Progress Report to the USFW – Willapa Wildlife Refuge

TOWARD SPARTINA ERADICATION: 2008 WILLAPA BAY SPARTINA SEED PRODUCTION & ERADICATION MODELS FOR THE UPPER TIDAL FLATS & SALTMARSHES

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Introduction

Achieving eradication of Spartina at Willapa Bay is dependent upon eliminating new vectors of infestation. The three vectors for the plant's continued spread in Willapa Bay are rhizomes, seeds sources from within Willapa Bay and seed sources from other PNW estuaries. Seeds from other estuaries entering and re-infesting Willapa are feasible, but not likely. Lateral spread from rhizomes is also a minimal threat owing to the recent massive and very successful control effort. Broken off rhizomes from mechanical control efforts and erosion are a minor threat that will need to be watched for. Seed production from clones that were either untreated or treated too late to affect seed viability are of greatest concern. Fortunately seeds only remain viable for about one year (Sayce, 1988). Thus the only real seed pool is from the previous season's production. Monitoring that production is important to assessing control needs.

Seed output from *S. alterniflora* is variable (Broome et al., 1974; Bertness et al., 1987; Sayce, 1988; Callaway and Josselyn, 1992) and unpredictable across years (Hubbard, 1970; Broome et al., 1974). Since self-pollinated seeds are not viable (Daehler and Strong (1994), the more isolated clones become the more important pollen limitation in seed production becomes (Davis et al., 2004). Although control efforts around Willapa have been very successful and have reduced total Spartina to under 30 net acres, there remain many untreated small isolated clones with the potential to re-infest much of Willapa. Even clones that are treated with imazapyr and glyphosate can produce viable seeds if treatments occur after mid-September (Patten 2006). He found that percent seed germination for 5pt Habitat + 3% Rodeo solution sprayed on 8/24/06, 9/7/06 and 9/21/06 was 0.03%, 1.5% and 4.5% respectively. To assure that the vector from new seed production is minimal, it is critical to assess the fecundity of isolated untreated or late treated Spartina clones.

To achieve eradication, it is also critical that every last clone/plant is found and treated. On vast open mudflats, spotting and then treating Spartina outliers is relatively easy. On the upper tidal marsh amongst the native salt marsh species and the deep tidal drainage channels, discovery is more problematic. Plants are difficult to see and treat. Applicators are likely to miss many plants and need to constantly re-visit previously treated areas. Achieving eradication is unlikely if outliers continue to be missed and those plants serve as vectors for new infestations.

What is the likelihood that applicators will miss a plant? How many times will a site need to be revisited to assure that all plants are treated? Answers to these are questions were critical in finding and eradicating *Caulerpa taxifolia* in Agua Hedionda Lagoon, a southern California

coastal bay over the past 10 years (Anderson, 2006). To achieve a similar eradication success for Spartina in the Willapa, those questions will need to be answer for our circumstances. The objectives of the studies reported here were to 1) assess the amount of viable seeds produced from isolated Spartina clones in Willapa Bay in 2008 2) assess the probability that a small Spartina clone will be skipped and not treated during a search and spray event, and 3) develop a model and a treatment protocol that will minimize the time and expense needed to eradicate Spartina from a site.

Methods

Viable Spartina seed production: Spartina stalks with mature seed heads were collected in the fall of 2007 and 2008 and placed in nylon screen bags. These bags were placed in a salt marsh in Willapa Bay at the 8' tide level for 2 months to allow for natural leaching of seed coat inhibitors. To test seed viability, germination was assessed by placing leached bags in an unheated greenhouse in aerated seawater. Water was changed weekly. The numbers of germinated and ungerminated seeds were counted after 8 weeks.

In 2007 seeds from 4 sites were collected. In 2008 seeds from 61 sites (isolated clones) were collected. In both years, site locations ranged throughout Willapa Bay. Notes were made at each site on clone size, seed maturity, plant vigor and height, nearest adjacent Spartina clones, and spray history. Total seeds collected at each sites varied from 10 to 5000^+ based on the number of seed heads that were available to harvest at each sites. Effort was made to collect all seed heads at each sites.

Probability of missed plants and eradication strategy: Five locations in Willapa Bay were surveyed one or two times between August 21 and October 7, 2008. The sites were in Spartina-infested upper tidal salt marsh at North Leadbetter, Oysterville, Nahcotta, Tarlatt slough, and Porter Point. Sites were sprayed by WSDA, WDNR or WNWR crews in 2008. Sites ranged in size from 2,500 to 14,000 ft^2 and had been sprayed one or two times prior to our surveying.

Spartina plants were recorded into three size classes (seedling = 1 stem, likely a seedling or small 2^{nd} year plant, small= < 0.5 ft^2 diameter, medium= > 0.5 ft^2 diameter) and whether they had been previous sprayed or overlooked. Large plants >3-4' diameter were usually ignored for this analysis. Plants were marked "sprayed" if there was some evidence of treatment (brown-down caused by glyphosate). In one instance, the survey was done immediately following spraying and dye was used as an indicator.

In another set of experiments, 12 sites (~5,000 $ft^{2/}$site) were surveyed on 10/21/2008 using a typical search pattern that would be employed during a spray operation. Each plant seen was marked with a red metal flag. This was a reasonably thorough search, but not a fine scale rigorous search. Similar to that done by backpack spray applicators. Immediately after each of these searches, a more rigorous search was employed to find all remaining unmarked plants, which were marked with white flags. Six of these searches were conducted with searchers spaced 17' apart and six used a 25' spacing. Data from the above search surveys were used to estimate the probability of detection of a typical plant and ultimately to devise an eradication model.

Results and Discussion

Viable Spartina seed production. In 2007, plants at two of the four sites examined produced seed (Table 1). One site, a ~ 20 m^2 untreated clone at the South Leadbetter parking lot surrounded by numerous other untreated clones, produced copious amounts of viable seed with an overall high % germination rate (~ 25%). The other site (just South of Nahcotta) produced a few viable seeds. In 2008, plants at only one of the 58 sites had viable seeds. That site had only 1 in 500 seeds germinate.

Numerous seedlings were noted in the Leadbetter area in 2008, suggesting that the seed count data from 2007 accurately reflected the re-infestation potential. The low fecundity of Spartina in Willapa in 2008 suggests that there will be very low levels of new seedlings in 2009. There could be several reasons for low seed production in 2008. First the monitoring protocol we used could have provided false negatives. Since we had reliable results in 2007 from this protocol, however, this seems unlikely. Second, production of viable Spartina seed is could be contingent on warm summer weather. Greatest seed production in the Willapa in past years has come during warm summers. The cool spring and summer of 2008 could be a big contributor to the lack of viable seed output in that year. Temperature conditions for both years were well below the 20 year average. The twenty year average for growing degree days (GGD) (base 45) from January to end of September is 2162. In 2007 it was 1992 and in 2008 it was 1663. Based on weather records at WSU Long Beach for the past 100 years, 2008 had the lowest GGD since 1963. Finally, it is also highly likely that seed output from isolated clones was pollen-limited, as has been proposed by Davis et al (2004) in earlier studies in the Willapa. That is, isolated plants don't receive enough pollen carried in the wind, to set seed. It is reasonable to concluded that both cool weather and isolation account for low production of viable seeds in Willapa Bay in 2008.

Probability of missed plants Multiple surveys across numerous sites and conditions resulted in data like those shown in Table 2 (from the studies of August 21, September 23, and October 7). Overall, applicators missed about 1/3 of the Spartina during any given search and spray event. This was consistent across all agencies and locations. If the target was only a small seedling then up to 50% of the plants were missed. These values are similar to values obtained in the study of October 21 and seem independent of whether the searcher was actually spraying or just marking plants with a flag. The difficulty in finding and treating all plants in an area is a problem that is central to the effort to achieve eradication of Spartina.

Based on our more detailed analysis of the data of August 21 and October 21, the probability of finding any given plant in a ~ 1/10 acre plot is about $p = 0.63$ (See Appendices A &B). Conversely, the probability that a plant will be overlooked is $1 – p = 0.37$. If the probability of detecting a single plant in a quad could be raised from 0.63 to 0.70, an improvement in detection of about 10% (by searching more slowly, or during more favorable light conditions, or by more workers per team), the ability to clear 95% of the ground in 4 searches improves by a disproportionate 33%. Aside from the number of searches, the probability of detection is one of the few parameters that we have any ability to manipulate to make eradication of Spartina easier.

At the present rate of discovery of Spartina plants, the density of plants must be less than about 15 per acre in order for searchers to find 95% or more of them in 5 or fewer searches. At greater plant densities, more than 5 searches will be required to find 95% or more of the plants. If the rate of discovery is improved by just 10%, then 95% or more of Spartina plants can be found in 5 or fewer searches even if the density of plants is as high as 20 per acre. If data from our studies sites in 2008 is representative of Spartina densities in Willapa Bay for 2009, increasing search events to find every last plant is not practical.

Based on these models and our previous efficacy studies, we have developed a detailed prescription and exacting protocols for achieving eradication suggest in Willapa Bay. Those are provided in a companion report entitled “Recommendations to expedite Spartina eradication in Willapa Bay: 2009 Report to Willapa National Wildlife Refuge” by Kim Patten and David Milne

The origins of the numbers used in this report and those used in our “Recommendations to expedite Spartina eradication in Willapa Bay” report are described and explained in Appendix A & B. Appendix A summarizes the considerations used in estimating the search effort needed to find 95% or more of the remaining Spartina plants in habitat like that searched experimentally during 2008.   Appendix B shows their mathematical derivations.

Table 3. Seed viability from isolated Spartina clones collected in Willapa Bay in 2007 and 2008.					
Location (year)	**Date sample**	**Size of clone**	**adjacent clones and sizes**	**germinated seeds (#)**	**~ % germination**
N.Willapa Meadow	10/5/2007	>500m^2	5 similar sized plants w/in 100'	0	0
N. Nahcotta	10/5/2007	>1m^2	5 similar sized plants w/in 100'	10	5
Leadbetter Point	10/5/2007	>20m^2	5 similar sized plants w/in 100'	500	25
S. Nahcotta	10/5/2007	>10m^2	5 similar sized plants w/in 100'	10	0
North Cove	9/26/2008	1 to 5 stems	5 similar sized plants w/in 100'	0	0
North Cove	9/26/2008	<1m^2	5 similar sized plants w/in 100'	0	0
North Cove	9/26/2008	1 to 5 stems	5 similar sized plants w/in 100'	0	0
North Cove	9/26/2008	1 to 5 stems	5 similar sized plants w/in 100'	0	0
North Cove	9/26/2008	1 to 5 stems	5 similar sized plants w/in 100'	0	0
Long Beach Pen.	9/29/2008	0.5m^2	5 similar sized plants w/in 100'	0	0
Long Beach Pen.	9/29/2008	1.5m^2	5 similar sized plants w/in 100'	0	0
Long Beach Pen.	9/29/2008	0.25m^2	5 similar sized plants w/in 10'	0	0
Long Beach Pen.	9/29/2008	3m^2	5 similar sized plants w/in 20'	1	0.002
Long Beach Pen.	9/29/2008	0.25m^2	12 similar sized plants w/in 50'	0	0
Long Beach Pen.	9/29/2008	<1m^2	12 similar sized plants w/in 50'	0	0
Long Beach Pen.	9/29/2008	0.25m^2	4 similar sized plants w/in 200'	0	0
Long Beach Pen.	9/30/2008	0.25m^2	5 similar sized plants w/in 200'	0	0
Porter Point	10/7/2008	0.25m^2	none	0	0
Porter Point	10/7/2008	1m^2	none	0	0
Porter Point	10/7/2008	1m^2	none	0	0
Porter Point	10/7/2008	0.25m^2	none	0	0
Porter Point	10/7/2008	1 to 5 stems	none	0	0
Porter Point	10/7/2008	0.25m^2	none	0	0
Porter Point	10/7/2008	0.25m^2	none	0	0
Giles Slough	10/7/2008	3m^2	5 similar sized plants w/in 200'	0	0
Giles Slough	10/7/2008	3m^2	5 similar sized plants w/in 200'	0	0
Giles Slough	10/7/2008	3m^2	5 similar sized plants w/in 500'	0	0
Porter Point	10/7/2008	<1m^2	ND	0	0
Oysterville	10/14/2008	<1m^2	none	0	0
Nahcotta	10/15/2008	1 to 5 stems	none	0	0
Nahcotta	10/15/2008	<1m^2	none	0	0
Willapa R.Meadow	10/15/2008	ND	ND	0	0
Willapa R.Meadow	10/15/2008	ND	ND	0	0
Tokeland	10/9/2008	ND	ND	0	0
Tokeland	10//8/2008	ND	ND	0	0
Northcove	10/6/2008	ND	ND	0	0
Northcove	10/7/2008	ND	ND	0	0
Porter Pt	10/21/2008	<1m^2	6 similar sized plants w/in 200'	0	0
Porter Pt	10/21/2008	<1m^2	6 similar sized plants w/in 200'	0	0
Porter Pt	10/21/2008	<1m^2	6 similar sized plants w/in 200'	0	0
Porter Pt	10/21/2008	<1m^2	6 similar sized plants w/in 200'	0	0
Nahcotta	10/21/2008	90m^2	none	0	0
Palix Meadow	10/17/2008	<1m^2	ND	0	0
Palix Meadow	10/16/2008	<1m^2	ND	0	0
Palix Meadow	10/27/2008	1 to 5 stems	6 similar sized plants w/in 200'	0	0
Palix Meadow	10/27/2008	0.25m^2	6 similar sized plants w/in 500'	0	0
Palix Meadow	10/28/2008	0.25m^2	6 similar sized plants w/in 500'	0	0
Palix Meadow	10/28/2008	0.25m^2	6 similar sized plants w/in 500'	0	0
Palix Meadow	10/28/2008	0.25m^2	6 similar sized plants w/in 500'	0	0
Palix Meadow	10/28/2008	0.25m^2	6 similar sized plants w/in 500'	0	0
Palix Meadow	10/28/2008	0.25m^2	6 similar sized plants w/in 500'	0	0
Palix Meadow	10/28/2008	0.25m^2	6 similar sized plants w/in 15'	0	0
Niawiakum River	10/17/2008	0.25m^2	ND	0	0
Johnson Slough	10/28/2008	2m^2	2 similar sized plants w/in 100'	0	0
Johnson Slough	10/28/2008	2m^2	none	0	0
Johnson Slough	10/28/2008	0.25m^2	2 similar sized plants w/in 5'	0	0
Johnson Slough	10/28/2008	1m^2	none	0	0
Palix River area	10/24/2008	ND	ND	0	0
Johnson Slough	10/27/2008	ND	ND	0	0
Palix River area	10/23/2008	ND	ND	0	0
Nemah area	10/21/2008	ND	ND	0	0

ND = No Data.

Table 4. Search pattern success for Spartina plants in the upper tidal salt marsh in 2008.

date	location	% missed plants				Calculated plants per acre**			
		Seedling*	small*	medium *	all	total plants/ acre	missed total plants	seedlings	missed seedling
8/21	leadbetter	100	83	--	88	70	61	17	17
8/21	leadbetter	17	52	0	41	357	148	52	9
8/21	leadbetter	50	39	67	46	845	392	505	253
8/21	leadbetter	85	68	29	65	741	479	287	244
8/21	leadbetter	100	100		100	44	44	9	9
8/21	leadbetter	50	45	50	47	166	78	52	26
8/21	oysterville	0	0	100	67	105	70	17	0
8/21	oysterville	100	27	67	54	941	505	261	261
8/21	oysterville	0	2	0	1	1255	17	70	0
8/21	113th	0		0	0	44	0	26	0
8/21	113th	--	--	--	--	0	0	0	0
8/21	113th	--	--	--	--	0	0	0	0
8/21	113th	63	67		64	96	61	70	44
8/21	113th	70	54	0	60	305	183	174	122
8/21	113th	44	100	63	56	157	87	78	35
8/21	porter pt	0	0	0	0	139	0	61	0
8/21	porter pt	81	24	40	50	331	166	139	113
8/21	porter pt	0	33	0	15	174	26	17	0
8/21	porter pt	--	100	0	50	17	9	0	0
8/21	porter pt	--	100	0	50	17	9	0	0
8/21	porter pt	100	100		100	44	44	17	17
8/21	porter pt	--	100	100	100	295	295	0	0
9/3	Leadbetter	57	25	0	28	13	3	3	5
9/3	Leadbetter	--	100		100	17	17	17	0
9/3	Leadbetter	75	100	0	69	189	131	131	58
9/3	Leadbetter	--	100	0	55	160	87	87	0
9/3	Leadbetter	--	100	0	63	139	87	87	0
9/3	Nahcotta	0	100	0	53	163	87	87	33
9/3	Nahcotta	0	100		50	44	22	22	22
9/3	Oysterville	40	100	0	60	490	294	294	163
9/3	Oysterville	70	100	0	75	218	163	163	109
9/3	Oysterville	100	100	0	56	196	109	109	44
9/3	Oysterville	75	100	0	63	174	109	109	44
10/7	Leadbetter	100	56	40	61	157	96	35	35
10/7	Leadbetter	27	41	12	28	470	0	131	0
10/7	Leadbetter	43	3	10	17	662	44	200	0
10/7	Leadbetter	40	63	0	47	131	61	44	17
10/7	Leadbetter	59	32	13	30	688	209	148	87
10/7	Leadbetter	29	8	0	14	192	26	61	17
average of all sites		47	64	19	52	263	108	92	46

*seedling = 1 stem, likely a seedling, small= < 0.5 ft^2 diameter, medium= > 0.5 ft^2 diameter

** Calculations based on # of Spartina plants/plot.

Appendix A.

In overview, thought was given to the problem of the number of searches needed to find a given plant, the number of searches needed to find some or all of the plants in a small experimental plot ("quad"), and the number of searches needed to find 95% or more of the plants in a whole region.

The numbers of plants found in a quad are described by the binomial distribution (see Appendix B). This gives the formulas shown in Table 1A below for estimating the probability of finding any given plant in a quad (= p [lower case] in this report) and the total number of plants originally present in a quad even though not all may have been found.

The more difficult challenge is estimation of the number of searches needed to find more than 95% of the plants in a whole region. To arrive at this estimate, it was necessary to (1) estimate the probability "p" of finding an individual plant; (2) assume that the undiscovered plants yet to be found occur in a Poisson distribution; (3) calculate the Bayesian probability that a searcher will discover (a) a subplot ("quad") with plants in it *and* (b) discover all of the plants in that quad. With these numbers, assumptions, and inferences, a model can be developed that shows the numbers of searches needed to find more than 95% of plants in a whole region. (The model may also be useful for estimating the number of searches needed to find *the last* plant in the region – considered as premature, here.)

The following illustrates this model development and its findings.

<u>1) The probability of finding (or overlooking) a given plant in a search area.</u>

The probability of finding an individual plant, p, is related to the probability of overlooking it, q, by the relationship $p + q = 1$. Table 1A shows data used to estimate the probability of overlooking a single plant during a search of a quad. Two methods of making this estimate are available (as shown in the Table caption). A "direct" method is applied to the August 21 data and a "binomial" method is applied to the October 21 data. As seen in Table 1A, both methods give values of that probability, "q", that are about the same.

The average probability of overlooking a plant during a standard search of a quad is about 0.37, a figure used for numerical examples throughout this report. For that value of q, the probability of finding an individual plant is 0.63. From binomial search theory, on average about 37% of plants in a quad will be overlooked by a team of searchers during a typical search of many quads and about 63% of the plants will be found.

<u>2) The distribution of the plants in nature.</u>

This analysis is concerned with a situation in which Spartina is no longer common. That is, the vast populations recently present in Willapa Bay have been reduced to scattered, isolated individuals and clumps, mostly widely separated.

In this "end game" situation, individual Spartina plants are assumed to have a Poisson distribution in the marsh. This distribution is common in nature for organisms that show neither contagion (presence of one attracts others, eg. barnacles) nor repulsion (presence of one repels others, eg. territorial birds). It is a truly random distribution in space that results from random dispersal of individuals or propagules, particularly applicable when organisms become rare. While huge meadows of Spartina existed, the density of grass would act to block seed dispersal and would invalidate this assumption. With the plants reduced to isolated individuals, their distribution in space is almost certainly Poisson. (Figure 1B in Appendix B illustrates a Poisson-like distribution of Spartina abundance, based on bay-wide Spartina surveys conducted by Pacific County in fall 2008. The distributions of plants in the quads with 50 or fewer per quad are also Poisson-like in Table 1A.)

The binomial theory described in the Appendix B applies to a single quad and allows us to calculate the probability of finding none, one, two … or all of the plants in the quad when it is searched. Poisson theory (described in Appendix B) describes the number of quads expected to contain none, one, two, … etc. plants and can be used to estimate the effort needed to clear the whole marsh, as summarized in the following.

<u>3) Estimating the number of searches needed to eradicate “all” (say, > 95%) of the Spartina plants.</u>

When a spray team searches for the plants, two probability factors are at work. First, the team must find places where the plants are still present. Having found such places, then the team must find all of the plants in those places. The probability that both will happen depends upon both p (the probability of finding any given plant) and the average number of plants in the region in which they are searching. A summary follows. (The mathematics of this situation are shown in Appendix B.)

To extrapolate mathematically from the study of quads to the whole marsh, we start by imagining the marsh divided up into a grid of quads. The challenge to the search team is to find the quads that actually have plants in them and, once located, to find all of the plants in each of those quads.

Suppose the mean number of plants per quad is “λ” (Greek letter lambda traditionally used in Poisson calculations, equivalent to population mean μ of statistics). In that case, the probability that a quad will contain x = zero, one, two, three … n plants is given by

$$p(x) = e^{-\lambda} \lambda^{x}/x!$$

In particular, the probability that a quad entered by the search team has no plants in it at all is given by p(0) or

$$p(0) = e^{-\lambda}$$

Conversely, the probability that there is at least one plant in a quad entered by a search team is 1 – p(0), or

$$p(1 \text{ or more}) = 1 - p(0) = 1 - e^{-\lambda}.$$

Table 1A. Estimates of the values of q = probability of overlooking a plant in a quad, using data from the August 21 and October 21, 2008 observations. August; direct estimate, October, binomial estimate.

Quad	Quad size (ft^2)	% missed	Initial # N_o of plants	
				August 21. Initial # of plants (N_o) is estimated from
1	5000	61	8	# found live Aug 21 (= n_2) + # found
2	5000	23	41	dead on Aug 21 (these were found
3	5000	52	97	alive
4	5000	60	85	and sprayed by previous searchers, =
5	5000	67	5	n_1).
6	5000	48	19	$N_o = n_1 + n_2$.
7	2500	33	6	q = % missed estimated from # found
8	2500	64	54	live (unsprayed) Aug 21 divided by
9	2500	1	72	estimated initial N_o of plants.
10	5000	0	5	q = % missed = (n_2/N_o)x100.
11	5000	0	0	
12	5000	0	0	
13	5000	43	11	
14	5000	41	35	
15	5000	69	18	
16	5000	0	16	
17	5000	48	38	
18	5000	11	20	
19	5000	33	2	
20	5000	33	2	
21	5000	67	5	
22	4280	67	29	
1	5000	61	18	October 21.
2	5000	38	63	Initial # of plants is estimated from
3	5000	21	86	binomial theory using formula
3A	5000	88	64	$N_o = n_1^2/(n_1-n_2)$ where n_1 = no. found
3B	5000	44	98	on first search, n_2 = no. found on
3C	5000	16	23	second search.
16	5000	0	9	For fraction q = probability of over-
17	5000	46	24	looking a plant, formula is
18	4000	67	27	(n_2/n_1)x100,
19	5000	33	5	also from binomial theory.
20	5000	0	3	For Quads 1 and 23, initial N_o and
21	5000	25	11	probability q are estimated using the
23	7950	24	45	method for August 21. This is due to
Values of n, q, N_o	all data	Oct 21 data	Aug 21 data	n_2>n_1 on those occasions, a bad-luck sampling anomaly to be expected
no. obs. = n =	35	13	22	occasionally.
mean q =	36.7	35.7	37.4	
sd dev q =	25.3	25.7	25.6	
mean N_o	--	36.6	25.8	

The probability that a team (1) finds a quad with at least one plant in it, *and* (2) finds all of the plants in that quad, can be calculated by the mathematics shown in Appendix B. In the following, that probability is designated as P (capital P). For a marsh with an average of λ plants per quad, each of whose probability of being found by a team of searchers in that quad is p, Appendix B shows that the value of P is given by …

$$P = e^{-\lambda}(e^{\lambda p} - 1)$$

This fraction P is also the fraction of quads (that is, of the whole area searched) in which the team will, by chance during the first search, find all of the plants and eliminate all of them. (The team will not know that all were found; they will know only that plants were found and treated there.)

The first search changes the situation in such a way that the same model cannot be used for searches, 2, 3, or more. A different model must be used. Appendix B shows the mathematics of that "overlay" model. The key factor is that the different searches must be *independent* of each other. That is, each team of searchers must be utterly uninfluenced by the team that preceded them. Some ways to do this would be to rotate spray teams that are used to treat each of the sites (i.e. – not have the same applicators always spraying the same site), and alternating direction of travel through a zone (north to south with east to west). It is also important that features of the topography – channels, logs, easy walking, and difficult walking – not be allowed to subtly channel all search teams over the same paths.

If independent searches are achieved, a new value of P – say, P_2, P_3, P_4, P_n – can be calculated for each search. As with search no. 1, each is the fraction of the ground that will be cleared of all plants by the end of that search. (These are cumulative; each includes the fraction cleared by all previous searches.) These are used in the way shown in the Appendix B to find the data shown in Tables 2A, 3A, and 4A below, which show the effort needed to find 95% or more of the Spartina in areas where the average density of plants is low.

Table 2A. Relationships between Spartina density, probability of discovery of a single plant, and *area cleared by searchers* after n = 1, 2 … 5 searches with p = 0.63.*

av. # plants per quad l	value of P $e^{-l}(e^{lp} - 1)$	initial % of quads with zero plants (e^{-l})	search # 1	2	3	4	5
0.005	0.003	0.995	0.003	0.004	0.005	0.005	0.005
0.050	0.030	0.951	0.030	0.042	0.046	0.048	0.048
0.500	0.225	0.607	0.225	0.321	0.362	0.380	0.388
1.0	0.323	0.368	0.323	0.481	0.558	0.596	0.614
1.5	0.351	0.223	0.351	0.543	0.649	0.707	0.738
2.0	0.342	0.135	0.342	0.548	0.673	0.749	0.795
3.0	0.280	0.050	0.280	0.477	0.616	0.715	0.784
5.0	0.150	0.007	0.150	0.278	0.387	0.478	0.556
10.0	0.025	0.000	0.025	0.049	0.072	0.095	0.117

*In this Table, analysis is by plants per quad (Col. 1); plants per acre is value in Col. 1 x 10. Lower case p = probability of discovery of a single plant. Upper case P = probability that a search team will find a quad with plants in it *and* find *all* of its plants. The plants are assumed to have a Poisson distribution (see text). As such, some quads have no plants in them when the search begins (Col. 3). The values in search columns 1, 2, … 5 are the cumulative fractions of the total quads cleared by searchers by the ends of search numbers 1, 2, … 5. This value does not include the fraction of quads with zero plants in them before the searches began. Example. After 3 searches of an area in which initial plant density was 20/acre (2.0/quad), searchers will have cleared 67.3% of the region. This cleared area is in addition to the 13.5% of the region that was already clear before the first search began. The total area clear of plants after the third search is 13.5% + 67.3% = 80.8%. See Table 3A.

Table 3A. Relationships between Spartina density and *the entire area free of Spartina* after n = 1, 2, … 5 searches.*

av. # plants per quad l	value of P $e^{-l}(e^{lp} - 1)$	initial % of quads with zero plants (e^{-l})	search # 1	2	3	4	5
0.005	0.003	0.995	0.998	0.999	1.000	1.000	1.000
0.050	0.030	0.951	0.981	0.993	0.997	0.999	0.999
0.500	0.225	0.607	0.832	0.928	0.969	0.987	0.995
1.0	0.323	0.368	0.691	0.849	0.926	0.964	0.982
1.5	0.351	0.223	0.574	0.766	0.872	0.930	0.961
2.0	0.342	0.135	0.477	0.683	0.808	0.884	0.930
3.0	0.280	0.050	0.330	0.527	0.666	0.765	0.834
5.0	0.150	0.007	0.157	0.285	0.394	0.485	0.563
10.0	0.025	0.000	0.025	0.049	0.072	0.095	0.117

* As in Table 2A, except that the values in search columns 1, 2, … 5 are the area cleared by the searches plus the area already free of Spartina before the first search (value in search col. 1, 2, … 5 added to the value in col. 3 of Table 2A). p = 0.63. The horizontal line separates cases in which less than 95% of the area is free of Spartina (below the line). Example. Where the initial plant density is 15 per acre (1.5/quad), 93% of the area will be clear of Spartina after 4 searches and 96.1% will be clear after 5 searches if the probability of detecting any given plant is p = 0.63.

the improvement in eradication ability that would accompany an improvement of plant detection success is shown in Table 4A. If the probability of detecting a single plant in a quad could be raised from 0.63 to 0.70, about 10% (by searching more slowly, or during more favorable light conditions, or by more workers per team), the ability to clear 95% of the ground in 4 searches improves by a disproportionate 25% (compare rows $\lambda = 1.5$ and $\lambda = 2.0$, 4 searches, Tables 3A and 4A). Aside from the number of searches and better herbicide efficacy, the probability of detection is the *only* parameter that we have any ability to manipulate to make eradication of Spartina easier.

Table 4A. Relationships between Spartina density and *the entire area clear of Spartina* after n = 1, 2, … 5 searches if probability of plant detection is improved from p = 0.63 to p = 0.70.*							
av. # plants per quad λ	value of P $e^{-\lambda}(e^{\lambda p} - 1)$	initial % of quads with zero plants ($e^{-\lambda}$)	search # 1	2	3	4	5
0.005	0.003	0.995	0.999	1.000	1.000	1.000	1.000
0.050	0.034	0.951	0.985	0.995	0.999	1.000	1.000
0.500	0.254	0.607	0.861	0.951	0.983	0.994	0.998
1.0	0.373	0.368	0.741	0.894	0.956	0.982	0.993
1.5	0.414	0.223	0.638	0.831	0.921	0.963	0.983
2.0	0.413	0.135	0.549	0.765	0.877	0.936	0.967
3.0	0.357	0.050	0.407	0.629	0.769	0.855	0.910
5.0	0.216	0.007	0.223	0.392	0.525	0.628	0.709
10.0	0.050	0.000	0.050	0.097	0.142	0.185	0.225

* Example. Where the initial plant density is2.0/quad (20 per acre), 93.6% of the area will be free of Spartina after 4 searches and 96.7% will be free after 5 searches. Compare with Table 3A. The density of Spartina for which 95% or more can be found in 5 searches has increased by 25% (from 1.5 to 2.0/quad) as a result of improving the detection of individual plants by about 10% (from .63 to .70).

Table 5A shows the numbers of searches that would be needed to clear 95% of the ground of Spartina at different improved levels of detection of individual plants. From that Table, it seems evident that the "end game" (in which we can eradicate 95% of the plants during a single season) begins after the plants have been reduced in numbers to about 50 plants per acre.

Table 5A. The number of searches needed to clear 95% of a region of Spartina at different levels of detection success. *					
mean # of plants per quad = λ	Values of p 0.63	0.70	0.75	0.80	0.90
0.005	0	0	0	0	0
0.05	0	0	0	0	0
0.5	3	2	2	2	1
1.0	4	3	3	3	2
1.5	5	4	4	3	2
2.0	6	5	4	3	2
3.0	9	7	5	4	3
5.0	>14	13	10	7	4
10.0	>14	>14	>14	>14	7
20.0	>14	>14	>14	>14	>14
30.0	>14	>14	>14	>14	>14
50.0	>14	>14	>14	>14	>14

*Includes fraction of area cleared by workers + fraction of area with zero plants before the first search. p = the probability of detection of any given plant.

Appendix B:

1) Estimates based on binomial theory.

We assume that the discovery of Spartina plants in a quad searched by field workers is a binomial process. This is the basis of the estimates of p and q from binomial theory in Table 4a, Appendix A. The binomial parameters are …

N = the number of plants in the quad before the search begins;
p = the probability that any particular plant will be discovered;
q = 1 - p = the probability that any particular plant will *not* be discovered;
p and q are the same for all plants and don't change as the search progresses.

The search of a quad is assumed to be a binomial process in which N "trials" are made, each with probability p of success. The probability of finding x of the N plants is given by
[1] $p(x;N,p) = N!\, p^x (1-p)^{N-x} / [x!(N-x)!]$ $(0<=x<=N)$

The Expected Number of discovered plants at the end of the search is given by

[2] $E(x) = N*p$

(*The number present when you started searching times the probability of finding each individual plant.)*

From properties of the binomial distribution we can estimate the numbers of plants remaining undiscovered after 1, 2, … n searches, the number of plants N initially present in a quad before the first search began, the probability p of discovering each plant during one search, and the number of searches needed to reduce the number of remaining undiscovered plants to less than one. Table 1b shows the basis of these derivations.

Table 1 b. Estimate of the number of plants remaining undiscovered in a quad after 1, 2, 3 or more searches using a binomial discovery mode		
Search No.	Expected Number found, this search.	Number remaining undis-covered after this search.
0	-	N (starting condition)
1	Np	$N - Np = N(1 – p) = Nq$
2	Npq	$Nq - Npq = Nq(1 - p) = Nq^2$
3	Npq^2	$Nq^2 – Npq^2 = Nq^3$
n	Npq^{n-1}	Nq^n

Estimate of N. The number N of plants initially present in the quad before the first search was made is estimated from the numbers discovered during the first two searches.

Let n_1 = the number discovered during the first search, n_2 = the number discovered during the second search (which were overlooked during the first search). From Table 1b …

$n_1 = Np$ $n_2 = Npq = Np(1-p)$

Substitute $p = n_1/N$ from 1st expression into 2nd expression to get $n_2 = N(n_1/N)(1 – n_1/N)$ and solve for N to get

[3] $N = n_1^2/(n_1 - n_2)$ for $n_1 > n_2$

(*Because this is a probability situation, n_2 may sometimes be larger than n_1, That can happen if a searcher has worse-than-average luck on the first search and average or better-than-average luck on the second search. When this is the case, this expression and those that follow can't be used.*)

<u>Estimate of p and q</u>. From Table 1A $n_1 = Np$ and $n_2 = Npq = Np(1-p)$.

Divide 1st expression by 2nd expression to get $(n_1/n_2) = Np/Npq = 1/q = 1/(1-p)$.

Solve for p and q to get [4] $p = 1 - (n_2/n_1)$ and $q = n_2/n_1$ for $n_1 > n_2$

<u>Estimate of the number of searches needed to find the last plant in a quad.</u> Suppose n searches are needed to reduce the number of plants still undiscovered to less than one. That is …

$Nq^n <= 1$ (from Table 1A, row n)

Solve for n to obtain …

[5] $n <= -\log(N)/\log(q)$

<u>The special case of searching a quad and finding all (or none) of the plants in it.</u>

Formula [1] above shows the following probabilities of finding all or none of the plants in a quad.

Table 2 b. Probabilities of finding none, or all, of the plants in a quad that has 1, 2, 3 or more plants in it.				
Number of plants in quad (n)	probability of finding none (x = 0)	numerical example if q = 0.37	probability of finding all (x = n)	numerical example if p = 0.63
1	q	0.37	p	0.63
2	q^2	0.14	p^2	0.40
3	q^3	0.05	p^3	0.25
4	q^4	0.02	p^4	0.16
5	q^5	0.01	p^5	0.10
n	q^n	--	p^n	--

<u>3) Finding all of the plants in a whole region.</u>

The following formulates the problem in Bayesian terms. We find the probability that a team will find a quad with some number of plants in it *and* then find all of the plants in that quad. We then sum these probabilities (pr(find quad with 1 *and* find 1) + pr(find quad w 2 *and* find 2) + pr(find quad w 3 *and* find 3) + etc … to find the value of P_1 used in the text.

P_1 is the probability that a team will find a quad with one or more plants in it and then find all of the plants in that quad. P_1 is also the fraction of quads in a region that the team will succeed in clearing during the first search of that region.

The Bayesian basis.

Probability of finding a given plant in a quad is p; Probability of overlooking it is q = 1 - p. The Bayesian model finds the probability that a searcher finds all (or none) of the plants in a quad, *given that he/she is searching a quad with plants in it*. p(x|x) = prob.(searcher finds all x plants given that x are in the quad) p(x) = prob.(there are x plants in the quad)

The probability that there are x in the quad being searched AND that the searchers find all x of them is given by the product p(x)*p(x|x). Likewise, p(x)*p(0|x) = the probability that the searcher will find a plot with x plants and overlook all of them. Given that there are 1, 2, 3, …. N plants in a quad, the probability of finding all (or none) of them is as follows:

x	p(x|x)	p(0|x)
0	--	--
1	p	q
2	p^2	q^2
3	p^3	q^3
n	p^n	q^n

Suppose that the number of plants in a quad is Poisson-distributed. If l is the mean number of plants per quad, the probability that a quad contains, 0, 1, 2, … n plants is given by p(x) = e-l lx/x! The probability that a searcher will find a quad with x = 1, 2, 3 etc. plants and find all of them is given in the following. The quantity P1 (sum at bottom of 4th column) is the “grand total” probability that a searcher will find a quad with any number of plants and find them all.

x	p(x)	p(x|x)	p(x)*p(x|x)
0	e^{-l}	--	--
1	e^{-l} l/1!	p	e^{-l} lp/1!
2	e^{-l} l^2/2!	p^2	e^{-l} l^2p^2 /2!
3	e^{-l} l^3/3!	p^3	e^{-l} l^3p^3/3!
n	e^{-l} l^n/n!	p^n	e^{-l} l^np^n/n!

[6] P_1 = Sp(x)p(x|x)

To visualize this, imagine that the rectangle in Figure 1 b represents the acreage A to be searched. If “a” is the size of one quad, there are A/a quads in this area. If the average number of plants per quad is l and if they are Poisson-distributed among the quads, fraction e^{-l} of the quads (or acres) have no plants in them when the search begins. All of the rest have plants. In fraction P_1, the searchers will find all of the plants. In fraction F_1 the searchers will find some of the plants but not all. In fraction Q_1, the searchers will find no plants, even though plants are present

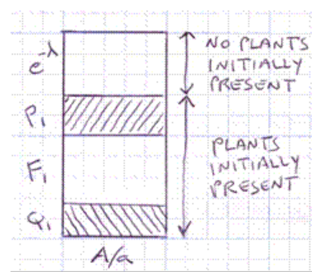

Figure 1b

Table 3b shows a few numerical values for P_1 and Q_1 for situations in which the mean number of plants per quad ranges from 0.5 to 20.0 per quad and the probability of discovery of any given plant in a quad is 0.63. (For 1/10 acre quads, the mean number of plants ranges from 5 to 200 per acre.)

At 20 plants/quad (= 200/acre), a searcher will find and inadvertently clear almost no quads at all. That is because with so many plants per quad, the chance of finding a quad with very few is small and the chance of overlooking at least one in most quads is very large. Likewise, in only 3 in a million cases will searchers enter a quad with plants in it and fail to find even one of them (last line of Table 3b).

Table 3b. The fraction P_1 of quads cleared of plants by observers at regional plant densities shown in Column 1. Col. 2 shows quads expected to be “naturally” clear of Poisson-distributed plants. Q_1 shows fractions of quads whose plants are completely overlooked by observers. 10 quads = 1 acre.			
p =	0.63	probability that one or more plants are present AND the searcher …	
q =	0.37	finds all …	misses all …
Value of l (plants/quad)	% w zero plants	P_1	Q_1
	(= e^{-l})	e^{-l} [e^{lp} - 1]	e^{-l} [e^{lq} - 1]
0.0	1.00	--	--
0.05	0.95	0.03	0.02
0.5	0.61	0.22	0.12
1.0	0.37	0.32	0.16
1.5	0.22	0.35	0.17
2.0	0.14	0.34	0.15
3.0	0.05	0.28	0.10
4.0	0.02	0.21	0.06
5.0	0.01	0.15	0.04
10.0	4.54E-05	0.02	1.79E-03
20.0	2.06E-09	6.11E-04	3.37E-06

As the number of plants per acre decreases, the value of P_1 increases, reaching a maximum value of 0.35 at 1.5 plants/quad (= 15 plants/acre). At that plant density, 35% of all quads will contain plants that are discovered and eliminated during one search. Since 22% of all quads were already free of plants (2nd column, Table 3b); 57% of all quads will be clear by the end of the search. During that search, 17% of all quads will contain plants that are completely overlooked by the searchers. The rest of the quads (100% - [57% + 17%] = 26%) will have plants, some (but not all) of which were found by the searchers.

By the time the plants have been reduced to a density of 0.05/quad (= 1 plant per 2 acres) 95% of quads are clear (2nd data row, 2nd column, Table 3b). Searchers will find plants and clear them from only 3% of quads and overlook all plants in only 2% of quads. The plants are now so scarce that the most likely number in a quad is one, which is either found or overlooked.

<u>The “Overlay Model</u>.” The following shows the considerations used to arrive at an estimate of the amount of effort needed to find the “last” plants.

In Figure 1A, the quantities $e^{-l} + P_1 + F_1 + Q_1$ add up to one. Rearranged algebraically,

$$[7] \quad P_1 + F_1 + Q_1 = 1 - e^{-l}$$

and

$$[8] \quad (F_1 + Q_1) = 1 - e^{-l} – P_1 \quad \text{(both used in the following).}$$

In Figure 1A, the “height” of each compartment multiplied by its “width” is the number of quads initially empty, cleared, partially cleared, or whose plants were completely overlooked during the search (respectively, top to bottom). Thus …
P_1* A/a is the actual number of quads cleared during the first search. After this search, there are $(e^{-l} + P_1)$*(A/a) quads clear of plants.

After the first search, conditions are changed. The model that serves for the first search is invalidated for subsequent searches. Clearing all, some, or none of the plants in the quads that initially contained them during the first search drastically changes their distribution among the quads to something other than Poisson. (For example, in the $(F_1 + Q_1)*(A/a)$ quads that still have plants, none of these quads contain zero plants – a very non-Poisson situation.) We may envision the effect of a second search, however, by conducting the following "thought experiment."

Suppose that, the day after the first search, a second team inspected the area. Suppose that all evidence of the first team was gone and the second team thought they were the first ones to search it. (Thus there are no footprints to follow, plants sprayed yesterday have not yet turned brown, etc.) Like the first team, the second team would spray no plants in the quads that really don't have any, all of the plants in fraction P_1 of the quads, none of the plants in fraction Q_1 of the quads, and some-but-not-all in the rest (fraction F_1 of the quads). If the search were truly independent of the previous inspection, the *number* of quads cleared by crew 2 would, on average, be the same as the *number* cleared by crew 1 the day before, but these would not all be exactly the same quads (see Figure 2b).

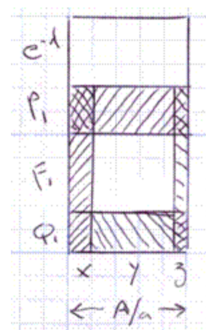

Figure 2b

The vertical strip of height $(P_1 + F_1 + Q_1)$ and width x shows the quads cleared by crew 2. Since this is the same number as were cleared by crew 1, then …

$(P_1 + F_1 + Q_1)*x = P_1*(A/a)$

from which $x = P_1*(A/a)/ (P_1 + F_1 + Q_1)$

or [9] $x = P_1*(A/a)/(1 - e^{-l})$

[substituting from equation [7] above].The *additional* area cleared by crew 2 has the dimensions $(F_1 + Q_1)*x$.

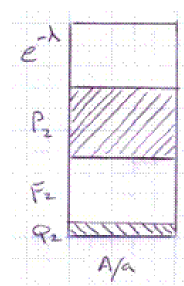

Figure 3b

Figure 3b shows the state of the marsh after the additional area cleared by the second search is added to the amount cleared by the first search. In this diagram, the fraction of cleared area P_2 is greater than P_1, and the fraction $(F_2 + Q_2)$ with plants remaining is smaller than $(F_1 + Q_1)$. P_2 is calculated as follows. The *total* area cleared by the two searches is given by

$P_2*(A/a) = P_1*(A/a) + (F_1 + Q_1)*x$

$= P_1*(A/a) + P_1*(A/a)* (F_1 + Q_1)/(1 - e^{-l})$

giving $P_2 = P_1*[1 + (F_1 + Q_1)/(1 - e^{-l})]$.

[substituting for x from equation [9] above]

Substituting $(F_1 + Q_1) = 1 - e^{-l} – P_1$ gives $P_2 = P_1*[1 + 1 – P_1/(1 - e^{-l})]$

[from equation [8] above]

which simplifies to $P_2 = P_1 + P_1 – (P_1*P_1)/(1 - e^{-l})$.

At the end of the second search and beginning of the third, the area now looks like Figure 3A. A larger fraction (P_2) has been cleared and the fraction in which some or all plants have been overlooked $(F_2 + Q_2)$ has shrunk. The same argument used to find P_2 is applied to this new situation, with the result that the fraction cleared by the end of the third search is given by …

$P_3 = P_1 + P_2 – (P_1*P_2)/(1 - e^{-l})$.

In general, repeating this argument gives this formula for P …

$P_n = P_1 + P_{n-1} – (P_1*P_{n-1})/(1 - e^{-l})$.

P_n is the cumulative percent of ground cleared by the end of the nth search. The *total* percent of plant-free area is this plus the ground that was free of plants before the first search, namely $P_n + e^{-l}$.

These formulas are the basis of the tables of results in Appendix A.

This analysis assumes all of the searches occur within a few days of each other, all cover the whole search region, and all are totally independent. In particular, the value of l is the same for all searches. If the searches are separated by days or weeks, conditions are changed. Subsequent teams will be searching ground in which l is lower every week by virtue of the clearance work of previous teams. Each search will seek fewer and fewer quads containing plants; within those quads the plants will not be Poisson-distributed. If the searches are conducted within a few days of each other, this model is valid.

What if the searches are conducted over a whole season? There are not likely any factors that could make the effort needed to find the "last" plants greater than predicted by this model. A few factors point in the other direction. For example, if areas in which no plants were found during three consecutive searches were then disregarded for the rest of the season, the amount of ground needing searching would diminish and/or the search could be concentrated on the remaining ground, increasing the probability of finding all of the plants there. Such factors suggest that the end game could go faster than this model indicates, but probably not more slowly. We tentatively regard its predictions as defining the upper limit of the effort that will be needed to get the "last" plants.

Table 4b. Estimate of the duration of effort needed to eradicate Spartina at ideal discovery and control levels of efficacy (90%) and typical levels of discovery and efficacy observed in 2008 (63 & 75% respectively). *

PLANTS/ACRE	No. searches to find and kill all of them (ideal efficacy @ p = .90, k = .90)**	Season of last search (eradication achieved) @ five searches/season	No. searches to find and kill all of them (observed efficacy @ p = .63, k = .75)	Season of last search (eradication achieved) @ five searches/season
0.05	2	2009	4	2009
0.50	3	2009	8	2010
5	5	2009	13	2010
10	6	2010	~14	2010
15	7	2010	>15	>2010
20	8	2010	>15	>2010
30	10	2010	>15	>2010
50	15	2011	>15	>2010

*Assumes 5 searches per season of sites inhabited by Spartina at densities ranging from 0.05 plants/acre (5 plants/100 acres) to 50 plants/acre.
**p=probability of finding, k=probability of killing (efficacy)

This applies to open or vegetated mid-intertidal habitats like those studied during the 2008 experiments. It almost certainly applies to low-intertidal habitats as well. It does *not* apply to creek channel habitats or to vast swards of upper intertidal native vegetation with rare, isolated spots of Spartina in their midsts.

The searches must examine every "plot" in the total of acreage suspected of containing Spartina. (A "plot" is one tenth of an acre, the unit used in modeling search outcomes.) The outcome assumes that there is *no* reseeding of the searched area by plants growing undetected elsewhere. (The plants in the searched area are assumed repressed and unable to go to seed in significant numbers, themselves.) Searches may be conducted from early in the growing season and treatment need not be 100% effective, as long as the condition "three (or two) consecutive searches that find zero plants" for stopping further searches of a quad for that season is strictly obeyed.

By the “eradication dates” shown in Table 4b, there is still an exceedingly remote chance that some Spartina has been overlooked; surveillance will be needed for a few more years. These estimates are valid only if *all* of the Spartina-infested area is searched in the manner described during 2009 and thereafter.

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