PROPAGATION, HYBRIDIZATION AND GENETIC MANIPULATION OF *OENOTHERA* SPECIES

By

STEPHEN D. STANPHILL

Bachelor of Science in Forestry

Oklahoma State University

Stillwater, Oklahoma

2004

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2013

PROPAGATION, HYBRIDIZATION AND

GENETIC MANIPULATION OF

OENOTHERA SPECIES

Thesis Approved:

Bruce L. Dunn

Arthur R. Klatt

Justin Q. Moss

ACKNOWLEDGEMENTS

I would like to acknowledge the help of my major professor and thesis advisor, Dr. Bruce Dunn for his guidance and instruction, my fellow graduate students as well as the faculty and staff of the department of Horticulture and Landscape Architecture of Oklahoma State University. I also would like to recognize the unending help and support of my family.

Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University.

Name: STEPHEN D. STANPHILL

Date of Degree: JULY, 2013

Title of Study: PROPAGATION, HYBRIDIZATION AND GENETIC MANIPULATION OF *OENOTHERA* SPECIES

Major Field: HORTICULTURE

Abstract: *Oenothera* L. (*Onagraceae*) is a perennial herb native to North and South America. Three species were treated with 0.6% solutions of ethyl methanesulfonate and five toxic heavy metals at two concentrations to induce phenotypic mutations. Addition of metals had some significant effects on seed germination and survival. Ethyl methanesulfonate treatments had varying effects on the seedling mortality rates of each *Oenothera* species. Phenotypic mutants were induced in all three species, but did not produce viable seed, nor maintain stable chlorophyll mutations. In the second experiment, a rooting protocol was developed for two *Oenothera* species. Cuttings were propagated in both vermiculite and perlite to determine the better rooting media. Three indole-3-butyric acid commercial rooting hormones were applied to cuttings to determine any difference in efficacy versus a control of non-treated cuttings. Vermiculite produced significantly more and longer roots in both species. Significantly more cuttings of *Oenothera pallida* rooted in vermiculite than in perlite. None of the three IBA treatments produced significantly greater effect than the control. Slightly significant effect was observed in the interaction of media and hormone. In the third experiment, twelve *Oenothera* species were crossed with allied genera of the *Onagraceae* family in order to incorporate desirable heritable characters. Interspecific crosses were also made among the twelve *Oenothera* species. Very few seeds were produced from the intergeneric *Onagraceae* crosses. Viable seeds which were produced showed no signs of the desired heritable characters. One interspecific cross from the *Oenothera* section *Hartmannia* (Spach) Munz produced a potential hybrid.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

LITERATURE REVIEW

The genus *Oenothera* L. belongs to the family *Onagraceae* and contains 145 species. The genus contains 15 subgenera which include *Anogra* (Spach) Jeps., *Chylismia* (Torr. & A. Gray) Jeps., *Eulobus* (Nutt. ex Torr. & A. Gray) Munz, *Oenothera* Torr. & A. Gray, *Gauropsis* (Torr. & Frém.) Munz, *Hartmannia* (Spach) Munz, *Heterostemon* Nutt., *Kneiffia* (Spach) Munz, *Lavauxia* (Spach) Jeps., *Megapterium* (Spach) Munz, *Pachylophus* (Spach) Jeps., *Raimannia* (Rose ex Britton & A. Br.) Munz, *Renneria* H.P. Fisch., *Sphaerostigma* (Ser.) Jeps. ex Munz, and *Taraxia* (Torr. & A. Gray) Jeps. (Missouri Botanical Garden, 2013). Evening primrose is the most often used common name for species in the genus *Oenothera*. Other common names include suncup or sundrop.

Light Absorption

Many of the *Oenothera* species are very tolerant of direct sunlight. Dement and Raven (1974) identified the pigments responsible for the contrasting ultraviolet patterns which identified several species of *Oenothera* to insect pollinators. The UV pattern interaction with nocturnal pollinators has also been investigated in conjunction with floral chemical attractants (Kawaano et al. 1995). The mechanisms for protection

from and acclimation to solar UV-B rays by *Oenothera stricta* Ledeb. ex Link were identified (Robberecht and Caldwell, 1983).

Range

The genus has a broad habitat range, with 66 species native to the U.S. and represented by at least one species in every state excluding Alaska. Although some species are considered weeds, many of the species are grown in gardens or have commercial interest. The spread of invasive *Oenothera spp.* throughout Europe over the last 200 years was studied by Mihulka and Pyšek (2001). Populations originating from South America were found largely in oceanic regions while North American species were found inland.

Evening Primrose Oil

Many varieties of the species *Oenthera biennis* L. are grown worldwide as an oil crop; *Oenothera* seed oil is used as a dietary supplement for the treatment of medical conditions ranging from eczema to rheumatoid arthritis (Immel, 2001). The oil content and chemical composition of evening primrose seed was described by B.J.F. Hudson (1984). Seeds were found to contain 15% protein, 24% oil and 43% cellulose plus lignin. The oil component fatty acids of *Oenothera* spp. seed contained up to 14% gamma-linolenic acid, an omega-6 fatty acid. Seed anatomy of 32 species representing the 15 sections of *Oenothera* were explored in a systematic and evolutionary study by Tobe et al. (1987). It was found that the distinct differences in seed coat tissues, particularly the exotesta, mesotesta and endotesta were indicative of the evolutionary lineages for each species.

Life Cycles

Oenothera is a ubiquitous and benign herbaceous genus of primarily perennials, though some species have an annual or biennial growth habit, depending on the individual drought tendencies of the native climate in which each species evolved (Evans et al., 2005). Evans et al. later investigated the impact of climate change on two subgenera of *Oenothera*: *Anogra* and *Kleinia* (2009).

Flowers and Pollinators

The flowers of *Oenothera* range from 2 to 5 cm in diameter. Flowers are also diverse and non-aromatic, with colors usually yellow, pink, or white. Many of the flowers are capable of blooming both day and night such as *Oenothera speciosa* Nutt., but several taxa including those in sections Lavauxia, Anogra, and Kleinia are exclusively night blooming (Raguso et al., 2007; Theiss et al., 2010). Pollinators of *Oenothera* include honeybees , flies, birds, and a variety of moths including the hawk-moths (Krakos, 2011). Hawk-moths are the primary pollinators of most of the night blooming evening primrose species. The honeybee and moth typically pollinate the day-bloomers. The behavior of these pollinators was shown to be significantly impacted by the structural changes in flowers due to grazing from herbivores (Mothershead and Marquis, 2000). Given ideal conditions found in nature, it was found that pollination from honeybees could achieve complete seed-set in *Oenothera fruticosa* L. (Silander and Primack, 1978).

Genetic Mutations

Much work has been done in the field of genetic research and breeding with regard to the *Oenothera*. The genus was studied extensively by renowned pioneer of plant genetics Hugo De Vries in 1886. De Vries (1915) determined the coefficient of mutation for *Oenothera biennis* L. and *Oenothera lamarckiana* Auct. In a culture of 8500 *O. biennis* specimens, 3 different types of mutants were observed to spontaneously emerge. He classified them *nanella* (0.1%), *semigigas* (0.05%) and *sulfurea* (0.3%). In *O. lamarckiana*, De Vries found higher numbers of *nanella* (1.5%) and *semigigas* (0.3%), but no evidence of the pigment mutation observed in *sulfurea*. Mass mutations of up to 50 percent were also observed in some strains of *Oenothera pratincola* Bartlett (Bartlett, 1915). The wide variety of species has been of special interest because of a tendency for genetic anomaly in nature of several sections (Cleland, 1936). The first spontaneous polyploid was discovered in *Oenothera lamarckiana*. The subsequent mutants and crosses were studied by R.R. Gates (1907). The most vigorous of these mutants was *Oenothera rubrinervis* Gates (1909). It was in *O. rubrinervis* that chromosome ring formation was first observed in *Oenothera*. Even numbers of four to 14 chromosomes often form varying numbers of linked rings in most species of *Oenothera*. Odd numbers of five or nine chromosomes, however, have been observed in trisomic specimens (Cleland, 1967). Chromosome catenation in *Oenothera* was expounded on later in other works (Darlington, 1929).

The structure and function of pollen formation in *Oenothera gigas*, as well as other *O. lamarckiana* mutant hybrids was outlined by Gates. It was explained how the tetraploid cells would reduce to diploid gametes, and yet retain the same volume (Gates, 1907,

1911). Gates showed the cytological makeup of the tetraploid hybrid *O. lata gigas* to be an uneven distribution of its 21 chromosomes. In somatic cells that were investigated, fourteen were paternally originated, and 7 were maternal (Gates, 1909). Incompatibility in *Oenothera* has been the subject of many studies. Many of the species of *Oenothera* have been found to be self-incompatible or incompatible with closely related genotypes. It has been observed that in several populations of a given *Oenothera* species (*Oenothera laciniata* Hill), as many as 15 genotypes, with a mean of 10.5 can exist within one population (Ellstrand and Levin, 1982). A series of four genotypically different populations of *Oenothera organensis* Munz were polycrossed to find that all four were completely self-sterile, and partially incompatible with their related genotypes (Emerson, 1939). Emerson determined that the self-sterility was due to the stylar rejection of pollen tube growth from any pollen cell carrying either of its allelomorph components (Emerson, 1940). Clonal propagation from vegetative cuttings was possible, but seed was found to be completely inviable. This self-sterility was later overcome by irradiating *O. organensis* pollen, resulting in self-compatible mutants (Lewis, 1949). The pollen tube growth of *O. organensis,* as in most *Oenothera* species, is more rapid in competition. Though it was found to be clonally repeatable, it has a low heritability rate (9.4%) in *O. organensis* (Havens, 1994).

Phylogenetic Revisions

Several newly discovered combinations were shown following a detailed botanical review of three *Oenothera* sections around the United States (Wagner, 1983). Within *Oenothera* section *Megapterium,* three new subspecies of *Oenothera macrocarpa* Nutt. (*O. macrocarpa* subsp. *incana, O. macrocarpa* subsp. *oklahomensis* and *O. macrocarpa*

subsp. *fremontii*), and one new species, *Oenothera howardii* A. Niels., (previously *Lavauxia howardii* A. Niels.) were defined from related populations around the southwest United States and northern Mexico. Two distinct *Oenothera* populations in Colorado from section *Pachylophus* were also identified. *Oenothera harringtonii* Wagner was determined as its own distinct species. It had previously been classified a variety of *Oenothera caespitosa* Nutt. *eximia* sensu Munz. Conversely, the species *Pachylophus macroglottis* Ryb. was redefined as *Oenothera caespitosa subsp. macroglottis* due to its distinguishing leaf morphology and habitat. In section *Oenothera*, *O. elata* H.B.K. *hirsutissima* (A. Gray ex S. Wats.) Dietrich was distinguished from its previous classification as *Oenothera biennis* L. var. *hirsutissima* A. Gray. Populations previously included in *Oenothera rhombipetala* sensu Munz. from around the eastern United States were found to have a continuous ring of 14 chromosomes, distinguishing them from the rest of the species. These populations were redefined as *Oenothera clelandii* Dietrich, Raven and W.L. Wagner. Also in section *Oenothera*, three populations from Arkansas, Nevada and Alabama of *Oenothera heterophylla* Spach. were found to be distinct and separate from the main species and reclassified as *Oenothera herophylla* subsp. *orientalis* Dietrich, Raven and W.L. Wagner. Wagner et al. (1985) outlined the systematics and evolution of the species *Oenothera caespitosa*, which includes five subspecies. The systematic of section *Kneiffia*, and its five recognized species were outlined by Straley (1977). Wagner collaborator, Werner Dietrich (1977) had previously published an account of the South American *Oenothera* section *Oenothera*, primarily a revision of the 57 taxa in subsection *Munzia* Munz, as well as a revision of subsection *Emersonia* (1985). Wagner published several revisions to the

existing taxa of *Oenothera*, including one new species (*Oenothera coryii* W.L. Wagner), three new sections (*Ravenia, Eremia* and *Contortae*), a subsection of *Pachylophus* called *Australis* (Wagner, 1986), as well as several species of a new subsection isolated from subsection *Raimannia* called *Oenothera* section *Oenothera* subsect. *Nutantigemma* Dietrich and Wagner (1987). Raven et al. (1979) had previously outlined the systematics of subsection *Euoenothera,* finding it to contain basic A, B, and C genomic complexes which remain homozygous or interbreed to form heterozygotic combinations of the three original genotypes composing all species within the subsection. The complete nucleotide sequence of a plastid chromosome was mapped from *Oenothera elata* Kunth, one of the major species of subsection *Euoenothera* (Hupfer et al., 2000). This map covered plastome one of the five distinguishable *Euoenothera* plastomes and provided a greater understanding of how many *Oenothera* species evolved their unique abilities for interspecific hybridization and complex heterozygosis.

Limited work has been done with regard to commercial breeding of *Oenothera* and its tendency for mutation has not been fully explored. Arnold and Kressel (1965) utilized several chemical and physical mutagens in an attempt to induce stable plasma mutations in several species of *Oenothera*, without success. Many of the *Oenothera* species show potential for cultivation as commercial ornamentals; however there have been only a few commercially available cultivars developed as bedding plants in the United States. The appeal of this genus as a commercial ornamental crop would be increased greatly by the incorporation of leaf morphology changes such as leaf variegation, or the incorporation of novel flower colors from related genera of plants.

LITERATURE CITED

Bartlett, H.H. 1915. Mass mutation in *Oenothera pratincola*. Bot. gaz. 60(6): 425-456.

Catcheside, D.G. 1933. Chromosome configurations in trisomic *Oenotheras*. Genetica, 15(3): 177-201.

Cleland, R.E. 1924. Meiosis in pollen mother cells of *Oenothera franciscana sulfurea*. Bot. Gaz. 149-170.

Cleland, R.E. 1929. New evidence bearing upon the problem of the cytological basis for genetical peculiarities in the *Oenotheras.* Amer. Nat. *63*(689): 497-510.

Cleland, R. E. 1936. Some aspects of the cyto-genetics of *Oenothera*. Bot. Rev. 2(6): 316-348.

Cleland, R.E. 1951. Extra, diminutive chromosomes in *Oenothera*. Evol. 165-176.

Cleland, R.E. 1967. The origin of closed circles of five chromosomes in *Oenothera.* Am. J. Bot. 993-997.

Davis, B.M. 1916. Hybrids of *Oenothera biennis* and *Oenothera franciscana* in the first and second generations. Genet. 1(3): 197.

Darlington, C.D. 1929. Ring-formation in *Oenothera* and other genera. J. Genet. 20: 345-64.

Dement, W.A. and P.H. Raven. 1974. Pigments responsible for ultraviolet patterns in flowers of *Oenothera (Onagraceae)*. Nature. 705-706.

DeVries, H. (1915). The coefficient of mutation in *Oenothera biennis* L. Bot. Gaz. 169-196.

Dietrich, W. 1977. The South American species of *Oenothera* sect. *Oenothera* (*Raimannia, Renneria; Onagraceae*). Ann. Misso. Bot. Gard. 425-626.

Dietrich, W., P.H. Raven, and W.L. Wagner. 1985. Revision of *Oenothera* sect. *Oenothera* subsect. *Emersonia (Onagraceae*). Syst. Bot. 29-48.

Dietrich, W. and W.L. Wagner. 1987. New taxa of *Oenothera* L. sect. *Oenothera* (*Onagraceae*). Ann. Misso. Bot. Gard. 144-150.

Ellstrand, N.C. and D.A. Levin. 1982. Genotypic diversity in *Oenothera laciniata (Onagraceae*), a permanent translocation heterozygote. Evolution, 63-69.

Emerson, S. 1939. A preliminary survey of the *Oenothera* organensis population. Genet. 24(4): 524.

Emerson, S. 1940. Growth of incompatible pollen tubes in *Oenothera organensis*. Bot. Gaz. 890-911.

Evans, M.E., D.J. Hearn, W.J. Hahn, J.M. Spangle, and D.L. Venable. 2005. Climate and life-history evolution in evening primroses (*Oenothera, Onagraceae*): a phylogenetic comparative analysis. Evolution. 59(9): 1914-1927.

Evans, M.E., S.A. Smith, R.S. Flynn, and M.J. Donoghue. 2009. Climate, Niche Evolution, and Diversification of the "Bird-Cage" Evening Primroses (*Oenothera*, Sections *Anogra* and *Kleinia*). Am. Nat. 173(2): 225-240.

Gates, R.R. 1907. Pollen Development in Hybrids of *Oenothera lata*× *O. Lamarckiana*, and Its Relation to Mutation. Bot. Gaz. 81-115.

Gates, R.R. 1909. The Behavior of the Chromosomes in *Oenothera lata* x *O. gigas*. Bot. Gaz. 179-199.

Gates, R.R. 1911. Pollen formation in *Oenothera gigas*. Ann. Bot. (4): 909-940.

Gates, R.R. 1933. The general bearings of recent research in *Oenothera*. Amer. Nat. 67(711): 352-364.

Havens, K. 1994. Clonal repeatability of in vitro pollen tube growth rates in *Oenothera organensis (Onagraceae*). Am. J. Bot. 161-165.

Hudson, B.J.F. 1984. Evening primrose (Oenothera spp.) oil and seed. J. Am. Oil Chem. Soc. 61(3): 540-543.

Hupfer, H., M. Swiatek, S. Hornung, R.G. Herrmann, R.M. Maier, W.L. Chiu, and B. Sears. 2000. Complete nucleotide sequence of the *Oenothera* elata plastid chromosome, representing plastome I of the five distinguishable *Euoenothera* plastomes. Mol. Gen. Genet. 263(4): 581-585.

Immel, D.L. 2001. Common evening primrose USDA-NRCS fact sheet. USDA NRCS Nat. Plant Data Cent.

Jacob, K.T. 1940. Chromosome catenation in *Oenothera*. Bot. Gaz. 143-155.

Kawaano, S., M. Odaki, R. Yamaoka, M. Oda-Tanabe, M. Takeuchi, and N. Kawano. 1995. Pollination Biology of *Oenothera (Onagraceae*). The Interplay between Floral UV-Absorbancy Patterns and Floral Volatiles as Signals to Nocturnal Insects. Plant Species Biol. 10(1): 31-38.

Kevan, P.G. 1972. Insect pollination of high arctic flowers. J. Ecol. 831-847.

Krakos, K. 2011. The evolution and reproductive ecology of *Oenothera (Onagraceae*). openscholarship.wustl.edu.

Mihulka, S. and P. Pyšek. 2001. Invasion history of *Oenothera* congeners in Europe: a comparative study of spreading rates in the last 200 years. J. Biogeogr. 28(5): 597-609.

Mothershead, K. and R.J. Marquis. 2000. Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. Ecology. 81(1): 30-40.

Ogden, S., and L.S. Ogden. 2009. U.S. Patent No. PP19,663. Washington, DC: U.S. Patent and Trademark Office.

Raguso, R.A., A. Kelber, M. Pfaff, R.A. Levin, and L.A. McDade. 2007. Floral biology of north American *Oenothera* sect. *Lavauxia* (Onagraceae): advertisements, rewards, and extreme variations in floral depth 1, 2. Ann. Misso. Bot. Gard. *94*(1): 236-257.

Raven, P.H., W. Dietrich and W. Stubbe. 1979. An outline of the systematics of *Oenothera* subsect. *Euoenothera* (*Onagraceae*). Syst. Bot. 242-252.

Robberecht, R. and M.M. Caldwell. 1983. Protective mechanisms and acclimation to solar ultraviolet-B radiation in *Oenothera stricta*. Plant, Cell & Environment. 6(6): 477-485.

Silander, J.A. and R.B. Primack. (1978). Pollination intensity and seed set in the evening primrose (*Oenothera fruticosa*). American Midland Naturalist. 213-216.

Straley, G.B. 1977. Systematics of *Oenothera* sect. *Kneiffia (Onagraceae*). Ann. Misso. Bot. Gard. 381-424.

Tobe, H., W.L. Wagner, and H.C. Chin. 1987. A systematic and evolutionary study of *Oenothera (Onagraceae*): seed coat anatomy. Bot. Gaz. 235-257.

Tropicos.org. Missouri Botanical Garden. Jun 2013 <http://www.tropicos.org>
Turker, A.U., E.C. Mutlu, and A.B. Yıldırım. 2008. Efficient in vitro regeneration of fireweed, a medicinal plant. Acta Physiol. Plant. 30(4): 421-426.

Wagner, W.L. 1983. New species and combinations in the genus *Oenothera (Onagraceae*). Ann. Misso. Bot. Gard. 194-196.

Wagner, W.L., R.E. Stockhouse, and W.M. Klein. 1985. The systematics and evolution of the *Oenothera caespitosa* species complex (*Onagraceae*). Mono. Syst. Bot. 12.

Wagner, W.L. 1986. New taxa in *Oenothera (Onagraceae*). Ann. Misso. Bot. Gard. 475-480.

CHAPTER I

GENETIC MUTATION OF THREE OENOTHERA SPECIES

Oenothera L. is a perennial flowering herb which has seen limited cultivar development. The introduction of mutant characteristics such as leaf variegation may increase the appeal of the genus. The three species of *Oenothera* used in this study were *Oenothera speciosa, Oenothera pallida,* and *Oenothera missouriensis.* These three species represent the phenotypic diversity of the genus in flower color, texture, and growth habit. Seeds were treated with ethyl methanesulfonate and metal ion solutions in order to induce mutation.

Hypothesis 1 : *Oenothera* seeds treated with EMS will yield desirable mutations.

Hypothesis 2: The addition of metal ions into the EMS solution will increase the rate of mutation.

Introduction

Mutation breeding has produced many valuable plant characteristics in a number of crops, including dwarf plants in bell pepper (Alcantara et al. 1996); 'naked-tufted' seed coat mutants in cotton (Bechere et al. 2009); semidwarfism, waxy endosperm and other heritable characters in rice (McKenzie and Rutger, 1986); and the formation of adventitious buds in *Chrysanthemum morifolium* (Broertjes et al., 1976). Methods for inducing plant mutations include physical mutagens and chemical mutagens. Physical

mutagens include various types of radiation including alpha, beta, gamma, and X-rays which are ionizing radiations. The mode of action for ionizing radiations is the breakage of hydrogen bonds in DNA strands, causing cross-linkages of genes. Non-iodizing radiations such as UV rays induce purine or pyrimidine dimers.

Chemical mutagens are a diverse group of agents which cause genetic changes in a variety of ways. Acridine dyes such as proflavine and acridine orange bond between nucleotides, disrupting their arrangement and causing additions and deletions of bases. Base analogues such as 5-bromo-deoxyuridine induce base pair substitutions. Sodium azide is a potent chemical mutagen which converts cytosine to a modified base and can induce chromosome breakages. Alkylating agents are another group of chemical mutagens commonly used in plant mutagenesis and their mode of action is alkylating phosphate groups causing mis-pairing or loss of bases. They include ethylene amine (EI), ethylene oxide (EO), ethyl ethane sulphonate (EES), diethyl sulphate (DES), N-nitroso-N-ethyl urea (NEU), N-nitroso-N-methyl urea (NMU) and ethyl methanesulfonate (EMS) (Toker et al., 2007).

The alkylating agent EMS has proved to be an effective chemical mutagen as a seed treatment in many species such as maize (Neuffer and Fiesor, 1963), eucalyptus (McManus et al., 2006) and in cowpea (Girija and Dhanavel, 2009). Ethyl methanesulfonate is an alkylating agent which was the first chemical found to cause unambiguous mutation in T2 phages (Loveless, 1958). Ethyl methanesulfonate causes point mutations by, among other similar mechanisms, ethylating guanine nucleotides into O-alkylguanine. This transformed nucleotide no longer bonds with cytosine, but with uracil, during RNA synthesis before mispairing with thymine during DNA replication.

This change prompts a functional switch from guanine to adenine in the DNA strand (McManus et al., 2007). Ethyl methanesulfonate has been shown to have induced successful point mutations and even chromosome breakages in large amounts of plants of various crop types (Emmanuel and Levy, 2002). Chromosome aberrations in *Vicia faba* L. have also been observed as a result of EMS treatment (Michaelis and Rieger, 1963). The visible effects of EMS treatment on seedlings of horticultural crops include leaf and cotyledon distortions, chlorophyll deficiencies and abnormalities, loss of height or vigor, and delayed or deformed flowers (Girija and Dhanavel, 2009). The most common effect of EMS on seeds is failure to germinate or early seedling death. The mortality rate caused by EMS can vary greatly among plant species.

Ethyl methanesulfonate-treated individuals are not competitive in relation to non-treated plants because of their lack of vigor and other abnormalities. The M2 and further generations are used to establish elite lines, which carry the chemically mutated genes that may be desirable (Alcantara, 1996). Bhatia and Narayanan (1965) were able to increase the mutation rate of EMS on *Arabidopsis thaliana* (L.) Heynh. through the addition of copper and zinc ions. Similar results were seen in *Vicia faba* (Moutschen-Dahmen, 1963), *Triticum* L. (Bari, 1963) and *Hordeum* L. (Moutschen and Degraeve, 1965).

A concentration of 20 mM EMS has been proven to successfully produce variegated seedlings of *Oenothera hookeri* Torr. & A. Gray (Epp, 1973). Chlorophyll mutants have been one of the primary results of EMS treatment in higher plants (Gaul et al., 1966). Ethyl methanesulfonate has often been used to induce variegated leaves in ornamental plants (Pan and Upadhyaya, 1998; Smith and Brand, 2012). Leaf variegation can

introduce phenotypic variety into an already commercially successful cultivar which makes it a popular trait among ornamental growers (Koh and Davies, 2001). Variegation has also been observed to occur spontaneously in *Oenothera*. The 'LISHAL' cultivar was discovered as an unintended variegated offspring of the unpatented *Oenothera* 'Crown Imperial' cultivar at a nursery in Great Britain that was the result of spontaneous mutation (Catt, 2008).

The goal of this research is to discern the best procedure or set of procedures for inducing variegated leaves or other morphological changes in three *Oenothera* species using varying concentrations of solutions of EMS and five metal ions.

Materials and Methods

Oenothera pallida Lindl., *O. speciosa* Nutt., and *O. missouriensis* Nutt. seed were purchased from Everwilde Farms (Bloomer, WI) in spring of 2010. On 10 December 2010, seeds of each species were counted into 22 groups of 100 seeds. Each group represented a mutagen treatment and there were three replications for each treatment.

Treatments included a control (de-ionized water); a solution of de-ionized water and 0.6% EMS (Thermo Fischer Scientific, Bridgewater Township, NJ); 10 μM zinc sulfate heptahydrate (Sigma-Aldrich Corp., St. Louis, MO), 10 μM zinc sulfate heptahydrate plus 0.6% EMS, 20 μM zinc sulfate heptahydrate, 20 μM zinc sulfate heptahydrate, 10 μM manganese sulfate monohydrate (Sigma-Aldrich Corp., St. Louis, MO), 10 μM manganese sulfate monohydrate plus 0.6% EMS, 20 μM manganese sulfate monohydrate, 20 μM manganese sulfate monohydrate plus 0.6% EMS, 10 μM chromium trioxide (J.T. Baker Chemical Co., Phillipsburg, NJ), 10 μM chromium trioxide plus 0.6% EMS, 20

μM chromium trioxide, 20 μM chromium trioxide plus 0.6% EMS, 10 μM cupric sulfate (Sigma-Aldrich Corp., St. Louis, MO), 10 μM cupric sulfate plus 0.6% EMS, 20 μM cupric sulfate, 20 μM cupric sulfate plus 0.6% EMS, 10 μM aluminum potassium sulfate (Sigma-Aldrich Corp., St. Louis, MO), 10 μM aluminum potassium sulfate plus 0.6% EMS, 20 μM aluminum potassium sulfate, and 20 μM aluminum potassium sulfate plus 0.6% EMS. Each of the five metals were diluted in deionized water. Each treatment was placed into an individual empty tea bag (Lipton, Unilever Co., Englewood Cliffs, NJ). The teabags were then grouped by treatment into plastic Ziploc (236mL) containers with lids (SC Johnson, Racine, WI).

Seeds were left in each solution (15.55°C) for 24 hours. On 11 December 2010, seeds were removed from solution. Each individual teabag was rinsed with tap water for 10 seconds and the seeds were taken out and left to dry for two hours at 15 to 16°C. Seeds were then spread evenly over Metro-mix 902 potting media (Sun Gro Horticultural, Vancouver, BC, Canada) in six inch azalea pots (ITML Elite, Myers Industries Inc., Akron, OH). Seeds were then lightly covered with 1 cm more of pottingmedia. Seeds were watered daily as necessary to maintain soil moisture. Germination numbers were then counted for each treatment for 30 days following initial germination. Mutagenic affects such as leaf distortions and chlorophyll abnormalities recorded based on visual observations. Data was analyzed with the GLM procedure using t-tests ($p<0.05$) of the least squared differences in SAS 9.3 (SAS Institute Inc., Cary, NC). The experimental design was completely random by species.

Results and Discussion

Significant differences in germination rate were seen among the mutagen treatments (Tables 1.1). The control group of *O. missouriensis* seed germinated at a rate of 60%, while the seed treated with 0.6% EMS solution had a 5% germination rate, which was significantly lower ($P<0.05$). The germination rate of *O. missouriensis* seed treated solely with metal ions was not significantly different than control. Similarly, the seeds treated with EMS and metals did not produce significantly different results than the EMS treatment alone (Table 1.1).

The control group of *O. pallida* seed germinated at a rate of 46.33%, which was higher than the germination of seeds treated with 0.6% EMS solution (11.66%). Only one of the metal ion treatments (Cupric sulfate 20μM + EMS) produced significantly less germination than control. All of the rest of the *O. pallida* treatments were statistically equal to the untreated seed (Table 1.1).

The control group of *O. speciosa* seed germinated at a rate of 52.33%, which was higher than seed treated with 0.6% EMS (36.66%). Two treatments: the lower concentration of zinc alone (10μM) and chromium (10μM) plus EMS produced germination equal to that of control. The lower concentration of chromium treatment resulted in significantly higher germination than control ($p<0.05$). The majority of the other treatments were statistically similar. The low aluminum concentration plus EMS resulted in the lowest average *O. speciosa* germination (16.7%). Average germination rates after 30 days of growth for each treatment can be seen in Table 1.1. This is consistent with results from an experiment to induce mutation on *Oenothera hookeri* (Epp, 1974). Seeds which were

treated with 0.04 and 0.08 M solution of EMS resulted in viability rates which were significantly lower than untreated seed. The 0.08 M EMS treatment also resulted in significantly lower viability (9%) than the 0.04 M treatment (29%).

The 10 and 20 μM addition of the metal ions were not significantly different from each other across the three treated species (Table 1.1). For *O. missouriensis* and *O. pallida*, these treatments were not significantly different from control. In the experiment performed by Bhatia and Narayanan (1965), equal concentrations of metal ions were added to a 10 x 10^3 μM solution of EMS and distilled water. Seeds were then soaked in the solutions for 24 hours at 24°C. The result on *Arabidopsis* showed that EMS in the presence of metal ions produced significantly more chlorophyll deficient mutants. The exposure to heavy metals alone has been known to cause chromosome aberrations in *Vicia faba* and *Allium cepa* root tips (Minissi et al., 1998). Low concentrations of copper (25 ppm), zinc (200 ppm), and chromium (40 ppm) were found in polluted streams in which chromosome aberrations were observed in plants at a higher rate than non-polluted areas. The results of this experiment may differ from Minissi et al. (1998) due to the prolonged exposure of the streamside plants and the differing forms of the metals. Metals such as chromium are more soluble in water, and therefore more easily absorbed by plants (Cervantes et al., 2001).

One potential cause for the different results would be the concentration of EMS. In this study, the concentration of EMS was much higher than in Bhatia and Narayanan, (0.6% EMS: roughly equal to 0.058μM). With a much higher concentration of EMS, the comparatively smaller concentrations of metal ions may not have been enough to influence the mutagenic effect of EMS. In a similar study by Moutschen-Dahmen and

Moutschen-Dahmen (1963), 10 μM copper and zinc were added to increasing concentrations of EMS (from 0 to 0.16 mM solutions). The results of this experiment proved similar to Bhatia and Narayanan (1965). The percentage of chromosome breakages of EMS treated seedlings was increased from 1% to 20% when zinc and copper ions were added. The concentrations of EMS used in this study were far lower than that used in Bhatia and Narayanan.

Mutants

Ethyl methanesulfonate had significantly different ($P<0.05$) effects on the three cultivars of treated *Oenothera* seeds, because the mutant seedlings seen in *O. pallida* occurred at a higher rate than the other two species when compared to the surviving germinated seedlings (Table 1.2). The germination rate of *Oenothera speciosa* was affected the least, only resulting in a 30% mortality rate compared to control. *Oenothera missouriensis* had a mortality rate of 91.7% among EMS treated seed compared to control. These variations in EMS affecting different *Oenothera* genotypes is consistent with previous findings (Kressel & Arnold, 1967). Most seedlings showed the effect of the treatment as soon as cotyledons opened. Leaves, including cotyledons, had distorted shape and often showed regions of chlorophyll deficiency. (Figures 1.1 and 1.2) The shape distortions were often caused by a shortened leaf midrib or veins. These mutation effects were often expressed as periclinal chimeras.

Conclusions

The treatment of *Oenothera* seeds in a 0.6% solution of EMS for 24 hours most often results in an increase in seed and seedling mortality. Differences in the seed coat morphology of some species may alter the absorption rate of the mutagen.

Ethyl methanesulfonate treatments and treatments including both EMS and metal ions of *Oenothera missouriensis* yielded an average mutation rate of 1.6%. Phenotypic mutants of *O. missouriensis* did not survive to produce offspring. Treatments of *Oenothera pallida* which included EMS resulted in an average mutation rate of 2.82%. The majority of these *O. pallida* mutations were chlorophyll-related and desirable, but very few survived to sexual maturity and no viable seed was produced. *Oenothera speciosa* seed treatments which included EMS resulted in a mutation rate of 0.484%. Phenotypic mutations primarily resulted in slightly distorted leaves. These mutations were not stable, undetectable at flowering stage. Both hypotheses were proved invalid by these results.

Table 1.1 Effect of ethyl methanesulfonate and five metal ions on the germination rate of three *Oenothera* species 30 days following initial germination (n = 300).

Treatment[z]	Germination					
	O. missouriensis		***O. pallida***		***O. speciosa***	
Control	60.0	a[y]	46.3	a	52.3	ab
EMS 58x10^{3}µM	5.0	cd	11.7	b	36.7	abcd
Zn 10µM	61.3	a	47.7	a	50.3	ab
Zn 20µM	62.0	a	52.0	a	46.7	abcd
Zn 10µM + EMS 58x10^{3}µM	5.7	bcd	49.3	a	44.3	abcd
Zn 20µM + EMS 58x10^{3}µM	5.3	bcd	64.7	a	40.3	abcd
Cu 10µM	57.7	a	41.7	a	43.7	abcd
Cu 20µM	60.3	a	62.7	a	38.7	abcd
Cu 10µM + EMS 58x10^{3}µM	10.3	b	38.7	a	43.7	abcd
Cu 20µM + EMS 58x10^{3}µM	6.0	bcd	30.0	b	31.0	abcd
Al 10µM	57.3	a	54.0	a	39.0	abcd
Al 20µM	54.3	a	60.3	a	31.3	abcd
Al 10µM + EMS 58x10^{3}µM	4.0	d	54.3	a	16.7	e
Al 20µM + EMS 58x10^{3}µM	8.3	bcd	51.7	a	26.3	de
Mn 10µM	62.3	a	41.3	a	34.3	abcd
Mn 20µM	55.7	a	38.3	a	47.3	abcd
Mn 10µM + EMS 58x10^{3}µM	7.3	bcd	56.7	a	21.3	cde
Mn 20µM + EMS 58x10^{3}µM	4.0	d	51.3	a	41.3	abcd
Cr 10µM	50.3	a	50.0	a	56.3	a
Cr 20µM	54.7	a	45.3	a	44.7	abcd
Cr 10µM + EMS 58x10^{3}µM	9.0	bc	49.3	a	54.0	ab
Cr 20µM + EMS 58x10^{3}µM	5.0	cd	49.3	a	47.0	abc

[z]Metal ion treatments: Zinc = ZnSO4, Copper = CuSO4, Aluminum = AlKSO4, Manganese = MnSO4, Chromium = CrO3. Solutions of 10 and 20 millimolar metal ions were diluted in deionized water.

[y]Treatments in the same letter group within columns are not significantly different at a confidence level of alpha = 0.05.

Table 1.2 The effect of metal ions and ethyl methanesulfonate on seedling morphology of *Oenothera* species.

Treatment[z]	Average of Observed Phenotypic Mutants (%)[y]					
	O. missouriensis		***O. pallida***		***O. speciosa***	
Control	0	c[x]	0	d	0	b
EMS 58x10^{3}µM	1.33	ab	1.67	bc	1.00	a
Zn 10µM	0	c	0	d	0	b
Zn 20µM	0	c	0	d	0	b
Zn 10µM + EMS 58x10^{3}µM	1.00	ab	3.00	ab	0.33	ab
Zn 20µM + EMS 58x10^{3}µM	2.00	ab	3.00	ab	0.33	ab
Cu 10µM	0	c	0	d	0	b
Cu 20µM	0	c	0	d	0	b
Cu 10µM + EMS 58x10^{3}µM	2.67	a	3.00	ab	0.33	ab
Cu 20µM + EMS 58x10^{3}µM	1.00	bc	1.67	ab	0.00	b
Al 10µM	0	c	0	d	0	b
Al 20µM	0	c	0	d	0	b
Al 10µM + EMS 58x10^{3}µM	0.67	bc	3.33	ab	0.67	ab
Al 20µM + EMS 58x10^{3}µM	1.67	ab	2.00	bc	0.67	ab
Mn 10µM	0	c	0	d	0	b
Mn 20µM	0	c	0	d	0	b
Mn 10µM + EMS 58x10^{3}µM	2.00	ab	3.67	ab	0.67	ab
Mn 20µM + EMS 58x10^{3}µM	1.33	ab	3.00	ab	1.00	a
Cr 10µM	0	c	0	d	0	b
Cr 20µM	0	c	0	d	0	b
Cr 10µM + EMS 58x10^{3}µM	2.67	a	2.33	ab	0.00	b
Cr 20µM + EMS 58x10^{3}µM	1.33	ab	4.33	a	0.33	ab

[z]Metal ion treatments: Zinc = ZnSO4, Copper = CuSO4, Aluminum = AlKSO4, Manganese = MnSO4, Chromium = CrO3. Solutions of 10 and 20 millimolar metal ions were diluted in deionized water.

[y]Phenotypic Mutants: Seedlings showing any leaf distortion, chlorophyll inconsistency or variegation.

[x]Treatments in the same letter group within columns are not significantly different at a confidence level of alpha = 0.05.

Figure 1.1 Phenotypic EMS mutants of *Oenothera missouriensis* showing chlorophyll deficiency and leaf distortion at five weeks after germination.

Figure 1.2 Phenotypic EMS mutants of *Oenothera pallida* showing chlorophyll deficiency and leaf distortion at two weeks after germination

Figure 1.3 Mature EMS mutant of *Oenothera pallida* with distorted leaf structure and shortened stem internodes

Figure 1.4 Mature EMS treated chlorophyll mutant of *Oenothera pallida*

LITERATURE CITED

Alcantara, T.P., P.W. Bosland, and D.W. Smith. 1996. Ethyl Methanesulfonate-Induced Seed Mutagenesis of *Capsicum annuum.* J. Hered. 87(3): 239241.

Arnold, C.G. and M. Kressel. 1965. Attemps at causing plasma mutations in *Oenothera*. Z. Verebungsl. 96.

Bari, G. 1963. The mutagenic effect of ethylmethanesulfonate alone and in combination with copper on wheat. Cariologia 16: 619-624.

Bartlett, H.H. 1915. Mass mutation in *Oenothera pratincola*. Bot. gaz. 60(6): 425-456.

Bechere, E., D.L. Auld, and E. Hequet. 2009. Development of 'naked-tufted'seed coat mutants for potential use in cotton production. Euphytica 167(3): 333-339.

Bhatia, C.R. and K.R. Narayanan. 1965. Genetic effects of ethyl methanesulfonate in combination with copper and zinc ions on *Arabidopsis thaliana*. Genet. 52(3): 577.

Cervantes, C., J. Campos-García, S. Devars, F. Gutiérrez-Corona, H. Loza-Tavera, J.C. Torres-Guzmán, and R. Moreno-Sánchez. 2001. Interactions of chromium with microorganisms and plants. FEMS Microb. Rev. 25(3): 335-347.

DeVries, H. (1915). The coefficient of mutation in *Oenothera biennis* L. Bot. Gaz. 169-196.

Emmanuel, E., and A.A. Levy. 2002. Tomato mutants as tools for functional genomics. Curr. Op. in Plant Bio. 5(2): 112-117.

Epp, M.D. 1973. Nuclear gene-induced plastome mutations in *Oenothera hookeri*. I. Genetic analysis. Genet. 75(3): 465-483.

Epp, M.D. 1974. The homozygous genetic load in mutagenized populations of *Oenothera hookeri* T. and G. Mutat. Res.-Fund. Mol. M. 22(1): 39-46.

Gaul, H., K. Bender, E. Ulonska, and M. Sato. 1966. EMS-induces genetic variability in barley; the problem of EMS-induced sterility; and a method to increase the efficiency of EMS treatment. International Atomic Energy Agency.

Girija, M. and D. Dhanavel. 2009. Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combined treatments in cowpea (*Vigna unguiculata* L. Walp). Global J. Mol. Sci. 4: 68-75.

Harshberger, J.W. 1905. Mutants and Hybrids of the *Oenotheras.* Torreya. 5(8): 147-149.

Kressel, M. and C.G. Arnold. 1967. The differential sensitivity of the genome of *Oenothera berteriana* to ethylmethanesulfonate. Mut. Res. 4(6): 797.

Loveless, A. 1958. Increased rate of plaque-type and host-range mutation following treatment of bacteriophage in vitro with ethyl methane sulphonate. Nature. 1212-1213.

McKenzie, K.S., and J.N. Rutger. 1986. A new semidwarf mutant in a long-grain rice cultivar. Crop Sci. 26(1): 81-84.

McManus, L.J., J. Sasse, C.K. Blomstedt, and G. Bossinger. 2006. Pollen treatment for mutation induction in *Eucalyptus globulus* ssp. *globulus* (*Myrtaceae*). Austral. J. Bot. 54(1): 65-71.

McManus, L.J., J. Sasse, C.K. Blomstedt, and G. Bossinger. 2007. The effects of ethyl methanesulfonate treatment on *Eucalyptus* pollen behaviour in vitro. Trees. 21(3): 379-383.

Mehra-Palta, A., H.U. Koop, S. Goes, E.M. Troidl, G. Nagy, S. Tyagi and, R.G. Herrmann. 1998. Tissue culture of wild-type, interspecific genome/plastome hybrids and plastome mutants of evening primrose (*Oenothera*): controlled morphogenesis and transformation. Plant Cell Rpt. 17(8): 605-611.

Michaelis, A., and R. Rieger. 1963. Interaction of chromatid breaks induced by three different radiomimetic compounds. Nature. 1014-1015.

Minissi, S., D. Caccese, F. Passafiume, A. Grella, C. Eleanora, and M. Rissoni. 1998. Mutagenicity (micronucleus test in *Vicia faba* root tips), polycyclic aromatic hydrocarbons and heavy metal content of sediments collected in Tiber river and its tributaries within the urban area of Rome. Mut. Res. 420: 77–84.

Moutschen-Dahmen, J. and M. Moutschen-Dahmen. 1963. Influence of Cu++ and Zn++ ions on the effects of ethyl methanesulfonate (EMS) on chromosomes. Experientia. 19(3): 144-145.

Moutschen, J. and N. Degraeve. 1965. Influence of thiol-inhibiting substances on the effects of ethyl methane sulphonate (EMS) on chromosomes. Experientia. 21(4): 200-202.

Neuffer M.G. and G. Fiesor. 1963. Mutagenic action of ethyl methanesulfonate in corn. Sciences. 139: 346.

Pan, T.K., and P.P. Upadhyaya. 1998. Improvement of wild ornamental species of *Reinwardtia* through mutagenesis. Phytodiversification and Human Welfare. 307.

Smith, W.A. and M.H. Brand. 2012. 'Summer Skies' *Buddleja davidii*. HortScience. 47(1): 126-127.

Toker, C., S.S. Yadav, and I.S. Solanki. 2007. Mutation breeding. In Lentil (pp. 209-224). Springer Netherlands.

CHAPTER II

ASEXUAL PROPAGATION OF TWO OENOTHERA SPECIES

A rooting protocol was developed for two species of *Oenothera*. The goal of this study was to determine the ability of two species of *Oenothera* stem cuttings to produce adventitious roots under intermittent mist irrigation for varying conditions. The experiment tested two variables: media type and rooting hormone application. Perlite and vermiculite rooting media were tested to determine which better facilitated root growth in these two *Oenothera species.* Three commercially available rooting hormones were also tested versus a control of untreated stem cuttings to determine if one hormone was more effective at producing adventitious roots or callus material. Effects tested for were: ability of stem cuttings to produce roots, average number of roots per cutting, and average length of the longest root produced.

Hypothesis 1: The rooting media will have significantly different results in the effects tested.

Hypothesis 2: The application of rooting hormones to the *Oenothera* cuttings will result in more rooted cuttings, higher number and longer roots than the control.

Hypothesis 3: The effect of the three rooting hormones will not be significantly different.

Introduction

Oenothera spp., also known as evening primrose, can be propagated both sexually and asexually using several different propagation methods (Cleland, 1924). Tissue culture has been a successful means of vegetative reproduction of *Oenothera* (Mehra-Palta, 1998), but the rapid rate of adventitious root formation enables most species to be rooted from stem cuttings. Supplementation of rooting media with indole-butyric acid (IBA) produced a higher percentage of rooted in vitro explants than control in commercial cultivars of *Oenothera* (De Gyves et al., 2001). Incorporation of 1mg/l IBA into a tissue culture substrate has proven to produce 100% rooting of callus material for in vitro culture of five species of *Oenothera* (Thiem et al., 1999).

While the in vitro cultures employ a much lower concentration throughout the agar to induce root initiation, production of large softwood cuttings of *Gaura lindheimeri* Engelm. & Gray required use of a much higher concentration of IBA. The dipping of *Gaura* cuttings in a 1000 ppm powder before being stuck is recommended (Anderson & Peters, 2002). Indolebutyric acid is a synthetic auxin which has been used commercially and in research as a plant growth regulator and adventitious root stimulator. Natural and synthetic auxins have been used extensively in plant cell, tissue, and organ cultures to elicit specific morphogenetic responses (Nissen and Sutter, 1990). An experiment on *Pennisetum setaceum* Forsk. tested the rooting of herbaceous perennials with or without IBA, and the use of differing media types including perlite and vermiculite (Cunliffe et al., 2001). The researchers found that peat and perlite produced a significantly higher percentage of rooted cuttings than sand, vermiculite and a mixture of peat and perlite.

Little information is available concerning an effective protocol for vegetative reproduction of stem cuttings of *Oenothera* species. In one study of *Oenothera* micropropagation, IBA was applied as part of the rooting substrate mixture at low rates (0.2 to 1.0 mg/L) (De Gyves et al., 2001). This experiment also only tested the treatments on three commercial varieties of *O. biennis* L. The rooting experiment utilized other rooting hormones, such as Naphthaleneacetic acid (NAA), within the substrate at varying concentrations. De Gyves et al. also only used one standard IBA treatment and the same rooting media within the experiment.

Materials and Methods

On 9 June 2011, cuttings of *Oenothera speciosa* Nutt. and *O. drummondii* Hooker were taken from existing plant material growing in the Oklahoma State University horticulture research greenhouses in Stillwater, Oklahoma. The *O. speciosa* cuttings were taken from 6 month old plants grown from seed purchased from Everwilde Farms (Bloomer, WI) in spring of 2010. The *O. drummondii* cuttings were taken from 2 ½ year old plants grown from seed from ARS-GRIN. *Oenothera speciosa* cuttings were taken from the tip of each stem, while cuttings of *O. drummondii* were taken from both tip and midsection of the stem. All cuttings were 14 cm long. The cuttings were grouped in bundles of 25 and placed in individual four gallon plastic buckets filled with clear tap water. The buckets were then placed in a walk-in cooler (International Cold Storage Inc., Wichita, KS) for 15 hours at 4°C.

For each media type, the cuttings were divided into four rooting hormone treatments plus a control with no hormone treatment. The three rooting hormones tested were: Dip'N

Grow Liquid Rooting Concentrate (Dip'N Grow Inc., Clackamas, OR), Hormex Rooting Powder (Brooker Corporation, Hollywood, CA) and Hortus IBA Water Soluble Salts (Hortus USA Corp., New York, NY). In total, eight treatments were tested for each species. Each treatment consisted of three repetitions of 20 cuttings each.

The 1000 ppm IBA Hormex Rooting Powder concentration was used in this experiment. The other two rooting hormones were mixed in solution of 1000 ppm concentration. Leaves were removed from the bottom 2 cm of each cutting. Any flower buds or open flowers were also removed from the tips of cuttings. The bottom 2 cm of each cutting was then dipped into the rooting hormone solution or powder for a period of 5 seconds before being stuck into the plug tray. The cuttings were stuck into 98-cell hex plug trays (McConkey Co., Sumner, WA) that had been filled with coarse vermiculite (Sun Gro Horticultural, Vancouver, BC, Canada) or perlite (Sun Gro Horticultural, Vancouver, BC, Canada). The cuttings were then placed under an intermittent mist set to water for 8 seconds every 2 minutes.

On 17 June 2011, all rooted cuttings were measured for number of roots present, as well as the length in centimeters of the longest root. Each cutting was removed from the plug tray and the rooting media was washed away under tap water for 10 to 15 seconds. Data for the *O. speciosa* cuttings was recorded after eight days under the mist irrigation, while *O. drummondii* cuttings were recorded after 14 days. The experimental design of the experiment completely randomized by treatment and blocked by species. Type III test of fixed effects was used to determine significance using SAS 9.3. Three levels of significance were tested for ($p<0.05$, $p<0.01$ and $p<0.001$).

Results and Discussion

The number of cuttings rooted was significantly lower in perlite for *O. drummondii* ($P<0.01$), but not significantly different for *O. speciosa* (Table 2.1). Perlite rooting media also proved to be the significantly less effective for these two species of *Oenothera*, with regard to number of roots produced and length of the longest root (*O. speciosa* $P<0.001$, *O. drummondii* $P<0.05$) (Table 2.2, 2.3). In a similar experiment, several rooting substrates were tested on five varieties of *Fuchsia hybrida* hort. ex Siebold and Voss (*Onagraceae*). The number of roots produced was found to have been higher in a mixture of peat and perlite compared to perlite alone as well as a mixture of perlite and sand (Erzsebet et al., 2012). That study's results align with the results of this *Oenothera* study. A substrate medium of only perlite is likely to drain quicker than a media with more porous space for water to absorb. Compared with this experiment, the more slowly draining rooting media, vermiculite, produced a higher average number of significantly longer roots.

After eight days, 91.67% of the *speciosa* cuttings had rooted (Table 2.1). These results are comparable to those of previous IBA supplemented media in the propagation of *Oenothera spp.* (De Gyves, 2001). Supplementation of rooting media with IBA was proven to produce 100% rooted plant material from related species *Epilobium angustifolium* L. (*Onagraceae*) versus a control which produced no roots (Turker et al., 2008). Adventitious root initiation had been observed four days after cuttings were placed under the mist. Rooting percentage of *O. speciosa* was not significantly higher in either media type. There was no significant difference in rooting percentage of *O. speciosa* between IBA treatments compared to control. No significant media × hormone

interaction was observed compared with the rooting percentage of the control. After 14 days, 43.1% of all treatments of the *drummondii* cuttings had rooted. Adventitious root initiation had been observed 12 days after cuttings were placed under the mist. Rooting percentage of *O. drummondii* was significantly higher ($P<0.01$) in vermiculite than in perlite. There was no significant difference in rooting percentage of *O. drummondii* between IBA treatments compared to control. No significant media × hormone interaction was observed compared with the rooting percentage of the control.

The average length of the longest *O. speciosa* root was longer in vermiculite than in perlite with a high level of significance $P<0.001$ (Table 2.2). No rooting hormone treatment produced any significantly longer roots than that of the control group. No significant media × hormone interaction was observed compared with the average longest root of the control group. The average length of the longest *O. drummondii* root was significantly longer in vermiculite than in perlite $P<0.05$ (Table 2.2). No rooting hormone treatment produced any significantly longer roots than that of the control group. The Dip'N Grow treatment produced longer roots in vermiculite than the control group with a low level of significance.

The average number of *O. speciosa* roots was higher in vermiculite than in perlite $P<0.001$ (Table 2.3). No rooting hormone treatment produced significantly more roots than that of the control group. No significant media × hormone interaction was observed compared with the average number of roots of the control. The average number of *O. drummondii* roots was higher in vermiculite than in perlite $P<0.05$ (Table 2.3). No rooting hormone treatment produced significantly more roots than that of the control

group. The Hortus treatment produced a higher number of roots in vermiculite than the control group $P<0.05$ (Table 2.3).

Conclusions

From these results it can be concluded that vermiculite is a better rooting media for both *O. speciosa* and *O. drummondii*, producing significantly more and longer roots. It can also be concluded that IBA rooting hormone treatments of this concentration do not produce significantly more or longer roots for these two *Oenothera* species. Hypotheses 1 and 3 were proved to be valid. Hypothesis 2 was proved invalid.

Table 2.1 Effect of media and hormone treatment on percentage of *Oenothera* cuttings rooted after being under mist for 8 and 14 days (n=60)

Species[z]	**Treatment**	**Rooted (%)**	**Probability**[y]
O. speciosa	Media		
	Perlite	91.25	NS
	Vermiculite	92.10	
	Hormone		
	Control	91.66	NS
	Dip'N Grow	93.33	
	Hormex	85.00	
	Hortus	96.65	
	Media × Hormone		NS
O. drummondii	Media		
	Perlite	27.10	**
	Vermiculite	59.15	
	Hormone		
	Control	44.16	NS
	Dip'N Grow	40.00	
	Hormex	45.83	
	Hortus	42.50	
	Media × Hormone		NS

[z]*Oenothera speciosa* rooted quickly and was removed from mist six days earlier than *O. drummondii*, which was removed after 14 days.

[y]NS = not significant, *, **, and *** for P<0.05, P<0.01, and P<0.001, respectively.

Table 2.2 Effect of media and hormone treatment on length of two *Oenothera* species' roots.

Species	Treatment		Root lenght (cm)	Probability[z]
O. speciosa	Media			
		Perlite	1.82	***
		Vermiculite	3.57	
	Hormone			
		Control	2.75	NS
		Dip'N Grow	3.26	
		Hormex	2.28	
		Hortus	2.49	
	Media × Hormone			NS
O. drummondii	Media			
		Perlite	2.29	*
		Vermiculite	5.39	
	Hormone			
		Control	4.05	NS
		Dip'N Grow	4.26	
		Hormex	3.45	
		Hortus	3.59	
	Media x Hormone			*
	Dip'N Grow × Vermiculite			

[z]NS = not significant, *, **, and *** for P<0.05, P<0.01, and P<0.001, respectively.

Table 2.3 Effect of media and hormone treatment on number of *Oenothera* roots (n=60)

	Treatment	# of roots	Probability[z]
O. speciosa	Media		
	Perlite	12.82	***
	Vermiculite	23.19	
	Hormone		
	Control	16.88	NS
	Dip'N Grow	22.66	
	Hormex	13.47	
	Hortus	19.00	
	Media × Hormone		NS
O. drummondii	Media		
	Perlite	8.88	*
	Vermiculite	14.99	
	Hormone		
	Control	10.49	NS
	Dip'N Grow	11.75	
	Hormex	11.34	
	Hortus	14.15	
	Media × Hormone		
	Hortus × Vermiculite		*

[z]NS = not significant, *, **, and *** for P<0.05, P<0.01, and P<0.001, respectively.

LITERATURE CITED

Anderson, N. and W. Peters. 2002. Potted plant production of *Gaura lindheimeri*. Dept. of Hort. Sci. Bul., Univ. of Minn..

Cleland, R.E. 1924. Meiosis in pollen mother cells of *Oenothera franciscana sulfurea*. Bot. Gaz. 149-170.

Cunliffe, B.A., M.H. Meyer, and P.D. Ascher. 2001. Propagation of *Pennisetum setaceum* ‘rubrum' from cuttings. J. Envir. Hort. 19(1): 1-3.

De Gyves, E.M., C.A. Sparks, A.F. Fieldsend, P.A. Lazzeri, and H.D. Jones. 2001. High frequency of adventitious shoot regeneration from commercial cultivars of evening primrose (*Oenothera* spp.) using thidiazuron. Ann. of App. Bio. 138(3): 329-332.

Erzsebet, B., C. Maria, H. Denisa and M. Buta. 2012. The influence of different types of substrates on rooting of *Fuchsia* cuttings. J. Hort., For. and Biotech. 16(2): 116-122.

Mehra-Palta, A., H.U. Koop, S. Goes, E.M. Troidl, G. Nagy, S. Tyagi and, R.G. Herrmann. 1998. Tissue culture of wild-type, interspecific genome/plastome hybrids and plastome mutants of evening primrose (*Oenothera*): controlled morphogenesis and transformation. Plant Cell Rpt. 17(8): 605-611.

Nissen, S.J., and E.G Sutter. 1990. Stability of IAA and IBA in nutrient medium to several tissue culture procedures. HortScience. 25(7): 800-802.

Thiem, B., L. Skrzypczak, and E, Lamer-Zarawska. 1999. Micropropagation of selected *Oenothera* species and preliminary studies on their secondary metabolites. Acta Societatis Botanicorum Poloniae. 68(1):15-20.

Turker, A.U., E.C. Mutlu, and A.B. Yıldırım. 2008. Efficient in vitro regeneration of fireweed, a medicinal plant. Acta Physiol. Plant. 30(4): 421-426.

CHAPTER III

INTERGENERIC HYBRIDIZATION OF OENOTHERA SPECIES

Twelve species of *Oenothera* L. were reciprocally crossed with eight species from allied genera within family *Onagraceae* Juss. The goal of this program was to breed an intergeneric hybrid which would display traits from the non-*Oenothera* species which would be desirable for the *Oenothera* genus. The creation of interspecific hybrids of *Oenothera* spp. was also a goal of this research.

Hypothesis 1: Intergeneric hybrids of *Onagraceae* can be created through crossing of *Oenothera* with species from the *Clarkia* Pursh, *Camissonia* Link, and *Calylophus* Spach genera.

Hypothesis 2: Interspecific hybrids of *Oenothera* can be created through the crossing of twelve distinct *Oenothera* species.

Introduction

Oenothera 'Fransiscana Sulfurea' was created by crossing of *Oenothera biennis* L. and *Oenothera fransiscana* Bartlett (Davis, 1916). This hybrid was the basis for significant cytological discoveries in the *Oenothera* genus (Cleland, 1924). Cleland noted the distinct chromosome configurations of the hybrid and its backcrossed progeny. Cleland

found that a negative correlation was illustrated between inherited red pigments of *Oenothera rubricalyx* Gates hybrids with *Oenothera lamarckiana* Auct. and *Oenothera rubrinervis de Vries*, a *lamarckiana* mutant. In a later study, Cleland examined the unique chromosome configurations of 11 F1 hybrids of the following species and varieties: *O. biennis L.*, *O. hookeri* Torr. & A. Gray, *O. lamarckiana, O. suaveolens* Desf., *O. muricata* L., *O. grandiflora* Ruiz. & Pav., *O. fransiscana* Bartlett, *O. fransiscana 'sulfurea'* Gates, *O. latifrons* Cleland, *O. aurata* Cleland, and a mutant of *O. fransiscana 'Sulfurea')* (Cleland, 1927). The catenation of chromosomes from 32 hybrids of R.R. Gates (King's College, London, UK) collection were outlined by D.G. Catcheside (1933).

The chromosomes of *Oenothera* are catenated, forming linked rings end to end during prophase of meiosis (Jacob, 1940) which often produce obstacles to breeding between related species (Gates, 1933). The base number of chromosomes for *Oenothera* is 2n=14. The majority of the species are diploid, but there are a few exceptions. Hugo DeVries' historic discovery of the first spontaneous tetraploid species in nature is one example, finding that *Oenothera lamarckiana* Auct. had mutated to become *Oenothera gigas* (Harshberger, 1905). Other species, such as *Oenothera speciosa* Nutt. can be found as haploid, diploid, or triploid. Many species and most hybrids of *Oenothera* have chromosome number notations which are uniquely affected by catenation. Catenated circles of chromosomes can be as small as 4 or contain all 14 chromosomes in one ring. For each species, the number and ring configuration is constant (Cleland 1929). A diploid *Oenothera* which possessed five pairs of chromosomes and a small circle of four chromosomes would carry the notation 4 + 5ii (Catcheside, 1933). It was theorized that

these chromosome circles are the result of segmental interchange. The evolution of *O. lamarckiana* included the combination of two complexes, *Velans* and *Gaudens.*

Velans complex = **1-2, 3-4, 5-8, 7-6, 9-10, 11-12, 13-14**

Gaudens complex = 1-2, 3-14, 5-6, 7-4, 12-10, 11-8, 13-9

If the 14 chromosomes of each complex are numbered accordingly, the matching end arrangements combine to form the one pair and one circle of 12 chromosomes of *O. lamarckiana*. Each chromosome represents a pair from the parent complex "…with the *velans* chromosomes in bold type-face" (Cleland and Blakeslee, 1930). In this catenated form, the lethal genes become balanced resulting in self-incompatibility or incompatibility with closely related species.

1-2 **3-4** – 4-7 – **7-6** – 6-5 – **5-8** – 8-11

| | | |

1-2 3-14 – **14-13** – 13-9 – **9-10** – 10-12 – **12-11**

Cleland later found (1951) that aside from the polyploidy of the constituents of subsections *Hartmannia* and *Kneifa*, naturally occurring aneuploidy and polyploidy are rare in *Oenothera.* Cleland described two true-breeding lines of mutants of *Oenothera hookeri* Torr. & A. Gray which carried a pair of extra and diminutive chromosomes, sometimes finding one or three in each plant. The two mutants had the notation 4 + 5ii +

2. Significant work has been done more recently to breed *Oenothera* oil seed crops with higher gamma linolenic acid (GLA) content. Early flowering cultivars were developed so that hotter temperatures would increase the production of GLA (Levy et al., 1992). Of these ornamental and oil-crop cultivars, crosses have only been made intraspecifically. The day-blooming 'Gold Evening Primrose' was produced through the hybridization of *Oenothera lamarckiana* and the island evening primrose (*Oenothera sp.)* (Kim, 2009). Self-incompatability and pollination requirements of *O. speciosa* are described by CL Wolin (1984). This research describes the necessity of *O. speciosa* pollinators to facilitate outcrossing. Plants were subjected to seven pollination treatments. Control flowers which were left untouched and open to pollinators had a >450% increase in seed set than those which were selfed by hand. LK Crowe (1955) studied the self-sterility and self-fertility of 10 species of *Oenothera* (*O. acaulis* Cav., *O. speciosa, O. organensis* Munz, *O. missouriensis* Sims, *O. rhombipetala* Nutt. ex Torr. & A. Gray, *O. trichocalyx* Nutt., *O. pallida* Douglas ex Lindl., *O. latifolia* [Rydb.] Munz, *O. deltoides* Torr. & Frém., *and O. runcinata* [Engelm.] Munz), the latter five being of the same sub-genus *Anogra*. In a polycross of the ten species he proved that those species belonging to *Anogra* were compatible with each other, yet self-incompatible (with the exception of *O. trichocalyx*). All non-*Anogra* species were both self-incompatible and incompatible with each other or produced inviable seed (with the exception of *O. acaulis* which was self-fertile but incompatible with all other species).

Despite the difficulties posed to interspecific breeding, some *Oenothera* cultivars have been produced commercially. Several intraspecific *Oenothera* progeny have been patented in the last decade. In addition to the variegated 'LISHAL' cultivar, a branch

offshoot of *Oenothera speciosa* was selected for patent named *Oenothera* 'TURNER01'. The leaves of 'TURNER01' were more variegated and its growth habit was lower than the parent plant (Morum, 2006). A cross made of two unpatented cultivars of *Oenothera hybrida* Michx. resulted in a German cultivar named 'INNOENO131' (Hofmann, 2006). An open pollination of *Oenothera fremontii* resulted in the cultivar 'Shimmer', which has narrower ribbon-like foliage which differentiate it from the parent material (Ogden and Ogden, 2009).

The genes for purple flower color are carried by the genus *Clarkia* Pursh. which also belongs to the family *Onagraceae.* Other desirable characteristics exist in close relatives of *Oenothera* such as the flower shape of genus *Calylophus* Spach and the flower clustering of genus *Camissonia* Link. The phylogenetic tree in Figure 1 shows the genera comprising *Onagraceae,* and the relationship between *Oenothera, Clarkia,* and *Camissonia.* The genus *Calylophus* is incorporated into *Oenothera.*

In order to increase the appeal of *Oenothera spp.* as an ornamental crop, the introduction of new flower morphology into the genus was tested through interspecific and intergeneric hybridization within the family *Onagraceae.*

Materials and Methods

Populations of several species of *Oenothera* plants (*Oenothera drummondii* Hooker, *Oenothera pallida* Lindl., *Oenothera speciosa* Nutt. (Everwilde Farms, Bloomer, WI), *Oenothera missouriensis* Nutt., *Oenothera hookeri* Torr. & A. Gray, *Oenothera coronopifolia* Torr. & A. Gray (Plants of the Southwest, Santa Fe, NM), *Oenothera rosea*

L'Hér. ex Aiton, *Oenothera biennis* L., *Oenothera flava* [A. Nelson] Garrett (USDA – GRIN), *Oenothera caespitosa* Nutt. *ssp. marginata* [Nutt. ex Hook. & Arn.] Munz (Alplains, Kiowa, CO), *Oenothera elata* Kunth *ssp. hirsutissima* [A. Gray ex S. Watson] W. Dietr.(The Theodore Payne Foundation, Sun Valley, CA), *Oenothera longifolia cv.* Lemon Sunset (Diane's Flower Seeds, Ogden, UT), were grown from seed in six inch azalea pots (ITML Elite, Myers Industries Inc., Akron, OH) using a soilless growing media Metro-mix 902 (Sun Gro Horticulture, Vancouver, BC, Canada). Seeds of *Clarkia amoena* Lehm., *Clarkia pulchella* Pursh., *Clarkia unguiculata* Lindl., *Clarkia purpurea* W. Curtis, *Calylophus hartwegii* Benth., *Camissonia ignota* Jeps., *Camissonia boothii* Douglas ex Lehm. and *Camissonia cheiranthifolia* Hornem. ex Spreng from three other genera were purchased from JL Hudson Seedsman (La Honda, CA) for the hybridization program.

Flowers were emasculated one or two days prior to crossing. Sepals were carefully cut open with forceps and premature anthers were removed in order to prevent self-fertilization. Fertilization was performed using forceps one day after emasculation. If the stigma of the female plant remained turgid for a second day, pollination was performed again in the same manner. Eight distinct species of *Oenothera* were employed as female parents in crosses: *O. drummondii, O. pallida, O. speciosa, O. missouriensis, O. rosea, O. caespitosa* ssp. *marginata, O. elata ssp. hirsutissima, and O. hookeri.* All of the *Oenothera* species listed were also used as male parent plants in addition to four others: *O. biennis, O. flava, O. longifolia cv.* Lemon Sunset, and *O. coronopifolia*). These *Oenothera* plants had flowers which bloomed sparser or within a much shorter time frame. In order to be utilized as female parent, a plant needed at least five blooms in

order to complete a reciprocal cross. Those with less than five blooms in a given time period were only able to be pollen donors. When the seed capsules matured, the seed was placed into a coin envelope to be sown later. Collected seed was sown into seedling trays with 18-cell plastic inserts filled with metro-mix 702 on 14 March and 26 September, 2011 and moved to the Oklahoma State University Horticulture Research Greenhouses (Stillwater, OK), which were set to a day temperature of 24°C and 18°C night temperature.

Results and Discussion

During the spring and summer of 2010 and 2011, 721 crosses were made among the *Oenothera* species and with the allied *Onagraceae* genera. The majority of seed capsules of the parent plant immediately began to wither days after pollination and most fell off before any seed could develop. All capsules, however, that were recovered were opened and the undeveloped seeds were sown.

From the 86 crosses using *O. drummondii* as the female parent, ten of the crosses produced seed. The majority of seed was sown on 26 September, 2011. One cross with *O. caespitosa ssp. marginata* produced seed earlier and was sown on 2 June, 2011. Of the seed collected, 64% germinated. After germination the seed was evaluated until maturity. All seedlings looked identical to *O. drummondii* and bore no similarities to male parents. All plants had the distinctive yellow flowers of the female parent.

From the 110 crosses using *O. pallida* as the female parent, only one cross produced seed. The seed was produced by the cross *O. pallida x Clarkia purpurea*. The seed was planted and the resulting seedling was evaluated to show no resemblance to *C. purpurea*.

The suspected selfed seed was produced for a couple of likely reasons. Western flower thrips (*Frankliniella occidentalis* Pergande) were a common pest problem in the greenhouse populations of *Oenothera*. These thrips likely caused some unintended pollination of previously emasculated flowers. Flowers being pollinated by thrips is a common occurrence in nature (Kevan, 1972). Additionally, many of the individuals of each species were grouped close together and hand-watered. Accidental selfing was likely due to the movement resulting from these cultural practices.

From the 162 crosses using *Oenothera speciosa* as the female parent, zero viable seed was produced. Immature seeds recovered from capsules of three crosses with *O. longifolia* 'Lemon Sunset' and *O. caespitosa marginata* were sown resulting in no germination. The crosses using *O. missouriensis, O. caespitosa ssp. marginata, O. elata ssp. hirsutissima and O. hookeri* as the female parent produced no viable seed.

Of the 44 crosses of *Calylophus hartwegii,* no seed was produced. The crosses of the three species of *Camissonia* produced seed which was aborted or underdeveloped. All of it failed to germinate. Similarly, the crosses of *Clarkia pulchella, Clarkia unguiculata* and *Clarkia purpurea* produced no viable seed. Of the 12 crosses of *Clarkia amoena,* one produced a total of 70 seeds, all of which failed to germinate.

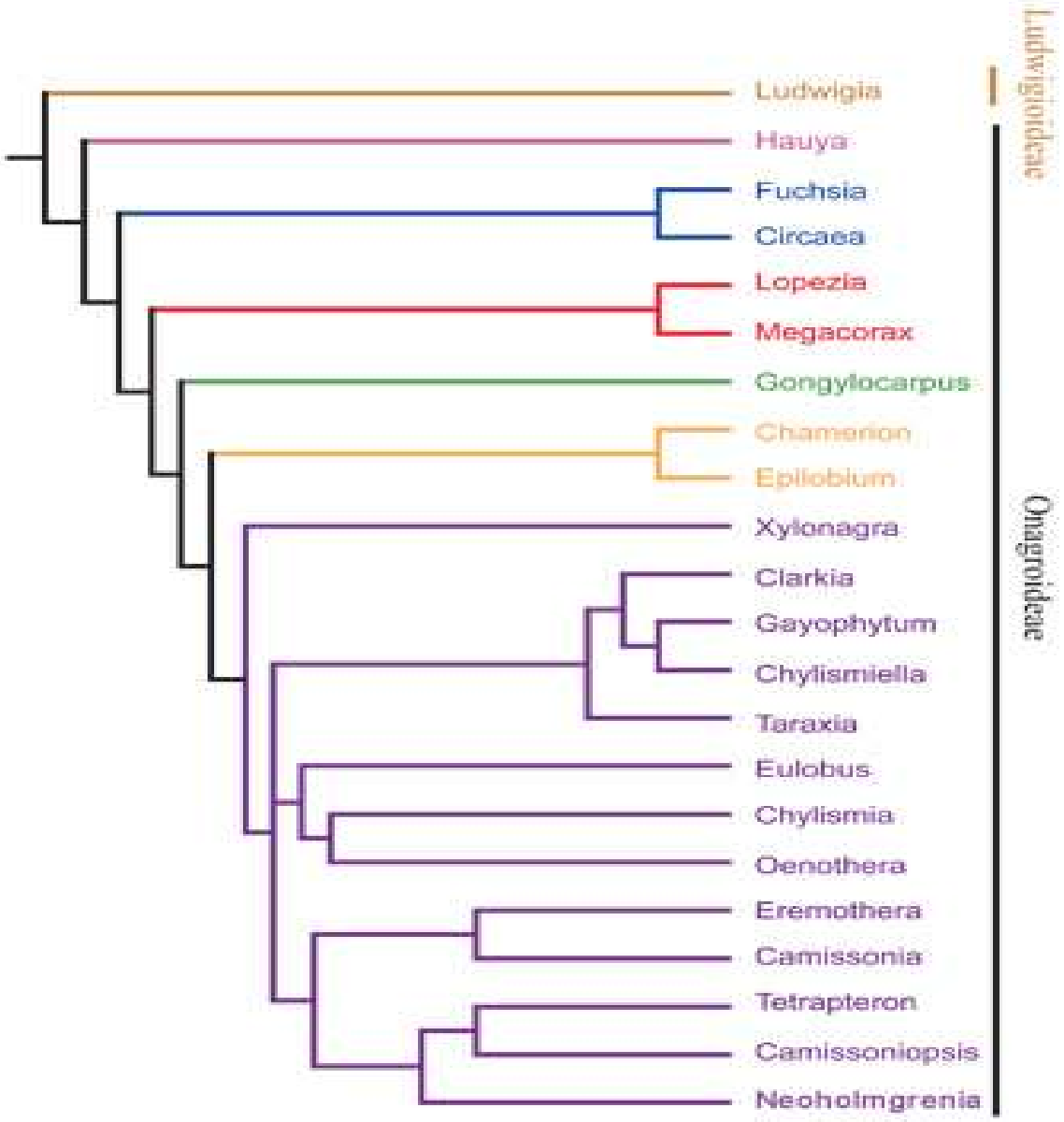
There were 43 crosses using *Oenothera rosea* (Figure 3.2) as female parent with seven distinct male parents. Only the cross *O. rosea x O. speciosa* produced viable seed. Of the 24 seeds only 12 germinated. Eleven of the seedlings resembled *O. rosea* and one seedling resembled *O. speciosa*. The flowers of *Oenothera rosea* are rose-colored and have an average diameter of 1.6 cm. Its leaves average 6 cm long and 2 cm wide and are

narrowly ovate. The pink or white flowers of *O. speciosa* (Figure 3.3) can range from 5 to 8 cm in diameter. Fully expanded adult leaves have an average length of 5 cm and a width of 2 cm. The leaves vary in shape from lanceolate and lobed to ovate (Richardson, 1995). The suspected hybrid (Figure 3.4) has flowers with the rose-coloration of *O. rosea* and the flower size (5.5 cm diameter) of *O. speciosa. Oenothera speciosa* typically has blooms which are cupped when fully open. The progeny has blooms resembling that of *Oenothera rosea,* which open completely to form a flat surface perpendicular to the capsule. The leaves of the progeny have an average width of 2 cm, common to both species. The average length of mature leaves is 4.5 cm which are ovate and slightly lobed toward the petiole. This is a shorter leaf than that of *O. rosea,* from which the seed was taken.

Conclusions

The resulting lack of success in crosses resulting from an interspecific *Oenothera* cross could be due to the differences in sections of the two plants. Consistent with the work of LK Crowe (1955), none of the plants from differing *Oenothera* sections were capable of producing any viable seed. The differences in chromosome arrangement between the various sections are likely the cause. The only two plants which showed potential for compatibility were *O. rosea* and *O. speciosa,* both of which were members of section *Hartmannia.* Other species which may have been compatible: *O. drummondii* and *O. hookeri* (*Raimannia*) or *O. elata*, *O. biennis,* and *O. longifolia (Oenothera).* Crosses were not made among these plants due to the sparseness of flowers or incompatible flowering periods. Hypothesis 1 was proved to be invalid. Hypothesis 2 was proved valid, but only in one case.

Figure 3.1. The phylogenetic tree of family *Onagraceae*



Levin et al. 2003.

Figure 3.2. Photograph of *Oenothera speciosa*.

Figure 3.3. Photograph of *Oenothera rosea*

Figure 3.4. Photograph of suspected cross *Oenothera rosea* × *speciosa*

Table 3.1 The sections of *Oentothera* species used in this breeding study.

Species	**Section**
O. drummondii	*Raimannia*(Rose ex Britton & A. Br.) Munz
O. hookeri	*Raimannia*(Rose ex Britton & A. Br.) Munz
O. pallida	*Anogra* (Spach) Jeps.
O. speciosa	*Hartmannia* (Spach) Munz
O. rosea	*Hartmannia* (Spach) Munz
O. missouriensis	*Megapterium* (Spach) Munz
O. caespitosa marginata	*Pachylophus* (Spach) Jeps.
O. elata hirsutissima	*Euoenothera* Torr. & A. Gray
O. biennis	*Euoenothera* Torr. & A. Gray
O. longifolia	*Euoenothera* Torr. & A. Gray
O. flava	*Lavauxia* (Spach) Jeps.
O. coronopifolia	*Chylismia* (Torr. & A. Gray) Jeps.

Table 3.2 The interspecific and intergeneric crosses of twelve species of *Oenothera*.

Female parent	Male parent	# of Crosses made	Date of cross	Date sown	# of seeds	Germ.
O. drummondii	*Camissonia cheiranthifolia*	1	4/8/2010	-	-	-
	O. rosea	2	4/9/2010	-	-	-
	Clarkia unguiculata	2	10/7/2010	-	-	-
	Clarkia unguiculata	6	10/8/2010	-	-	-
	O. speciosa	2	2/21/2011	-	-	-
	Clarkia purpurea	3	2/24/2011	-	-	-
	Clarkia purpurea	4	3/4/2011	-	-	-
	Camissonia cheiranthifolia	5	3/7/2011	-	-	-
	O. speciosa	2	3/7/2011	-	-	-
	O. caespitosa marginata	5	3/8/2011	6/2/2011	50	22
	O. speciosa	4	3/8/2011	-	-	-
	Camissonia cheiranthifolia	5	3/14/2011	-	-	-
	O. caespitosa marginata	6	3/14/2011	-	-	-
	Clarkia purpurea	5	5/26/2011	9/26/2011	37	34
	Clarkia unguiculata	5	5/26/2011	9/26/2011	12	7
	O. rosea	4	6/6/2011	9/26/2011	61	56
	Clarkia pulchella	4	6/6/2011	9/26/2011	34	22
	Clarkia amoena	5	6/16/2011	9/26/2011	51	32
	Calylophus Hartwegii	5	7/27/2011	9/26/2011	39	30
	O. biennis	5	7/27/2011	9/26/2011	33	0
	Clarkia purpurea	1	8/3/2011	-	-	-
	O. speciosa	5	8/4/2011	9/26/2011	15	9
O. pallida	*O. speciosa (wh)*	5	4/8/2010	-	-	-
	Camissonia cheiranthifolia	1	4/9/2010	-	-	-

Female parent	Male parent	# of Crosses made	Date of cross	Date sown	# of seeds	Germ.
O. pallida	*Clarkia amoena*	5	5/13/2010	-	-	-
	Camissonia cheiranthifolia	2	5/17/2010	-	-	-
	Camissonia cheiranthifolia	5	5/18/2010	-	-	-
	Clarkia pulchella	5	5/19/2010	-	-	-
	Clarkia amoena	5	5/19/2010	-	-	-
	Clarkia unguiculata	6	5/21/2010	-	-	-
	Clarkia purpurea	1	5/21/2010	3/14/2011	1	1
	Clarka amoena	3	6/2/2010	-	-	-
	Camissonia ignota	6	6/4/2010	-	-	-
	Clarkia purpurea	7	6/7/2010	-	-	-
	Camissonia ignota	4	6/10/2010	3/14/2011	0	-
	O. speciosa	3	6/10/2010	3/14/2011	0	-
	O. sp	2	6/10/2010	-	-	-
	O speciosa	1	9/17/2010	-	-	-
	Clarkia unguiculata	1	11/22/2010	3/14/2011	0	-
O. speciosa	*Camissonia cheiranthifolia*	1	11/22/2010	-	-	-
	Camissonia cheiranthifolia	1	2/21/2011	-	-	-
	Camissonia cheiranthifolia	2	2/23/2011	-	-	-
	Clarkia unguiculata	6	5/25/2011	9/26/2011	0	-
	Clarkia pulchella	5	6/16/2011	9/26/2011	0	-
	Calylophus hartwegii	5	7/27/2011	9/26/2011	0	-
	O. biennis	5	7/28/2011	9/26/2011	0	-
	Camissonia cheiranthifolia	5	8/1/2011	9/26/2011	0	-
	O. flava	5	8/4/2011	9/26/2011	0	-
	O. caespitosa marginata	8	8/5/2011	9/26/2011	0	-

Female parent	Male parent	# of Crosses made	Date of cross	Date sown	# of seeds	Germ.
O. speciosa	*O. caespitosa marginata*	3	4/9/2010	-	-	-
	Camissonia boothii	3	4/19/2010	-	-	-
	Clarkia pulchella	5	4/20/2010	-	-	-
	Clarkia purpurea	5	4/30/2010	-	-	-
	O. macrocarpa	2	4/30/2010	-	-	-
	Clarkia pulchella	2	5/1/2010	-	-	-
	Camissonia ignota	4	5/13/2010	-	-	-
	Clarkia pulchella	5	5/17/2010	-	-	-
	Clarkia purpurea	8	5/17/2010	-	-	-
	Clarkia amoena	5	5/19/2010	-	-	-
	Clarkia unguiculata	5	5/19/2010	-	-	-
	Camissonia boothii	1	5/20/2010	-	-	-
	Clarkia unguiculata	3	5/21/2010	-	-	-
	Camissonia boothii	3	5/21/2010	-	-	-
	Clarkia amoena	10	5/21/2010	-	-	-
	Camissonia cheiranthifolia	7	6/7/2010	-	-	-
	Camissonia boothii	3	6/9/2010	-	-	-
	Calylophus hartwegii	5	6/9/2010	-	-	-
	O. Lemon Sunset	1	6/10/2010	3/14/2011	0	-
	O. missouriensis	3	8/19/2010	-	-	-
	O. Lemon Sunset	4	8/24/2010	3/14/2011	0	-
	O. rosea	4	8/25/2010	-	-	-
	Camissonia cheiranthifolia	3	8/25/2010	-	-	-
	O. caespitosa marginata	10	8/27/2010	3/14/2011	0	-
	O. elata hirsutissima	1	8/30/2010	-	-	-
	Camissonia cheiranthifolia	6	9/1/2010	-	-	-

Female parent	Male parent	# of Crosses made	Date of cross	Date sown	# of seeds	Germ.
O. speciosa	*O. rosea*	3	9/16/2010	-	-	-
	O. rosea	2	9/17/2010	-	-	-
	Calylophus hartwegii	3	9/20/2010	-	-	-
	Clarkia unguiculata	2	9/30/2011	-	-	-
	(X) self	3	10/7/2010	-	-	-
	Clarkia unguiculata	1	10/8/2010	-	-	-
	O. drummondii	2	2/21/2011	3/14/2011	0	-
	Camissonia cheiranthifolia	2	2/23/2011	3/14/2011	0	-
	Clarkia purpurea	2	2/24/2011	3/14/2011	0	-
	Camissonia cheiranthifolia	10	3/2/2011	3/14/2011	0	-
	O. caespitosa marginata	14	3/9/2011	3/14/2011	0	-
	Clarkia purpurea	5	3/10/2011	3/14/2011	0	-
	Clarkia purpurea	2	3/17/2011	3/14/2011	0	-
O. missouriensis	*Camissonia boothii*	1	4/14/2010	-	-	-
	Camissonia boothii	1	4/19/2010	-	-	-
	Camissonia cheiranthifolia	2	5/17/2010	-	-	-
	Calylophus hartwegii	6	7/22/2010	-	-	-
	Camissonia ignota	5	7/29/2011	-	-	-
	Clarkia unguiculata	3	8/4/2010	-	-	-
	O. speciosa	1	9/30/2010	-	-	-
	Camissonia cheiranthifolia	1	10/7/2010	-	-	-
	Clarkia unguiculata	6	10/8/2010	3/14/2011	0	0
	Clarkia unguiculata	5	5/25/2011	-	-	-
	Clarkia pulchella	5	5/25/2011	-	-	-

Female parent	Male parent	# of Crosses made	Date of cross	Date sown	# of seeds	Germ.
Clarkia pulchella	*O. speciosa*	2	4/20/2010	-	-	-
	O. speciosa	3	5/1/2010	-	-	-
	O. pallida	5	5/17/2010	-	-	-
	O. pallida	3	6/9/2010	-	-	-
Clarkia purpurea	*x O. missouriensis*	5	5/14/2010	-	-	-
	x O. speciosa	3	5/14/2010	-	-	-
	x O. rosea	1	5/17/2010	-	-	-
	x Clarkia amoena	5	5/19/2010	-	-	-
	x O. pallida	5	5/21/2010	-	-	-
	x O. speciosa	2	6/9/2010	-	-	-
	x O. speciosa	10	3/7/2011	9/26/2011	0	-
	x O. caespitosa marginata	8	3/16/2011	-	-	-
	x O. rosea	2	3/17/2011	-	-	-
Clarkia unguiculata	*O. missouriensis*	5	5/14/2010	-	-	-
	O. speciosa	5	5/14/2010	-	-	-
	Camissonia ignota	5	5/14/2010	-	-	-
	O. missouriensis	1	5/17/2010	-	-	-
	Camissonia ignota	1	5/17/2010	-	-	-
	O. speciosa	8	5/18/2010	-	-	-
	O. pallida	1	5/19/2010	-	-	-

Female parent	Male parent	# of Crosses made	Date of cross	Date sown	# of seeds	Germ.
Clarkia	*O. pallida*	1	5/21/2010	-	-	-
unguiculata	*O. missouriensis*	1	7/30/2010	-	-	-
	O. missouriensis	2	8/24/2010	-	-	-
	O. Coronopifolia	5	8/27/2010	-	-	-
	O. missouriensis	1	8/30/2010	-	-	-
	O. missouriensis	7	10/7/2010	-	-	-
	O. missouriensis	2	10/13/2010	-	-	-
	O. speciosa	2	5/26/2011	-	-	-
	O. lemon sunset	5	6/2/2011	-	-	-
Clarkia amoena	*O. pallida*	2	5/17/2010	-	-	-
	O. pallida	1	5/20/2010	8/3/2010	70	-
	O. speciosa	2	5/21/2010	-	-	-
	O. speciosa	2	6/10/2010	-	-	-
	O. pallida	2	6/11/2010	-	-	-
	O. pallida	3	6/14/2010	-	-	-
Camissonia	*O. pallida*	5	5/17/2010	-	-	-
boothii	*O. speciosa*	5	5/17/2010	-	-	-
				-	-	-
O. rosea	*Clarkia purpurea*	1	5/17/2010	-	-	-
	Clarkia purpurea	3	5/18/2010	-	-	-
	Clarkia purpurea	6	6/15/2010	-	-	-
	Clarkia unguiculata	6	6/15/2010	-	-	-

Female parent	Male parent	# of Crosses made	Date of cross	Date sown	# of seeds	Germ.
O. rosea	*O. speciosa*	1	6/15/2010	3/14/2011	24	12
	Camissonia cheiranthifolia	3	3/7/2010	-	-	-
	Clarkia purpurea	5	5/27/2011	-	-	-
	Clarkia unguiculata	5	5/27/2011	-	-	-
	O. lemon sunset	5	5/27/2011	-	-	-
	O. sp	3	6/3/2011	-	-	-
	Calylophus hartwegii	5	8/5/2011	-	-	-
O. caespitosa marginata	*Clarkia purpurea*	5	5/19/2010	-	-	-
	Camissonia boothii	1	5/20/2010	-	-	-
	O. speciosa	1	6/10/2010	-	-	-
	O. Missouriensis	3	8/24/2010	-	-	-
	Calylophus hartwegii	1	8/27/2010	-	-	-
	Clarkia unguiculata	2	9/16/2010	-	-	-
	Clarkia purpurea	2	2/23/2011	-	-	-
	Clarkia purpurea	3	3/2/2011	-	-	-
	Camissonia cheiranthifolia	5	3/4/2011	-	-	-
	O. speciosa	2	3/8/2011	-	-	-
	Clarkia unguiculata	1	5/25/2011	-	-	-
	Clarkia pulchella	1	6/3/2011	-	-	-

Female parent	Male parent	# of Crosses made	Date of cross	Date sown	# of seeds	Germ.
Calylophus hartwegii	*O. speciosa*	2	6/9/2010	-	-	-
	O. pallida	2	6/11/2010	-	-	-
	O. speciosa	3	6/14/2010	-	-	-
	O. pallida	2	6/16/2010	-	-	-
	O. caespitosa marginata	3	8/3/2010	-	-	-
	O. missouriensis	8	8/3/2010	-	-	-
	Camissonia cheiranthifolia	4	8/3/2010	-	-	-
	O. elata hirsutissima	5	8/3/2010	-	-	-
	O. speciosa	8	8/25/2010	-	-	-
	O. caespitosa marginata	1	8/25/2010	-	-	-
	O. speciosa	1	9/30/2010	-	-	-
	O. sp	2	6/20/2011	-	-	-
	O. flava	2	7/28/2011	-	-	-
	O. biennis	1	7/29/2011	-	-	-
Camissonia cheiranthifolia	*O.speciosa*	4	6/7/2010	-	-	-
	O. pallida	2	6/9/2010	-	-	-
	O. pallida	3	6/10/2010	-	-	-
	O. speciosa	2	6/14/2010	-	-	-
	O. missouriensis	8	7/28/2010	-	-	-
	O. rosea	2	11/22/2010	-	-	-
	O. pallida	3	11/22/2010	-	-	-
	O. drummondii	3	2/23/2011	-	-	-
	O. drummondii	4	2/24/2011	-	-	-

Female parent	Male parent	# of Crosses made	Date of cross	Date sown	# of seeds	Germ.
Camissonia cheiranthifolia	*O. speciosa(white)*	1	3/7/2011	-	-	-
	O. speciosa	3	3/10/2011		-	-
	O .speciosa	1	3/14/2011	-	-	-
	O. coronopifolia	5	5/25/2011	-	-	-
	O. missouriensis	5	6/16/2011	-	-	-
	O. pallida	5	7/27/2011	-	-	-
Oenothera elata hirsutissima	*Clarkia unguiculata*	3	5/21/2010	-	-	-
	Calylophus hartwegii	4	8/24/2010	-	-	-
Camissonia ignota	*O. pallida*	5	6/3/2010	-	-	-
	O. speciosa	7	6/7/2010	-	-	-
	O. missou	9	7/28/2010	-	-	-
O. hookeri	*O. coronopifolia*	1	8/30/2010	-	-	-

LITERATURE CITED

Blakeslee, A.F. and R.E. Cleland. 1930. Circle formation in *Datura* and *Oenothera*. Proceedings of the National Academy of Sciences of the United States of America. 16(3): 177.

Catt, P. 2008. U.S. Patent No. PP18,402. Washington, DC: U.S. Patent and Trademark Office.

Cleland, R.E. 1924. Meiosis in pollen mother cells of *Oenothera franciscana sulfurea*. Bot. Gaz. 149-170.

Cleland, R.E. 1929. New evidence bearing upon the problem of the cytological basis for genetical peculiarities in the *Oenotheras*. Amer. Nat. *63*(689): 497-510.

Cleland, R. E. 1936. Some aspects of the cyto-genetics of *Oenothera*. Bot. Rev. 2(6): 316-348.

Cleland, R.E. 1951. Extra, diminutive chromosomes in *Oenothera*. Evol. 165-176.

Cleland, R.E. 1967. The origin of closed circles of five chromosomes in *Oenothera.* Am. J. Bot. 993-997.

Crowe, L.K. 1955. The evolution of incompatibility in species of *Oenothera*. Heredity. 9: 293-322.

Davis, B.M. 1916. Hybrids of *Oenothera biennis* and *Oenothera franciscana* in the first and second generations. Genet. 1(3): 197.

Darlington, C.D. 1929. Ring-formation in *Oenothera* and other genera. J. Genet. 20: 345-64.

Ellstrand, N.C. and D.A. Levin. 1982. Genotypic diversity in *Oenothera laciniata (Onagraceae*), a permanent translocation heterozygote. Evolution, 63-69.

Emerson, S. 1940. Growth of incompatible pollen tubes in *Oenothera organensis*. Bot. Gaz. 890-911.

Gates, R.R. 1907. Pollen Development in Hybrids of *Oenothera lata*× *O. Lamarckiana*, and Its Relation to Mutation. Bot. Gaz. 81-115.

Gates, R.R. 1909. The Behavior of the Chromosomes in *Oenothera lata* x *O. gigas*. Bot. Gaz. 179-199.

Gates, R.R. 1911. Pollen formation in *Oenothera gigas*. Ann. Bot. (4): 909-940.

Harshberger, J.W. 1905. Mutants and Hybrids of the *Oenotheras*. Torreya. 5(8): 147-149.

Havens, K. 1994. Clonal repeatability of in vitro pollen tube growth rates in *Oenothera organensis (Onagraceae*). Am. J. Bot. 161-165.

Hofmann, S. 2006. U.S. Patent No. PP16,393. Washington, DC: U.S. Patent and Trademark Office.

Hupfer, H., M. Swiatek, S. Hornung, R.G. Herrmann, R.M. Maier, W.L. Chiu, and B. Sears. 2000. Complete nucleotide sequence of the *Oenothera* elata plastid chromosome, representing plastome I of the five distinguishable *Euoenothera* plastomes. Mol. Gen. Genet. 263(4): 581-585.

Jacob, K.T. 1940. Chromosome catenation in *Oenothera*. Bot. Gaz. 143-155.

Kim, B.H. 2009. Gold evening primrose and method of breeding the same. WIPO Patent 2009017372.

Levin, R.A., W.L. Wagner, P.C. Hoch, M. Nepokroeff, J.C. Pires, E.A. Zimmer, and K.J. Sytsma. 2003. Family-level relationships of *Onagraceae* based on chloroplast rbcL and ndhF data. Amer. J. Bot. 90(1): 107-115.

Lewis, D. 1949. Incompatibility in flowering plants. Biol. Rev. 24(4): 472-496.

Morum, L.A. 2006. U.S. Patent No. PP17,230. Washington, DC: U.S. Patent and Trademark Office.

Ogden, S., and L.S. Ogden. 2009. U.S. Patent No. PP19,663. Washington, DC: U.S. Patent and Trademark Office.

Raguso, R.A., A. Kelber, M. Pfaff, R.A. Levin, and L.A. McDade. 2007. Floral biology of north American *Oenothera* sect. *Lavauxia* (Onagraceae): advertisements, rewards, and extreme variations in floral depth 1, 2. Ann. Misso. Bot. Gard. *94*(1): 236-257.

Richardson, A. 1995. Plants of Southernmost Texas. University of Texas Press. 85-86.

Silander, J.A. and R.B. Primack. (1978). Pollination intensity and seed set in the evening primrose (*Oenothera fruticosa*). American Midland Naturalist. 213-216.

Theiss, K. E., K.E. Holsinger, and M.E. Evans. 2010. Breeding system variation in 10 evening primroses (*Oenothera* sections *Anogra* and *Kleinia*; *Onagraceae*). Am. J. Bot. 97(6): 1031-1039.

Wagner, W.L. 1983. New species and combinations in the genus *Oenothera (Onagraceae*). Ann. Misso. Bot. Gard. 194-196.

Wolin, C.L., C. Galen and L. Watkins. 1984. The breeding system and aspects of pollination effectiveness in *Oenothera speciosa* (*Onagraceae*). Southw. Nat. 15-20.

VITA

Stephen D. Stanphill

Candidate for the Degree of

Master of Science

Thesis: PROPAGATION, HYBRIDIZATION AND GENETIC MANIPULATION OF *OENOTHERA* SPECIES

Major Field: Horticulture

Biographical:

Education:

Completed the requirements for the Master of Science/Arts in Horticulture at Oklahoma State University, Stillwater, Oklahoma in July, 2013.

Completed the requirements for the Bachelor of Science in Forestry at Oklahoma State University, Stillwater, Oklahoma in May, 2004.

Experience:

Staff Forester I, Texas Forest Service. Idalou, Texas. June, 2004 – August, 2008.

Senior Research Specialist, Department of Horticulture and Landscape Architecture, Oklahoma State University. Stillwater, Oklahoma. October, 2008 – Present.

Professional Memberships: American Society for Horticultural Science