowers are produced having no petals and only four long stamens. These flowers are likely equivalent to the cleistogamous flowers described for *C. corymbosa* by Schulz (1903). Fruit is a two-valved silique which dehisces forcefully, though not as explosively as close relatives *C. flexuosa* or *C. hirsuta*

Representative Specimens Examined: **NEW ZEALAND:** *Laird s.n* (K); *Philipson 10145* (K).

Cardamine flexuosa With., Arr. Brit. Pl., ed. 3: 578. 1796.—Type: “Rookery at Edgebaston”

(fl & fr). [Lectotype, designated by Post et al., *in review*: Curt. 277!]

Erect annual to 30 cm tall. *Stems* one to many, angular, ribbed, flexing at the nodes (zigzag). *Basal rosette* upon germination. *Leaves* odd pinnately compound, 7–13 leaflets, the terminal one being up to 2 times larger than the lateral leaflets. *Basal leaflets* ovate to orbicular in shape and petiolulate, rarely sessile, terminal leaflet may approach a reniform shape. *Cauline leaves* odd pinnately compound, leaflets lanceolate to oblanceolate in shape. All leaflets are irregularly lobed having 3–5 lobes sometimes terminating in a short tooth. At onset of flowering many basal leaves die back and the rosette is no longer visible. *Flowers* four green (sometimes purplish) sepals half the length of petals. *Petals* four white, cuneate to 4 mm long. *Stamens* six (four long and two short) the four long ones being generally longer than the blossom. *Inflorescence* a raceme. *Fruit* a two valved capsule 1.5–2.5 cm long held at an angle from the rachis, dehisces forcefully releasing 20–24 seeds (Figure 1.5).

Representative Specimens Examined: **AUSTRALIA:** *Lepschi* 1936 (K). **CHINA:** *Hu* 209 (K); *Hu* 9417 (K); *Yao* 8885 (K). **JAPAN:** *coll. ign* 2320/51 (K); *Furuse* 10770 (K). **USA:** **CT** *Tucker & Tucker* 13815 (MO); **GA** *Duncan* 23704 (NCSC); *Adams* 17603 (NCSC); *Cusick* 32229 (MO); **FL** *Correll & Popenoe* 51473 (NY); *Correll & Popenoe* 51504 (NY); *Burch* 6502 (NY); **IL** *Cusick* 35226 (NY); *Cusick* 35228 (MO); **NJ** *Mackenzie* 3129 (MO); **OH** *Cusick* 33305 (MO); *Cusick* 32647 (NY); **MO** MBG1-2 *Post* 73 (NCSC); MBG2-1 *Post* 74 (NCSC); **NC** *Post* 45 (NCSC); *Post* 46 (NCSC); *Post* 47 (NCSC); *Post* 48 (NCSC); *Post* 49 (NCSC); *Post* 50 (NCSC); *Post & Adkins* 84 (NCSC); *Post & Adkins* 84 (NCSC); *Post & Adkins* 75 (NCSC); *Post & Adkins* 75 (NCSC); *Post & Adkins* 76 (NCSC); *Post & Adkins*

78 (NCSC); *Post* 58 (NCSC); *Post* 59 (NCSC); *Post* 60 (NCSC); *Post* 61 (NCSC); *Post* 62 (NCSC); *Post* 63 (NCSC); *Post* 64 (NCSC); *Post* 65 (NCSC); *Post* 66 (NCSC); *Post* 67 (NCSC); *Post* 56 (NCSC); *Post* 56 (NCSC); *Post* 56a (NCSC); *Post* 52 (NCSC); *Post* 53 (NCSC); **NY** *Post* 87 (NCSC); *Post* 88 (NCSC); **OR** *Altland s.n.* (NCSC); **PA** *Cusick 31707* (MO); **SC** *Barkley 23* (MO).

Cardamine hirsuta L., Sp. Pl.: 655, 1753.—Type: “*In Europae areis, hortis, arvis*”. (fl & fr)

[Lectotype, designated by Fawcett and Rendle 1914; LINN, no. 835.13!; isoelectotype: LINN, no. 835.14!])

Erect annual 10–17 cm. *Stem* usually only one. *Basal rosette* persists throughout the season. *Basal leaves* odd pinnately compound 9–13 leaflets, the terminal one being up to 2 times larger than the lateral ones. *Basal leaflets* ovate to orbicular in shape and petiolulate, having 3–5 lobes sometimes terminating in a short tooth. *Terminal leaflets* may approach a reniform shape. *Cauline leaves* odd pinnately compound, 5–7 leaflets, lanceolate to oblanceolate in shape. *Cauline leaflets* are irregularly lobed except for the terminal leaflet which is rarely lobed. *Flowers* four green sepals alternating with four white, spatulate petals. *Stamens* four and generally longer than the blossom. *Inflorescence* a raceme. *Fruit* a two valved capsule 1.8–2.5 cm long held upright and nearly parallel to the rachis. *Fruits* dehisce forcefully releasing 16–24 seeds (Figure 1.6).

Representative Specimens Examined: **POLAND:** *Zelazny s.n.* (NCSC). **USA:** **AL** *Kral 88031* (MO); **KY** *Athey 537* (MO); **MS** *Bryson 20610* (NCSC); *Bryson 20611* (NCSC); *Bryson 20615* (NCSC); **NC** *DeLoach & Dukes 61* (NCSC); *Godfrey 49011* (NCSC); *Ingle 23*

(NCSC); *Ittenbach* 17 (NCSC); *Jones* 41 (NCSC); *Pultorak* 692 (NCSC); *Post* 51 (NCSC); *Smith* 169 (NCSC); *Thomas & Spell* 165086 (NY); **OR** *coll. ign. s.n.* (K); **SC** *Angerman s.n.* (NCSC); *Nelson & Chicone* 21022 (MO); *Wooten* 3 (MO); **TN** *Kelly* 83 (NCSC); *McNeilus* 99-47 (NY); *Phillippe* 2520 (NCSC); **VA** *Churchill s.n.* (NCSC); *Selden* 25 (NCSC).

Cardamine oligosperma Nutt., Fl. N. Amer. i. 85. 1838.—Type: “Oregon woods” (fr).

[holotype: *Nuttall s.n.*, NY!; Isotype: *Nuttall s.n.*, GH!, K!]

Erect annual 20–30. *Stem* usually single. *Basal rosette* persists throughout the season. *Basal leaves* odd pinnately compound, 5–9 leaflets, the terminal one being up to 2 times larger than the lateral ones. *Basal leaflets* are ovate to orbicular in shape and petiolulate, having 3–5 lobes sometimes terminating in a short tooth. *Cauline leaves* odd pinnately compound, 3–5 leaflets, ovate to obovate in shape. *Cauline leaflets* irregularly lobed and may terminate in a short tooth. *Flowers* four green sepals alternating with four white, spatulate petals. *Stamens* six (four long and two short). *Inflorescence* a raceme. *Rachis* typically straight sometimes curving. *Fruit* a two valved capsule 2.2–2.8 cm long and 1.5–2.0 mm wide, held upright, dehisce forcefully releasing 12–16 seeds (Figure 1.7).

Representative Specimens Examined: **AK** *Zika* 16967 (MO); *Zika* 16971 (MO); *Zika* 16990 (MO); **NV** *Morefield* 3087 (NY); **CA** *Hansen* 94 (K); **OR** *Constance & Rollins* 2966 (K); *coll. ign s.n.* (Isotype-NY); **UT** *Goodrich* 16272 (NY); *Holmgren* 274 (NY); **WA** *Eyerdam s. n.* (MO); *Halse* 3576 (MO); *Suksdorf s.n.* (Isotype-MO, US); *Suksdorf* 723 (Isotype-MO, NY); *Suksdorf* 7452 (Isotype-CAS, GH, MO, NY, WTU).

Cardamine scutata Thunb., Trans. Linn. Soc. London 2: 339. 1794.—Type: “Japan”. (fr)

[holotype: *Thunberg s.n.*, UPS–Thunb.!]

Erect annual 15-50 cm tall. *Stem*. *Basal leaves* not rosulate. *Basal leaflets* odd pinnately compound 3–9 the terminal one being up to 2 times larger than the lateral ones, and obovate to reniform in shape. *Cauline leaves* odd pinnately compound, 3–5 leaflets. *Cauline leaflets* having 3-5 lobes, terminal leaflet sometimes unlobed. *Flowers* four green sepals alternating with four white, spatulate petals to 4.5 mm. *Stamens* six (four long and two short). *Inflorescence* a raceme. *Rachis* straight. *Fruit* a two valved capsule 1.5–2.8 cm long and 1.0–1.5 mm wide, held upright, dehisce forcefully releasing 20–36 seeds (Figure 1.8).

Representative Specimens Examined: **AUSTRALIA:** *Kissane s.n.* (K). **JAPAN:** *Furuse 9050* (K); *Furuse 11112* (K); *Ohba 71505* (K); *Taquet 563* (K); *Tsugaru 12964* (MO); *Tsugaru 13005* (MO); *Tsugaru 22220* (MO). **RUSSIA:** *Gage 2176* (NY).

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Figure 1.1. Strict consensus of 700,000 most parsimonious trees resulting from parsimony analysis of nuclear gene region *CIP7* for *Cardamine* species in United States nurseries (Length=59; CI= 0.814; RI=0.875). Bootstrap support (BS) is given above branches and Bayesian posterior probabilities (PP) are given below branches. Species identifications shown are based on morphological examinations. State abbreviations are given in parenthesis next to accessions. UK denotes fresh accessions from England and H denotes herbarium specimens.

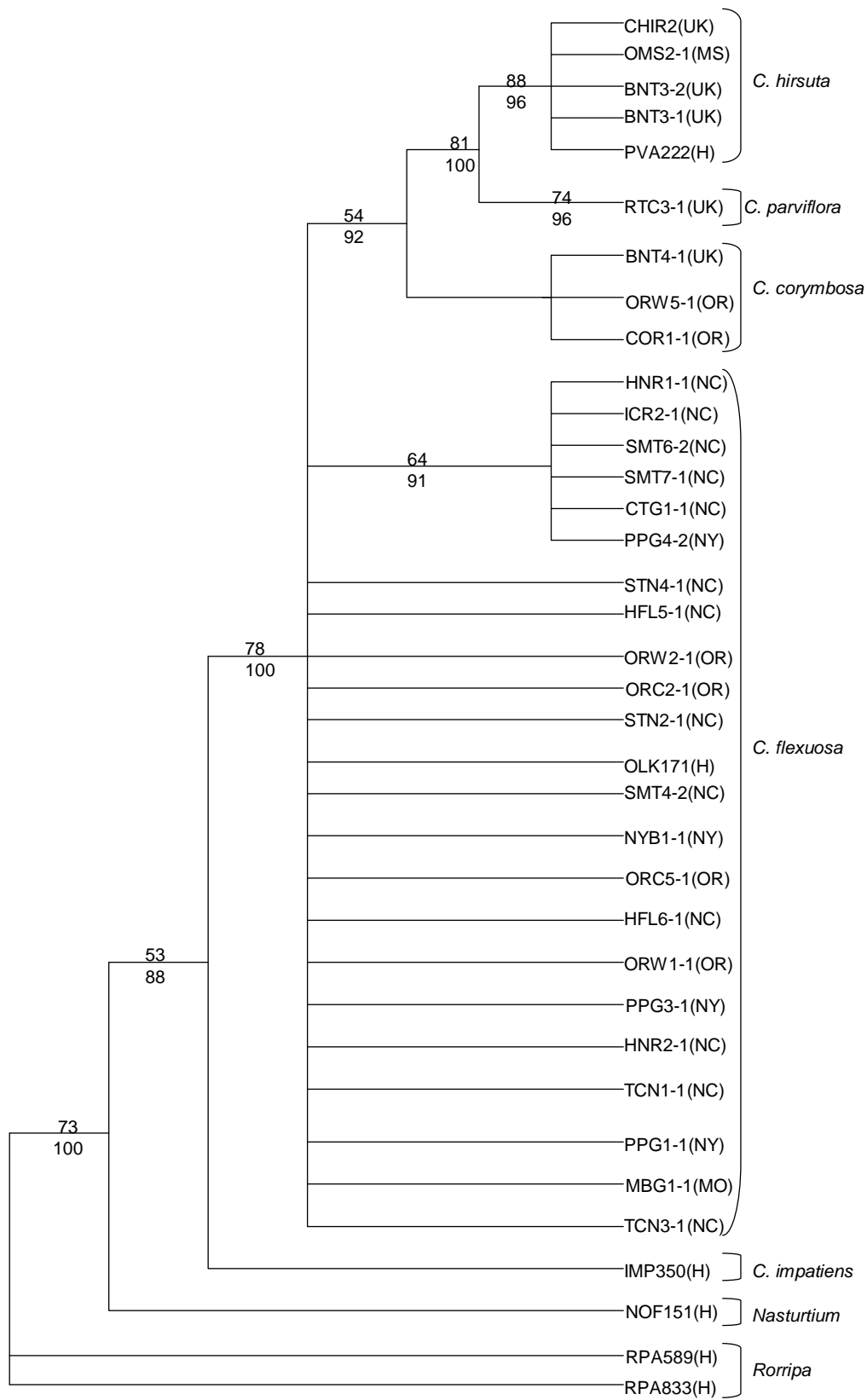


Figure 1.2. Majority Rule consensus for *ITS* for *Cardamine* species in United States nurseries including our fresh accessions labeled with state acronyms and selected ones, labeled “Lih”, from Lihova et al. (2006). UK denotes fresh accessions from England and OLK and HIR are herbarium sheets. Bayesian posterior probabilities (PP) are given above branches. Species identifications shown are based on morphological examinations.

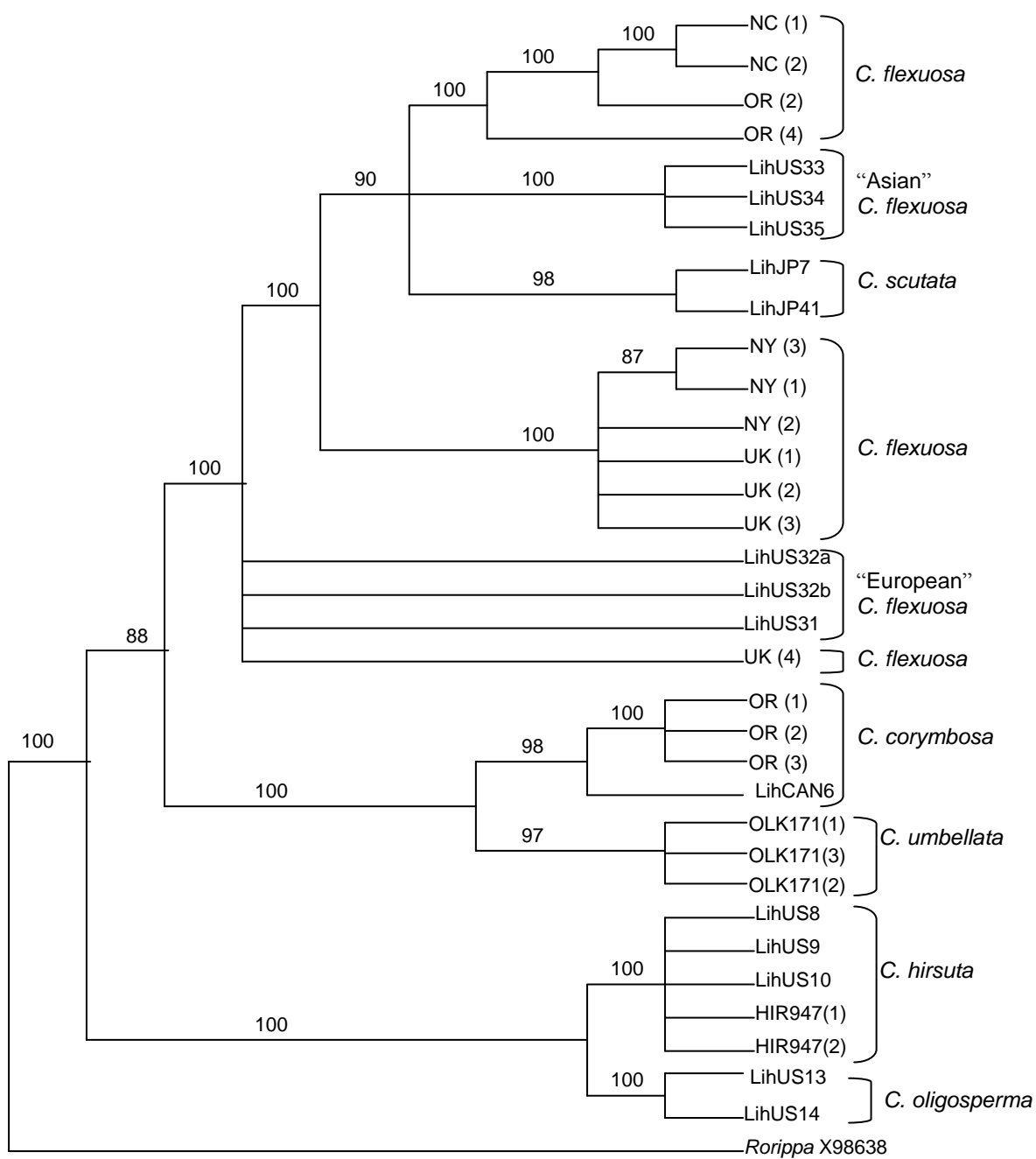


Figure 1.3. Strict consensus of 13 most parsimonious tree resulting from parsimony analysis for combined matrix of *CIP7* and *ITS* for *Cardamine* species in United States nurseries (Length=73; CI= 0.922; RI=0.978). Bootstrap support (BS) is given above branches and Bayesian posterior probabilities (PP) are given below branches. Species identifications shown are based on morphological examinations. Morphological characters are given under species identification. State abbreviations are given in parenthesis after each accession. UK denotes fresh accessions from England and H denotes herbarium specimens.

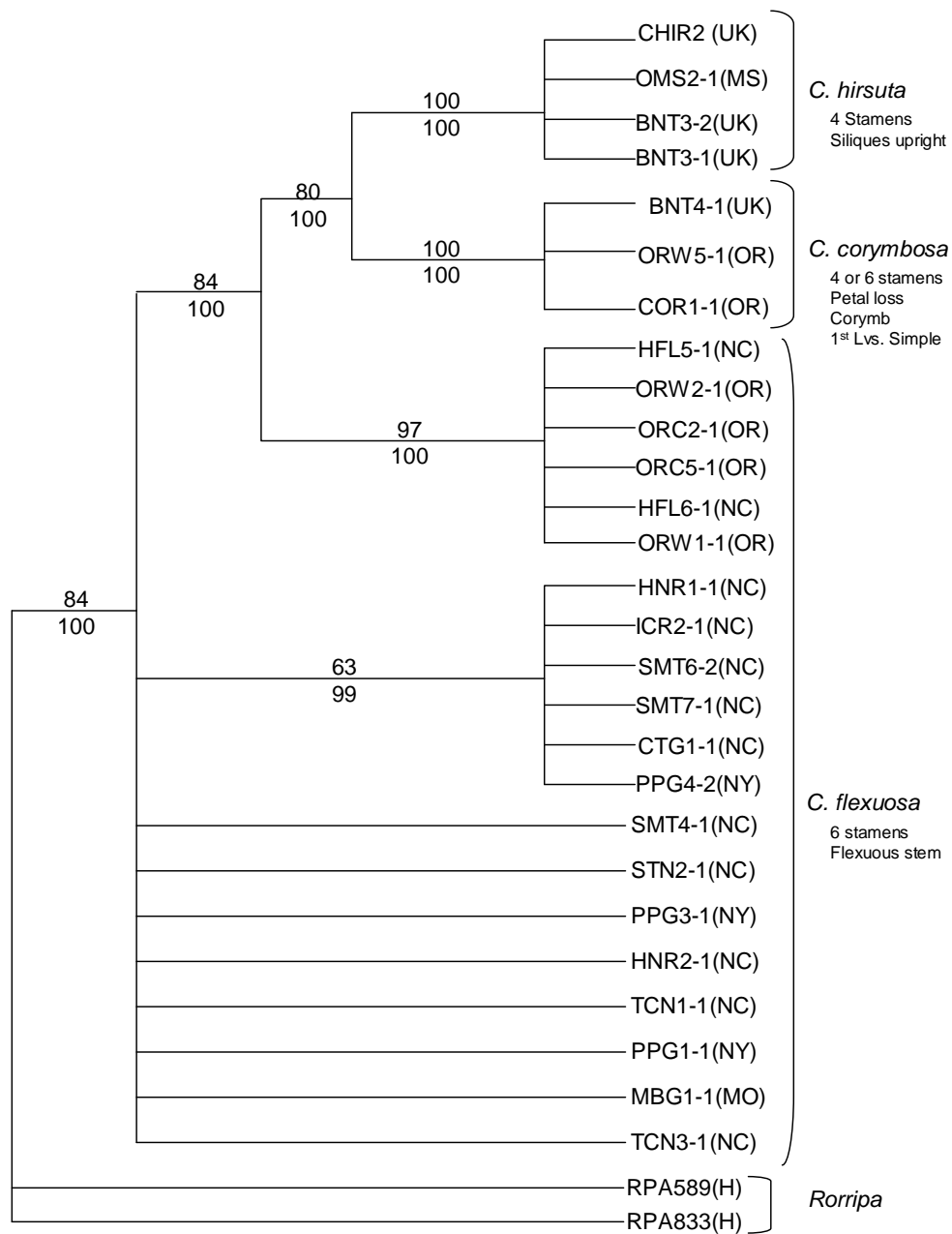


Figure 1.4. Illustration of *Cardamine corymbosa* Hook.f. a) habit, b) basal leaf, c) petaliferous flower-cutout, d) petaliferous flower-top view, e) apetalous flower, f) apetalous flower-top view, g) apetalous flower-cutout, h) stamen, i) silique, j) silique-cutout, k) seed.
[Illustrations by Nancy C. Routh]

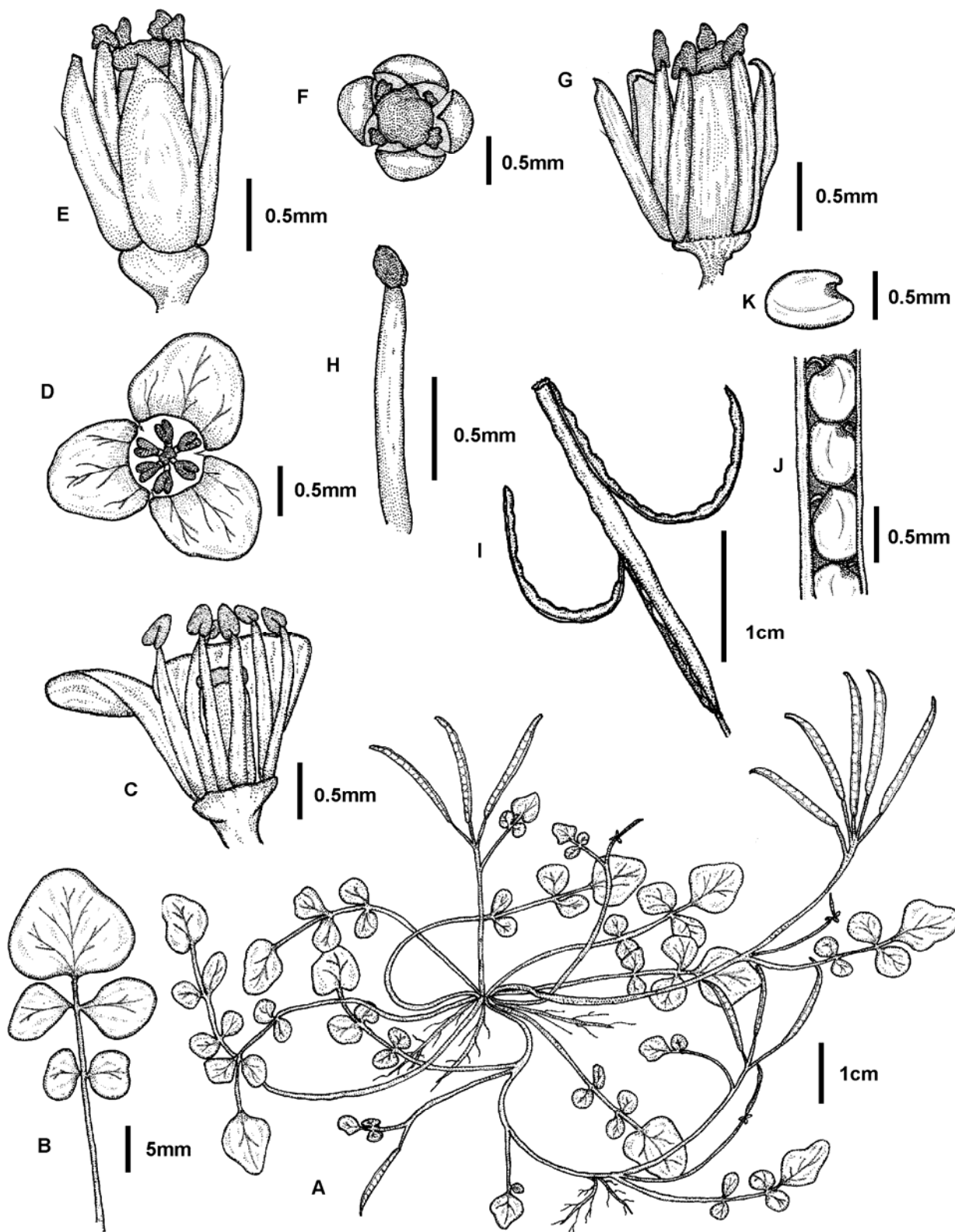


Figure 1.5. Illustration of *Cardamine flexuosa* With. a) habit, b) basal leaf, c) cauline leaf, d) flower- cutout, e) flower-side view, f) flower-top view, g) petal, h) stamens, i) seed.
[Illustrations by Nancy C. Routh]



Figure 1.6. Illustration of *Cardamine hirsuta* L. a) habit, b) basal leaf, c) cauline leaf, d) flower-cutout, e) flower-side view, f) flower-top view, g) petal, h) stamen, i) silique, j) silique-cutout, k) seeds. [Illustrations by Nancy C. Routh]

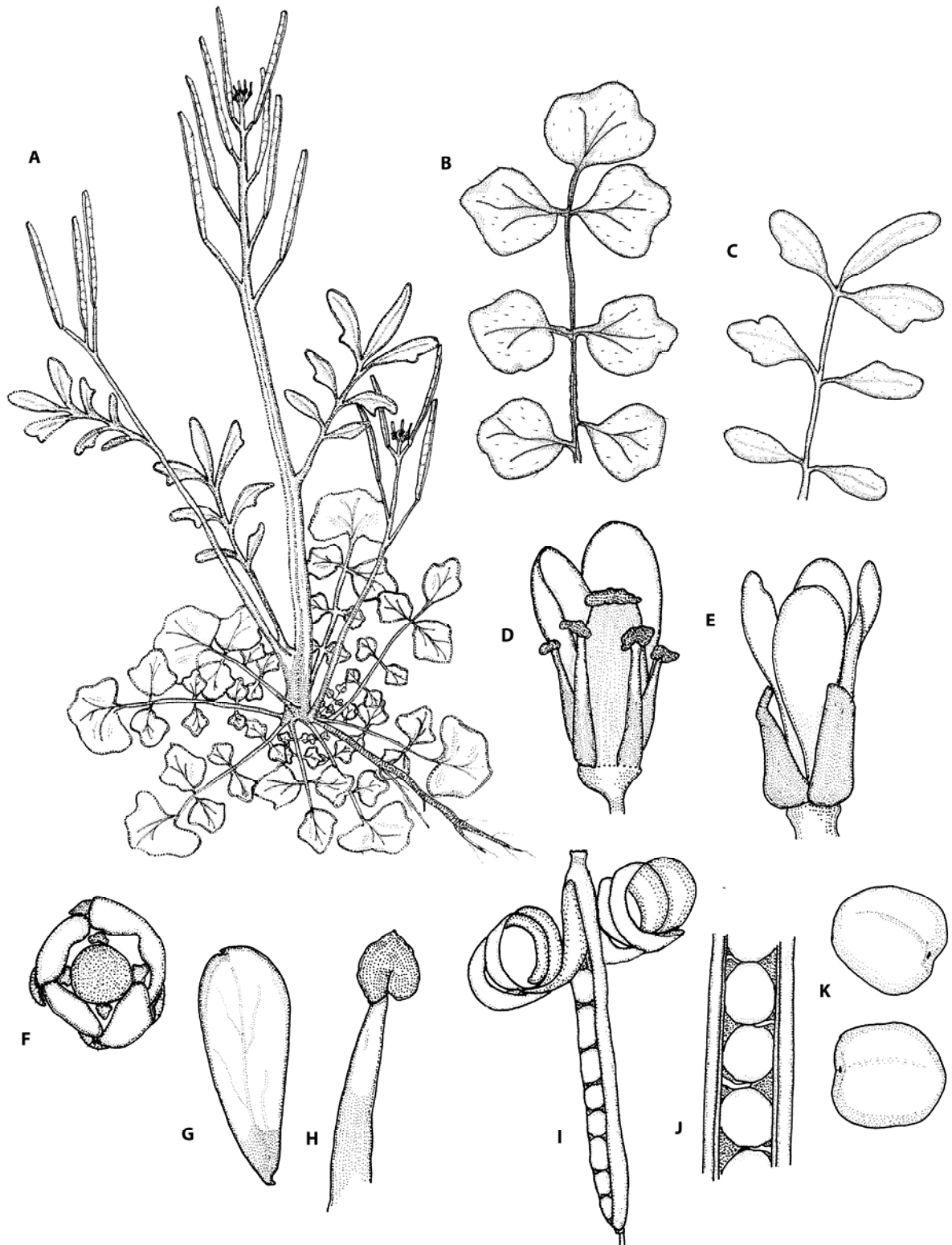


Figure 1.7. Illustration of *Cardamine oligosperma* L. a) habit, b) basal leaf, c) cauline leaf, d) flower-cutout, e) flower-side view, f) flower-top view, g) petal, h) stamen, i) silique. [Illustrations by Nancy C. Routh].

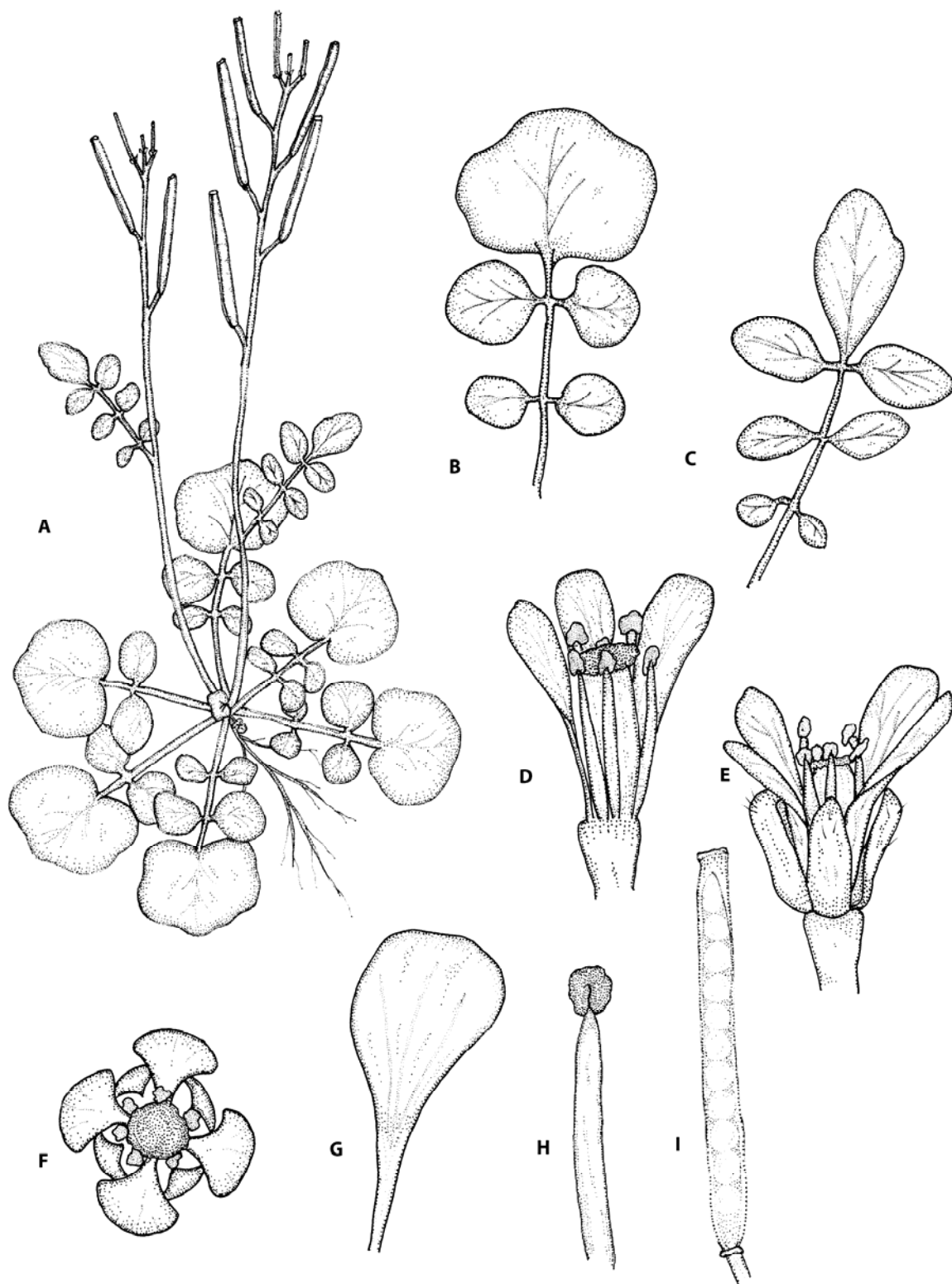
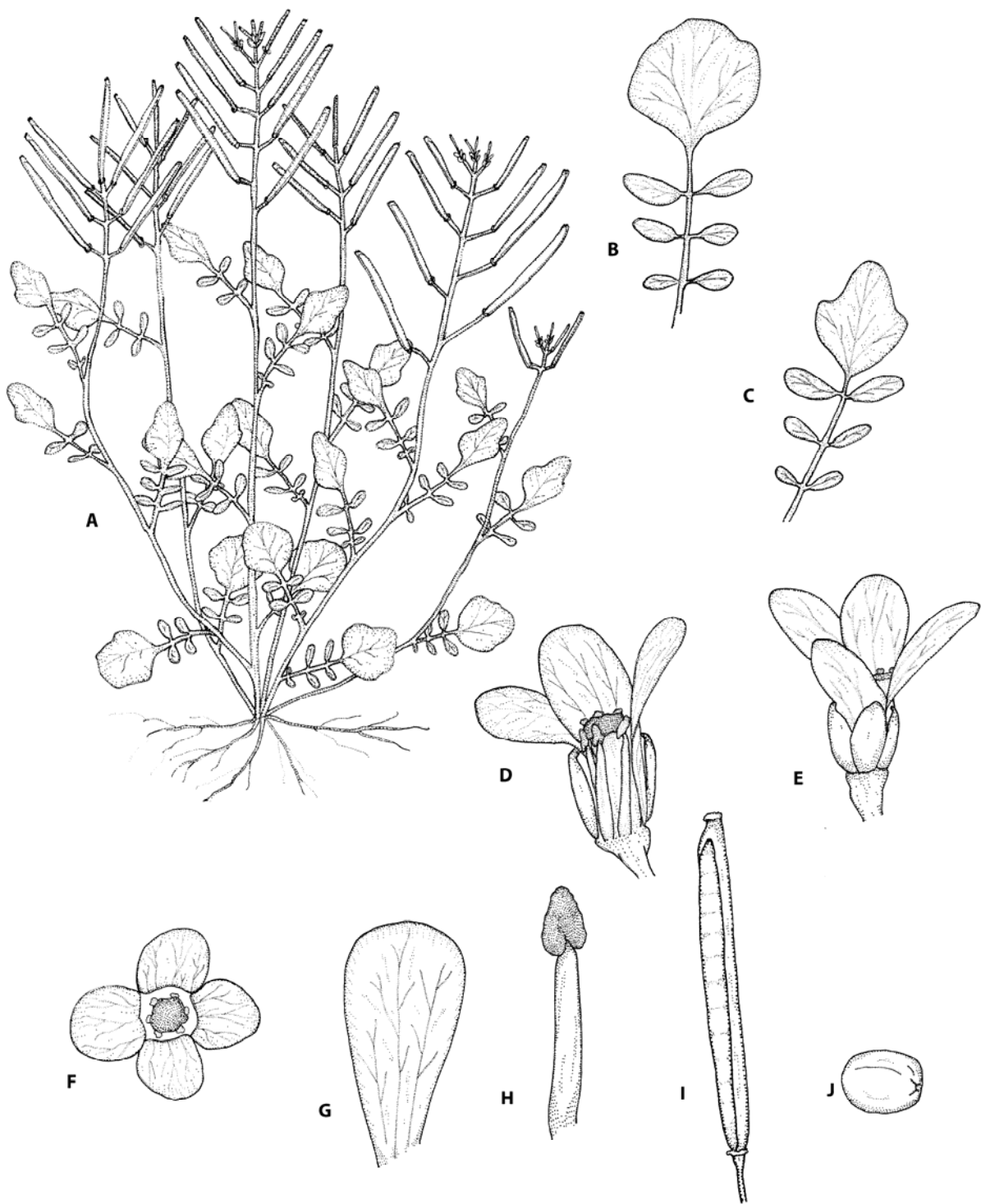


Figure 1.8. Illustration of *Cardamine scutata* Thunb. a) habit, b) basal leaf, c) cauline leaf, d) flower-cutout, e) flower-side view, f) flower-top view, g) petal, h) stamen, i) silique, j) seed.
[Illustrations by Nancy C. Routh]



Chapter 2

Lectotypification of *Cardamine flexuosa* (Brassicaceae).

(In the format appropriate for submission to Journal of the Botanical Institute of Texas)

Lectotypification of *Cardamine flexuosa* (Brassicaceae)¹

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In 1796, William Withering (1741–1799) described a new species from England as *Cardamine flexuosa* With. The primary set of Withering's collections are held at BM (Stafleu and Cowan 1976). However, upon inspection of *Cardamine* L. held by the museum we found no specimen bearing his name or hand for any taxa. In addition, there is no record of the type collection being on loan at the time. There are also none of his own *Cardamine* specimens or those of others he may have studied in the herbaria known to house his material: BR, K, LINN, LIV, UPS-Thunb (Stafleu and Cowan 1976). Loss of this material has been previously reported (Marhold 1995; Lihova et al. 2006); however, a lectotype remains undesignated. In the absence of the holotype we refer to illustrations cited in the protologue to lectotypify *Cardamine flexuosa*. Withering cited the following three illustrations which serve as syntypes in the absence of other original material: *Flora Londinensis* or, Plates and descriptions of such plants as grow wild in the environs of London. 1777. t. 277.; *Enumeratio plantarum florae Danicae*. 1770. t. 735.; *Flora Britannica Indigena*: or plates of the indigenous plants of Great Britain. 1778. *C. hirsuta*.

Here we designate one of these illustrations to serve as the lectotype for *Cardamine flexuosa* With.:

Cardamine flexuosa With. Arr. Brit. Pl. ed. 3: 578. 1796. –Type: “Rookery at Edgebaston”.

(Lectotype: Curt. 277., designated here).

The lectotype for *Cardamine flexuosa* With. (Fig. 3.1) is a historical illustration from Curtis' *Flora Londonensis* (1777). This work is a collection of loose plates most of which are hand

colored; however, plate 277 is one of the few to remain uncolored. It is accompanied by a brief but thorough description in Latin with the English translation given. Plate 277 is labeled as the closely related species *C. hirsuta*, but clearly illustrates the siliques angled away from the inflorescence and flexing nodes typical of *Cardamine flexuosa* With. It undoubtedly refers to the taxon in Withering's original description of the species. Basal leaflets in the illustration are not totally congruent with what we have observed in the field and on herbarium specimens, but the description accompanying the plate reads: "Lobes of the radical leaves vary much in shape and are frequently much rounder than the figure represents." The only other caveat to our determination here is that a detailed illustration of the androecium in the bottom right corner of the plate labeled #3 cannot refer to *C. flexuosa* because it illustrates only four stamens when *C. flexuosa* always has six. Information given in the description accompanying the plate also sheds some light on this problem. The main morphological difference between *C. hirsuta* and *C. flexuosa* is stamen number, *C. hirsuta* having four and *C. flexuosa* six. Curtis' illustration shows four stamens and gives the following information in the description: "The same plant, early in the spring, when the weather is cold, has only four stamina; as the summer advances, it has constantly six." We believe that Curtis was encountering two different taxa during the course of the growing season. Our evidence suggests that *C. hirsuta* has a winter annual life cycle and is only encountered in flower during the cooler months of Spring. *Cardamine flexuosa* on the other hand does not have such a set life cycle in the landscape and can be seen flowering later in the year. So we suggest that in the early Spring, Curtis was observing *C. hirsuta* with four stamens and later, when he observed six stamens, it was almost certainly *C. flexuosa* which has only subtle differences in vegetative morphology. The two remaining illustrations cited

in the protologue (Walc. *C. hirsuta* and Fl. Dan. 735) are hardly representative of the taxon in question and more closely resemble the taxa they were meant to illustrate: *C. hirsuta* and *C. impatiens* respectively. The Walcott illustration of *C. hirsuta* (1778) depicts a plant with a very straight stem and rachis covered in fine hairs. This is more typical of *C. hirsuta* than *C. flexuosa*. Table 735 (Oeder 1770) does exhibit a flexing stem but the stem and rachis are very thick compared to *C. flexuosa* and there are numerous cauline leaves with leaflet shapes more typical of *C. impatiens*. *Cardamine flexuosa* has sparser cauline leaves which exhibit fewer leaflets per leaf than the basal leaves.

ACKNOWLEDGEMENTS

I would like to thank the curators of BM, K, and LINN for the courtesies extended during my visits. I would also like to thank curators at BR, LIV, and UPS-Thunb. for their assistance in searching for holotype material of this taxon, and those at MO for providing high resolution images of the designated lectotype.

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Figure 2.1. Digital image of the lectotype for *Cardamine flexuosa* in W. Curtis' *Flora Londinensis* or, *Plates and descriptions of such plants as grow wild in the environs of London*. t. 277. [Courtesy of Missouri Botanical Garden Library.]



Cardamine hirsuta.

Figure 2.2. Digital image of the description accompanying type illustration for *Cardamine flexuosa* in Curtis' Flora Londinensis or, Plates and descriptions of such plants as grow wild in the environs of London. Page previous to t. 277. [Courtesy of Missouri Botanical Garden Library.]

CARDAMINE HIRSUTA. HAIRY LADIES-SMOCK.

CARDAMINE Lin. Gen. Pl. TETRADYNAMIA SILIQUOSA.

Siliqua elastice diffiliens valvulis revolutis. Stigma integrum. Cal. subhians.

Raii Syn. Gen. 21. HERBÆ TETRAPETALÆ SILIQUOSÆ ET SILICULOSÆ.

CARDAMINE foliis pinnatis, floribus tetrandris. Lin. Syst. Vegetab. p. 497. Sp. Pl. p. 915. Fl. Suec. n. 587.

CARDAMINE foliis pinnatis hirsutis, pinnis subrotundis, flaminibus quaternis. Haller Hist. 472.

CARDAMINE hirsuta. Scopoli Fl. Carn. n. 817. t. 38.

NASTURTIUM aquaticum minus. Bauh. Pin. 104.

CARDAMINE impatiens altera hirsutior. Raii Syn. p. 300. The lesser Hairy, impatient Cuckow-Flower or Ladies-Smock.

CARDAMINE foliis pinnatis, foliolis radicalibus subrotundo-cordatis; caulinis ovatis dentatis petiolatis. Hudson. Fl. Ang. ed. 2. p. 295. Lightfoot Fl. Scot. p. 348.

RADIX annua, fibrosa, fibris albidis.	ROOT annual and fibrous, the fibres whitish.
CAULIS spithameus, et ultra pro ratione loci, in fossis humidis reperitur etiam sesquipedalis, solidus, erectus, flexuosus, sulcato-angulosus, prope basin purpureus, et sæpius hirsutissimus, superne fere glaber, ramosus, ramosissimus etiam occurrit.	STALK about a span high, or more, according to the situation in which it grows; in wet ditches it is sometimes found even a foot and a half in height, solid, upright, crooked, grooved or angular, purple near the base, and most commonly very hairy, above nearly smooth, branched, sometimes very much so.
FOLIA radicalia plurima, in orbem posita, pinnata, foliolis petiolatis, rotundato angulatis, plerumque quinque lobatis, hirsutis, punctis prominulis scabriusculis, lobis inæqualibus, nunc obtusis, nunc acutis, caulina angustiora et magis profunde incisa, lobis paucioribus.	LEAVES next the root numerous, forming a circle, pinnated, the small leaves standing on foot-stalks, round yet angular, generally divided into five lobes, hirsute, roughish with little prominent points, the lobes unequal, sometimes blunt and sometimes pointed; those of the stalk narrower, and more deeply indented, with fewer lobes.
FLORES parvi, albi, primo vere tantum, tetrandri.	FLOWERS small, and white, early in the spring having only four stamina.
CALYX: PERIANTHIUM tetraphyllum, foliolis ovato-oblongis, obtusis, concavis, deciduis, pilis paucis albidis instructis, fig. 1.	CALYX: a PERIANTHIUM of four leaves, of an ovate, oblong shape, obtuse, hollow, deciduous, furnished with a few white hairs, fig. 1.
COROLLA: PETALA quatuor, alba, calyce duplo fere longiora, patentia, integerrima, obtusa, fig. 2.	COROLLA: four white PETALS, almost twice the length of the calyx, spreading, entire and obtuse, fig. 2.
STAMINA: FILAMENTA plerumque sex, quorum duo, breviora, alba. ANTHERÆ minimæ, lutescentes, fig. 3.	STAMINA: for the most part six FILAMENTS, of which two are shorter than the rest, of a white colour. ANTHERÆ very small and yellowish, fig. 3.
PISTILLUM: GERMEN oblongum, tenue, flaminibus paulo brevior. STIGMA capitatum, fig. 4.	PISTILLUM: GERMEN oblong, slender, a little shorter than the stamina. STIGMA forming a small head, fig. 4.
PERICARPIUM: <i>Siliqua</i> erecta, uncialis, subcompressa, bivalvis, elastice diffiliens, valvulis revolutis, fig. 5.	SEED-VESSEL: an upright <i>Pod</i> , about an inch in length, somewhat flattened, of two valves, which burst with an elastic force, and roll back, fig. 5.
SEMINA duodecim circiter, suborbiculata, compressa, glabra, e flavo fusca, fig. 6.	SEEDS about twelve in number, nearly round and flattened, smooth, and of a yellowish-brown colour, fig. 6.

We were inclined to believe with our ingenious friend Mr. LIGHTFOOT, that the *Cardamine hirsuta* and *parviflora* were distinct species; but repeated observation and culture have convinced us, that they are both the same, varying only in size, in hairiness, and in the number of their stamina.

In wet situations, where the soil is luxuriant, it grows a foot or two in height, and loses in a great degree its hairiness; in exposed places it seldom reaches more than six or eight inches, and is generally much more hairy; and, when it grows singly, much more branched. The same plant, early in the spring, when the weather is cold, has only four stamina; as the summer advances, it has constantly six. The lobes of the radical leaves vary much in shape, and are frequently much rounder than the figure represents.

This species is by no means general about London, but abounds in particular places; as by Chelsea water-works, in wet ditches about Hampstead, Highgate, and elsewhere.

It flowers in April and May. In the garden, if the situation in which it is sown be shady, and the season not uncommonly dry, it continues flowering and seeding during the whole of the summer.

According to Mr. LIGHTFOOT, the young leaves are a good ingredient in a salad, and may easily be obtained in the spring, when Mustard and Cress are not to be had.

Chapter 3

Cardamine corymbosa (Brassicaceae): New to the United States

(In the format appropriate for submission as a Note to Weed Technology)

Cardamine corymbosa (Brassicaceae): New to the United States¹

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Abstract: *Cardamine corymbosa* (Brassicaceae)—New Zealand Bittercress—is reported as new to the United States. While collecting specimens to determine what *Cardamine* species occur in the nursery trade, *C. corymbosa* was discovered in a container nursery in Wilkes County, North Carolina. The nursery tracked the shipment of contaminated plants to a wholesale nursery in Washington County, Oregon. It was subsequently confirmed that *C. corymbosa* also occurs in a Clackamas County, Oregon nursery and has likely been distributed throughout the United States as a contaminant in container grown ornamental plants. Thus far there have been no reports of naturalized populations outside of container nursery crop production facilities.

Nomenclature: New Zealand Bittercress, *Cardamine corymbosa*, Brassicaceae

Introduction

Cardamine corymbosa Hook.f. (New Zealand bittercress) has not been reported for the United States and is not currently cited by USDA PLANTS database (USDA:NRCS 2008). The vouchers cited here constitute the first reports of the occurrence of this species in the United States and, specifically, North Carolina and Oregon.

Voucher specimens: **U.S.A. North Carolina. Wilkes Co.:** growing in 10-L containers with weeping Colorado blue spruce recently delivered from an Oregon nursery, 4 Aug 2006, Post & Adkins 84 (NCSC). **U.S.A. Oregon. Clackamas Co.:** growing in containers at a nursery, Oct 2006, Altland *s.n.* (NCSC).

Cardamine corymbosa is a winter annual native to New Zealand and several sub-Antarctic islands (Cheeseman 1925; Wace 1960). It has been introduced to other parts of the world including Australia, the United Kingdom, and Ireland (Rozeffelds et al. 1999; Reynolds 2002; Yeates & Williams 2006). More recently, it was discovered in the United States in container grown nursery crops in Wilkes County, North Carolina [Post & Adkins 84 NCSC]. *Cardamine corymbosa* plants were observed growing in 10-Liter containers with weeping Colorado blue spruce (*Picea pungens* Engelm.). The 10-L stock plants were distributed to North Carolina from a larger nursery located in Washington County Oregon. Subsequently, we were able to confirm the presence of *Cardamine corymbosa* in a nursery in Clackamas County, Oregon by examining a specimen collected and provided by Dr. James Altland [Altland s.n., NCSC]. A composite illustration from these specimens is provided (Figure 4.1)

Description

Cardamine corymbosa was first described by Joseph Dalton Hooker in *Icones Plantarum* (1857) as “a small and very distinct species of *Cardamine*, wiry and fragile in every part.” The species has a decumbent habit with wiry, unbranched stems, which creep along the ground. It is capable of rooting at the nodes to produce daughter plants. Basal leaves are compound with 3–5 leaflets, the terminal leaflet being up to 2 times larger than lateral ones. Leaflets are sessile or nearly so, and obovate to orbicular in shape. Terminal leaflets may approach a reniform shape. Flowers are dimorphic. The first flowers produced in spring follow the typical Brassicaceae form: four sepals alternating with four clawed petals. In these petaliferous flowers, one or more of the petals may be fused. There are six stamens, four long and two short. Later in the life cycle flowers are

produced having no petals and only four long stamens. These flowers are likely equivalent to the cleistogamous flowers described for *C. corymbosa* by Schulz (1903). Inflorescences are formed in a corymb rather than the typical cruciferous raceme. Each flower is borne on a pedicel 0.8–1.5cm long. Inflorescences are indeterminate and may elongate past the first corymb to produce another corymb after siliques have formed on the prior inflorescence. Fruit is a two-valved silique which dehisces forcefully, though not as explosively as close relatives *C. flexuosa* or *C. hirsuta*. Siliques are 1.8–2.7 cm and produce 6–7 mature seed in each valve (or 12–14 per fruit). Preliminary evidence suggests that seed is approximately 70% viable as soon as it is shed, and germinates readily (Post et al. *unpublished data*).

Discussion

In 2007, Oregon was reported to have the 2nd largest number of nursery operations and total value of wholesale nursery crops sold in the U.S., and to have the greatest area under nursery crop production (USDA:ERS 2007). As a major producer of container nursery stock, Oregon has several large nurseries that ship plants throughout the U.S. Consequently, it is likely that *C. corymbosa* occurs throughout the United States, at least in container nurseries. The species has not been reported in the landscape or as a naturalized plant in any U. S. location to date. However, as contaminated nursery stock is planted into the landscape by homeowners and professional landscapers, *C. corymbosa* establishment is likely. The species has already been reported as a troublesome weed of polytunnels in Australia, New Zealand, England, and Ireland (Anon 1999) and was

subsequently reported as a garden weed in these same regions (Rozefelds et al. 1999; Reynolds 2002).

Cardamine corymbosa is of concern to the container nursery industry because of its close relationship to two other troublesome nursery crop weeds, *Cardamine flexuosa* With. (flexuous bittercress) and *Cardamine hirsuta* L. (hairy bittercress). *Cardamine corymbosa* has the potential to become particularly problematic for nurseries because of its ability to produce stolons, which would make it difficult to effectively hand weed containers. Despite the availability of numerous herbicides labeled for bittercress control, it remains one of the most common and costly weeds in container nurseries (Mathers 1996). In 2007, USDA IR-4 Ornamental Horticulture annual survey reported bittercress to be the most prominent weed of United States greenhouse and nursery production systems, and the third most prominent weed in landscapes. An additional bittercress species to deal with, particularly one such as *C. corymbosa*, which spreads by stems which root at the nodes, may further increase the cost of bittercress control in nursery crops.

Acknowledgements

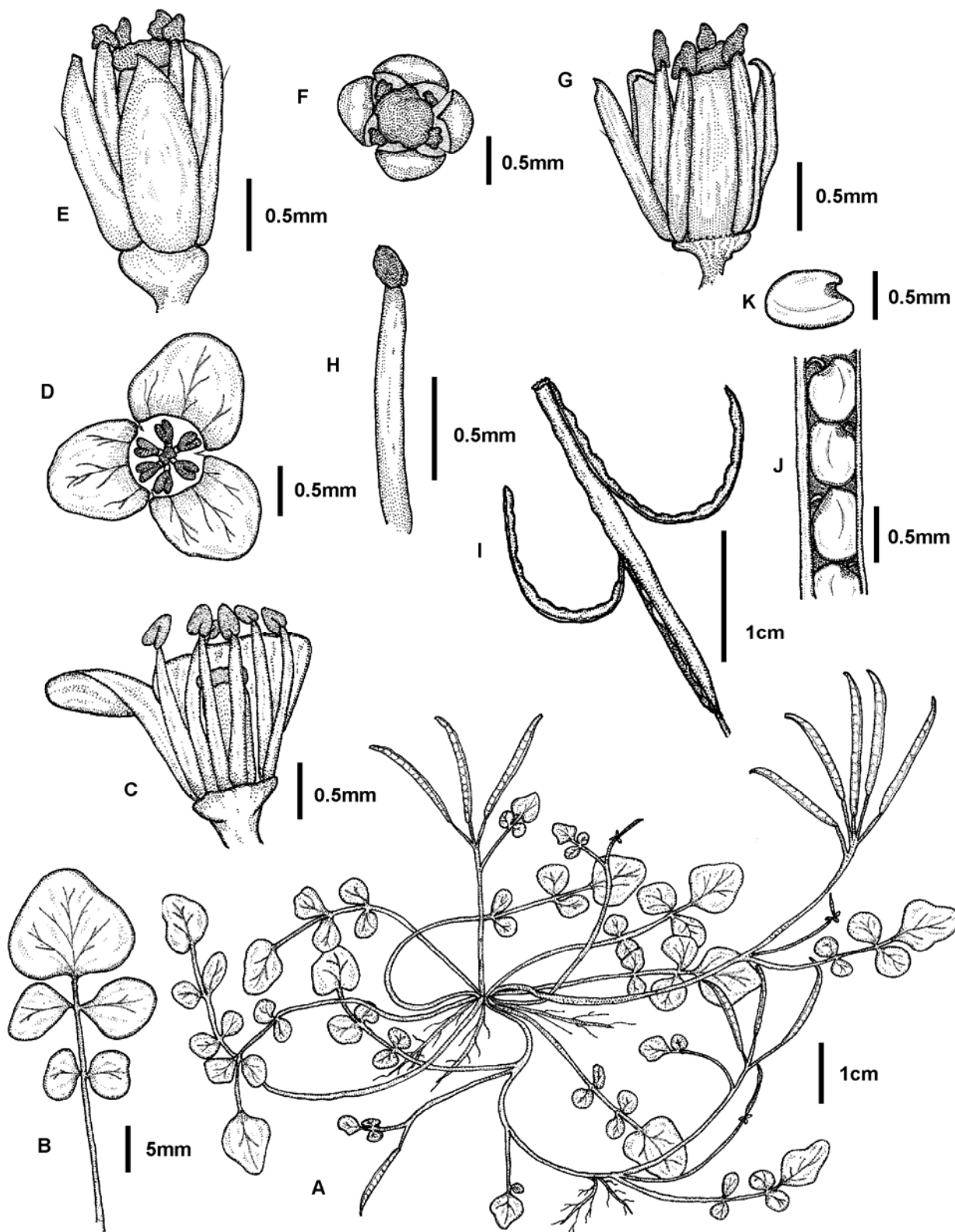
We are grateful to the many nursery managers who allowed us to scout their nurseries for bittercress. Dr. James Altland provided voucher specimens he collected from Oregon nurseries and Mr. Craig Adkins accompanied me to several Wilkes county North Carolina nurseries to scout for bittercress species. Dr. John Atwood also spent many hours accompanying me to nurseries in England, which ultimately led to the positive identification of the U.S. vouchers as *Cardamine corymbosa*.

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Figure 3.1. Illustration of *Cardamine corymbosa* Hook.f. a) habit, b) basal leaf, c) petaliferous flower-cutout, d) petaliferous flower-top view, e) apetalous flower, f) apetalous flower-top view, g) apetalous flower-cutout, h) stamen, i) silique, j) silique-cutout, k) seed. [Illustrations by Nancy C. Routh]



DISCUSSION AND CONCLUSIONS

Previously, most bittercress infesting container nursery crops was thought to be hairy bittercress or *C. hirsuta* L. (Fain et al. 2005, *personal communication*). Fain et al. (2005) reported three other species to occur in the trade including *C. flexuosa* With., the native *C. oligosperma* Nutt., and *C. scutata* Thunb. (2005). Based on our field collections and examination of herbarium material, four species of *Cardamine* occur in United States container nurseries. They include *Cardamine corymbosa* Hook. f. (New Zealand bittercress), *Cardamine flexuosa* (wavy or flexuous bittercress), *Cardamine hirsuta* (hairy bittercress) and *Cardamine oligosperma* (little western bittercress). We did not encounter the reported *C. scutata* (See Chapter 1). A previously unreported species for the United States was discovered: *Cardamine corymbosa* (New Zealand bittercress). *Cardamine flexuosa* and *C. corymbosa* are exotic to the United States and therefore do not appear together in any key to *Cardamine* for the United States, resulting in frequent misidentification (Hickman 1925; Hitchcock et al. 1955–1969; Detling 1937; Rollins 1993). A new key for *Cardamine* documented or reported in container nurseries in the United States is provided (See Chapter 1).

The following descriptions are based on personal observations of plants growing in the field and greenhouse as well as herbarium specimens. Throughout the course of this research over 4000 herbarium sheets were consulted. When examining herbarium material we focused on those characters traditionally used for species circumscription including flowers and fruiting structures, but also examined leaf, stem and root morphology, as well as surface hairs, in order to capture the morphological variability among these species.

Cardamine flexuosa is the most commonly encountered species in United States nurseries comprising 90% of specimens collected from U.S. container nurseries. Though most bittercress in nurseries was previously thought to be hairy bittercress, this species only comprised 6% of our collected specimens. *Cardamine oligosperma* and *C. corymbosa* accounted for 2% each.

Cardamine corymbosa is a new report for the United States. It is native to New Zealand and was likely introduced through the nursery trade. There are confirmed records from nurseries in Wilkes County, North Carolina and Washington and Clackamas Counties, Oregon. The North Carolina population was distributed from a nursery located in Washington County, Oregon. Given that nurseries in Washington County, Oregon distribute plants nationally, the species has likely been distributed throughout the United States. *Cardamine corymbosa* has been reported as a troublesome weed of polytunnels Australia, New Zealand, England, and Ireland (Anon 1999) and was subsequently reported as a garden weed in these same regions (Rozefelds et al. 1999; Reynolds 2002). It has not been reported in U.S. landscapes to date; however, as contaminated container crops are planted into landscapes by homeowners and professionals, establishment will likely occur.

Cardamine corymbosa is most easily distinguished from the other three species. It has a decumbent habit with wiry, unbranched stems capable of rooting at the nodes to produce daughter plants which creep along the ground. Upon germination it has a loose rosette form but the first true leaves are simple rather than compound. Basal leaves become compound a few weeks after germination with 3–5 leaflets which are sessile or nearly so, and obovate to orbicular in shape. This species also exhibits dimorphic

flowers. The first flowers produced in spring follow the typical Brassicaceae form: four sepals alternating with four petals and six stamens; however, one or more of the petals may be fused in these early flowers. Later in the life cycle, flowers are produced having no petals and only four long stamens. Inflorescences are also formed in a corymb rather than the typical cruciferous raceme.

Cardamine flexuosa was the most commonly encountered and is also the most morphologically variable of the four species. It germinates as a basal rosette with compound leaves having 7–13 leaflets, the terminal one being up to 2 times larger than the lateral ones. Leaves become deciduous as the plant begins to flower and basal leaves do not maintain a rosette form. The plant can be up to 30 cm tall and is usually many branched with stems that flex at the nodes. The main identifying character is flowers having 6 stamens (four long and two short), the long stamens being longer than the blossom.

The next most common species, *Cardamine hirsuta*, is the one most easily confused with *C. flexuosa*, morphologically (Appendix B). In the vegetative state, when both have a basal rosette, they are essentially identical. Leaves have a similar range of morphologically variability and may be glabrous or have short hairs covering the surface. Overall plant height is 10–17 cm. The distinguishing character is flowers having only four long stamens and petals held in a more upright position. Fruits are also held upright and tend to be almost parallel to the flowering stem. Through field observations we also noted that *C. hirsuta* seems to have a more static life cycle than *C. flexuosa*. We only observed *C. hirsuta* germinating in late fall to winter and flowering/fruiting February to April before senescence, indicating it may be a true winter annual species. This is in

contrast to *C. flexuosa*, which seems to germinate and grow any time of year, completing several life cycles in the typical growing season and continuing to proliferate over the winter months, especially in covered production areas.

Cardamine oligosperma is similar to *C. hirsuta* in habit but exhibits the more common six stamens of Brassicaceae. This species also forms a persistent basal rosette upon germination. Its overall height is taller than *C. hirsuta* usually >20 cm tall and fruits are typically wider, being >1 mm but held in a similar arrangement as *C. hirsuta*. Another difference is in the cauline leaves which are nearly ovate to obovate. In contrast, *C. hirsuta* and *C. flexuosa* have cauline leaves which are lanceolate to oblanceolate in shape.

Previous research has reported differential herbicide efficacy on bittercress in container nurseries (Smith et al. 1997; Altland et al. 1998; Altland et al. 1999). This may be due to the fact that several species occur in the trade and the most common species, *C. flexuosa*, has been reported resistant to isoxaben (Gallery), a commonly used preemergence herbicide in container nurseries (Eelen & Bulcke 1997). It is possible that inconsistent bittercress control could be associated with a differential species response to herbicides, or that differential responses to herbicides exist within a species. However, it is equally possible that differential control is due to improper herbicide application or other environmental factors. The specimens documented in this report will serve as a seed source of accurately identified species accessions for such efficacy comparisons using common nursery herbicides.

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Appendix A. Exsiccatae.

Appendix A. List of exsiccatae. Herbarium abbreviations follow Holmgren et al. (1990). Accession information, Collector, Number (Herbarium);

C. corymbosa- **NC** HKR2-1 *Post & Adkins 84* (NCSC); **OR** COR 1-1 *Altland s.n.*(NCSC).

C. hirsuta- **USA: MS** OMS1-1 *Bryson 20610* (NCSC); OMS2-1, *Bryson 20611*, (NCSC); WMS 1-1 *Bryson 20615* (NCSC); HFL7-1 *Post 51* (NCSC);

***C. flexuosa* Withering-** **USA: NC** HFL1-1 *Post 45* (NCSC); HFL2-1 *Post 46* (NCSC); HFL3-1 *Post 47* (NCSC); HFL4-1 *Post 48* (NCSC); HFL5-1 *Post 49* (NCSC); HFL6-1 *Post 50* (NCSC); (NCSC); HKR2-2 *Post & Adkins 84* (NCSC); HNR1-1 *Post & Adkins 75* (NCSC); HNR1-2 *Post & Adkins 75* (NCSC); HNR2-1 *Post & Adkins 76* (NCSC); ICR2-1 *Post & Adkins 78* (NCSC); SMT1-1 *Post 58* (NCSC); SMT2-1 *Post 59* (NCSC); SMT3-2 *Post 60* (NCSC); SMT4-2 *Post 61* (NCSC); SMT5-1 *Post 62* (NCSC); SMT6-1 *Post 63* (NCSC); SMT7-1 *Post 64* (NCSC); STN1-1 *Post 65* (NCSC); STN2-1 *Post 66* (NCSC); STN3-2 *Post 67* (NCSC); TGA1-1 *Post 56* (NCSC); TGA1-2 *Post 56* (NCSC); TGA2-3 *Post 56a* (NCSC); ZKA1-3 *Post 52* (NCSC); ZKA2-3 *Post 53* (NCSC); **NY** PPG3-1 *Post 87* (NCSC); PPG4-1 *Post 88* (NCSC); **OR** ORY2-1 *Altland s.n.* (NCSC);

Appendix B. Photographs.

Cardamine corymbosa



A.



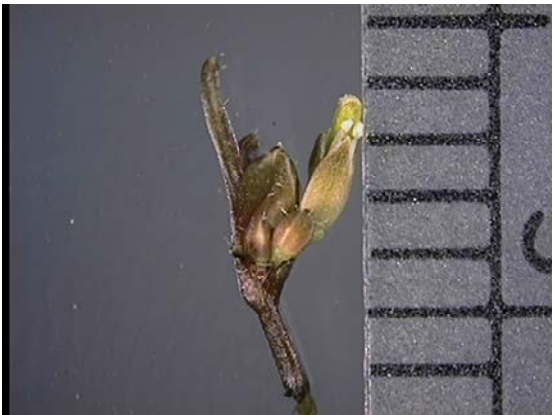
B.



C.



D.



E.



F.



G.



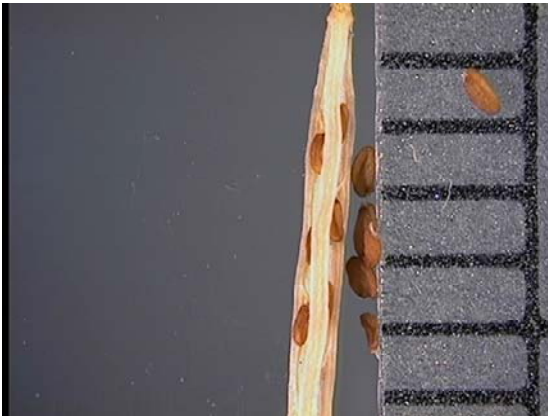
H.



I.



J.



K.



L.



M.



N.

Figure B.1. Photographs of *Cardamine corymbosa*. A) Habit; B) Seedling; C) Basal leaves; D, E) Floral rosette; F) Flower side view G) Flower with one sepal removed; H) Elongating silique with sepals and stamens still attached; I) Elongating silique with sepals removed; J) Silique showing valves rolled up on each side; K) Silique showing seeds on either side of replum; L) Seed attachment; M) Seeds; N) Corymb.

Cardamine hirsuta



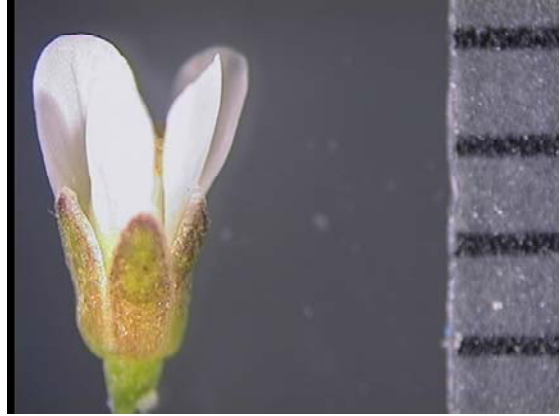
A.



B.



C.



D.



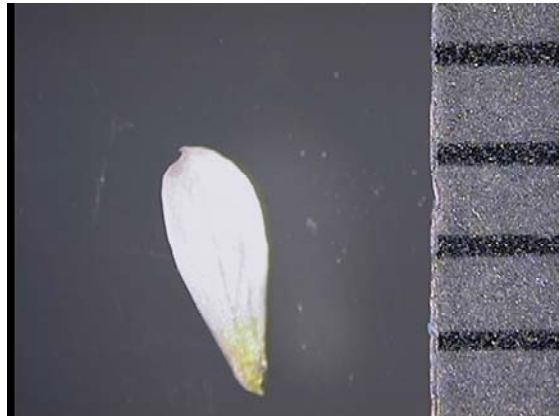
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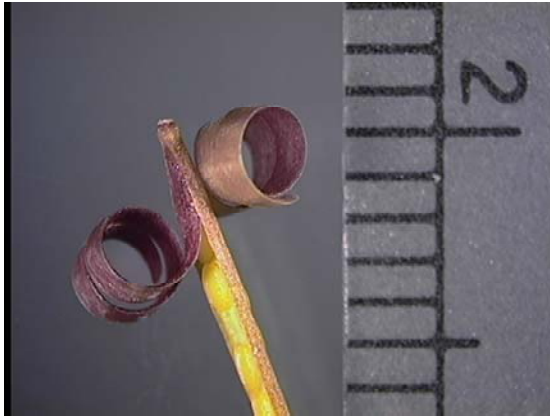
F.



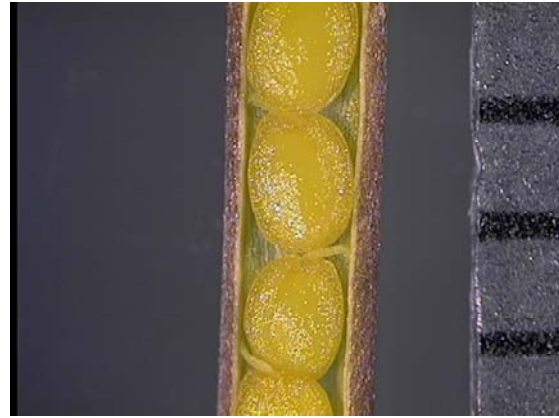
G.



H.



I.



J.



K.

Figure B.2. Photographs of *Cardamine hirsuta* L. A, B) Cauline leaves showing surface hairs; C) floral rosette; D) Flower side view; E) Flower top view; F) Flower with one petal removed; G) Flower with two petals removed; H) Petal; I) Silique showing valves rolled up on each side; J) Seed attachment; K) Seeds.

Cardamine flexuosa



A.



B.



C.



D.



E.



F.

Figure B.3. Photographs of *Cardamine flexuosa* A) Terminal leaflet of basal leaf; B) Floral rosette; C, D) Flower top view; E) Flower side view; F) Flower with one petal and sepal removed.

Cardamine oligosperma



A.



B.



C.



D.



E.



F.

Figure B.4. Photographs of *Cardamine oligosperma* Nutt. A) Habit; B) Basal rosette; C) Floral rosette D) Siliques; E) Flower side view; F) Flower top view.

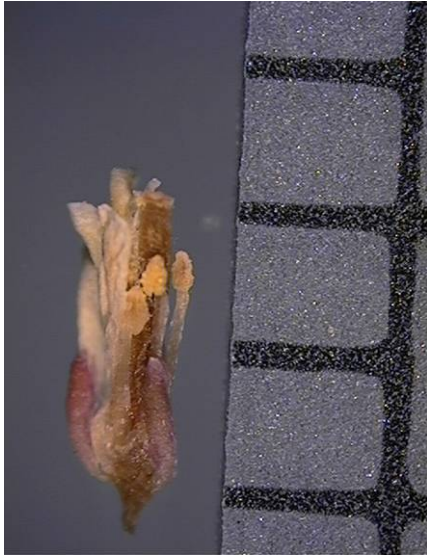
Cardamine scutata



A.



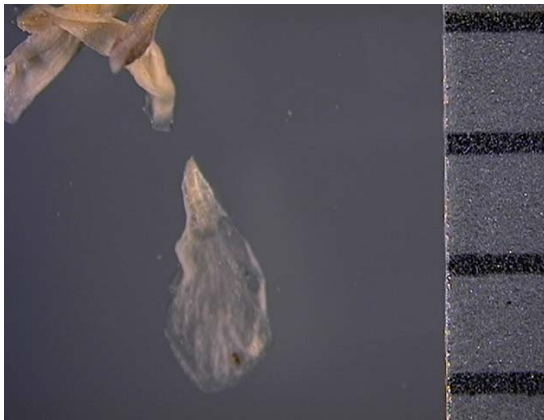
B.



C.



D.



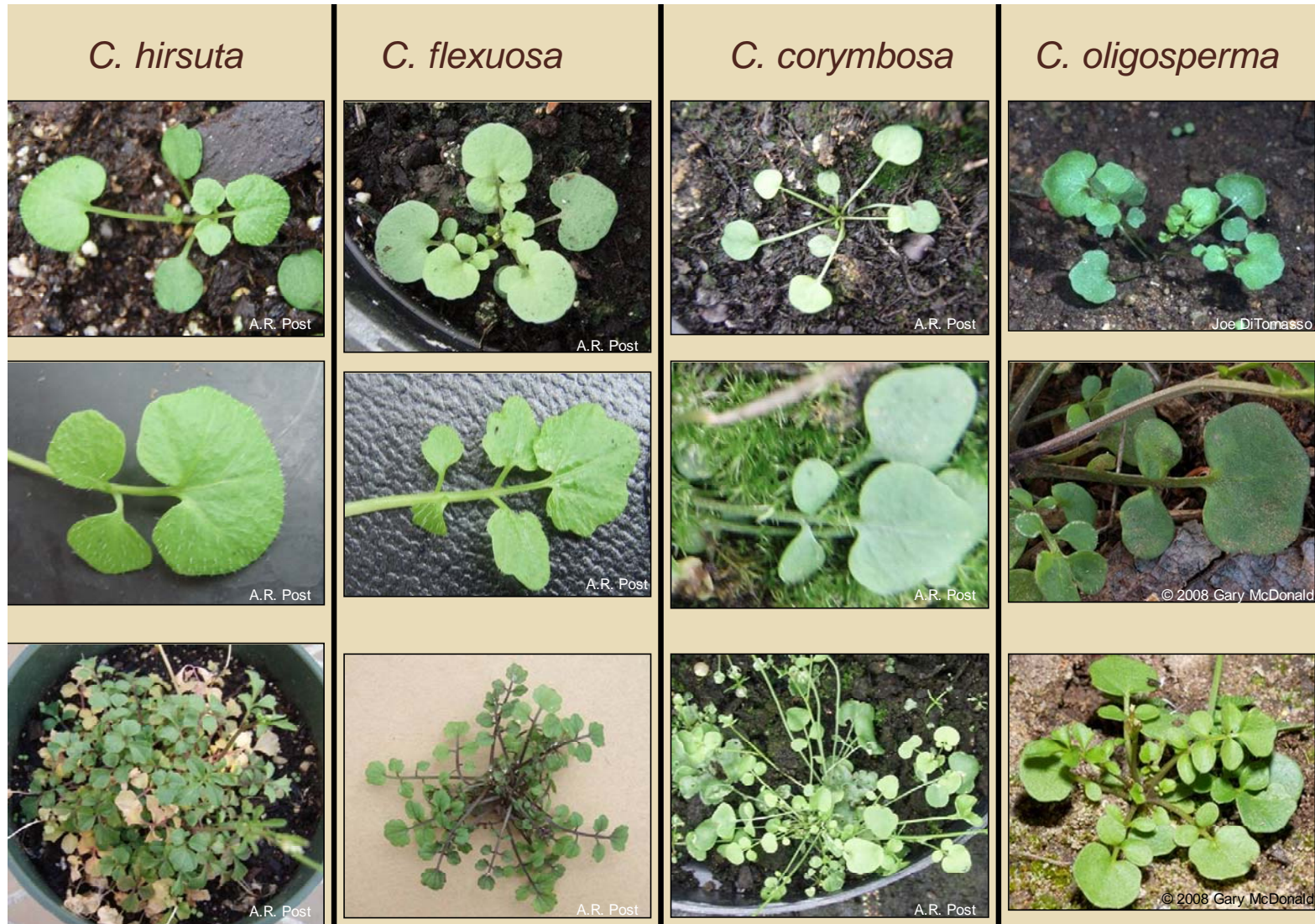
E.



F.

Figure B.5. Photographs of *Cardamine scutata* Thunb. A) Cauline leaves; B) Floral rosette; C, D) Flower side view with one petal removed; E) Petal; F) Silique and seed. Photographs from pressed and re-hydrated material.

Vegetative Comparison



Reproductive Comparison

