

# **SANDPLAIN GERARDIA**

*(Agalinis acuta)*

## **POPULATION AND HABITAT VIABILITY ASSESSMENT**

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### **WORKING DRAFT REPORT**

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# **SANDPLAIN GERARDIA**

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## **EXECUTIVE SUMMARY AND RECOMMENDATIONS**

### Searches for new populations

1. Even if official surveys are not conducted using USFWS recovery money, surveys for new populations will be continued by many botanists (including those who would otherwise be paid). These searches should be encouraged and all discoveries should be documented and sites protected by appropriate agencies.
2. Searches, at this time, for new populations of *Agalinis acuta* are a low priority.

### Seedbank study needs

1. Try experiments with other possible host plants and compare results.
2. Test viability of seeds presently stored in artificial seedbanks.
3. Test freshly collected seed that is immediately dried and frozen (and store seeds from all populations).
4. Determine long-term reproductive success with seed.
5. Investigate the question of self-compatibility.

### Census

1. Annual population status surveys should be conducted at all sites with *Agalinis acuta*.
2. Census methods should be standardized for all states to allow comparisons among sites, to assess progress toward recovery goals, to improve demographic models, to assess natural trends, and to determine if population remain extant.
3. Counts should be made while plants are in flower (estimated between Sept 1 and Oct 15), which will vary among states. Each individual stem should be counted. The location of clusters of plants should be mapped within the site to document aerial extent of available/occupied habitat.
4. Data should be presented in an annual report to the USFWS detailing population size, distribution of plants (patches) and details of disturbances, general weather patterns and

observed habitat conditions.

### Distribution

1. The general distribution map for *Agalinis acuta* is out of date and inaccurate. This map should be updated and available for presentations and use to direct searches.
2. Detailed distribution maps should be prepared for the Massachusetts/Rhode Island/Connecticut sites and analyzed for soils, vegetation and land use to determine patterns and possibilities for establishing a new metapopulation in that vicinity.
3. The potential for appropriate habitat in New Jersey is unknown. Botanists in New Jersey should be consulted to determine whether detailed searches have been conducted in the state and whether appropriate habitat exists.
4. Rainfall and temperature data should be assembled from stations nearest extant sites for *Agalinis*. Correlations should be made to population sizes.

### Soil and Habitat

1. To test the hypothesis that micro-habitat conditions play a crucial role in the success of germination, we recommend that additional soil analysis should be done in a standardized way sampling the upper few centimeters because the upper surface soils appears to be the most critical for seedling establishment. Any experimental design should consult with 1992-93 Maryland study.
2. Statistical analysis of soil samples should be done.
3. Recommend identifying algal organisms present at New York sites and possible contribution to seedling germination establishment. Determine if blue green algae with nitrogen fixing cells are present.

### Propagation and Seedbanks

1. Start with existing sites and augment with seed from within that population.
2. Try experiments with other possible host plants and compare results.
3. Test viability of seeds presently stored in artificial seedbanks.

4. Test freshly collected seed that is immediately dried and frozen (and store seeds from all populations).
5. Determine long-term reproductive success with seed.
6. Investigate the question of self-compatibility.

### Genetics

1. Undertake exploratory molecular genetic studies to provide information on:
  - (a) Differentiation of the Maryland population from those in the other states.
  - (b) Levels and distribution of heterozygosity within and between the 10-11 identified sites with populations.
  - (c) Possible markers to follow outcome of augmentation, reintroduction, and translocation studies.
  - (d) Contribution of the seedbank to the retention of genetic diversity through time at individual sites.
  - (e) Dispersal of genetic material between subpopulations at sites.
  - (f) Effective population sizes of individual populations and effect of seedbank.

## **PROGRAM GOALS**

### **Long Term Program Goals**

1. Establish at least 3 long term evolutionarily viable populations each of which may contain subpopulations which may function as a natural metapopulation. At least 1 each needs to be located in New York and New England both of which may have additional populations given available habitat and funds. One such population is already established in Maryland (Soldier's Delight site). Establishment of these recovery sites may occur through the augmentation of existing sites that have suitable, unoccupied habitat, or the reintroduction of the species to historic areas which may need to be managed to become suitable habitat.

Potential sites for establishing such viable populations:

NY - Montauk Point, Sayville;  
MA - Martha's Vineyard, Nantucket.

The suitability of a selected site must be viewed as tentative until it has been shown to be successful based on predefined criteria.

2. Criteria for choosing sites for development of viable populations:

- a. Potential for long term protection and management (look at Recovery Plan for definition of protection)
- b. Site to be large enough to have minimal single species (AG AC) management and fire management should be an option.
- c. Preferably site has historic records of species
- d. Appropriate microhabitat needs to be available or there is the potential for restoration of microhabitat.

### **Short and Long Term Goals by Site**

1. NEW ENGLAND

Short term - All cemetery sites need to maintain or enhance existing populations with

purpose of providing genetic material for the establishment of the viable metapopulations. Long term - Willing to let sites go from a global vantage point when the 2 proposed reintroduced viable populations, with at least one in New England, have been successfully established and they include material from the current sites.

## 2. NEW YORK

Belport Avenue/Bellport Railroad/Montauk Downs - For short and long term goals, same as New England cemetery sites.

Belport Ave. site - Continue to push for management agreement with Town of Brookhaven for maintenance but not expansion of site.

Hemstead Plains - Not considered to be a possibility for use as an augmented or reintroduced larger viable population.

Short term - Maintain population at minimum and expand the population.

Long term - Might have to be maintained in perpetuity if none of the other, metapopulation sites are successfully established. This will involve active management of genetic flow with other New York populations if it is to be the "fallback population".

Sayville - Strong candidate as site for augmentation and establishment as one of the viable metapopulations.

Short term - Maintain and expand population distribution and size at the site. It is publicly owned, it can be managed, there is sufficient acreage and it meets the other criteria.

Long term - Consider as site for metapopulation establishment.

Shadmore (Ditch Plains) -

Short term - Maintain and expand population, may use secondary means of protection (i.e. allow partial development, incorporate zoning in protective measures for AG AC).

Long term - If 3 viable metapopulations become established, this site may not be of long term importance.

3. MARYLAND

Soldier's Delight - Maintain population. Unless new evidence, current preserve appears to be sufficient for maintenance of a viable metapopulation. Long term protection and management need to be assured (if not already).

Number and Composition of Proposed Viable Metapopulations

1. What are the minimum number and sizes of viable metapopulations of *Agalinis acuta* needed to secure the species and allow its down listing?

Suggested minimum criteria for securing the species are to:

(a) provide demographic security with less than 5% probability of extinction of the species in natural sites over the next 100 years.

(b) provide genetic security by retention of current levels of heterozygosity and diversity present in the wild populations.

(c) provide and manage sufficient natural habitat with naturally reproducing populations of the species to meet criteria (a) and (b) and to allow continuing natural selection.

(d) provide guidelines for recognizing the loss of one of the populations and for its re-establishment by translocation of materials from one or more of the other populations.

2. Sources of plants for the Metapopulations

(a) Based upon the assumption that the goal of the program is to secure the species and the present genetic diversity, each of the known small populations within the region of the proposed large populations are to contribute to its establishment. However each of the regional populations are proposed to be managed separately, except in the event of catastrophe.

(b) The techniques for selecting and using the seeds from each of the small populations for the establishment of the large populations need to be determined. The simplest technique might be the collection and mixing of seed from each of these remnants to place at the selected new sites.

(c) Molecular genetic studies would provide useful information on the amount and

distribution of genetic diversity in each of the small remnant populations. Information might be obtained on the:

- (1) depth of divergence of the more widely separated small populations and of the regional populations,
- (2) presence or lack of heterozygosity in the populations,
- (3) possible presence of markers that could be used to follow the establishment of the new populations and as an indicator of possible founder effects, and
- (4) distribution of heterozygosity among the populations as a possible guide to the quantitative contribution of each as sources for the new population.

### Life History - Summary

*Agalinis acuta* is a member of the Schophulariaceae. It is an annual. It is probable hemi-parasitic on little bluestem (*Schizachyrium scoparium*). It occurs with little bluestem at all known sites. Root connections between these species have been documented. *Agalinis acuta* individuals will form root connections to other species and even to other *A. acuta* plants if little bluestem is not present. It is unknown what nutrients are associated with these connections, nor if ineffective connections are made with some species. When *A. acuta* was grown in pots without little bluestem, plants were depauperate, but did survive to produce seed (Brumback, pers. obs.). *Agalinis*/host species relationships need to be identified, since there may be limitations associated with hemi-parasitism in terms of establishing new metapopulations.

In the field, *Agalinis acuta* has been seen as early as June 4, when it still held cotyledons, but also had three pairs of true leaves (Zaremba, pers. obs.). It is believed to germinate in the field in mid May, but may continue to germinate through the summer with favorable moisture conditions. Some extremely small plants are seen each fall in New York. It is unknown if these remained extremely small all summer or if they germinated late and attained only small size before the end of the growing season. Even the smallest plants are known to flower.

*Agalinis acuta* flowers between mid-August and mid-October. Earliest observed flowering date in New York is August 14, when plants were seen that had already shed some flowers. Flowering is probably associated with plant size.

Eleven species of flower visitors from 12 orders (Lepidoptera, Coleoptera, Diptera, Hymenoptera) were observed in 1988-89 (Blanchard, pers. obs.). This is consistent with the diversity of visitors reported in the literature for other *Agalinis* species. Although all visitors are probably somewhat effective as pollinators, pollen-gathering bees are probably most effective, since they transport large amounts of pollen and generally visit numerous *Agalinis* flowers during a short interval. Pollen loads from bees collected at *Agalinis* plants were examined and consist of from 46% to virtually 100% of *Agalinis* pollen (Blanchard, pers. obs.). Overall, pollinators and pollination do not seem to offer potential bottlenecks in the life of the plant.

Flowers open in the early morning and are usually shed by midday the first day they open. Flowers that are artificially isolated from pollinators hold their corollas up to three days, suggesting a strategy to favor outcrossing. Literature on self-compatibility and self-incompatibility in related members of the scrophulariaceae is ambiguous. Steven Carroll tentatively concluded that *Agalinis acuta* was self-incompatible based on a few experimental data; Judith Canne-Hilliker speculated that anthers brush against stigmas when the corollas fall, thus effecting pollination. Self-incompatibility in an annual like *A. acuta* would seem to be a bad strategy, but as yet we really don't know.

Capsules per plant number roughly 8-10 (?) (ref??). In one Maryland study, an average of 12 capsules (SD =  $\pm 1.6$ ) was documented from 22 plants (Maddox, pers. obs.) In one New York study,

an average of 4 capsules per plant in a population was observed. Plants in this population were considered to be very small (Blanchard, pers. obs.) At another New York site, one plant with 104 capsules was noted. Large plants at New York sites often have 60 to 80 capsules (Zaremba, pers. obs.).

In the wild, the average number of seeds per capsule is estimated at 20-70 (ref ??). For one wild population, an average of 55-60 seeds was documented per capsule (Brumback, pers. obs.). Good seedbanks therefore appear to exist in the wild.

It is not known how far seed is capable of dispersing. Most plants are clumped around little bluestem in the vicinity of plants from the previous year, suggesting low dispersal distances. In a test to determine dispersal distances, 3 x 3-inch plastic squares coated with Tangle-Trap were set at a range of distances from a population (Blanchard, pers. com.) One seed was found 10 feet from the nearest *Agalinis* plant (20 feet from the center of the *Agalinis* plot). Wind probably accounts for some dispersal of seeds (and even whole plants); small mammals could contribute to seed dispersal.

*Agalinis acuta* produces abundant seed at each population. How long seed remains viable in the soil remains unknown. At one isolated site in New York, (Bellport Avenue) plants were not seen for three years, then in the fourth year 18 plants were observed (Zaremba, pers. obs.). Seeds can survive at least four years.

### **Propagation Efforts**

Percent germination under cultivation was measured for three different groups in three separate tests using little bluestem as a host plant. All seeds were collected in 1988. One group of seed from Massachusetts was sown in 1988; other seed from the same Massachusetts sites was sown in 1989; New York seed in the third group was sown in 1989. In all cases, seed germination occurred after a cold, moist period (over-wintered), although instances of germination without a cold period are known. The first Massachusetts group germinated at a rate of 34% (22 plants) (November sowing, outdoors); the spring-sown Massachusetts group (subdivided into fertilized and unfertilized) germinated 8% and 4%, respectively, the following year; and the NY seeds (spring sowing, also fertilized and unfertilized) germinated 20% and 9% the following year. Two factors that appear to affect germination rate are sowing freshly collected seed and treating seed to a cold, moist period. In the first MA group, 61% of seedlings died before flowering. The other two groups died due to predation by slugs.

In these tests, the total number of seeds produced under cultivation came to approximately 15,000: 16 (out of the 22 plants from the first group) produced 173 capsules with an average number of 87 seeds per capsule. Samples of this seed were sown, and 44% germinated; 87% of the seedlings experienced mortality, leaving 25 plants that produced 60 flowers; no capsules were counted on

these plants. Since then, the pots have been left alone and have been seeding in ever since, producing many seedlings, but only within the seed frame. In general, this demonstrates the survival strategy of this species: it produces a lot of seed and the most tenacious individuals germinate and survive. It should also be noted that while it is possible to produce a lot of seed under cultivation, that after several generations the genetic variability may be lower than in the wild.

### **Seedbanking:**

In another test, seeds collected from NY were sown in soil collected from within the population. They were sown in June, 1989, and no germination had occurred by July, so the seeds and soil were mixed in half of the flats. Only the seed in the mixed flats germinated the following year. Implications are unknown.

In another test, soil samples were taken from the Waquoit, MA site and one NY site from (a) within the extant populations, (b) one meter outside the populations, and (c) four meters outside the populations. For the NY population, four seedlings were produced, all from soil blocks taken from within the populations. The seedlings were removed from the flats and placed in pots with little bluestem. Two seedlings survived the transplant, producing a total of two capsules with 33 seeds per capsule; i.e., the plants were depauperate.

For the Waquoit population, *Agalinis* seedlings were seen adjacent to one of the holes made by the collection. Two seedlings germinated from soil samples from within the population. One survived transplanting to a pot and produced two flowers and two capsules, with an average of 40 seeds per capsule. Unfortunately, all the remaining flats were then accidentally discarded, thwarting plans to assess long-term seedbanking.

This test indicated that the seedbank occurred only within the boundaries of the extant population, and that transplanting can be done, even though the transplants are depauperate (still, there is some production).

Artificial seedbanking: Two groups of five-year old seed are still undergoing germination testing, but germination rates are much lower (one seedling from 50 1988 seeds, and 3 seedlings from 99 1989 seeds). The seed had been dried in a desiccation chamber for three weeks and stored under refrigeration (not freezing) since receipt. Some seed has since been re-dried and placed into a freezer awaiting further testing. Artificial seedbanking has not yet proven to be a reliable method for preservation of material, although freezing of freshly collected seed may prove much more efficacious. Some seed from other populations is in Bill's seedbank; seed from the MD population is also in Ft. Collins.

### Further study needs

1. Try experiments with other possible host plants and compare results.

2. Test viability of seeds presently stored in artificial seedbanks.
3. Test freshly collected seed that is immediately dried and frozen (and store seeds from all populations).
4. Determine long-term reproductive success with seed.
5. Investigate the question of self-compatibility.

### Translocations

In an attempt to create additional subpopulations at two NY sites, Bob Zaremba scattered 50 seeds at 10 locations in some proximity ( $> 1/10$  mile) to occupied *Agalinis* areas (November 1989). There was no germination during 1990. All locations were revisited in 1991; one location supported one *Agalinis* plant (near a little bluestem plant). In 1992, there were nine plants at the locations where one plant was seen in 1991, although we cannot necessarily infer whether these plants germinated from the original seeds sown or from the one plant or from a mix of both sources. This year, there were seedlings seen in early July.

As a part of the 1989 *Agalinis* disturbance project, 25 seeds each were moved to 18 small plots (25 x 25 cm) at two sites (700 seeds total, all near little bluestem). Sown on the surface after the disturbance treatment. No germination seen.

These experiments did not have a high level of success. This could be attributed to the small number of seeds that were moved, or their presumed failure to find host species, or other unknown factors.

1. Assumptions for conducting future translocations:
  - (a) It is not possible to achieve non-managed, wholly self-sustaining populations in the future. The goal of population maintenance is therefore minimal management.
  - (b) The likelihood of long-term survival for any given population is significantly diminished if the population does not occur on protected lands, because necessary management intervention could be precluded at any point.
  - (c) Augmentation or reintroduction is an inevitability for this species, because a significant proportion of existing populations are on the verge of loss or will require excessive management attention.
  - (d) Suitable habitat for translocations is available or can be created/restored.

(e) Genetic information is necessary when conducting augmentations/ reintroductions to determine founder effects. Further, while it may not be necessary for other reasons, an initial genetic overview may be highly desirable in order to eliminate duplication and maximize effectiveness of recovery efforts.

2. Selection criteria:

- (a) Presence of host plants,
- (b) Mineral soils and other key habitat characteristics,
- (c) Long-term commitment to necessary management/monitoring,
- (d) Known genetics,
- (e) Research benefits.

3. Basic requirements of any translocation project:

- (a) Careful documentation of baseline conditions,
- (b) Long-term monitoring,
- (c) Strong controls on projects, and
- (d) Careful definition of both project goals (e.g., population viability standard) and all possible outcomes,
- (e) All reintroductions are treated as experiments

4. Conditions under which augmentation should be considered:

- (a) Populations should be on protected land.
- (b) After a population viability standard is defined, all protected populations which do not meet the standard will be augmented, except for those that have insufficient available habitat and available habitat cannot be created/restored.

5. Possible approaches to augmentation:
- (a) Expanding population extent and numbers by spreading seeds within the population and also spreading them to areas near the population.
    - Plot a. sow seed and let it go
    - Plot b. sow seed and snip seed heads
    - Plot c. sow seed and pull plants before they go to seed
    - Plot d. control -- don't do anything

Site preparation would be identical for all plots. May need to flood new plots with seed, rather than just putting out a small sample. In conjunction with project, determine founder effect by analyzing genetics of parent plants and seedlings. Apply these findings to possible reintroduction efforts.

- (b) Transplanting cultivated *Agalinis* plants, along with host plants, to plots within or near existing populations. Examine genetics as above.

6. Conditions under which reintroductions should be considered:

- (a) All reasonable protection measures have been taken to protect existing viable populations.

- (b) Augmentation has been initiated at all targeted populations, and augmented populations have shown some evidence of success or failure.

- (c) Efforts should be taken to maintain all remnant populations until viable reintroduced populations (definition to be determined) have been established in protected natural habitat and requiring minimal management intervention.

- (d) Reintroductions may be considered where

- (1) suitable historical habitat is available in which a reintroduced population could be maintained with minimal management intervention (e.g., Martha's Vineyard and Nantucket), thereby alleviating the need to intensively manage remnant populations;  
or

- (2) it has been determined that a wider distribution is needed to ameliorate catastrophic loss or to "fill in" between disjunct populations.

7. Possible approaches to reintroductions:

- (a) Sowing seeds around host plants at selected sites.
- (b) Transplanting *Agalinis* and host plants.

**Distribution, Census History, and Search Effort**

Table 1. Locations and reported numbers of plants by year.

Site	80	81	83	84	85	86	87	88	89	90	91	92
NY-Hemp Plains				1K-2K#	NA	89	529	51	948	172	109	164
Sayville					300-500#	379	200	76	227	56	3	253
Bellport Ave					29#	0	0	0	18	4	2	4
Bellport RR				8#	12	54	134	266	6	3	2	33
Shadmoor				140	44	134	175	349	519	183	398	63
Montauk Downs					230#	52	84	72	40	150	1	5
CT-Plainfield										10#	0	0
MA-Percival	40#	35	44	26	8	11	1	NA	45	340	150	306
Waquoit		73#	317	220	NA	130	18	NA	330	1362	NA	42
RI-Richmond								56#	40	??	??	??
MD-Soldiers Delight						30?	1K?	150?	10500 ++1K	9500 *	7K-8K *	> 20,000 *

# the year that the plants were first known from the site

?? data exist but are not available for this table

\* Maryland data from 1990-1992 are estimates for the entire site; data exist for some sections of the population, but have not yet been analyzed.

**Search effort**

### Connecticut

#### 1. Populations

Historical: 2 in 2 counties.

Extant: 1 (last seen in 1990) in a county not represented in historical collections.

2. Searches have been conducted at all historical sites in Rhode Island. C. Raithel, RI Natural Heritage Program, looked at approximately 30 cemeteries in the towns bordering Rhode Island. He targeted cemeteries for searches, since *Agalinis acuta* has been located in three other cemeteries and at no other types of sites in New England. Raithel used indicator species, such as little bluestem and *Viola pedata*, to identify sites with high potential for *Agalinis*. Additional searches may be productive in the Plainfield & Voluntown area for more "natural areas". Nancy Murray and Ken Metzler spent 5-6 days/year over the past three years looking for *Agalinis* in Connecticut. C. Raithel spent 4 or 5 days over the 3 years (mostly in 1990).

3. Who has searched: Nancy Murray, Ken Metzler, and Chris Raithel.

4. Search time 1988-1992: approximately 20-25 days.

5. Total search time: approximately 20-25 days.

### Massachusetts

#### 1. Populations

Historical - 16 in 5 counties.

Extant - 2

2. Searches have been conducted at all historical sites, although some of these searches with negative results have not been documented. Bruce Sorrie, Massachusetts Natural Heritage Program botanist, looked extensively over a period of several years at historic sites, particularly on Cape Cod, Nantucket, and Martha's Vineyard. No new sites for *Agalinis acuta* have been located since 1981 despite many days of searching.

3. Who has searched: B. Sorrie, J. Richburg, P. M. Brown, M. DeGreggorio, R. Zaremba, P. Dunwiddie, C. Caljouw, T. Rawinski, J. Bruno, and R. Dyer.

4. Search time 1988-1992: approximately 5 days.

5. Total search time: approximately 100 days.

### Rhode Island

1. Populations

Historical - 5 in 4 counties.

Extant - 1

2. The status of efforts to locate *Agalinis acuta* in Rhode Island is unknown, although it is believed that extensive searches have been conducted in all appropriate habitat, focusing on historical sites and cemeteries. All cemeteries within sections of the state with sandy soil are believed to have been searched.

3. Who has searched: C. Raithel, R. Enser, B. Sorrie, and C. Caljouw.

4. Search time 1988-1992: approximately 5 days. ??

5. Total search time: approximately 10 days. ??

### New York

1. Populations

Historical - 11 in 2 counties.

Extant - 6

2. All historical sites and seemingly appropriate habitat have been visited repeatedly by several botanists over a period of several years. Despite continued searches, no new sites have been located since 1985.

3. Who has searched: S. Clements, P. Zika, S. Young, R. Zaremba, C. Mangles, J. Beitel, and many local botanists.

4. Search time 1988-1992: approximately 15 days.

5. Total search time: approximately 100 days.

### Pennsylvania

1. Populations

Historical - 0

Extant - 0

2. Although there are no historical records for ***Agalinis acuta*** in Pennsylvania, there are records from sites in Md. within serpentine barrens similar to those found in Pennsylvania. All 19 serpentine barrens in Pennsylvania have been searched over many days without finding any ***Agalinis***.
3. Who has searched: R. Latham and A. Rhoads.
4. Search time 1988-1992: 5 days.
5. Total search time: 5 days.

### Maryland

1. Populations
  - Historical - 2 in 1 county.
  - Extant - 1.
2. The extent of surveys in Maryland is uncertain. Both historical sites have been searched. It is unknown if all historical sites have been searched.
3. Who has searched: R. Bartgis, C. Ludwig and D. Maddox?, others?
4. Search time 1988-1993: approximately 5 days ?
5. Total search time: 5 days (probably much more)

### **Recommendations for further searches**

From 1988 to 1993, 55-60 days were spent searching for new locations for *Agalinis acuta* throughout its range. Two new sites located in cemeteries were discovered during this period. These sites were disjunct from previously known sites.

It is generally agreed that there are probably additional extant populations of *A. acuta* within the vicinity of currently known populations, since *A. acuta* can, and often does, occur at extremely disturbed sites that cannot be identified as "natural areas." There are numerous cemeteries, roadsides, railroadsides and other disturbances in areas with sandy soil within the range of *A. acuta* current and historical sites. All known natural areas within this range are believed to have been

adequately searched for *A. acuta*. Any additional populations located are believed to occur at disturbed sites. If new populations were located in disturbed habitat, the value of these sites would be to provide additional genetic material for reintroduction projects and information on *Agalinis* habitat requirements. It is not believed that new sites will be located that support *Agalinis* and can be effectively managed as a part of metapopulation long term.

If, following genetic analysis of populations, it is determined that some of the smaller populations are genetically distinct, it would be valuable to obtain additional material for translocation and additional searches would be warranted. If, however, these smaller known populations are not genetically distinct, additional surveys will be of lower value relative to other necessary tasks to recover the species.

Even if official surveys are not conducted using USFWS recovery money, surveys for new populations will be continued by many botanists (including those who would otherwise be paid). These searches should be encouraged and all discoveries should be documented and sites protected by appropriate agencies.

Searches, at this time, for new populations of *Agalinis acuta* are a low priority.

### **Current census methods**

In New York, annual counts have been conducted since each site was first located, except for Hempstead Plains in 1985. Counts have emphasized individuals that set seed. All counts were conducted between Sept. 9 and Oct 15 each year. At three New York sites, Hempstead Plains, Sayville and Montauk Downs, *Agalinis* occurs in patches scattered over the site. Individual patches of plants separated by more than 10 meters without plants have been identified, mapped, and counted separately. Patches within some sites are as far as 1000 meters apart and are believed to have pollen exchange but not seed exchange. "Subpopulation" data for these three New York sites have been collected since 1989.

In Massachusetts, at least during the last three years, total number of stems were counted regardless of capsule production. Dates of counts vary by state. Methodology for counting is not available for Rhode Island and Maryland surveys.

### **Census considerations**

1. Seeds lost from the seedbank: It is unknown how many seeds germinate, but do not survive to reproduce. No program has followed a population through an entire season from germination to seedset. Plant mortality and loss of seed from the seedbank due to germination could be evaluated in an isolated "subpopulation" without following an entire population. While knowing

general plant mortality (prior to seedset) would improve our understanding of *Agalinis* demography, it is considered a low priority. It is believed that there is abundant seed present in the seedbank and that loss of seed is probably a result of depletion primarily through other means (eg desiccation, being eaten by small mammals) than germination.

2. Annual seed production: Several studies have examined either the number of capsules produced/plant or the number of seeds/capsule. Although knowing seed production would assist in the development of a population model, it is believed that even small numbers of individuals produce large numbers of seed. Census work focusing on capsule and/or seed production does not at this time seem needed unless a situation develops that suggests reduced capsule numbers or seeds.

3. Flowers that produce capsules: Fifteen percent of flowers do not produce a capsule (Blanchard, pers. obs.). Since adequate numbers of seed to replenish the seedbank seem to be produced on only a few plants, it seems unnecessary to count flowers or capsules.

4. Plant number as an assessment of habitat quality/condition: Populations of *Agalinis acuta* fluctuate dramatically from year to year in response to site disturbance and general habitat condition and, presumably, weather (general rainfall and temperature). While low plant numbers for a year may only indicate unfavorable weather or excessive browse, large populations do indicate that adequate habitat continues to exist at the site. Further, the distribution of plants at a site indicates the extent to habitat available and can indicate, when distribution is limited, that management is necessary to maintain the population.

5. Plant number as an evaluation of progress toward the recovery goal: The recovery goal for *Agalinis acuta* is written in terms of effective population size (numbers of plants seen) and cannot be evaluated without at least periodic counts. Each population is likely to exhibit distinctive trends in relation to variable site conditions. Census data are needed to describe these trends, determine when a "successful" population is established, and evaluate management needs.

### **Recommendations for census surveys**

Annual population status surveys should be conducted at all sites with *Agalinis acuta*. Census methods should be standardized for all states to allow comparisons among sites, to assess progress toward recovery goals, to improve demographic models, to assess natural trends, and to determine if population remain extant. Census information is also a good indicator of habitat quality when areal extent of a population is included and may indicate the need for management to maintain habitat.

All sites should be visited each year to conduct counts of all individuals. Counts would be made while plants are in flower (estimated between Sept 1 and Oct 15), which will vary among states. Each individual stem should be counted, even if it does not have flowers or if it is extremely

small (some mature individuals may be only 1 cm tall). It is not necessary to determine that seeds are produced by each of these plants. (That would require revisits until seedset and dispersal.) The location of clusters of plants should be mapped within the site to document aerial extent of available/occupied habitat. Maryland may, because it has a huge population, need to modify these census techniques. Data should be presented in an annual report to the USFWS detailing population size, distribution of plants (patches) and details of disturbances, general weather patterns and observed habitat conditions.

### **Other recommendations related to distribution**

1. The general distribution map for *Agalinis acuta* is out of date and inaccurate. This map should be updated and available for presentations and use to direct searches.
2. Detailed distribution maps should be prepared for the Mass./RI/CN sites and analyzed for soils, vegetation and land use to determine patterns and possibilities for establishing a new metapopulation in that vicinity.
3. The potential for appropriate habitat in New Jersey is unknown. Botanists in New Jersey should be consulted to determine whether detailed searches have been conducted in the state and whether appropriate habitat exists.
4. Rainfall and temperature data should be assembled from stations nearest extant sites for *Agalinis*. Correlations should be made to population sizes.

## **MANAGEMENT**

### **Habitat Characterization**

Sayville, Bellport Ave, Bellport RR sites on Long Island are found in human-created grassy openings in pine barrens. Montauk Downs and Shadmoor sites are within maritime grassland. Hempstead plains is treated as a different one than maritime grasslands. Rhode Island, Massachusetts and Connecticut sites are old cemeteries dating back to 1700's that are within pitch pine barrens with scrub oak present. Cemeteries are 1/2 to 1 acre in size. Maryland's Soldiers' Delight site is in an area of about 1700 acres of *Pinus/Quercus* woodlands. Site has up to 100 openings about one half acre in size.

Table 2 Plant taxa with *Agalinis acuta* by state (frequency as %). Only taxa with frequencies of over 10% are listed.

TAXON	STATES				
	NY (92)	MA (89)	CT <sup>1</sup>	MD <sup>2</sup>	RI <sup>3</sup>
"Bare" Ground	100	100	-	-	
<i>Agalinis acuta</i>	100	44	P	P	
<i>Andropogon scoparius</i>	100	100	P	P	
<i>Sorghastrum nutans</i>	45	-	-	-	
<i>Potentilla simplex</i>	45	50	P	-	
<i>Panicum</i> ( <i>Dichanthelium</i> ) spp.	45	63	-	-	
<i>Aletris farinosa</i>	35	-	-	-	
<i>Solidago juncea</i>	35	-	-	-	
<i>Solidago nemoralis</i>	30	-	P	-	
<i>Danthonia spicata</i>	25	13	P	-	
<i>Chrysopsis falcata</i>	20	-	-	-	
<i>Corex pensylvanica</i>	15	81	-	-	
<i>Aster dumosus</i>	15	-	-	-	
<i>Baptisia tinctoria</i>	15	6	-	-	
<i>Carex umbellata</i>	15	-	-	-	
<i>Lespedeza stipulacea</i>	15	-	-	-	
<i>Solidago tanvifolia</i>	15	-	-	-	
<i>Linum intercursum</i>	15	-	-	-	
<i>Antennaria neglecta</i>	-	69	-	-	
<i>Arctostaphylos uva-ursi</i>	-	44	-	-	
<i>Aster linarifolius</i>	-	94	P	-	
<i>Aster paternus</i>	-	44	-	-	

TAXON	STATES				
	NY (92)	MA (89)	CT <sup>1</sup>	MD <sup>2</sup>	RI <sup>3</sup>
<i>Aster spectabilis</i>	10	25	-	-	
<i>Bartonia virginica</i>	-	19	-	-	
<i>Cladonia</i>	-	44	-	-	
<i>Festuca ovina</i>	10	63	-	-	
<i>Helianthemum dumosum</i>	-	38	-	-	
<i>Hypericum gentianoides</i>	10	38	-	P	
<i>Lechea maritima</i>	-	18	-	-	
<i>Pinus rigida</i>	10	18	P	-	
<i>Polytrichum</i>	5	69	-	-	
<i>Vaccinium angustifolium</i>	10	31	-	P	
<i>Viola pedata</i>	-	50	P	P	
<i>Potentilla canadensis</i>	-	25	-	-	
<i>Aristida purpurea</i>	-	-	-	P	
<i>Aristida dichotoma</i>	-	-	-	P	
<i>Asclepias verticillata</i>	-	-	-	P	
<i>Senecio smalli</i>	-	-	-	P	
<i>Agalinis purpurea</i>	-	-	-	P	
<i>Talinum teretifolium</i>	-	-	-	P	
<i>Cerastium arvensa</i>	-	-	-	P	
<i>Phlox subulata</i>	-	-	-	P	
<i>Oenothera frutosa</i>	-	-	-	P	
<i>Arabis lyrata</i>	-	-	-	P	

TAXON	STATES				
	NY (92)	MA (89)	CT <sup>1</sup>	MD <sup>2</sup>	RI <sup>3</sup>
<i>Polygala verticillata</i>	-	-	-	P	

1 = partial list only. 2 = partial list from literature. 3 = no data currently available

P = present.

Exposed mineral soils were present in 100% of New York and Massachusetts samples in greater or lesser amounts. It appears that there is plenty of available habitat based on associated species and general soil conditions. Dominated by one of three grasses: little bluestem, broome grass or Indian grass. All sites have been maintained as grassland somehow. Sites have history of disturbance: mowing, digging/scraping, paths, fire, herbicide. Many are in depression areas such as in aisles between the graves. It has been speculated that these areas are wetter. However it could be that the plants just escaped mower more. The rest of the cemetery is mowed on a regular schedule.

## Soils

All sites have droughty mineral soils, but it's been observed that in some sites where *Agalinis* is found the soils have a layer of clay or silt or may have slightly higher moisture content due to slight depression. The serpentine soils are predictably different from those collected in coastal grassland-type communities. Maryland soils are higher in Mg, Mn, pH, NO<sub>3</sub>, and Ca and lower in SO<sub>4</sub>-S. Massachusetts soils had a higher percentage of sand. RI had a higher percentage of organics. Both New York and Rhode Island were high in SO<sub>4</sub> relative to Md and Ma. RI had higher levels of organics outside the *Agalinis* sites and lower SO<sub>4</sub>. New York and New England sites seem to be nutrient poor. Maryland soils are very different (higher in pH, potassium etc).

In Maryland, Massachusetts and Long Island a number of sites (n=7) found along edges of trenches/paths) or in slight depressions. In Massachusetts greater than 90 percent of plants were found in aisle-like depressions between graves. in trough but don't know if this is result of seeking out trough or non-mowing. Moisture may be the key factor. Thin, slimy algal mat layer on soil surface of New York sites. Recommend identifying organisms present and possible contribution to seedling germination establishment. Determine if blue green algae with nitrogen fixing cells are present. Determine possible significance of clavarioid fungi and lichens present at New York sites.

Questions concerning burning: What nutrients are released by burning? What are the benefits of

burning to this species? Clearing?, Nutrient release?

### Recommendations

1. To test the hypothesis that micro-habitat conditions play a crucial role in the success of germination, we recommend that additional soil analysis should be done in a standardized way sampling the upper few centimeters because the upper surface soils appears to be the most critical for seedling establishment. Any experimental design should consult with 1992-93 Maryland study.
2. Statistical analysis of soil samples should be done.
3. Recommend identifying algal organisms present at New York sites and possible contribution to seedling germination establishment. Determine if blue green algae with nitrogen fixing cells are present.

### Past Habitat Manipulations

These activities include research management, management activities, and accidental manipulations

#### Massachusetts

Sites discovered in 1980. They have been monitored for 12 years. Since 1983 TNC has had cooperative management with cemetery which calls for annual mowing between October 15 and July 1.

1988 soil disturbance study implemented to \_\_\_\_\_???. Study consisted of 10 treatment plots plus 10 control plots at Waquoit(Bay view) Cemetery and 4 treatment and 4 controls at Percival Cemetery. A third site at Waquoit Bay National Research Reserve Area received two treatments and two controls. In treatment plots the litter was removed and soil scarified to 2 cm with hand rake in 1/2 square meter plots. Soil disturbance was done in late 1988, with resampling in October of 1989.

Results were inconclusive. Study did not include enough replication and the plots may have been too small. Some seedlings were found in both treatment and control plots with highest number of seedlings in control plot (in proximity to ant mound). Scarified plots in Massachusetts formally monitored for only one year in 1989.

In 1991 there were two severe storms in August and October that \_\_\_\_\_???

### Rhode Island

The Rhode Island site was discovered in 1988 in a cemetery. Rhode Island has established mowing schedule to exclude the period from July 1 to October 15. The dates were chosen based upon recommendations by Bob Zaremba with the assumption that seed capsules have matured and seeds dispersed by then. No additional management has been done.

### Connecticut

The Connecticut site was discovered in 1990. The only management was the establishment of a mowing schedule with the same restrictions as Rhode Island.

### Long Island, New York

#### *Hempstead Plains-North*

Plants were first found in 1984. In 1988 TNC entered into a management agreement with Nassau Community College and in 1989 entire preserve fenced to prevent further dumping and control access.

In 1989 two manipulative studies were undertaken to test the effects of vegetation removal and soil disturbance on the germination of *Agalinis*. The first study (Long Island Study 2, Dec. 15, 1990 Final Report) consisted of six treatments with 3 replicates arranged in randomized block design within 5 m. of edge a 1998 plant. Treatments were removal of vegetation clipping, removal of duff and soil scarification to 1 cm and 3 cm.--combined in various ways along with a control.

Results: Many seedlings came up, but no *Agalinis acuta*. In early July three *Agalinis* seedlings were noted within the over-all study plot, but not within any of the treatment plots.

The second study (Long Island Study 3) in 1989-90 consisted of disturbance plots which were sprinkled with seeds. Two treatments (stomping and scratching) plus control plots. Half of each treatment and control plots received seeds and half of each treatment and control plots did not receive. Seed collected in early 1989 and sprinkled into treatment plots in January 1990. Monitored in 1991, 1992 and 1993. No *Agalinis acuta* were located in any of the units.

In 1991 a management unit containing no *Agalinis* was burned in April. In 1992 subpopulation 2 was burned in April. In mid November of 1992 subpopulation 1 was burned. In the same vicinity fire breaks were mowed. In 1993 two management units with *Agalinis* were burned.

*Hemstead Plains-South*

No soil disturbance work has been done comparable to studies done on the north site. Has not been managed--either mowed or burned--but immediate vicinity was fenced. An arson fire occurred in 1992 within fencing, but Agalinis was not burned. In 1989 seeds were scattered into promising sites and one site had germination in 1991 of one plant (two years after first scattering).

*Sayville Grasslands*

Management by FAA prior to decommissioning is unknown. Herbicide use is suspected.

In Fall 1988 test plots were established to examine the role of soil disturbance in *Agalinis* recruitment. All plots were 50 cm square and within 6 - 10 meters of existing populations. Treatments were placed in an area that burned in 1988 as well as unburned areas. Soil scarification and addition of seeds on some plots were done in a previously burned area and an unburned area.

Until recently has been no active management. Some plants founds at edge of active sand road. In May of 1991 brush hogged approximately 2 acres around the Agalinis plots and mowed the plots all brush removed. In September of 1992 the Agalinis populations were fenced with predator exclosures. In May of 1993 a slightly reduced area was brush hogged.

*Bellpot Avenue*

In 1991 (?) a narrow trench was dug through the site by LIRR. In 1991 the immediate area around *Agalinis* was fenced by Town and mowing regime changed to annual mowing between Nov. 1 and June 1. In Spring of 1992 telephone company moved telephone pole within the fenced area. That same spring were four plants. Previous year were two.

*Bellport Railroad*

Plants are adjacent to maintained railroad managed with herbicides until approx 1989. All active management by LIRR is currently suspended. In 1989 in April 1 inch bluestone was dumped on the site in April. It was immediately removed. Six plants flowered that year. Spring of 1991 a narrow trench was dug through the likely population. May of 1993 area was brush hogged. Site supported a low number of plants despite these activities.

*Shadmoor/Ditch Plains*

Site is within direct influence of ocean spray and winds. The plants are found along a narrow trail used by horses. There is no management.

### *Montauk Downs*

Studies, similar to the 3 described for Hempstead Plains-north and Sayville were undertaken in 1989. No *Agalinis* appeared in any treatment plots. In the winter of 1991-92 the immediate area around *Agalinis* populations were mowed. In winter of 1992-93 area was mowed again and black pines were removed. Before 1988 a fence was put around subpopulation adjacent to residences.

### Maryland

Research studies conducted in 1989, 1990, 1991 and 1992 at Soldier's Delight. Scarification in 1989 of 38 plots, .85 meter squared and divided into four quadrants. Two disturbed three times and two left. Group has no data results.

were relatively few plants in the 38 plots so ability to detect differences is not good. In 1991 conducted a fall burn. Fall fires, but not spring fires appear to improve reproductive output (in terms of number of fruits per plant).

In 1992 four different treatment types

1. Spring burns with six replicates. 30 meter by 3 meter (were originally fire lanes).
2. Post growing season or fall burns. Three approximately 1/2 acre burns with three 1/2 acre controls.
3. Fall burns with tree cutting added in as an additional factor. Two cuts with no burn. Two cuts with burn. No size information provided.
4. Tree removal only. Three one hectare cuts. And three no cut controls.

Strongest statement from these studies is that fall burns had a strong effect on reproductive output.

### **Summary of Habitat Manipulation Results**

1. In all cases, habitat manipulation (burning, mowing, tree cutting, scarification) has at least maintained an open grassy habitat which essential for *Agalinis*.
2. We were unable to correlate manipulations with increase in plant numbers due to the

naturally high population variability.

3. No new sub-populations were created through scarification or scarification with addition of seeds. Establishment of one new sub-population in the Hempstead Plains-South was along a heavily used trail. In this case the soil was not intentionally scarified as part of an experiment.
4. Even if there appears to be a strong positive result (more plants) following manipulation, it is possible that the high numbers are due to other unknown factors (weather is a possibility).
5. Possible flaws in experimental design and incomplete follow-up have led to weak conclusions and no clear understanding of what the results mean.
6. The strongest statement from the Maryland work is that the fall burn had a strong effect on reproductive output. This however was based upon only one event. Spring burns on Long Island show that germination still occurs after spring burns. Subsequent plants appeared to be larger.
7. Overall conclusion on the soil scarification studies in both New York and Massachusetts is that the size of the treatment plots were too small.

In Massachusetts there was a large increase in the number of plants at the sites between 1989 and 1990. The plot data, however, show no apparent difference in the germination rate between the treatment and the control plots in either year.

### **Recommendations**

1. No comprehensive written summary of what management for all sites has been done exists. Recommend that summary be compiled.
2. Natural variance in the populations requires much more replication, increased plot size and longer term studies. Lack of consistent staff commitment in different states is also a problem given funding and time available.
3. A better understanding of seed bank potential is needed.
4. We need to know more about microhabitat conditions.
5. Numbers of plants and how they are distributed over a plot. Need measure of this.

6. Consider additional green house studies with more controls looking at moisture, nutrient addition etc. Evaluate how results are relatable to field conditions.

7. Individual states:

New York: Continue burning at one current *Agalinis* site and one historical site; do follow-up of *Agalinis* stems and fruit production.

Massachusetts: Do block design burn, scarification and nutrient studies at Waquoit Bay Research National Reserve adjacent to Bay View Cemetery. Experiment with introduction of plants with little bluestem grown together in pots. Test for evidence of seedbanking at Manny Corellus State Forest on Martha's Vineyard following sandplain grassland restoration efforts, including the establishment of fire lanes and experimental test plots where woody vegetation is removed, soil are scarified or vegetation is burned. Possibly experiment with reintroduction of *Agalinis* seed obtained from cultivation or from natural populations on Cape Cod.

Another management technique worth testing at Martha's Vineyard (or possibly one of the other sites) is grazing. Livestock grazing was common on the Vineyard at the time that this species was reported to be 'frequent along roadsides'. Testing the effects of sheep, goats, cattle, and horse grazing in experimental treatment plots in conjunction with restoration of sandplain grasslands efforts at the 4000 acre Manny Corellus State Forest on Martha's Vineyard represents an opportunity to pursue this. Checking for the appearance of seedlings from an inactive seed bank in years 1 and 2 following initiation or cessation of a period of grazing would be an first approach. Should this prove unsuccessful, seed or plants could be introduced to treatment areas.

Maryland: Continue experimental burns to see if results such as seemingly successful fall burns can be replicated. Look for changes in soil chemistry in conjunction with burns.

## Population Biology

### Populations: Census and Distribution

The numbers of plants in a subpopulation varies widely from year to year and many do not produce any plants. Averaged over all of the populations and years, there were  $248 \pm 456$  plants (mean  $\pm$  SD) with a range of 0 to 1390 plants (Tables 3 & 4). About 12.1% of the subpopulations had no observed plants in individual years. The return of plants to the subpopulation site must come from the seed bank in most if not all cases. These statistics served to guide ranges of values used in the models and in particular the estimated variances.

The historical distribution of this species is summarized in another section. It has been locally abundant and widely distributed in appropriate grassland habitats which include Andropogon (the little blue-stem grass). This species appears to be an obligate associated species perhaps because of a hemi-parasitic relationship. Another undefined but possible association is with mycorrhizae.

Annual census data are available for individual sites for periods of 3 to 13 years. Some sites include more than 1 population (patch or stand) which may vary independently of one another from year to year. The data presented here are for the entire site. The number of patches varies from 1 to 11 except in the Maryland population in which perhaps 80 patches (stands) may be occupied. The currently available Maryland census data, based upon individual stands, are presented in Table 2.

The 3 populations that have had zero plants observed in some years have been recorded in the single stands. Zaremba noted that in the multiple patch sites (outside of Maryland) that individual patches in the multiple patch sites had been seen with no plants in some years (Table 3). The importance of even a single plant may be its proportional genetic contribution to the next years flowering plants and to the seed bank for following years. However if the species is self-incompatible, a single plant will produce no seeds and the subpopulation could be considered in the zero category.

Table 3. *Agalinis*: Distribution and Census History

Site	80	81	83	84	85	86	87	88	89	90	91	92
NYHemp				1-2K	NA	89	529	51	948	172	109	164
Sayville					300-500	379	200	76	227	56	3	253
Bell A					29	0	0	0	18	4	2	4
Bell RR				8	12	54	134	266	6	3	2	33

Site	80	81	83	84	85	86	87	88	89	90	91	92
Shad				140	44	134	175	349	519	183	398	63
Mont					230	52	84	72	40	150	1	5
CT- Plain										10		
MA-Perc	40	35	44	26	8	11	1	NA	45	340	150	306
Waqu		73	317	220	NA	130	18	NA	330	1362	NA	42
RI-Rich								56	40			
MD-Sold									10K±	10K±	8K±	>20K
MD-1									41	77	44	11
MD-2									1656	616	419	229
MD-3									185	196	110	1674
MD-4									360	606	175	3137
MD-5									5	10	207	412
MD-6									319	259	57	1481

**Population Spatial Structures**

The sites that have been identified are widely separated and unlikely to exchange genetic material (pollen) or seeds. These sites are likely to have been completely disjunct for about 50 or more years based upon historical collection information. The potential for exchange of pollen or seeds between patches at individual sites would be much greater given that insects are likely involved in pollination for the species and that evidence has been found for dispersal of seeds to at least 20 feet from the plant. The distance between patches at several of the sites varies from 30 meters to 200 meters.

The patches within a site are likely part of a local meta-population with a possibility for the exchange of genetic material but an uncertain and lesser potential for demographic recolonization. Indeed there is little evidence for progressive spreading of the local patch into immediately adjacent areas that appear to offer suitable habitat. There is a strong local site fidelity with plants each year tending to be associated with the same little bluestem plants. Each of the populations in these small patches would be subject to random loss of genetic variation by genetic drift as well as selection.

Table 4. Subpopulations (stands, patches) identified and censuses at each of the sites.

SITE	Subpops	Years	Mean	SD	N=0 Years
Hempstead	4	8	445	524	0
Sayville	7	8	199	147	4+
Bell Ave	1	8	7	11	3
Bell RR	1	9	58	89	0
Shadmoor	1	9	223	162	0
Montauk	11	8	79	77	16+
CT	3?	3	3	6	2
Perc-MA	+	11	91	121	1
Waqu-MA	+	8	312	441	0
Rich-RI	3?	2	48	8	0
Summary	(30+)?	74	162	268	26
MD-1	1	4	43	27	0
MD-2	1	4	730	637	0
MD-3	1	4	541	756	0
MD-4	1	4	1070	1390	0
MD-5	1	4	159	193	0
MD-6	1	4	529	644	0
Summary	6	24	512	749	0
Overall Summary	36+?	98	248	458	26

The establishment of larger populations in suitable protected habitat could be accomplished

by selection and mixing of seed samples from each of the sites in the area for initiation of the new population as a reintroduction into a historically occupied site. Alternatively seed from each site could be used to start patches within the site which then might provide the opportunity for exchange of genetic material by pollination events. The choice between these scenarios depends in part upon the uncertainty about the depth of genetic divergence that may have occurred between the populations at the several sites. If there are differences, then one or another of the seed groups might be favored to germinate and to serve as the founder source for the new population. If seeds from all of the several sites germinate and flower then there is an opportunity for exchange of genetic material and broadening the genetic material available for the new population.

Molecular genetic studies could provide additional information for evaluation of these possibilities as discussed further in the genetics section.

Table 5. Subpopulations and population numbers at 3 of the New York Sites.

SITE	85	86	87	88	89	90	91	92
Hempstead Plains								
1. Big	+	+	466	30	824	92	47	92
2. Small	+	+?	26	3	31	0	4	28
3. Small	-	-	37	18	93	81	49	37
4. Z - made					Seed	0	1	9
Montauk Downs								
1. Easement	+	+?	0	+	62	6	1	1
2. Big	51	+	24	+	13	140	0	3
3. Dump	9	1	0?	0?	0	0	0	0
4. Fairway-E	-	-	+	19	9	4	0	0
5. Fairway-W	25	+?	+?	+?	2	0	0	0
6. Scrape	-	-	2	0	0	0	0	0
Sayville								
1. Tower	+	+	55	33	52	3	0	41
2. Woodedge-1	+	+	97	7	88	7	2	129

SITE	85	86	87	88	89	90	91	92
3. Woodedge-2	+	?+	13	0	0	0	0	4
4. Roadbed	+	+	35	36	84	44	1	77
5. Open				-	3	0	0	2
6. Hecht					-	2	0	0
7. Cement				-	1	0	0	0

0 = Site surveyed.    + = Site surveyed.    - = site not surveyed.  
 ? = data uncertain.

## Reproduction

The plant is an annual with seed dispersal in the fall and germination in the late spring. Seeds bank in the soil and may be viable for at least 4 years. The seeds probably germinate in May and flowering in late August and September. Each plant forms from 4 to 50 pods with each pod containing from 35-100 seeds. Seed dispersal appears to primarily around the plant with evidence of wind dispersal up to at least 20 feet. It is unknown if they are dispersed by rodents or ants. The plants are hermaphroditic with limited evidence for self incompatibility.

All plants that survive to the flowering season do flower regardless of size. The yield of pods per plant varies from 4 to 12 with values up to 100 reported in some cases. Each pod may contain about 80 seeds. Thus an individual plant may produce 300 to thousands of seeds in a season. The bulk of these seeds fall within inches of the parent plant. The production of flowers and pods appears to be moisture dependent and varies from plant to plant and season to season in individual patches. Crowding may also reduce plant size.

The only quantitative data on reproductive success are from propagation studies. Seeding of natural outdoor sites has had a low success rate and provides little information. Brumback, in a propagation study, found in a sample of 87 seeds that 39% germinated and 34% of the seedlings survived to flowering plants.

These observations suggest that seed production is not limiting for the growth of populations of this species. However the loss of all of a population in a given year would mean that any plants appearing in the next season would be derived from the seed bank from seeds produced in an earlier year.

## **Mortality**

All of the plants die in the fall after seed production has occurred, thus adult mortality is 100% at 1 year. Estimates of mortality at several stages are available from the propagation studies. They reported that ??% of seeds germinated and that ??% of these seedlings survived to flower and produce seeds. These values are likely to be lower mortality limits for events in the natural habitat for the seeds from the source population and were used to provide a base for the models. We initialized the models at the stage of seedling survival with the production of 15, 20, or 25 seedlings per parent plant. Additional annual mortality, ranging from 40% to 85%, prior to seed production was estimated from field observations and included in groups of scenarios. Additional mortality was imposed stochastically through catastrophe events as discussed in that section.

We have not attempted to separately treat over winter seed survival, seed germination, or seed bank loss rates in the models. We have tested the supplementation and restoration of populations from the seed bank at various intervals by additional of individuals to the population. This allows continuity of populations at individual patches despite years of total population mortality prior to seed production.

## **Habitat and Carrying Capacity**

The habitat of this species is subject to losses from successional changes and requires continuing management to maintain the necessary grassland with open patches of ground. The plants also tend to be present in disturbed areas. Inferences about carrying capacity might be made from area data for each of the patches in relation to their population census history. Given the high seed production, high potential germination rates, high potential seedling survival rates and the presence of seed banks, it would appear that local population densities are a function of fluctuations in habitat characteristics from year to year. Moisture appears to play an important role in this variation and certainly was important for the high rates of germination and seedling survival in the propagation studies.

## **Seed Bank and Inbreeding**

Evidence for seed banking by this species has been found in both the wild populations and in the propagation studies of Brumback. Careful observations at one site (Bell Avenue) with only a single population patch recorded a 3 year interval with no flowering plants observed. There is no known site in the vicinity that could have recolonized this site. This would indicate a minimum four year seed bank survival of at least 18 viable seeds.

## **Threats and Catastrophes**

Threats to the species in local populations could come from complete destruction of the site, from a failure to manage the habitat for a grassland community, and from events that directly reduce reproduction in a year.

The concern about complete habitat loss leads to an emphasis in recovery planning on protected sites for long term conservation of the species and the establishment of 'viable' populations. Failure to manage might result from funding losses, a change in priorities as a result of political events, or local neglect. The effect of these events would depend upon the number of years of neglect and the rate of closure of the patch. Neither of these threats have been included in the simulation modelling scenarios.

Potential events directly reducing reproduction in a given year include herbivory, storms, and human disturbances. The primary herbivores are rabbits and rodents appears to occur at a given sites about every 3-5 years. Loss of plants, before they can reproduce, ranges from 20 to 80% in a given year. This event at a 20% probability, with either 20, 50, or 80% loss, was included as catastrophe 1 in the scenarios.

Dry wind storms that deposit salt on the plants in some populations occur about every 20 years. This results in a 100% loss for reproduction in that year. This event, at a 5% probability of occurrence, was included as catastrophe 2 in the scenarios.

A variety of human disturbances have resulted in the reduction of plants and reproduction. These include mowing at the wrong season and the application of herbicides. One of these appears to occur about once in 5 years at individual sites with a resultant 50 - 100% loss of reproduction for that year. This event, at a 20% probability, with either 50 or 100% loss, was included as catastrophe 3 in the scenarios.

Some human disturbances of individual sites appear to have resulted in an actual increase in reproduction. These may occur on the average of once every 5 years at some of the sites and may yield a doubling of reproduction either in that or the following year. This event, at a 20% probability, has been included as 'catastrophe' 4 in the scenarios.

## **Augmentation and Reintroduction**

### **Results of Population Modelling**

#### General

The VORTEX models were run on a machine with a 486DX CPU and 20 mgb of RAM. VORTEX version 5.1 dated, 7 May 1992 was used. The exploratory scenarios were run 100 times and the group of scenarios thought to represent the dynamics of the species most closely were run

500 times.

Scenarios were run for either 20 years with annual reports or for 100 years with 5 year interval reports. The shorter interval with frequent reports was used to allow comparison of annual changes with the annual census data which swings widely in the wild populations. Some short runs were also done with collection of reports on the individual runs by year collected.

All scenarios assumed no effects of inbreeding depression which may not be a safe assumption for the small isolated populations), no density dependence effects, and polygyny between plants. We assumed no self fertilization (self incompatibility) in these hermaphroditic plants which then requires at least 2 plants for reproduction to occur that year at a patch or site. The scenarios usually were initialized with populations of 100 individuals.

Data reported include (1) deterministic and stochastic 'r' and lambda values, (2) number of populations 'extinct' at each time interval, (3) mean population size and sd of the surviving populations at each time interval, (4) remaining expected heterozygosity at each time interval and at the end of the 20 or 100 year period, (5) number of runs or populations that went extinct at least once (maximum of 100 for 100 runs), (6) total number of runs going to extinction during the 20 or 100 years, (7) number of recolonizations (from the seedbank) after the extinctions, and (8) total number of re-extinctions that occurred, and the mean time to the first extinction and to re-extinction after recolonization.

## Basic Scenario

### Initial Population Size

Comparison of initial population sizes of 10 and 100 (Table 6) and 50 (Table 8) had no effect on the deterministic or stochastic r values, number of populations going extinct in 20 years or the population sizes at 18 and 19 years (before recolonization from the seedbank). There did not appear to be any effect of K or the SD in carrying capacity.

### Population Growth Rates (r)

The r values were greater than 2 in most of the scenarios examined, thus the population should be capable of 5-20 fold increases from one year to the next as seen in the census results and as would be expected from an annual plant producing large numbers of viable seeds. The stochastic r values were generally lower than the deterministic values as would be expected from the generally high variation in mortality and carrying capacity from year to year as well as the potential impact of

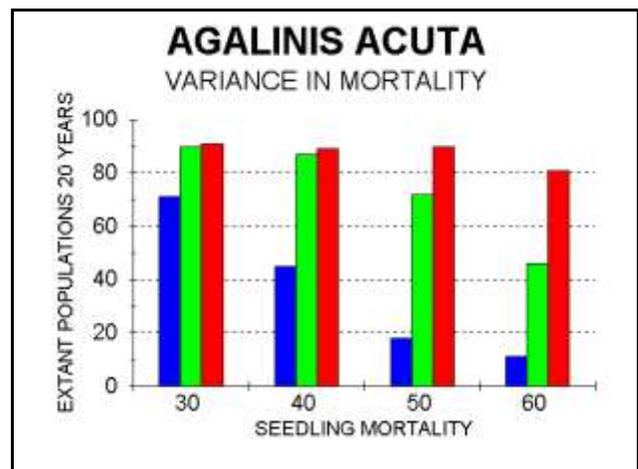
multiple challenges or catastrophes. Seedling mortality rates of 50-60% or greater resulted in  $r$  values below 2 but still highly positive.

### Sex Ratio

Since the model requires 2 sexes for reproduction but the plant is hermaphroditic we used a low proportion of males. Variation in this value from 0.05 to 0.2 in effect reduced the proportion of females in the population and provided a small proportional reduction in the  $r$  value (Table 8). This however had no effect on the extinction values, recolonization rates, or the rapid fluctuations in the population sizes. This compromise will work for this life history strategy until a module is added to the model to allow direct treatment of hermaphroditic plant and invertebrate species.

### Seedling Mortality

Variation of seedling mortality rates from 25 to 75% with their standard deviations set at 1/3, 1/2, and 1 of the mean was explored to determine the impact of mortality variance upon the numbers of extant populations after 3 years with no additions from the seed bank, and on the population sizes and their variance as well as population growth rates and their variance. (Figures 1 and 2). The 3 columns for each mortality rate represent the results for the respective standard deviation values.



Carrying Capacity

Catastrophes

Seed Bank

Table 6. AGALINIS - EFFECTS OF VARIANCE IN K																				
F I L E #	Mortal		Results*																	
	X	SD	Population Growth			Extinctions					18 Years			19 Years			20 Years			HET
			Det r	Stochastic r	SD	NE #	TE Yr	ReC #	ReE #	TE Yr	PS	N	SD	PS	N	SD	P S	N	SD	
K=100, SD=0, N=10																				
105	25	25	2.52	2.28	.549	60	9.0	73	29	5.4	100	101	11	90	84	29	84	100	12	.72
106	30	32	2.45	2.24	.660	85	6.4	164	100	4.4	100	68	44	87	84	31	79	98	9	.75
93	35	34	2.38	2.13	.715	93	6.2	217	158	3.4	100	54	49	79	74	33	66	101	10	.79
94	40	35	2.30	2.09	.737	97	6.0	223	169	3.9	100	47	48	72	60	35	57	95	14	.79
95	45	50	2.21	2.38	.654	100	2.4	452	431	2.0	100	21	35	50	65	22	21	100	10	.84
K=100, SD=50, N=10																				
107	25	25	2.52	2.28	.608	84	8.1	128	73	4.2	99	79	56	81	84	47	71	98	40	.73
108	30	32	2.45	2.21	.591	98	6.0	197	128	4.3	97	67	60	85	75	42	71	111	42	.76
96	35	34	2.38	2.14	.676	99	6.1	224	171	3.8	97	51	55	74	77	47	54	95	48	.78
97	40	35	2.30	2.10	.653	98	4.2	272	220	3.4	99	54	61	72	70	45	54	107	47	.80
98	45	50	2.21	2.36	.752	100	2.4	424	398	2.1	98	11	22	48	58	32	26	96	41	.83
K=100, SD=50, N=100																				
109	25	25	2.52	2.25	.561	78	6.7	138	86	5.0	96	64	53	84	84	49	74	100	51	.75
110	30	32	2.45	2.19	.585	99	5.4	258	204	3.3	92	58	58	69	73	44	55	108	46	.80
99	35	34	2.38	2.16	.622	97	6.3	219	165	3.9	96	48	57	68	70	45	57	98	49	.79
100	40	35	2.30	2.15	.606	99	5.3	260	211	3.5	97	53	63	61	58	42	50	93	45	.79
101	45	50	2.21	2.40	.612	100	2.1	444	416	2.1	96	18	36	48	52	18	28	102	47	.83

Table 6. AGALINIS - EFFECTS OF VARIANCE IN K																				
F I L E #	Mortal		Results*																	
	X	SD	Population Growth			Extinctions					18 Years			19 Years			20 Years			HET
			Det r	Stochastic r	SD	NE #	TE Yr	ReC #	ReE #	TE Yr	PS	N	SD	PS	N	SD	P S	N	SD	
K=100, SD=0, N=100																				
113	25	25	2.52	2.31	.534	67	8.5	83	33	4.7	100	83	38	94	89	26	83	101	8	.75
114	30	32	2.45	2.28	.604	88	6.8	149	85	5.0	100	65	45	86	80	31	76	98	10	.77
84	35	34	2.38	2.17	.752	92	6.5	207	149	3.8	100	58	48	74	76	30	66	99	9	.80
85	40	35	2.30	2.09	.720	97	6.2	231	176	3.6	100	52	49	75	68	35	58	99	11	.80
86	45	40	2.21	2.33	.928	100	2.1	456	428	2.0	100	19	33	47	60	18	28	100	7	.83
K=400, SD=100, N=100																				
115	25	25	2.52	2.27	.608	61	9.7	71	27	5.3	100	343	174	85	335	168	83	391	123	.84
116	30	32	2.45	2.20	.740	91	7.1	181	117	3.8	100	243	208	85	288	202	73	367	123	.83
87	35	34	2.38	2.14	.925	95	6.0	207	150	4.1	100	248	208	75	278	197	62	394	108	.84
88	40	35	2.30	2.04	1.00	95	5.8	218	165	3.8	100	214	222	74	244	195	58	376	113	.84
89	45	40	2.21	2.36	.868	100	2.6	427	396	2.2	100	86	171	56	114	148	31	425	92	.85
K=400, SD=200, N=100																				
117	25	25	2.52	2.27	.545	77	8.3	122	72	4.5	98	285	249	85	315	240	73	396	191	.82
118	30	32	2.45	2.19	.834	94	6.5	186	126	4.3	98	216	225	81	255	207	66	373	187	.82
90	35	34	2.38	2.07	.996	98	5.8	236	178	3.6	98	191	216	77	222	244	60	364	180	.83
91	40	35	2.30	2.00	1.17	99	5.7	242	191	3.5	99	208	232	72	183	181	52	349	207	.81
92	45	40	2.21	2.25	1.21	100	2.6	435	414	2.1	98	54	162	40	92	144	21	409	213	.84



<b>Table 7. AGALINIS - VARIANCE IN JUVENILE MORTALITY</b>																				
F I L E #	Mortal		Results*																	
	X	SD	Population Growth			Extinctions					18 Years			19 Years			20 Years			HET
			Det r	Stochastic r	SD	NE #	TE Yr	ReC #	ReE #	TE Yr	PS	N	SD	PS	N	SD	P S	N	SD	
K=400, SD=0, Mortality SD=Mean																				
68	30	32	2.45	2.17	.936	87	5.4	168	110	4.5	100	267	189	81	294	169	71	373	75	.82
69	40	35	2.30	2.02	1.08	99	5.6	252	208	3.6	100	187	198	79	210	182	45	310	121	.82
70	50	50	2.12	2.23	1.24	100	2.3	459	441	2.0	100	75	152	42	112	131	18	386	65	.86
71	60	50	1.89	2.15	1.21	100	1.7	533	522	1.5	100	21	75	28	76	95	11	364	72	.84
K=400, SD=0, Mortality SD=1/2 Mean																				
64	30	15	2.45	2.25	.450	50	10	60	20	4.3	100	335	151	92	355	125	90	388	52	.86
65	40	20	2.30	2.04	.551	56	9.0	73	30	5.0	100	354	130	95	357	119	87	381	60	.84
66	50	25	2.12	1.81	.704	85	6.4	173	116	3.8	100	272	185	82	294	170	72	339	106	.82
67	60	29	1.89	1.72	.794	99	5.4	264	219	3.2	100	169	196	63	205	190	46	297	136	.82
K=400, SD=0, Mortality SD=1/3 Mean																				
52	30	10	2.45	2.26	.419	47	8.2	64	26	4.2	100	344	138	98	353	125	91	389	57	.86
53	40	15	2.30	2.07	.489	55	9.2	70	26	4.4	100	348	134	93	359	116	89	386	61	.86
54	50	20	2.12	1.86	.657	59	7.3	85	36	5.2	100	299	170	92	317	153	90	360	106	.85
40	60	20	1.89	1.59	.815	76	9.0	127	70	3.7	100	282	176	87	311	162	81	341	121	.84
41	65	20	1.76	1.42	.911	89	7.6	184	125	3.3	100	173	193	85	182	187	70	243	159	.81
42	70	20	1.61	1.25	1.11	99	4.7	281	239	3.2	100	151	185	61	212	188	43	259	160	.80
43	75	20	1.42	1.16	.988	97	3.7	349	305	2.5	100	103	164	63	162	182	47	218	170	.79



<b>Table 9. AGALINIS - EFFECTS OF CATASTROPHES</b>																				
F I L E #	Mortal		Results*																	
	X	SD	Population Growth			Extinctions					18 Years			19 Years			20 Years			HET
			Det r	Stochastic r	SD	NE #	TE Yr	ReC #	ReE #	TE Yr	PS	N	SD	PS	N	SD	PS	N	SD	
No Catastrophes																				
120	30	10	2.53	2.52	.152	0					100	405	21	100	400	21	100	400	18	.92
121	40	15	2.38	2.34	.286	0					100	367	19	100	400	17	100	399	21	.91
127	40	28	2.38	2.26	.690	68	8.1	87	30	4.0	100	337	146	94	345	129	89	389	43	.85
122	50	20	2.20	2.11	.458	16	11.2	18	5	3.8	100	383	79	97	385	67	97	396	39	.90
48	60	20	1.97	1.84	.631	51	10	61	21	2.7	100	341	143	92	351	133	89	367	95	.86
49	65	20	1.84	1.70	.682	66	7.7	107	54	3.7	100	327	157	91	336	146	87	356	106	.84
50	70	20	1.69	1.55	.931	95	6.4	196	139	4.1	100	22	193	74	261	182	62	287	150	.82
51	75	20	1.50	1.37	1.00	99	5.0	288	242	3.2	100	119	175	68	144	178	47	202	148	.83
Catastrophes 1, 3, & 4																				
60	30	10	2.51	2.29	.412	0		0	0		100	398	20	100	400	20	100	402	20	.91
61	40	15	2.35	2.11	.482	0		0	0		100	395	18	100	405	19	100	396	20	.92
63	40	28	2.35	2.00	.865	62	8.4	84	38	4.7	100	316	166	90	334	142	84	377	76	.83

<b>Table 9. AGALINIS - EFFECTS OF CATASTROPHES</b>																				
F I L E #	Mortal		Results*																	
	X	SD	Population Growth			Extinctions					18 Years			19 Years			20 Years			HET
			Det r	Stochastic r	SD	NE #	TE Yr	ReC #	ReE #	TE Yr	PS	N	SD	PS	N	SD	PS	N	SD	
No Catastrophes																				
62	50	20	2.17	1.88	.576	22	11	29	9	1	100	371	109	98	377	92	98	386	53	.88
123	60	20	1.95	1.63	.778	56	9.1	85	44	3.4	100	338	144	93	353	129	85	363	102	.85
124	65	20	1.81	1.52	.751	74	9.6	115	62	3.3	100	265	181	86	304	161	79	338	123	.83
125	70	20	1.66	1.33	.927	97	6.5	238	178	3.0	100	173	189	71	225	188	63	274	156	.81
126	75	20	1.48	1.41	1.021	100	5.2	308	263	2.6	100	116	161	57	200	192	45	238	174	.79
Catastrophes 1, 2, & 4																				
56	30	10	2.56	2.36	.335	52	8.3	68	24	4.8	100	328	158	93	337	137	92	396	32	.86
57	40	15	2.40	2.20	.406	44	9.1	51	17	5.6	100	341	142	92	350	131	90	381	55	.86
58	50	20	2.22	1.95	.539	55	9.8	80	38	3.3	100	322	160	92	331	1	87	370	77	.86
44	60	20	2.00	1.70	.745	80	8.6	128	71	4.0	100	266	185	86	287	173	77	336	122	.83
45	65	20	1.87	1.51	.835	87	7.6	178	120	3.5	100	199	199	82	238	189	71	274	150	.82
46	70	20	1.71	1.39	.887	96	6.0	260	201	3.2	100	146	184	74	172	184	63	224	152	.82

<b>Table 9. AGALINIS - EFFECTS OF CATASTROPHES</b>																				
F I L E #	Mortal		Results*																	
	X	SD	Population Growth			Extinctions					18 Years			19 Years			20 Years			HET
			Det r	Stochastic r	SD	NE #	TE Yr	ReC #	ReE #	TE Yr	PS	N	SD	PS	N	SD	PS	N	SD	
	No Catastrophes																			
47	75	20	1.53	1.27	1.00	100	4.2	334	295	2.8	100	121	166	64	176	185	39	226	156	.81

## **PROPAGATION AND SEEDBANK**

Start with existing sites and augment with seed from within that population. Which sites? Anywhere that has suitable, available habitat for eventually reaching the viability standard.

Attempts have already been made to scatter seeds, but low numbers of seed were used.

### **Experimental design**

1. Flood an appropriate site with seed. Collect seed from a population during a really good year. Take 1 capsule from each (or a good sample) plant on the site and mix seeds for sowing.
2. Pick out 2 or 3 populations, then "artificially" disperse seed by taking seed from the existing population and using it to create new patches. Take best site first: Sayville seems to meet the requirement for available habitat. The next year, repeat the experiment at another site, if possible.
3. Choose plots based on area of bare ground, density of host plants, similarity of all habitat characteristics.
4. Should soil from within the population also be move in case there is an intermediary agent for the hemi-parasitism?
5. Water plots as needed, but at the same level among all plots.
6. Time frame for experimental augmentation: at least 5 years.
7. Plots should be 20 feet apart.
8. 1m x 1m plot size.
9. Pre-treatment (possibly using burn boxes or mowing) to maximize uniformity?
10. Any site preparation for sowing (scratching)? May add more variables than desired.
11. Replicates?
12. In response to the question about developing these plots on suitable habitat in an area not adjacent to an existing population (i.e., an experimental re/introduction), Bob Z. felt that he couldn't identify such habitat on Long Island right now, and plant numbers in MA may preclude such an experiment there at this time. Also, by focusing on augmenting existing populations, questions such

as microhabitat requirements and genetic issues do not need to be resolved to the same extent.

## Plots

### 1. Control plot (a).

Positive results in this plot (which would be watered and habitat maintained) may indicate effective habitat management scenarios for future consideration

### 2. Plot b.

(a) scatter 1000 seeds around host plant (very close to plant)

(b) let go and monitor for seedling mortality, number of plants that survive to fruit, number of capsules

### 3. Plot c.

(a) transplant 5-10 cultivated plants/hosts

(b) let the plot seed in

(c) monitor

### 3. Plot d.

(a) scatter 1000 seeds around host plants

(b) remove (and capsules) seedheads

(c) monitor for seedbank survival

### 4. Plot e.

(a) transplant plants/hosts directly from site to the plot with soil block

(b) let plot seed in

(c) monitor

### 5. Plot f.

(a) scatter 1000 seeds around host plants

(b) supplement with seeds in following years

(c) monitor

Criterion for failure: Don't see any initial germination for three years.

### **Seedbank Experiments**

1. Measure seedbank survival in MD by removing fruits from one of the patches at Soldiers Delight.
2. Measure seedbank survival at plot c.
3. Monitor NY patches that don't currently have plants and note the time span over which plants again appear.
4. Make comparisons.

## Genetics

The group discussed a series of scenarios concerning the possible contributions of molecular genetic studies to development and implementation of a recovery strategy for *Agalinis acuta*. The initial conclusion was that genetic studies are not a mandatory priority recovery action for the proposed recovery strategy. However there is a potential substantial contribution of genetic studies to our understanding of the population dynamics of this annual plant and they would address directly some of the assumptions made in this analysis.

Ho: The New York - New England *Agalinis acuta* populations do not differ significantly at a genetic level.

Ho: None of the *Agalinis acuta* populations are genetically depauperate.

Lines of evidence in support of hypotheses:

1. Evidence of recent widespread occurrence in New York -New England not associated with any particularly specific habitat. Suggests that it is an adaptable species, not a specialist.
2. Seedbank strategy prevents bottlenecks by allowing species to bridge extreme reproductive conditions, allows multi-generational mixing, may increase size of effective population beyond observed individuals.
3. Reproduces sexually, many different pollinators, not a clonal species.
4. Easily propagated under greenhouse conditions, no fitness problems. (However this provides an uncertain test of fitness in field conditions and in fact many subpopulations have shrunk to low or zero numbers in recent years.)
5. Host is common and widespread, plant appears to do well whenever host is present. (However there are many sites - near sites with the plant and elsewhere - that appear to provide appropriate habitat and the presence of host plants yet do not have *Agalinis acuta*.)
6. Appears to have mechanisms to promote outcrossing.
7. By and large, the larger populations are on the larger sites, while the smaller populations are on the smaller sites (although larger New York sites, i.e. Ditch Plains and Sayville are not "saturated.") This provides some evidence that lack of fitness is not what is limiting population sizes.

Ho: Other factors (presence of host plants, catastrophes, stochastic events affecting very small

populations, small sites, competition from other species, etc.) overwhelm any genetic factors affecting either viability of individual pops. or overall species security.

Conclusions:

1. Genetic studies are not important for setting recovery goals for *Agalinis acuta*.

(a) The need to protect against catastrophes requires populations in at least three geographically separate areas not subject to simultaneous wipe-out. Militates for preservation of site(s) in Maryland, New York, and New England regardless of genetic similarity (or diversity).

(b) "Minimum viable pop." will be determined by ratio of effective population to observed population sizes. If populations are outbred, we will want large populations in order to conserve full genetic complement as a program goal. If they are inbred (which we don't think they are), we will want large population to increase opportunities for accumulation of new variation by mutation. Therefore, no difference in action, regardless of inbred/outbred status.

2. Genetic studies are not important prerequisite for re-introduction to historic *Agalinis acuta* sites.

(a) New York - New England: *Agalinis acuta* was widespread only 50 generations ago and there is no evidence of adaptation to differing local conditions since fragmentation occurred.

(b) Maximizing genetic diversity of founding stock for reintroductions may be dealt with most effectively by using stock from many populations over several (eg. 5) years.

(c) Noting allelic differences among populations will not necessarily indicate how stock will do on an unoccupied site. Field trials are the most efficient and reliable indicators of fitness of stock to a given site.

3. Genetic differences between Maryland and New York - New England sites are likely, due to great intervening distance and observed habitat differences (serpentine). However, this potential variability is not of management interest unless we intend to introduce MD stock to NY-NE. This is not likely to be necessary as long as any NY-NE populations and/or seed source remain. [If NE-NY pops. were all extinct, this would be moot!]

4. Genetic studies are important if we decide to manage for one or more New England -New York metapopulations.

- a. There is no place on Long Island where management of a remotely self-sustaining ecosystem is even a potential option. The largest New York sites (Sayville and Ditch Plains, maybe Hempstead) could be managed with periodic artificial mixing of material among sites to simulate the "original" (circa 1930's) large, presumably panmictic populations. We should know what we have now on the extant sites before we start this operation and should monitor the resultant composition of the "metapopulation" that we create.
  - b. Re-establishment of sandplain *gerardia* using extant New England stock should be considered for incorporation into sandplain restoration on Martha's Vineyard and/or Nantucket. This offers potential opportunities for establishment of viable metapopulations in "natural" communities similar to those in which the species once occurred. To diversify genetic basis, introduction stock should be taken from several NY-NE pops. But even in this case, is the genetic composition of this established population of consequence, or should we just be pleased if it does well?
5. Exploratory molecular genetic studies would provide information on the points discussed above and thus reduce the level of speculation needed to make the other recommendations for management to recover this species. They would provide useful information to follow the course of any augmentation and reintroduction projects undertaken.
- (a) Differentiation of the Maryland population from those in the other states.
  - (b) Levels and distribution of heterozygosity within and between the 10-11 identified sites with populations.
  - (c) Possible markers to follow outcome of augmentation, reintroduction, and translocation studies.
  - (d) Contribution of the seedbank to the retention of genetic diversity through time at individual sites.
  - (e) Dispersal of genetic material between subpopulations at sites.
  - (f) Effective population sizes of individual populations and effect of seedbank.

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