Demographic Studies of Endemic Plants at the Antioch Dunes National Wildlife Refuge.

I. Seed Production and Germination

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Prepared for

Endangered Plant Project
California Department of Fish and Game
1416 Ninth Street, Room 1225
Sacramento, CA 95814

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Abstract

This study was designed to identify environmental and genetic factors that constrain the growth of populations of Erysimum capitatum (Dougl.) Greene var. angustatum (Greene) G. Rossb.) (Contra Costa Wallflower = ECA) and Oenothera deltoides Torr. & Frem. ssp. howellii (Munz) Klein (Antioch Dunes Evening Primrose = ODH), both endemic to Antioch Dunes National Wildlife Refuge in Contra Costa County, California. Herein we report on the relevant aspects of seed production and germination. A second report will deal with seedling demography, seedling emergence in the lab and field and seed bank dynamics.

With respect to seed production in situ; l) large numbers of seeds were produced by individuals of ODH and ECA in 1987, with average-sized plants producing about the same amount of seed as non-endangered relatives, 2) there appears to be no convenient method for artificially increasing seed production in ODH and ECA using supplements of water and mineral nutrients, and 3) a lack of suitable pollinators was implicated as the most serious limitiation imposed on seed production in ODH, with fertilization and pre-dispersal predation relatively less important in both taxa. Based on these findings we conclude that seed production does not limit ODH or ECA at this time but that 1) all populations of these taxa must continue to receive protection in order to insure maximum seed production and 2) identification, protection and enhancement of pollinator populations must be given management priority at Antioch Dunes NWR, especially those insects responsible for ODH pollination.

Germination studies showed that; 1) the overall germination potential of new ODH and ECA seeds was high, with 40-60% typical (depending on germination conditions) and 2) establishing artificial populations of ODH and ECA from seed will require different treatments to maximize germination, including stratification of ODH seeds. These results are in accordance with studies of common and rare plants and underscore the fact that seed germination per se is not necessarily a constraint on the growth of endangered populations.

Preface

Over the last 15 years, rare plant research has concentrated on providing information on the distribution and synecological relationships of endangered populations. Such research has greatly benefitted from standardization of survey and documentation techniques (Barkley 1981, Goff et al. 1982, Nelson 1984), thereby allowing the development of reliable data bases for identifying and protecting the most threatened taxa. In some cases, the result has been establishment of rare plant preserves in one form or another (e.g. "areas of critical environmental concern" such as Eureka Dunes in Inyo County, CA, "wildlife refuges" such as Antioch Dunes National Wildlife Refuge in Contra Costa Co., CA, and "botanical areas" like Cuesta Ridge in San Luis Obispo County, CA).

Although these preserves and other forms of land-use restriction are absolutely essential, they do not, in and of themselves, ensure the recovery and persistence of endangered populations. It has become increasingly apparent that these populations must be actively managed, beyond passive protection, in order to slow and eventually reverse their decline. Endangered species management requires quantitative, autecological data that can readily be generated from field monitoring programs.

Monitoring is a quantitative assessment of the status of a population over time using data derived from individual plants. Excluded are conservation efforts related to rare plant inventory (a geographically-based assessment of entire taxa) and survey (an ecologically-based assessment of entire taxa). These definitions differ from those presented by Palmer (1987), who included all three activities under the term "monitoring". We would prefer to limit the definition of monitoring in order to be consistent with existing policies and procedures for conserving endangered species. Traditionally, federal and state agencies generate lists or inventories based on the political geography of rare taxa (e.g. Ayensu and Defilipps 1978, Smith et al. 1984) rather than with the degree or causes of endangerment at any one locality. Indeed, these inventories are conducted without even visiting the known or historical sites of the taxa in question. Surveys, in contrast to inventories , involve field searches for populations of individual taxa based on ecological relationships (Nelson 1984). During a survey, populations are mapped, characterized with respect to habitat and endangerment factors, and individuals may be counted. A survey census can be quite sporadic (e.g. only once a year) and does not follow the fates of specific plants. A monitoring census, however, is accomplished by marking individual plants in a population and repeatedly measuring characteristics of their performance in situ (Davy and Jefferies 1981). Monitoring is, therefore, capable of identifying the timing and causes of poor performance (e.g. mortality prior to reproduction) and providing specific management recommendations for amelioration.

Rare plant monitoring has been discussed in general terms by Davy and Jefferies (1981), Williams (1981) and Huenneke et al. (1986). It is clear that a large number of demographic, genetic, and physiological characteristics of populations could be chosen for assessing performance over long or short periods of time, depending on the biology of the taxon in question. This creates a number of problems for the resource agency or biologist charged with designing, executing and interpreting a monitoring program under the usual time and monitary constraints imposed by the "real" (political) world. What kind of monitoring is appropriate? Which attributes need to be monitored under most circumstances? How will the quantitative parameters derived from a monitoring program be interpreted and used for managing a population? How does monitoring "fit in" to the established proceedures for recovery of an endangered species? Most managers with training in plant ecology recognize that life history traits, demographic attributes and genetic heterogeneity are important to plant conservation efforts, but they also need to know how these aspects of rare plant biology can be incorporated into monitoring programs and used to generate management decisions.

Demographic studies of plants indicate that each population possesses unique attributes that ultimately determine local abundance and/or persistence through time (Harper 1977). A thorough analysis of these attributes is of primary importance in the management of endangered populations, simply because abundance and persistence are at the center of all recovery efforts. Such efforts are usually undertaken once the threat of human disturbance has been minimized (preservation by land-use restriction). This is because preserve management requires a knowledge of the population's status (whether it is declining, stable, or growing) under the most "natural" of conditions. This determination can be made by selecting a few, relevant population attributes and conducting a demographic monitoring program (Pavlik and Barbour 1988). The purpose of our research program as a whole is to illustrate the use of a few selected population attributes, particularly seed production, germination, seed bank, and seedling survivorship, for determining the post-preservation status of endangered plant populations.

We sought to identify those environmental and genetic factors that constrain the growth of populations of *Erysimum capitatum* (Dougl.) Greene var. *angustatum* (Greene) G. Rossb.) (Contra Costa Wallflower) and *Oenothera deltoides* Torr. & Frem. ssp. *howellii* (Munz) Klein (Antioch Dunes Evening Primrose), both endemic to Antioch Dunes National Wildlife Refuge in Contra Costa County, California. In doing so, practical recommendations for active management were made in order to promote the persistence of these two taxa. Herein we report the results of seed production and germination studies. A second report, dealing with seedling demography, seedling emergence in the lab and field and seed bank characteristics, will be available in late 1988.

Acknowledgements

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Seed Production

Introduction

The population size of a plant species within a given area of habitat can be limited by the production of viable seeds, particularly when the species does not reproduce asexually. Erysimum capitatum (Dougl.) Greene var. angustatum (Greene) G. Rossb.), like most biennial plants, does not appear to propagate asexually (Harper 1977). Populations of this taxon, therefore, depend entirely on seed production. Oenothera deltoides Torr. & Frem. ssp. howellii (Munz) Klein may be capable of producing independent ramets from rosettes that form on the floral axis or on creeping underground rootstocks (Klein 1970, Pavlik l979a). The demographic significance of ramet production in Oenothera deltoides ssp. howellii is unknown and will not be addressed by the present study. It is assumed, however, that seed production is essential to the maintenance of the primrose population.

Both study species have the potential of producing large amounts of seed in an average year. This is because many ovules are usually formed within the ovaries of Oenothera and Erysimum flowers, regardless of species. However, the actual number of viable seeds produced per plant may be limited by a number of intrinsic and extrinsic factors. Intrinsic factors may include genetically-programmed fruit or ovule abortion (neither of which are known from these genera according to Wilson (1983)). Extrinsic factors include resource limitations, pollination limitations and predation on seeds or portions of the whole plant. Resource limitations (e.g. lack of water or mineral nutrients) inhibit vegetative growth, and therefore, the number of flower-bearing branches and ovule-containing ovaries. Inefficiencies in the system of pollination (pollinator availability and behavior, timing of floral anthesis) and in the ability of the pollen to affect fertilization (compatible pollen density on the stigma, pollen tube competition and growth) will allow only a fraction of the total ovules to be transformed into seeds. Further reductions in seed production may occur because of pre-dispersal predation by insects on the fruit of adult plants.

This investigation of *Erysimum capitatum* var. *angustatum* and *Oenothera deltoides* ssp. *howellii* at Antioch Dunes National Wildlife Refuge sought to 1) determine if *in situ* seed production is limited by water and mineral nutrient availability, 2) develop predictive relationships between plant size (canopy volume) and viable seed production, 3) compare seed production in *Erysimum capitatum* var. *angustatum* and *Oenothera deltoides* ssp. *howellii* to the closely-related but non-endangered *Erysimum capitatum* var. *capitatum* (Dougl.) Greene and *Oenothera hookeri* T. & G., and 4) examine the relative importance of pollination limitations, fertilization limitations and pre-dispersal predation on seed production in the endangered taxa.

Methods and Materials

Selection of plants. In early April 1987, 33 established plants of *Oenothera deltoides* ssp. *howellii* (ODH) and 33 of *Erysimum capitatum* var. *angustatum* (ECA) were marked and mapped at the Stamm and Sardis units of Antioch Dunes National Wildlife Refuge (ADNWR). These plants appeared to be vigorous and grew at microsites that were typical for the species at Antioch Dunes. ODH was studied on the sandy soils and clay pans of the Sardis Site; ECA on the sandy dune soils at the Stamm site. (A map of these localities will be included in the November 1988 report.)

Plants were also selected to insure that a wide variety of sizes (canopy volumes) were represented in the total sample. These plants either received supplements of water, Hoagland's mineral nutrient solution in water, or served as controls.

On 1 June 1987, a population of *Erysimum capitatum* var. *capitatum* was located along the summit trail of Mt. Diablo State Park (9.6 miles past the park entrance on North Gate Road) at an elevation of 3000'. Vegetation at the site was a mosaic of grassland, chaparral and pine-juniper woodland. A total of 9 plants were selected from undisturbed microsites along the beginning of the trail for *in situ* measurements of canopy volume. (This taxon was essentially the same growth habitat as ECA, so that canopy volume measurements were very comparable.) These plants were harvested and brought back to the laboratory for estimates of seed production, which was probably close to maximum for the species and locality (few unopened flowers remained on these robust individuals). On 7 September 1987 a population of *Oenothera hookeri* (OH) was located in Santa Cruz County near Manresa Beach State Park along Highway 1, in a disturbed ditch (elevation = 10'). Nine vigorous individuals were selected for *in situ* measurements of canopy volume. (This taxon has a distinctly different growth form that ODH, so that canopy volume measurements were not strictly comparable. Attempts to find a population of *Oe. deltoides* Torr. & Frem. var. *cognata* (Jeps.) Munz were unsuccessful. However, seed production in ODH can be compared with that of the closely related *Oenothera avita* (W. Klein) W. Klein ssp. *eurekensis* (Munz & Roos) W. Klein (Pavlik and Barbour 1986).) These plants were harvested and brought back to the laboratory for estimates of seed production, which was probably close to maximum for the species and locality (only a few unopened flowers remained on these robust individuals).

Water and nutrient supplements. Of the 33 marked plants of each species, six received supplements of water, six received supplements of all essential mineral nutrients in water and six were untreated and served as controls. The remaining 15 plants were also untreated and used to develop canopy volume-seed production relationships (see below). Although plants were assigned treatments randomly, an effort was made to include six plants of similar, average size (canopy volume) in every treatment class. Each week, beginning in April l987, one liter of distilled water or one liter of half-strength Hoagland's solution (Hoagland and Arnon 1949) were poured within a 5 cm radius at the base of the appropriate plants. The monthly water supplement was equivalent to 100 times the average yearly precipitation of Antioch Dunes (about 12.5"). The monthly nitrogen supplement was equivalent to 1200 times the concentration found in nutrient-poor dune substrates (Pavlik 1979a). These treatments were continued until 3 June l987.

Plant size. A non-destructive method was used to estimate the canopy volume (V_C) of individual plants. To estimate the canopy volumes of ODH and OH, the cover of each plant was mentally projected onto the ground surface and the average length and width of the canopy measured (± l cm). Isolated branches that protruded beyond the idealized outline were not included. Average height of the plant was recorded in the same way, ignoring isolated, tall branches and inflorescences that did not bear foliage. Assuming the canopy approximated a rectangular solid, V_C was the product of the three measurements. Other measures of canopy volume did not result in higher correlation coefficients and, therefore, were rejected on that basis. V_C was considered the best measure of plant size because of the potential of Oenothera species to grow vertically (Pavlik l979a, Pavlik and Barbour 1985) and because burial by sand could reduce the amount of illuminated leaf area and the number of exposed flowers without influencing estimates of (planar) canopy area.

The canopy volumes (V_C) of ECA and ECC were estimated by measuring the length of the infructescence (total plant height - height to the first fruiting branches) and multiplying this by the square of the mean infructescence radius times pi ((long radius + short radius/2)$^2\pi$). This treats V_C of ECA and ECC individuals as a cylindrical solid based solely on infructescence volume. Other measures of canopy volume did not result in higher correlation coefficients (V_C vs. seed output) and, therefore, were rejected on that basis.

Estimates of seed production. An enumeration model was used for each species in order to estimate actual seed output per plant at the time of maximum flower and fruit production (late April - mid May for ODH and ECA, early June - mid-July for ECC and OH). The basic procedure is amplified for each taxon below.

Oenothera deltoides ssp. howelli - V_C and the total number of all ovaries were measured in situ for each marked plant on 30 April 1987. The ovaries were categorized as either Type I (developed ovaries containing seed, = capsules) or Type II (undeveloped ovaries without seed). Type I ovaries were further divided into subtypes a (full-length fruits, approximately 4-6 cm long) and b (short fruits, approximately 2-4 cm long). A number of other measurements and observations were also recorded, including the number of branches/plant, open flowers/plant and the presence of predaceous insects. The April sampling data were used to represent peak seed production in ODH from counts of Type I capsules per plant (C_p) and the number of good seeds per capsule (S_c, see below).

On 30 June 1987, canopy volumes were again measured and two mature but non-dehisced capsules (one of Type Ia and one of Type Ib) were collected from each of the 33 individuals (n = 66 capsules). These capsules were dissected in the lab to determine the number of seeds per capsule of either Type 1a or 1b. Each seed was further categorized as good (completely filled, with typical shape), poor (partially filled, shrivelled or with irregular shape), or damaged (irregular shape or features due to predation). In addition, the number of funiculi were counted in each fruit to determine the original number of ovules formed in every fruit. The average number of good seeds per capsule (S_c) was used to calculate seed production per plant based on the number of Type I capsules per plant (S_c X C_p).

In order to determine if seed production was protracted beyond the 30 April 1987 date and to spot-check the validity of the S_c X C_p enumeration model, 9 previously-marked, control individuals of ODH were collected and brought back to the lab on 30 June 1987. The number of Type I capsules per plant (C_p) were counted and categorized into subtypes a and b. Two capsules of subtypes a and b from each of these individuals were sampled to determine the number of good, poor, and damaged seeds/plant. The average number of good seeds per capsule (S_c) was then used to calculate seed production per plant based on the number of capsules per plant (C_p). The product of S_c and C_p is an estimate of the total number of seeds per plant of size V_c. Oenothera is a non-determinant flower producer, so even this late estimate probably underestimates the total seed production for the year.

Oenothera hookeri - The ODH procedure was applied to OH, but with a sample of 18 Type Ia and Type Ib fruits. In addition, because so many more seeds were found in OH capsules relative to ODH capsules, a weight-number relationship was developed for counting the seeds once they were sorted into good, poor and damaged categories.

Erysimum capitatum var. angustatum - On 13 May 1987, canopy volumes were estimated and seed capsules counted and categorized in situ as an estimate of seed production during the period of maximum fruit production. The ovaries were categorized as either Type I (developed ovaries containing seed, = siliques) or Type II (undeveloped ovaries without seed). Type I ovaries were further divided into subtypes a (full-length fruits, approximately 4-6 cm long) and b (short fruits, approximately 2-4 cm long). A number of other measurements and observations were also recorded, including the number of branches/plant, open flowers/plant and the presence of predaceous insects. The May sampling data were used to represent peak seed production in ECA from counts of Type I siliques per plant (SI_p) and the number of good seeds per silique (S_{sI}, see below).

On 30 June 1987, canopy volumes were again measured and two mature but non-dehisced siliques (one of Type Ia and one of Type Ib) were collected from each of the 33 individuals (n = 66 siliques). These siliques were dissected in the lab to determine the number of seeds per silique in Type 1a or 1b fruits. Each seed was further categorized as good (completely filled, with typical shape), poor (partially filled, shrivelled or with irregular shape), or damaged (irregular shape or features due to predation). In addition, the number of funiculi and depressions in the ovary wall were counted in each fruit to determine the original number of ovules formed in every fruit. The average number of good seeds per silique (S_{sI}) was used to calculate seed production per plant based on the number of Type I siliques per plant (S_{sI} X SI_p).

In order to determine if seed production was protracted beyond the 13 May date and to spot-check the validity of the S_{sI} X SI_p enumeration model, 9 previously-marked, control individuals of ECA were collected and brought back to the lab on 30 June 1987. The number of Type I siliques per plant (SI_p) were counted and categorized into subtypes a and b. Two siliques of subtypes a and b from each of these individuals were sampled to determine the number of good, poor, and damaged seeds/plant. The average number of good seeds per silique (S_{sI}) was then used to calculate seed production per plant based on the number of capsules per plant (Sl_p). The product of S_{sl} and Sl_p is an estimate of the total number of seeds per plant of size V_c. Erysimum is a determinant flower producer, so these estimates account for most of the yearly seed production.

Erysimum capitatum var. capitatum - The ECA procedure was applied to ECC, but with a sample of 18 Type Ia and Type Ib fruits.

Assessing pollination, fertilization and predation limitations. Data gathered from the samples described above were used to calculate the number of ovules produced in all flowers on an individual (maximum reproductive potential). The number of good, poor, and damaged seeds were used to estimate limitations on reproductive potential due to pollination, fertilization and pre-dispersal insect predation. This analysis assumes that no intrinsic limitations exist in ODH and ECA (e.g. spontaneous fruit abortion), an assumption that will be tested in the 1987-1988 field season.

Oenothera (ODH only) - The 33 marked plants were also used to determine the total number of ovaries produced per plant (flower buds + flowers + developed fruits + undeveloped fruits) at the time of peak fruit production (30 April). The average number of ovaries per plant of size V_c multiplied by the average number of ovules per ovary (obtained by counting the average number of funiculi in each of the 66 capsules discussed above) gave an estimate of the average number of ovules per plant (O_p). The presence of undeveloped (Type

II) ovaries was attributed to a pollination limitation - that is, suitable pollen (from another individual in this obligate outcrosser) in any quantity was not deposited on the stigmatic surface. Type I ovaries received enough pollen to initiate seed and fruit development, but not all of these developed fruits formed the same number of good quality seed. The presence of poor quality (deformed) seeds was interpreted as resulting from limitiations during fertilization, zygote formation and embryogenesis. These "fertilization" limitations were derived from the average proportion of poor quality seeds in each of the 66 fruits dissected. Limits imposed by pre-dispersal insect predation were likewise derived from the average proportion of damaged seeds in these 66 capsules.

Erysimum (ECA only) - The 33 marked plants were also used to determine the total number of ovaries produced per plant (flower buds + flowers + developed fruits + undeveloped fruits) at the time of peak fruit production (13 May). The average number of ovaries per plant of size V_c multiplied by the average number of ovules per ovary (obtained by counting the average number of funiculi or ovary wall depressions in each of the 66 siliques discussed above) gave an estimate of the average number of ovules per plant (O_p). The presence of undeveloped (Type II) ovaries was attributed to a pollination limitation - that is, suitable pollen in any quantity was not deposited on the stigmatic surface. Type I ovaries received enough pollen to initiate seed and fruit development, but not all of these developed fruits formed the same number of good quality seed. The presence of poor quality seeds was interpreted as resulting from limitiations during fertilization, zygote formation and embryogenesis. These "fertilization" limitations were derived from the average proportion of poor quality seeds in each of the 66 fruits dissected. Limits imposed by pre-dispersal insect predation were likewise derived from the average proportion of damaged seeds in these 66 siliques.

Results and Discussion

Effects of water and mineral nutrient supplements. Despite the application of relatively large amounts of these resources throughout the reproductive season, no significant effect on seed production could be detected in Oenothera deltoides ssp. howellii (ODH) or Erysimum captitatum var. angustatum (ECA) (Table 1). In fact, both ODH and ECA exhibited reduced seed production when given water supplements alone. The addition of nutrient-supplemented water did not significantly improve seed production relative to control plants, but it did significantly improve it in ODH relative to the water only treatment ($P<0.05$, ANOVA).

The period of leaf and flower production of ODH was protracted well into July regardless of treatment. Additional vegetative and reproductive growth of ODH over the summer did not, however, result in additional seed production relative to that which occured by May and early June. This was probably because there were few pollinators available over the summer period which could affect pollination. The result was that many undeveloped capsules could be found on the uppermost portions of the branches while developed capsules, produced earlier, were confined to lower portions of the plant. Unlike the indeterminate ODH, the determinant flowering of ECA was not protracted and individuals were senescent by late May.

Table 1. Effects of water and nitrogen supplements on the seed production per plant (n=6 in control and treatment categories). Values (mean ± S.D.) within a taxon followed by the same letter (a, b) were not significantly different at $P<0.05$ (ANOVA).

	seed production (#/plant)		
	control	+ H_2O	+nutrients in H_2O
Oenothera	2527 ± 680^{a}	1654 ± 385^{b}	2250 ± 422^{a}
Erysimum	2002 ± 998^{b}	1034 ± 761^{b}	1859 ± 1077^{b}

The lack of response of these dune species to supplements of water and mineral nutrients is rather surprising, but there are several possible explanations. First, most dune species have very extensive root systems (Pavlik 1979b) and can draw upon the resources stored in rather large volumes of sand. In effect, water and nutrients do not limit the growth and seed output of these dune endemics. Secondly, the resources applied within a small area at the base of each plant may not be intercepted by an extensive root system. A third possibility is that distilled water supplements may have leached critical nutrients from portions of the root zone. This is supported by the fact that nutrient supplements enhanced seed production in both taxa relative to the water supplements, significantly so in ODH.

Seed production and plant size. Regressions of good seed output per plant (S_p) on plant size (canopy volume, V_c) were made using several transformations of the data. The best-fit relationships (those with the highest regression coefficients) are shown in Figures 1 and 2.

Oenothera deltoides ssp. howellii - There was a linear relationship between good seed output and canopy volume ($y = 1127 + 3.94\,x$, $r=0.58$, $P<0.01$, $n=33$) over the range of measured plant sizes at the end of April 1987 (Figure 1). S_c had a value of 66.0 ± 21.1 seeds per capsule from Types Ia and Ib fruits. The number of developed capsules per plant (C_p) ranged from 0 to 640. The maximum number of seeds from a single plant was 42,240 (not included in Figure 1), with an average sized plant ($V_c = 250$ dm^3) producing approximately 2100 seeds.

Oenothera hookeri - There was a linear relationship between good seed output and canopy volume ($y = 5934 + 3.18x$, $r=0.59$, $P<0.10$, $n=9$) over the range of measured plant sizes at the beginning of September 1987 (Figure 2). S_c had a value of 350.0 ± 181.2 seeds per capsule from Types Ia and Ib fruits. The number of developed capsules per plant (C_p) ranged from 8 to 113. The maximum number of seeds from a single plant was 39,550, with an average sized plant ($V_c = 2000$ dm^3) producing approximately 12,300 seeds.

Erysimum capitatum var. angustatum - There was a linear relationship between good seed output and canopy volume ($y = 1366 + 362.4x$, $r=0.78$, $P< 0.005$, $n=33$) over the range of measured plant sizes in mid-May 1987 (Figure 3). S_{sl} had a value of 22.2 ± 10.0 seeds per silique from Types Ia and Ib fruits. The number of developed siliques per plant (Sl_p) ranged from 16 to 337. The maximum number of seeds from a single plant was 7, 481, with an average sized plant ($V_c = 4$ dm^3) producing about 2800 seeds.

Erysimum capitatum var. capitatum - There was a linear relationship between good seed output and canopy volume ($y = 1486 + 1009.1x$, $r=0.97$, $P< 0.001$, $n=9$) over the range of measured plant sizes in early June 1987 (Figure 4). S_{sl} had a value of 28.4 ± 12.5 seeds per silique from Types Ia and Ib fruits. The number of developed siliques per plant (Sl_p) ranged from 16 to 375. The maximum number of seeds from a single plant was 10,650, with an average sized plant ($V_c = 2.0$ dm^3) produced approximately 3500 seeds.

Comparable measurements of seed production by other endangered or threatened plant species are rare in the published literature. At Eureka Dunes, Pavlik (1987) reported that the endangered Oenothera avita ssp. eurekensis had an S_c value of 77.8 ± 9.5, and an average sized plant produced 36,000 seeds in 1985 ($V_c = 60$ dm^3) and 7,000 in 1986 ($V_c = 12$ dm^3). The largest Eureka primrose individuals produced as many as 65,000 seeds. It is clear that ODH produces fewer developed capsules per plant and, therefore, has significantly lower seed output than the closely-related Eureka primrose. Capsules of Oenothera hookeri produced five times as many good seeds as those of ODH, but the plants tended to have fewer fruits. Overall, average-sized plants differed by a factor of 5.9 (OH/ODH).

Seed production by ECA compares favorably to that of other, non-endangered Erysimum species (Stevens 1932), including E. asperum (1,075 seeds/plant), E. parviflorum (4,600 seeds/plant) and the closely-related E. capitatum var. capitatum (3500 seeds/plant), but not when compared to E. cheiranthoides (30,500 seeds/plant).

Pollination, fertilization and predation limitations. Reductions in reproductive potential due to pollination, fertilization and pre-dispersal insect predation may be inferred from the relationships shown in Figures 3 and 4. In each case, curve d represents the actual output of good (filled, undamaged) seeds as shown in Figures 1 and 2. Curve a represents the total number of ovules produced per plant of size V_C. Curve b represents the total number of ovules found in developing (Type I) ovaries (e.g. those ovaries that were found on pollinated flowers). The difference between curves a and b is due, therefore, to limitations related to the pollination system. Curve c represents the total number of filled seed produced per plant of size V_C, including good and damaged seeds (that were presumably filled at one time). The difference between curves b and c is the result of some ovules not being transformed into seeds despite the fact that the flower was pollinated (i.e. other ovules became seeds), and represents the fertilization limitation. The difference between curves c and d reflects the fact that some of the filled seeds were eaten or damaged by insects while still attached to the parent plant.

Oenothera deltoides ssp. *howellii* - A considerable reduction (65%) in seed output of the Antioch Dunes Evening Primrose could be attributed to pollination limitations alone (Figure 5). Another 8% reduction resulted from seeds that did not fully develop within ripening fruits (poor quality seeds) and an additional 1% reduction in seed output was due to insect predation. The total reduction in reproductive potential was 74% in ODH.

Oenothera hookeri - The reduction in seed output due to pollination limitations was 17% in this non-endangered primrose. An additional 16% reduction occurred because some seeds did not fully develop in ripening fruits. Insect predation accounted for another 5% reduction in seed output. The total reduction in reproductive potential was 38%, the lowest measured in the present study.

Erysimum capitatum var. *angustatum* - The reduction in seed output due to pollination limitations was 18% in the Contra Costa Wallflower (Figure 6). An additional 20% reduction occurred because some seeds did not fully develop in ripening fruits. Insect predation, however, was higher in ECA than ODH, and accounted for a 13% reduction in seed output. The total reduction in reproductive potential was 51%.

Erysimum capitatum var. *capitatum* - The reduction in seed output due to pollination limitations was 28%. An additional 18% reduction occurred because some seeds did not fully develop in the ripening fruits. Insect predation was low compared to ECA, with an additional 2% reduction in seed output. The total reduction in reproductive potential was 48%.

The limitation imposed by pollination factors appears most critical with respect to seed production in ODH. Like many other members of the genus, ODH is vespertine and probably pollinated by large moths (perhaps hawkmoths, Gregory 1963) and three non-specific bees (two halictids and one melittid, U.S.F.W.S. 1983). Hawkmoths are not abundant (perhaps very rare) at Antioch Dunes, and neither is the melittid bee (U.S.F.W. S. 1983). A few bees have been observed in the vicinity of individual plants during the summer months. In Oenothera avita ssp. eurekensis at the Eureka Dunes, Pavlik and Barbour (1985) noted only a 21% reduction in seed output due to pollination limitations. This comparison strongly suggests that the disturbed and isolated nature of Antioch Dunes National Wildlife Refuge can negatively impact the endangered plants by negatively affecting their pollinators. Eureka Dunes and its immediate environment is relatively undisturbed and probably has abundant populations of pollinating insects. The widespread Oenothera hookeri experienced only 17% reduction in reproductive output due to pollination limitations at its Santa Cruz County locality. ECA is pollinated by a wider variety of non-specific halictid and apid bees (U.S.F.W.S. 1983), and therefore, seed production is less limited by pollination factors. This interpretation also applies to ECC, in which a 28% pollination limitation was measured.

The relatively small reductions in seed output due to fertilization limitations (8% , 16%, 20% and 18% in ODH, OH, ECA and ECC, respectively) suggest that once visited by pollinators carrying pollen, an ovary will have most of its transformed into seeds. These data do not provide evidence for post-pollination ovule abortion. Pavlik and Barbour (1985) reported 9% and 10% reductions due to fertilization limitations in Oenothera avita ssp. eurekensis and Astragalus lentiginosus var. micans at Eureka Dunes. Reductions in seed output due to pre-dispersal insect predation are negligible for ODH, but considerable in ECA. Observations made in June suggested that the proportion of insect-damaged seeds increased as the the year progressed, with perhaps as many as 30% of all good seeds affected by mid-summer.

Conclusions and Mangement Recommendations

I) Despite a number of potential limitations on seed production, large numbers of seeds can be produced by individuals of ODH and ECA each year (Table 1 and Figures 1 and 2). Yet, seedlings are rarely observed in abundance and the densities of established plants on the refuge certainly don't reflect the level of seed production. This strongly suggests that factors other than seed production limit the size and vigor of these populations. These factors include post-dispersal seed predation, mortality in the seed bank, low germination and mortality during seedling establishment. Separate studies, now in progress, are necessary to determine the relative impact of these factors on the population sizes of *Oenothera deltoides* ssp. *howellii* and *Erysimum capitatum* var. *angustatum* at Antioch Dunes.

2) There appears to be no convenient method for artificially increasing seed production in ODH and ECA. Supplements of seemingly critical resources had no significant effect on the species growing *in situ*. All reproductive populations of these species must, therefore, receive adequate protection in order to insure maximum seed production.

3) Pollination, fertilization and predation factors can reduce seed production relative to the maximum reproductive potential of these species. Of these, pollination appears to be most critical in *Oenothera deltoides* ssp. *howellii*. Protection and enhancement of insect pollinator populations must be given management priority at Antioch Dunes NWR. Identifying and protecting the sources of these insects are going to become even more crucial as land development continues to disturb adjacent properties and isolate the remnant dune ecosystem. Unfortunately, we know little or nothing about the biology of

these pollinators and even less about the floral and pollination biology of the Antioch Dune endemics. During the 1988 season we hope to make some in situ observations and collections of these insects.

Pre-dispersal insect predation may be significant in Erysimum captitatum var. angustatum. No immediate action is recommended to control the predators, but some additional estimates of seed predation must be made in order to monitor their effects from year to year. These are currently being made by the refuge staff and should be continued.

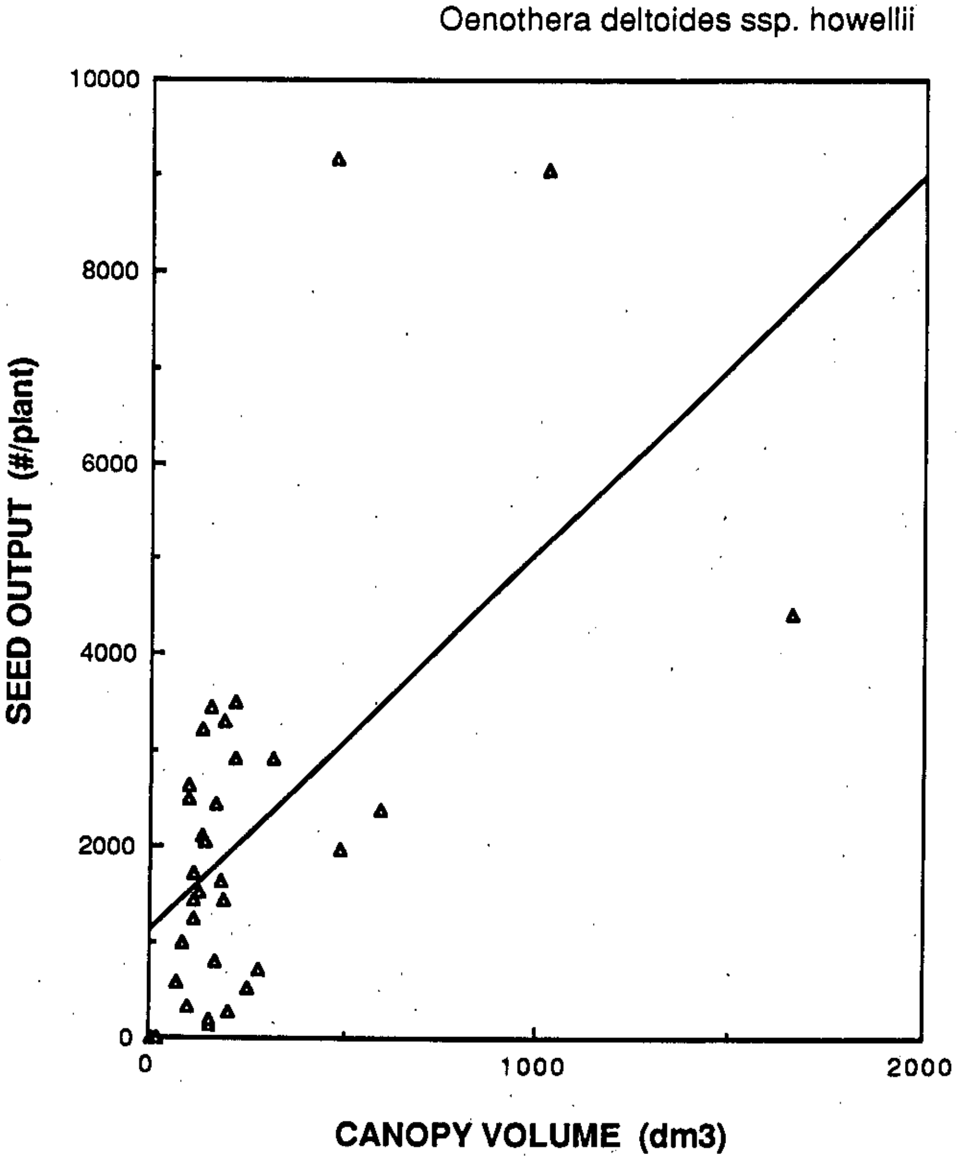

Figure 1. Seed output (number of filled, undamaged seeds per plant) as a function of plant size in Oenothera deltoides ssp. howellii at Antioch Dunes NWR, late April, 1987. See text for equation of the line.

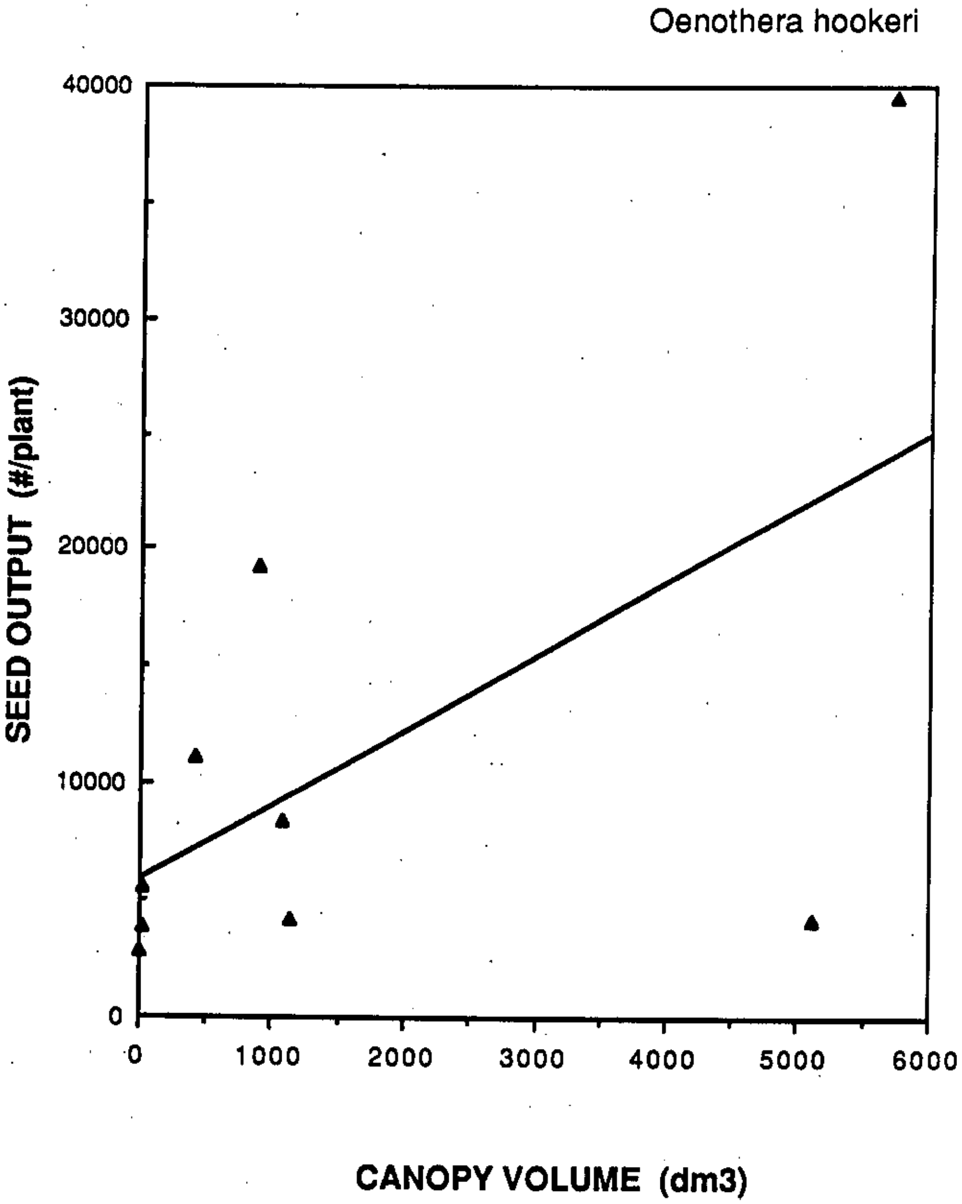

Figure 2. Seed output (number of filled, undamaged seeds per plant) as a function of plant size in *Oenothera hookeri* at Manresa Beach, Santa Cruz County, September 1987. See text for equation of the line.

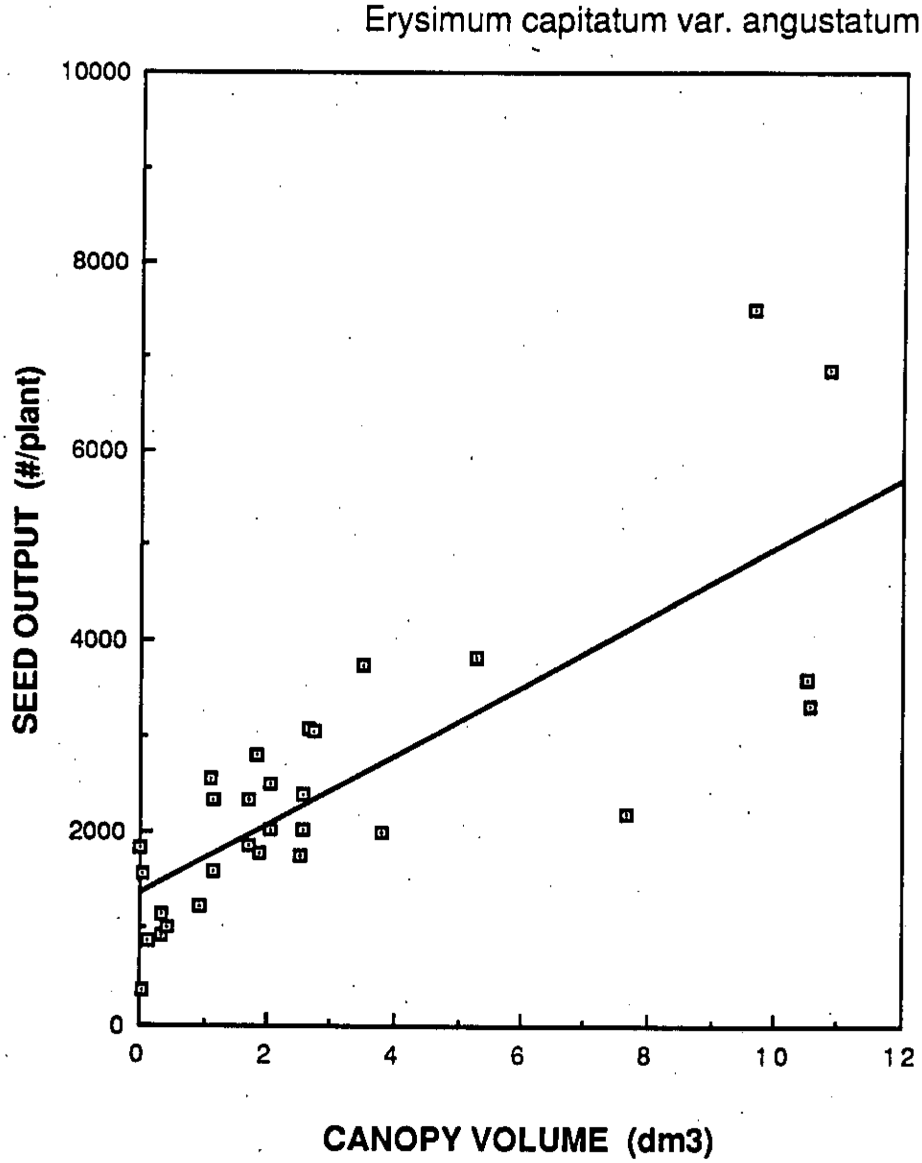

Figure 3. Seed output (number of filled, undamaged seeds per plant as a function of plant size in Erysimum capitatum var. angustatum at Antioch Dunes NWR, May 1987. See text for equation of the line.

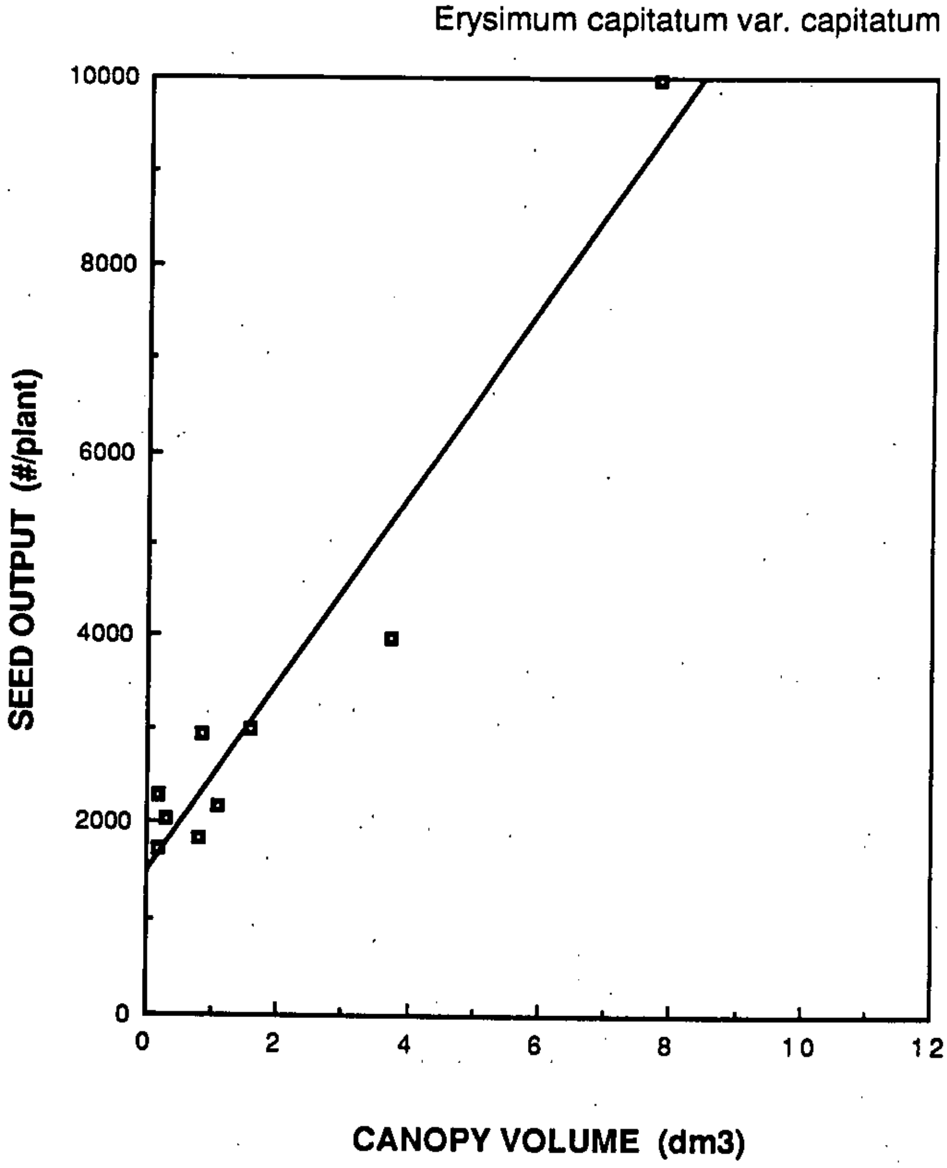

Figure 4. Seed output (number of filled, undamaged seeds per plant) as a function of plant size in Erysimum capitatum var. capitatum, Mt. Diablo State Park, June 1987. See text for equation of the line.

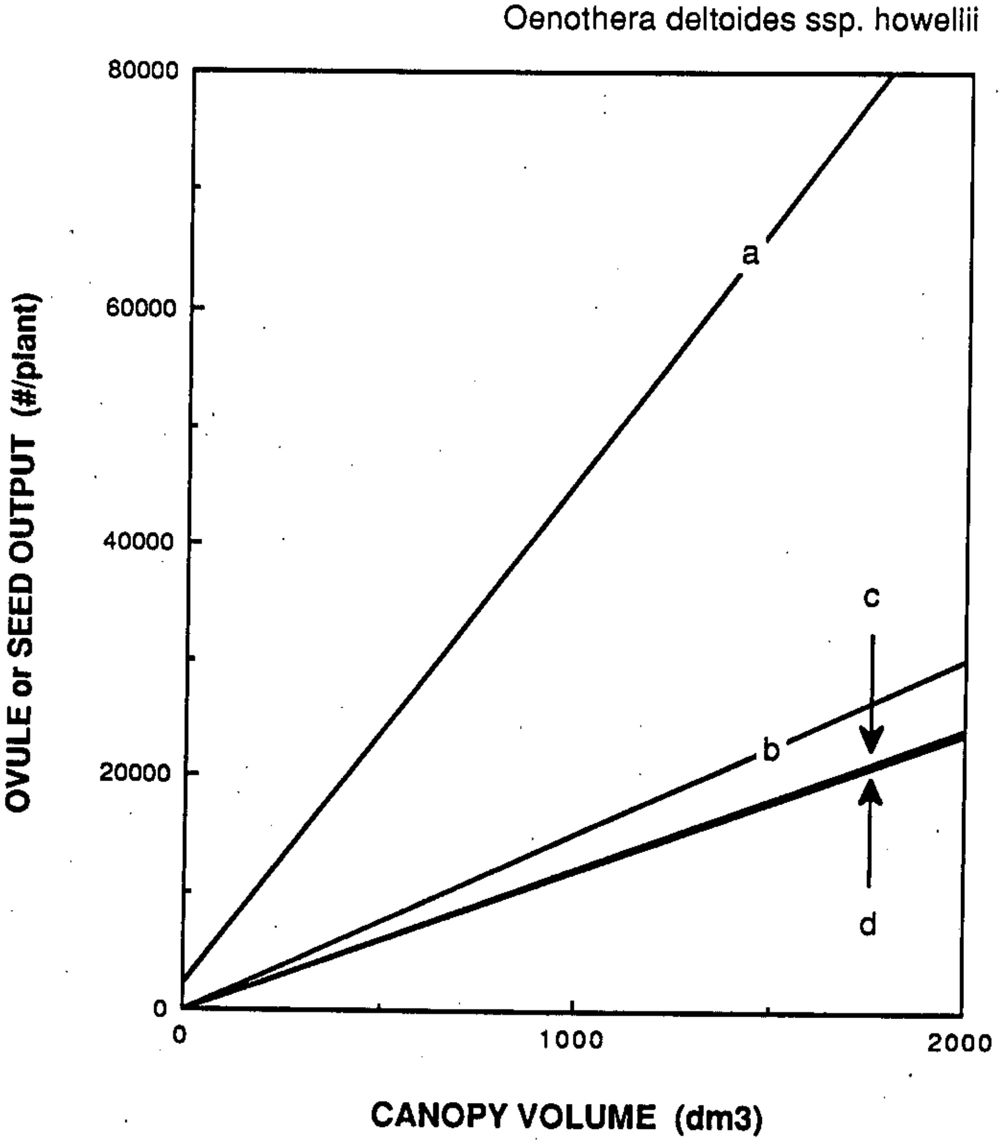

Figure 5. Effects of pollination, fertilization and pre-dispersal seed predation on reproductive (seed) output of *Oenothera deltoides* ssp. *howellii*. See text for details.

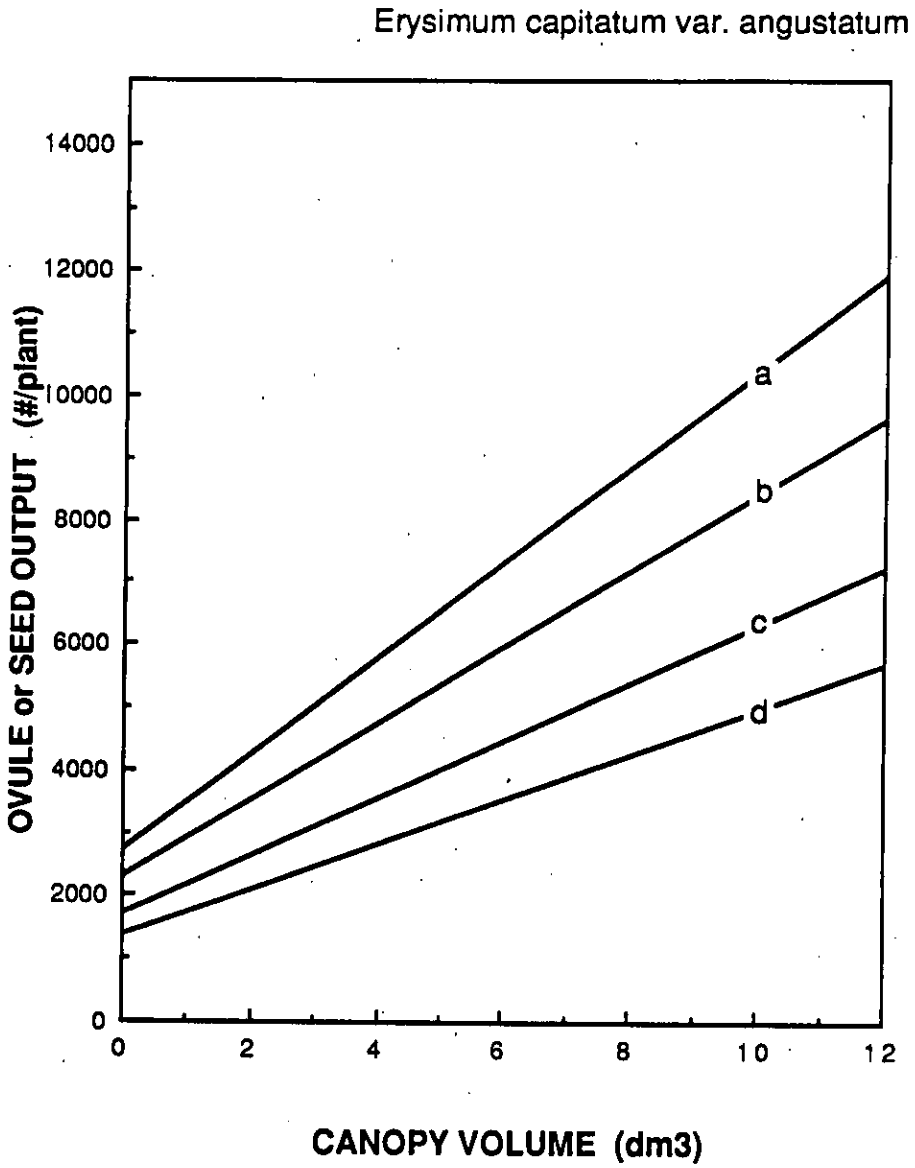

Figure 6. Effects of pollination, fertilization and pre-dispersal insect predation on reproductive (seed) output of *Erysimum capitatum* var. *angustatum*. See text for details.

Seed Germination

Introduction

Demographic studies of plants cannot ignore the fates of seeds because the highest rates of mortality often occur during this stage of the life cycle (Sharitiz and McCormick 1972, Leverich and Levin 1979). Life table analyses that exclude seed biology will not detect a number of major constraints on the population and lead to erroneous conclusions about its stability and ecological characteristics.

Despite the obvious importance of the seed stage in life table analyses, it is often difficult to obtain the relevant data . This is because of the potentially long period of time that seeds can exist in a cryptic, soil-borne state. A truely demographic analysis (one that follows the fates of individuals) is too laborious, time consuming, and uncertain to be of any practical significance in rare plant research. Instead, we may have to infer the relative importance of seed mortality factors from a series of parallel studies that examine each of several possible fates (Pavlik and Barbour 1986). One possible fate is that of germination and subsequent establishment of seedlings. The purpose of this study is to examine germination as one critical life stage that contributes to the population dynamics of <u>Oenothera</u> <u>deltoides</u> ssp. <u>howellii</u> and <u>Erysimum</u> <u>capitatum</u> var. <u>angustatum</u>.

Methods and Materials

In early July 1987, seeds collected during studies of the endangered *Oenothera deltoides* ssp. *howellii* (ODH), *Erysimum capitatum* var. *angustatum* (ECA), and the non-endangered *Erysimum capitatum* var. *capitatum* (ECC) were used to begin laboratory germination tests. Each lot of good quality seed (see chapter on seed production) was a mix of at least 12 different genotypes for a single taxon that had been stored dry at room temperature after removal from the fruits. Sublots of 50 seeds were counted and each sublot (replicate) was placed on a sterile filter paper disk in a sterile, plastic petri dish (5 cm diameter). These dishes (three replicates per variable) were kept at room temperature (25 C day, 18 C night) in a plant culture facility at Mills College. The species were given distilled water and checked daily for 10 days after the beginning of a trial. Germination was indicated by the protrusion of the radicle through the seed coat when observed with a dissection microscope (10X magnification). Once germinated, the seed was removed to a sterile culture dish and eventually used to try different transplant techniques. All species were subjected to tests for the effects of 1) light, 2) after-ripening, and 3) stratification on germination, as well as an assessment of overall seed viability. Comparison of treatment means was made by analysis of variance (ANOVA) on transformed data, when appropriate. The basic procedure for each test is amplified below.

Light In late July, 150 seeds in 3 sublots of 50 (3 replicates) were tested for the effects of light and dark conditions on the germination of each species. The light treatment consisted of two fluorescent lamps at a height of 15 cm above the petri dishes (total photon flux = 150 umol m^{-2} s^{-1}, about 1/12th of full sun). The light had a photoperiod of 12 hours. An additional set of three replicates of July seed were simultaneously kept in constant darkness under the same temperature and moisture conditions.

After-ripening In December 1987, 150 seeds from the same 1987 crop of each species were again tested for germination under conditions of total darkness. The seeds had been stored dry at room temperature for approximately 6 months. These tests coincided with field trials of germination on the same seed lot (data to be presented in the November 1988 report).

Stratification In late July 1987, 150 seeds from each species were stored under moist conditions at O C (a typcial stratification technique) for approximately 7 weeks. In September 1987, the seeds were brought to room temperature, given distilled water and allowed to germinate in complete darkness for a period of 10 days.

Results and Discussion

Responses to Light and Dark - Germination of ODH was significantly inhibited by light, with an average of 40% germination in the dark and 3% in the light (Figures 7 and 8). ECA showed a slight enhancement of germination by light (58 % vs. 62%), but this was not statistically significant. ECC seeds did not germinate at all, regardless of light-dark treatment.

Light-inhibited germination has also been observed in Oenothera avita ssp. eurekensis, another dune primrose that is closely-related to ODH (Pavlik 1979). This may be a characteristic of Oenothera taxa in general or it may have adaptive value for species of unstabilized sand dune habitats (Pavlik 1979b). December dark germination was again around 45%, but maximum germination occurred after 10 days in December as opposed to 5 days in July (Figure 7). It does appear that germination was suppressed due to an after-ripening effect.

Response to After-Ripening - Comparison of the dark germination trials of July and December1987 demonstrates that seed germination of the two endangered taxa is somewhat enhanced by after-ripening (Figures 8 and 9). The enhancement was a marginal 5% in ODH but a substantial 12% in ECA. Again, seeds of the widespread ECC did not germinate at all.

Response to Stratification - When compared to July dark germination trials, ODH seeds responded to stratification with a 36% improvement in germination (Table 2, F = 41.92, P<0.005). In contrast, stratification significantly reduced ECA germination by 34%. One ECC seed germinated under these conditions, but the response was statistically insignificant.

Table 2. Germination responses (% ± S.D.) of Oenothera deltoides ssp. howellii (ODH), Erysimum capitatum var. angustatum (ECA) and E. c. var. capitatum (ECC) to stratification. Values followed by the same letter are not statistically significant (ANOVA).

	ODH	ECA	ECC
unstratified 8/87	40.6 ± 5.0^a	58.0 ± 3.2^c	0 ± 0^e
stratified 9/87	76.0 ± 5.8^b	12.3 ± 4.2^d	0.3 ± 0.6^e

Conclusions and Management Recommendations

1) Establishing artificial populations of Oenothera deltoides ssp. howellii and Erysimum capitatum var. angustatum from seed will require different treatments to maximize germination. ODH seeds need to be stratified prior to planting and covered by at least 1 cm of soil in order to prevent light-inhibited germination. Since after-ripening only enhances ODH germination, it would be prudent to delay planting until November in order to minimize the probability of seed predation. ECA seeds should not be stratified and do not require burial for purposes of attenuating light (although burial would decrease predation).

2) In terms of overall germination potential, new seeds of Oenothera deltoides ssp. howellii are similar to thse of Oenothera eurekensis ssp. avita, with

40-60% typical (depending on stratification). Erysimum capitatum var. angustatum seeds tend to have higher germination under optimal conditions (dark, with 6 month after-rippening, no stratification), ranging between 58% and 72%. These percentages tend to compare well with both common and rare plants. The lack of seed germination in the non-endangered Erysimum capitatum var. capitatum remains unexplained, but it underscores the fact that seed germination is not necessarily an important factor in the population biology of rare plants. It is also a good example of how different two closed-related taxa can be in their physiological responses to the same environmental stimuli.

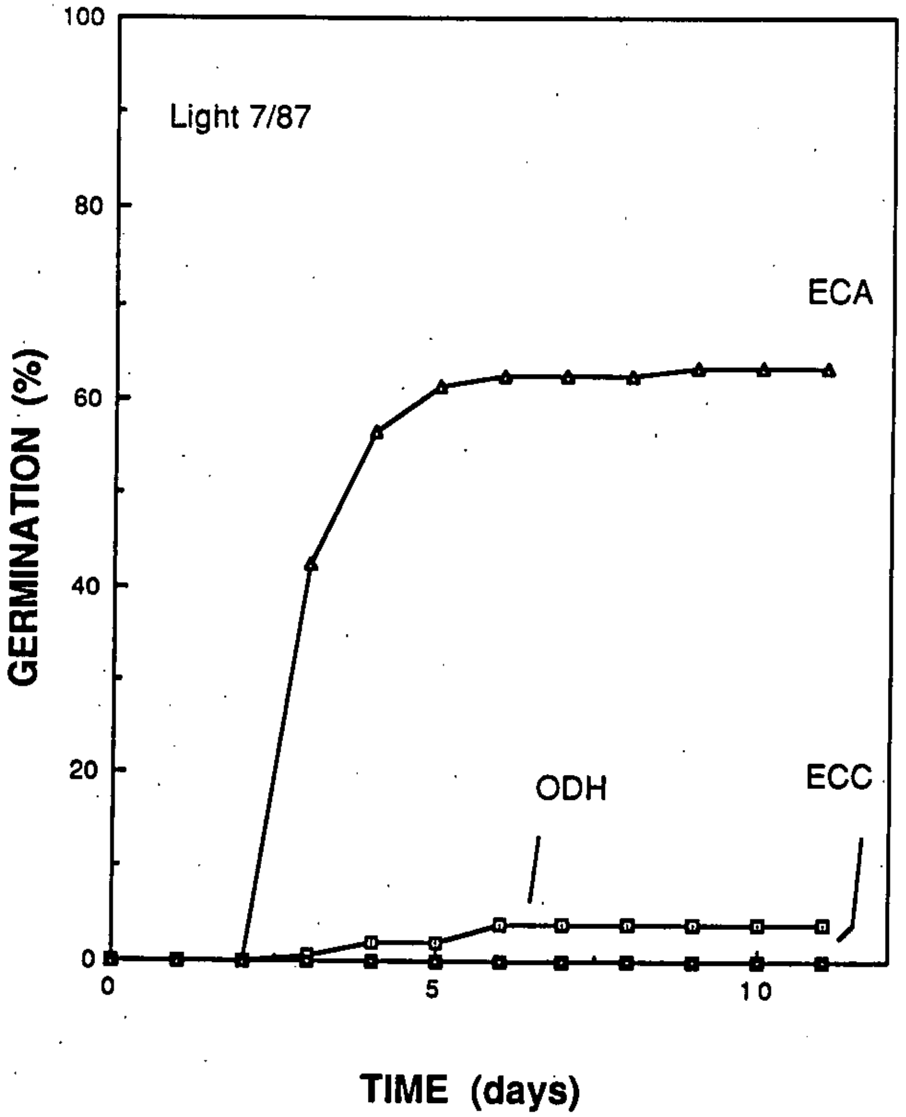

Figure 7. Effect of a 12 hr photoperiod on the germination of <u>Oenothera deltoides</u> ssp. <u>howellii</u> (ODH), <u>Erysimum capitatum</u> var. <u>angustatum</u> (ECA) and <u>E</u>. <u>c</u>. var. <u>capitatum</u> (ECC), July-August 1987 (compare with Figure 8). Each curve is the mean of 3 replicates.

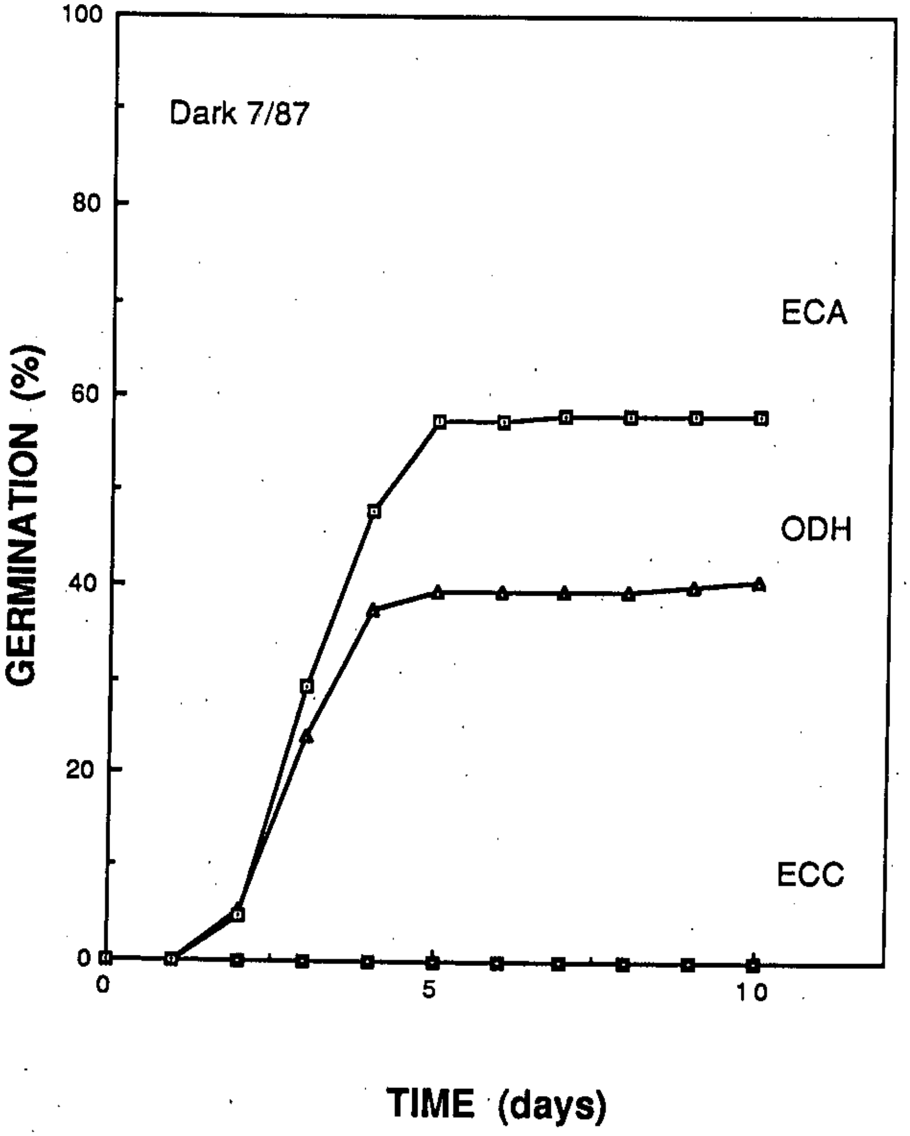

Figure 8. Effect of constant darkness on the germination of ODH, ECA and ECC, July-August 1987. Each curve is the mean of 3 replicates.

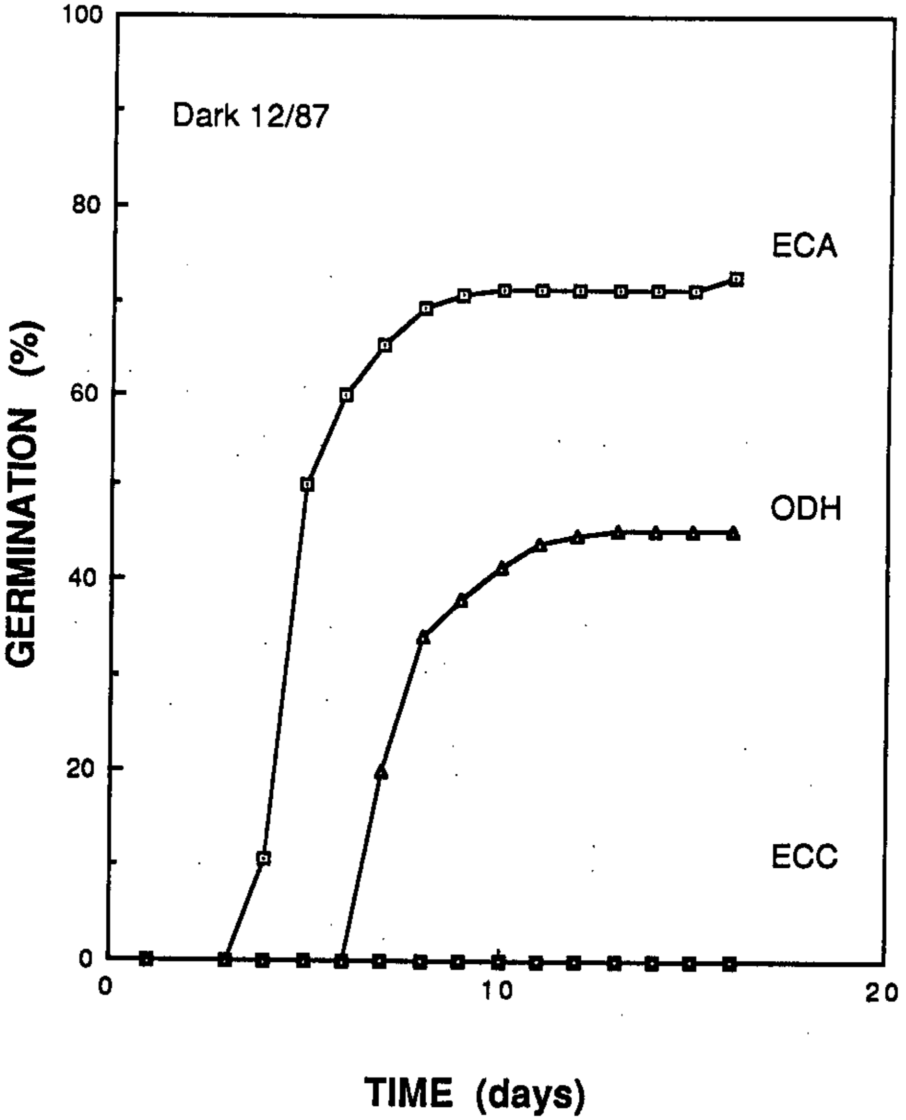

Figure 9. Effect of after-ripenning on the germination of ODH, ECA and ECC, December 1987 (compare with Figure 8). Each curve is the mean of 3 replicates.

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