

Development of Seedless Taxa of Popular Invasive Landscape Plants[®]

Alan G. Smith and Benjamin M. Clasen

Department of Horticultural Sciences, University of Minnesota, St. Paul, Minnesota 55108 U.S.A.

INTRODUCTION

The University of Minnesota Department of Horticultural Sciences has a long tradition of plant breeding and improvement of landscape plants. Our efforts balance the development of new plant introductions with mission-oriented research to solve problems and curiosity-driven discovery. Several publications review the history and productivity of the department's programs (Meyer, 2000; Davis and Gregor, 2008; West, 2009; <<http://www.extension.umn.edu/>>). This article will focus on our research efforts to produce non-invasive selections of popular plants used in the nursery industry.

Reichard and White (1997) define an invasive plant as "one that has or is likely to spread into native flora and managed plant systems, develop self-sustaining populations, and become dominant or disruptive (or both) to those systems." Invasive species are a primary threat to biodiversity on the planet, second only to habitat destruction, and are one of the least reversible of all human impacts on the environment. An invasive plant is one that is likely to spread to new areas and develop self-sustaining populations, which may disrupt the invaded ecosystem. Many non-native invaders have been intentionally introduced to new areas for cultivation as ornamental plants. The enthusiasm of growers and consumers for novel ornamental plants drives plant breeders and others to develop cultivars from non-native species. Several characteristics contribute to invasive potential; however a major determinant for many invasive plants is seed production. Plants that are sterile, or cannot set seed have a greatly reduced invasive potential. We discuss here two broad strategies to produce non-invasive selections of invasive plants using two approaches: biotechnology and mutagenesis breeding. An essential factor for the application of either strategy is that the aesthetic quality and horticultural characteristics of the plant are maintained or improved.

INVASIVE PLANTS AND THE GREEN INDUSTRY

The harm caused by invasive plants can be economic and ecological. Although many of the non-native invasive species thriving in North America were introduced many years ago, the enthusiasm for novelty by gardeners makes plant collection and introduction profitable for growers of woody and herbaceous plant material. Continued interest in development of novel landscape species of both herbaceous and woody types has led to a dramatic increase in the number of cultivated species offered by the vegetative and seed industries (Janick, 1999). The green industry has historically been a significant contributor of invasive plants (Wells et al., 1986). Many popular invasive landscape plants are still being produced and sold today.

Most cultivated horticultural species lack the ability to compete with native taxa and their continued existence in the landscape depends on remaining in cultivated sites. The majority of important horticultural crops do not develop into noxious weeds, despite worldwide cultivation for centuries. Although only a small propor-

tion of introduced or cultivated plants become invasive, the few that have become established outside cultivation can have significant environmental and economic impacts (Ruessink et al., 1995; Ewel et al., 1999). Examples of ornamental invasives inherently invasive or that have become invasive through adaptation or hybridization are shown in Table 1.

Table 1. Examples of ornamental invasives (inherently invasive or that have become invasive through adaptation or hybridization) [(Amrine and Stasny, 1993; Archibold et al., 1997; Carr and Crisci, 1988; Deering and Vankat, 1999; Egolf, 1970; Goldblatt and Raven, 1997; Hickman, 1993; Lindstrom et al., 2002; Pooler et al., 2002; Pyšek et al., 1995; Randall and Marinelli, 1996; Raven and Gregory, 1972; Westbrooks, 1998)].

butter and eggs	<i>Linaria vulgaris</i>
butterfly bush	<i>Buddleja</i>
Chinese privet	<i>Ligustrum sinense</i>
cleome	<i>Cleome hassleriana</i>
fountain grasses	<i>Pennisetum purpureum</i> , <i>P. polystachion</i> , <i>P. pedicellatum</i> , <i>P. setaceum</i>
giant hogweed	<i>Heracleum mantegazzianum</i>
hibiscus	<i>Hibiscus syriacus</i>
honeysuckles	<i>Lonicera maackii</i> , <i>L. morrowii</i> , <i>L. tatarica</i> , <i>L. japonica</i>
Hottentot fig	<i>Carprobrotus edulis</i>
Japanese knotweed	<i>Fallopia japonica</i>
jimson weed	<i>Datura stramonium</i>
kochia	<i>Kochia scoparia</i>
lantana	<i>Lantana camara</i>
Mexican mint	<i>Agastache rupestris</i>
oxeye daisy	<i>Leucanthemum vulgare</i>
pampas grass	<i>Cortaderia selloana</i>
perennial sweet pea	<i>Lathyrus latifolius</i>
purple loosestrife	<i>Lythrum salicaria</i>
Scotch broom	<i>Cytisus scoparius</i>
some maples	<i>Acer tataricum</i> subsp. <i>ginnala</i> , <i>A. platanoides</i> , <i>A. pseudoplatanus</i> , <i>A. negundo</i>
sunflower	<i>Helianthus annuus</i>
tamarix	<i>Tamarix ramosissima</i> , <i>T. chinensis</i> , <i>T. parviflora</i>
teasel	<i>Dipsacus laciniatus</i>
verbena	<i>Verbena bonariensis</i>
wild rose	<i>Rosa multiflora</i>

STERILITY TO ELIMINATE INVASIVENESS

Invasive plant species spread via sexual (seed) or asexual (vegetative) propagules. Invasive terrestrial plants are predominantly seed dispersed (Moreira, 1975). *Rhamnus cathartica*, formerly among the most popular landscape ornamentals and now one of the most infamous terrestrial invasive plants, can establish large seed banks with approximately 620 seeds/m² beneath mature shrubs (Archibold et al., 1997). It is likely that the high seed set of this and other species contributes greatly to their invasive potential. Seedlessness is therefore a target for reducing or eliminating invasiveness. Sheppard et al. (2002) theorized that a 62% reduction in *Cytisus scoparius* seed set would suppress spread in native grasslands. We are developing methods to minimize or eliminate seed production via genetic mechanisms and thereby prevent invasiveness of valuable landscape plants with invasive potential.

Sterility is also useful in preventing pollen production to reduce allergies (Ogren, 2003). In landscaping, male plants of dioecious crops are used to prevent messy fruit production in ginkgo (*Ginkgo biloba*) and kiwi [*Actinidia deliciosa* (syn. *A. chinensis*)]. Conventional breeding can create sterility in only a fraction of all crops, sometimes by crossing parents with different ploidy levels (tetraploid × diploid, 4x × 2x) to create sterile triploids (3x). Sterility must not interfere with the ornamental qualities (floral, foliar, etc.) conferring market value. Examples of plants where ornamental quality was not sacrificed in order to achieve non-invasiveness include sterile aspen, cottonwood, and pine trees that maintain their ornamental qualities and may have increased growth rates (Eis et al., 1965; Rishi et al., 2001).

GENETIC MODIFICATION FOR STERILE PLANT PRODUCTION

Genetic engineering of male and female sterility to reduce invasiveness can have broad applicability to many potentially invasive species. Broad applicability is a critical consideration when selecting a strategy to prevent invasive species in an industry where thousands of diverse genera have been introduced and are cultivated (Havey, 2004). Genetic engineering of plants for sterility can be accomplished without greatly altering the inflorescence or flower phenotypes in landscape crops.

Our goal is to use genetic engineering to produce sterile alternatives of well-known cultivars using a strategy to prevent the development of specific tissues in the pistil and the stamen that are required for seed production. This method uses the targeted expression of a cytotoxic gene to specific organs or tissues essential to reproduction. Both RNase-T1 from *Aspergillus oryzae* and barnase from *Bacillus amyloliquefaciens* have been used to prevent the development of specific reproductive tissue to cause sterility (Gardner et al., 2009). The key to producing sterile plants with a cytotoxic gene is targeting the gene to specific reproductive tissue. Fortunately, many reproductive and tissue-specific regulators are available with floral-specific expression. The ability to block the cytotoxicity of barnase using the inhibitor protein barstar (also isolated from *B. amyloliquefaciens*) is very advantageous. In an elegant set of experiments, Mariani et al. (1992) showed that expression of barnase in the tapetum caused male sterility, which could be reversed by hybridizing with plants that carried barstar gene.

Several male- and female-specific regulatory genes with stringent regulation have been isolated. For male sterility, the *Solanum esculentum* (syn. *Lycopersicon esculentum*) stamen-specific regulator from a gene (108-CRP; Aguirre and Smith, 1993; McNeil and Smith, 2005) was selected. Female sterility was accomplished

using a pistil-specific regulator in combination with the barnase gene to prevent the development of tissue essential for female reproduction. The SP41-related DNA sequence from *Nicotiana tabacum* 'Samsun' that drives expression specifically in styles (Sessa and Fluhr, 1995; Gardner et al., 2009) was used. The introduction of the male- and female-sterility genes into *N. tabacum* demonstrated that this combination of genes produces sterile plants (Gardner et al., 2009; and Fig. 1). The male-sterility gene inhibits pollen development very early. Introduction of the female-sterility gene resulted in a necrotic stigma that prevents pollen adherence and germination. The introduction of these genes is applicable to decreasing invasiveness through production of sterile plants. These data demonstrated the effectiveness of the strategy that prevents the development of essential tissue for male- and female-sterile plant production.

MUTAGENESIS BREEDING FOR STERILE PLANT PRODUCTION

Mutagenesis breeding is a variation of conventional plant breeding, where whole plants or their propagules are exposed to a mutagenic agent resulting in chromosomal mutations, some of which will result in sterility. In general, mutations provide plant breeders with a diversity of plant phenotypes. Our research tested the efficacy of γ -radiation mutagenesis with previously untested species to induce sterility. Sterility is achieved by exposing cells to high energy radiation and disrupting chromosome structure, or altering the number of chromosomes within cells, thereby preventing homologous pairing during meiosis. Chromosome structure can be altered in the form of deletions, additions, translocations, inversions, and rearrangements (van Harten, 1998).

Many mutagenic agents, including chemical and physical (radiation) agents have been tested and descriptions of their origin and use in plant breeding have been thoroughly reviewed (van Harten, 1998). X-radiation and γ -radiation are highly ionized, penetrating forms of radiation that are similar with respect to inducible mutation frequency, and are the most efficient systems for producing high frequency mutations in plants (Evans, 1960; Gunckel, 1957). Mutations including increased disease resistance, novel variegation, reduced lodging in grain crops, and sterility have been induced in a wide variety of crops using γ -radiation mutagenesis, and over 1800 mutant varieties have been released (Osborne and Lunden, 1961).

The type of propagule used, and the dose of radiation applied in a mutagenesis breeding project depends largely on the specific objectives of the project as well as the plant material and resources available. Seeds, vegetative propagules (dormant buds, stem cuttings, scions), pollen, and tissue culture callus are all suitable targets for irradiation experiments and each have their own advantages and disadvantages; seeds were the propagules selected for this study.

Seeds require very little preparation or space during irradiation, are easily stored, and can be directly sown into the field. These conveniences allow for irradiation of large numbers of seeds leading to a greater chance of finding a desirable mutant. However, in highly heterozygous crops, such as many woody perennials, there is often little chance that seeds will share all of the valuable phenotypes of their cultivated parent, so the selection of a desirable phenotype which carries all of the induced mutations of interest could be onerous.

Choosing efficient radiation doses to induce mutations can be determined systematically. Doses are selected that induce a high frequency of mutations while limiting lethality. A variety of factors including seed moisture content, dormancy stage, physical structure, chromosome size, and chromosome number can affect sensitivity to radiation, which makes *a priori* predictions about the sensitivity of plant material to radiation inexact (Konzak, 1957; Lunden, 1964; Sparrow et al., 1968).

The ornamental horticulture industry would benefit greatly from non-invasive taxa of *A. platanoides* 'Crimson King' and 'Emerald Lustre' and *A. tataricum* subsp. *ginnala* 'Compactum'. The sale of *A. platanoides* is prohibited in Massachusetts and is closely regulated in Connecticut. All of these species are also considered in-

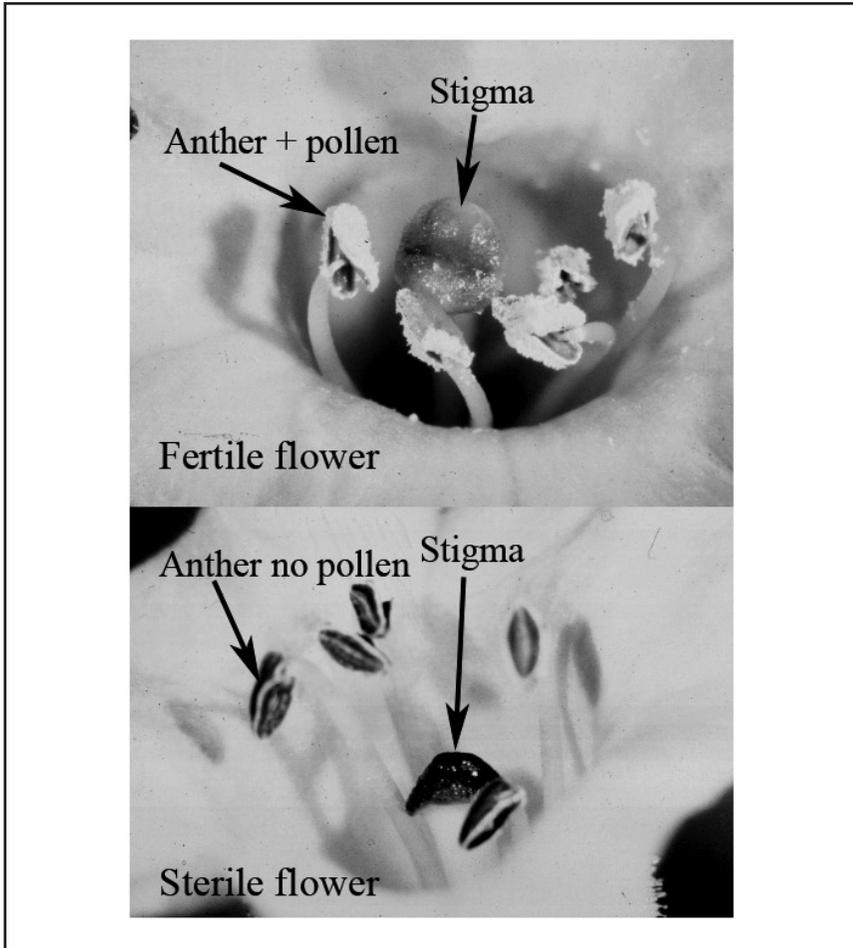


Figure 1. Photograph of a fertile flower (top photo) versus a sterile flower (bottom photo) of *Nicotiana tabacum*. The sterile flower is the result of introducing the male- and female-sterility genes into the plant. The male-sterility gene inhibits pollen development, which prevents pollen production. The female-sterility genes prevent full development of the stigma resulting in necrosis and the inhibition of pollen adherence and germination.



Figure 2. Seedlings with novel leaf shape phenotypes. Seeds were treated with varying doses of gamma radiation. Top Left: *Acer tataricum* subsp. *ginnala*. Top Right and Bottom: *A. platanoides*.

vasive in one or more states (USDA Plants Database, 2011). These plants have high ornamental appeal and are very popular crops, valued at over 7 million dollars per year to the Connecticut horticulture industry alone (Heffernan, 2004). Prohibiting these crops would be economically damaging to the horticulture industry and detrimental to consumers who would lose plant choices. The objective of this study was to assess the radiation sensitivity of specific genotypes based on median lethal dose (LD_{50}) and use that information to produce sterile and novel selections.

All plant material was irradiated in a Mark I $^{137}\text{Cesium}$ irradiator to determine sensitivity to radiation and to determine the LD_{50} of each species. The applied dose rate was 537 rads/min.

Seeds of *A. platanoides* 'Crimson King' and 'Emerald Lustre' and *A. tataricum* subsp. *ginnala* 'Compactum' were collected and irradiated within 2 days. Seeds were sown directly in the field and were subsequently covered with sand and hay. Seedling emergence was measured the following spring and survival rates were calculated as the proportion of plants that emerged throughout the growing season compared to the total number of seeds planted for each treatment/replication. Due to the protracted juvenile phase, sterility will be assessed as plants mature and flower. However, photographs of novel leaf-shape phenotypes generated by mutagenesis treatments show the effectiveness of these treatments (Fig. 2). We found that doses in excess of 50 kR were lethal to both species, and are unnecessary to use during the production of sterile plants. By further assessing survival over several years, the median lethal doses will be determined. The correlations calculated be-

tween radiation dose and survival for each species will be used to increase the efficiency of irradiation treatments used during the future production of sterile plants.

CONCLUSIONS

It is unlikely that plant exploration and the cultivation of non-native plants in new locations will be discontinued. However, it is likely that as new plants become problematic because of their invasiveness, there will be self-imposed or legislative restriction on their cultivation and sale. As sexual reproduction is one of the most important factors in determining the invasive potential of ornamental plants, producing sterile cultivars is a practical strategy. After the production of sterile varieties, it will be important to assess fertility levels, reduction in invasive potential, the effects of the introduced genes on plant growth and development, and the stability of the sterility. It is also incumbent on the green industry to educate consumers on the risks of invasive plants and the benefits of using non-invasive alternatives.

LITERATURE CITED

- Aguirre, P.J., and A.G. Smith.** 1993. Molecular characterization of a gene encoding a cysteine-rich protein preferentially expressed in anthers of *Lycopersicon esculentum*. *Plant Mol. Bio.* 23:477–487.
- Amrine, J.W., and T.A. Stasny.** 1993. Biocontrol of multiflora rose, pp. 9–21. In: McKnight, B.N. (ed) *Biological pollution: The control and impact of invasive exotic species*, Indiana Academy of Science, Indianapolis, Indiana.
- Archibold, O.W., D. Brooks, and L. Delaney.** 1997. An investigation of the invasive shrub European Buckthorn, *Rhamnus cathartica* L., near Saskatoon, Saskatchewan. *Can. Field-Nat.* 111:617–621.
- Carr, B.L., and J.V. Crisci.** 1988. Cladistic analysis of *Gaura*. *Amer. J. Bot.* 75:219.
- Davis, S., and J. Gregor.** 2008. *Northern treasure: The Minnesota Landscape Arboretum and Horticulture Research Center*, University of Minnesota. Afton Press, Afton, Minnesota, U.S.A.
- Deering, R.H., and J.L. Vankat.** 1999. Forest colonization and developmental growth of the invasive shrub *Lonicera maackii*. *Amer. Midl. Nat.* 141:43–50.
- Egolf, D.R.** 1970. *Hibiscus syriacus* 'Diana', a new cultivar [Malvaceae]. *Baileya* 17:75–78.
- Eis, S., E.H. Garman, and L.F. Ebell.** 1965. Relation between cone production and diameter increment of Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco), grand fir (*Abies grandis* [Doug.] Lindl.), and western white pine (*Pinus monticola* Dougl.). *Can. J. Bot.* 43:1553–1559.
- Ewel, J., D.J. O'Dowd, J. Bergelson, C.C. Daehler, C.M. D'Antonio, L.D. Gomez, D.R. Gordon, R.J. Hobbs, A. Holt, K.R. Hopper, C.E. Hughs, M. LaHart, R.B. Leakey, W.G. Lee, L.L. Loope, D.H. Lorence, S.M. Louda, A.E. Lugo, P.B. McEvoy, D.M. Richardson, and P.M. Vitousek.** 1999. Deliberate introductions of species: research needs. *BioScience* 49:619–630.
- Evans, H.J.** 1960. Chromatid aberrations induced by gamma irradiation. I. The structure and frequency of chromatid interchanges in diploid and tetraploid cells of *Vicia faba*. *Genetics* 46(3):257–275.
- Gardner, N., R. Felsheim, and A.G. Smith.** 2009. Production of male- and female-sterile plants through reproductive tissue ablation. *J. Plant Physiol.* 166:871–881.
- Goldblatt, P., and P.H. Raven.** 1997. *FSA contributions 9: Onagraceae*. *Bothalia* 27:149–165.
- Gunckel, J.E.** 1957. The effects of ionizing radiation on plants: Morphological effects. *Quart. Rev. Biol.* 32(1):46–56.
- Havey, M.J.** 2004. A new paradigm for the breeding of longer-generation hybrid crops. *Acta Hort.* 637:31–39.
- Hickman, J.C.** 1993. *The Jepson Manual: Higher plants of California*. Univ. of Calif. Press, Berkeley.

- Heffernan, B.** 2004. Economic impact of banning plants on the invasive list. A Report for the Connecticut Invasive Species Council.
- Janick, J.** 1999. Perspectives on new crops and new uses. Proceedings of the Fourth National Symposium New Crops and New Uses: Biodiversity and agricultural sustainability. American Society for Horticultural Science Press, Alexandria.
- Konzak, C.F.** 1957. III. Genetic effects of radiation on higher plants. The University of Chicago Press.
- Lindstrom, J.Y., G.T. Bujarski, M.J. Love, and B.M. Burkett.** 2002. *Buddleja* breeding at the University of Arkansas. Proc. Southern Nursery Assoc. 47:630–633.
- Lunden, A.O.** 1964. Seed embryo features and irradiation response. Radiat. Bot. 4:429–437.
- Mariani, C., M. DeBeuckeleer, J. Truettner, J. Leemans, and R.B. Goldberg.** 1990. Induction of male sterility in plants by a chimaeric ribonuclease gene. Nature 347:737–741.
- McNeil, K.J., and A.G. Smith.** 2005. An anther-specific cysteine-rich protein of tomato localized to the tapetum and microspores. J. Plant Physiol. 162:457–464.
- Meyer, M.H.** 2000. 150 years of hardy plants. University of Minnesota Agricultural Experiment Station, St. Paul, Minnesota, USA.
- Moreira, I.** 1975. Propagacao por semente do *Cynodon dactylon* (L.) Pers. Anais do Instituto Superior de Agronomia 35:95–112.
- Ogren, T.** 2003. Allergy reduction benefits of pollenless landscape plants, pp. 7–9. In: Meeting Summary, Modifying Reproduction in Urban Trees, 12–13 Feb. 2003. North Carolina Biotechnology Center.
- Osborne, T.S., and A.O. Lunden.** 1961. The cooperative plant and seed irradiation program of the university of Tennessee. Intl. J. Appl. Radiat. Isot. 10:198–209.
- Pooler, M.R., R.L. Dix, and J. Feely.** 2002. Interspecific hybridizations between the native bittersweet, *Celastrus scandens*, and the introduced invasive species, *C. orbiculatus*. Southeastern Naturalist 1:69–76.
- Pyšek, P., K. Prach, M. Rejmanek, and M. Wade.** 1995. Plant invasions: General aspects and special problems, SPB Academic Publishing, Amsterdam.
- Randall, J.M., and J. Marinelli.** 1996. Invasive plants: Weeds of the global garden, Brooklyn Botanic Garden, New York, New York.
- Raven, P.H., and D.P. Gregory.** 1972. A revision of the genus *Gaura* (Onagraceae). Mem. Torrey Bot. Club. 1:1–96.
- Reichard, S.** 1997. Prevention of invasive plant introductions on national and local levels, pp. 215–227. In: J.O. Luken and J.W. Thieret (eds.) Assessment and management of plant invasions, Springer-Verlag, New York.
- Rishi, A.S., N.D. Nelson, and A. Goyal.** 2001. Improvement of *Populus* through genetic engineering. Indian J. Plant Physiol. 6:119–126.
- Ruessink, J.L., I.M. Parker, M.J. Groom, and P.M. Kareiva.** 1995. Reducing the risks of nonindigenous species introductions. BioScience 45:465–477.
- Sessa, G., and R. Fluhr.** 1995. The expression of an abundant transmitting tract-specific endoglucanase (SP41) is promoter-dependent and not essential for the reproductive physiology of tobacco. Plant Mol. Biol. 29:969–982.
- Sheppard, A.W., P. Hodge, Q. Paynter, and M. Rees.** 2002. Factors affecting invasion and persistence of broom *Cytisus scoparius* in Australia. J. Appl. Ecol. 39:721–734.
- Sparrow, A.H., A.F. Rogers, and S.S. Schwemmer.** 1968. Radiosensitivity studies with woody plants-I Acute gamma Irradiation Survival Data for 28 species and Predictions for 190 Species. Radiat. Bot. 8:149–186.
- USDA, NRCS.** 2011. The PLANTS database (<<http://plants.usda.gov>>, 18 Feb. 2011). National Plant Data Center. Baton Rouge, Louisiana.
- van Harten, A.M.** 1998. Mutation breeding: theory and practical applications. Cambridge Univ. Press, Cambridge, United Kingdom.

-
- Wells, M.J., A.A. Balsinhas, H. Joffe, V.M. Engelbrecht, G. Harding, and C.H. Stirton.** 1986. A catalogue of problem plants in southern Africa, incorporating the national weed list of South Africa. *Memoirs of the Botanical Survey of South Africa* 53:1–658.
- West, A.** 2009. *Minnesota hardy: Showcasing new and enduring plants for your landscape.* University of Minnesota Agricultural Experiment Station, St. Paul, Minnesota, U.S.A.
- Westbrooks, R.** 1998. *Invasive plants: Changing the landscape of America: fact book,* Federal Interagency Committee for the Management of Noxious and Exotic Weeds, Washington D.C.