CIPM SEED Grant 2004 Report
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Original Abstract of Research from Thesis Proposal:

Invasive plants contribute to biodiversity loss and cause extensive economic damage and loss. Understanding the ecological impacts of invaders and why they are capable of displacing other species and communities is a priority. Much emphasis has been placed on the effects of invasives on ecosystem, community and population levels at a single point in time. However, these plants may continue to have effects as they stay resident. Gorse (*Ulex europaeus*) is invasive in many parts of the world, and alters the above and below ground ecosystem, biochemistry, and disturbance regimes. This research will examine gorse's ability to alter the soil ecosystem and biochemistry over time, including pH, organic matter content, nutrient mineralization/immobilization and soil and water chemistry. This study will lay the groundwork for further research into the alteration and impact of invasives to both above and below ground ecosystems over time. The current research will be done on the coast of Washington state, where gorse is found growing on sand. In order to determine the age of gorse stands, the progression of invasion over time is documented with aerial photos. Also, the technique "dendrochronology" estimates plant age by counting annual plant rings and will be used to accurately assess individual gorse plant age. An exploratory analysis, using multivariate techniques will also be done to begin to understand how these free dune systems are being changed by the succession of invasive plants across them. These results will be part of the empirical evidence needed to evaluate the current status of ecological impact models. With improvements in impact models and better understanding of the alteration of ecosystem processes, recommendations can be made for more precise prediction, assessment and management of invasives as well as improved recovery of processes to facilitate easier restoration once invasives are removed.

Summary of Research Done, Summer 2005:

The sand dunes on Grayland Beach in Western Washington have recently been invaded by gorse. The study site is just south of Westport on Highway 105, Grayland Beach Road, south of the state park restroom (approximately 46° 48' 565" N, 124° 05' 849" W). This site has received little to no management, so the gorse has been minimally disturbed, except next to the road where it was aerially sprayed in 2002. The area was invaded by gorse in the last 30 years, and there is no history of fire.

These dunes used to be open sand, and the beach is expanding in this area. Historically, the native grasses on these dunes were bunch grasses and the dunes moved more freely. Several invasive grasses, are now holding the dunes in place. Another major invasive of the area is *Cytisus scoparius* (Scotch broom), and although it is a nitrogen fixer and could effect the ecosystem in similar ways to gorse, it is sick or dead due to fungal attacks[1].

Questions Asked:

- Postulate 1: Gorse is changing the soil ecosystem over time.

  - *Hypothesis 1.1*: Gorse has no effect[2] on the soil parameters to be measured.
  - *Alternate Hypothesis 1.1*: Gorse at least one effect on the soil parameters to be measured.

  - *Hypothesis 1.2*: The measured soil parameters for gorse do not vary over time.
  - *Alternate Hypothesis 1.2*: At least one of gorse's effects on the measured soil parameters varies significantly over time.

- Postulate 2: Gorse's foliar nutrients are changing over time.

  - *Hypothesis 2.1*: Gorse's measured foliar nutrients do not vary over time.
  - *Alternate Hypothesis 2.1*: Gorse's measured foliar nutrients vary over time.

Experimental Design:

The specific area sampled started 26 meters south of the road, and continued 247 more meters south. The area sprayed by the Washington State Parks two years earlier was removed from the study area by starting so far south of the road. The east and west boundary were established by the extent of the gorse's spread.

Individual populations of gorse were determined spatially. Each population was spatially separated, either naturally or because there was a man-made path between them. There were two paths, and where there were paths, gorse was not sampled within the area disturbed by the path to avoid any complications the paths might cause. Initially, individual populations were roughly mapped using a GPS, and put on a scaled map (Appendix A: Figures, Figure 2). Next, individual populations were mapped using a compass and measuring tape. The populations were transcribed to graph paper, with each square representing 1 m^2. (Appendix A: Figures, Figure 3).

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[1] Nancy Ness (Grays Harbor Noxious Weed Control Board) and Marianne Elliot (UW graduate student, CFR) have been unable to confirm the type of fungus. However, all of the common symptoms of fungus disease are present. CSIRO has also had trouble diagnosing this fungus, but these die-backs are documented (personal communication, spring 2004).

[2] Unless otherwise stated, "an effect" is a statement of significance at the .05 α level.

Samples

All of the populations in the study area were used, except for one which was too small to support random sampling. That left eight populations. After each population was drawn on a grid, each grid intersection was numbered consecutively. Twelve random numbers were chosen, in case there was something unusable about the initial eight number. Eight populations were randomly sampled eight times, except for the first two populations which were sampled ten and nine times (Appendix A: Figures, Figure 3). The resulting number of samples taken was 67.

Controls

The area where gorse was not growing had high homogeneity and was mostly covered in grasses. Because of this, a different sampling technique was used. Random numbers were selected on a 10 m^2 grid in a similar fashion to how they were chosen within the populations for the sample. The random spots were then truthed by locating them within the study site. Random spots were not used if they were on a path or had gorse within a meter (Appendix A: Figures, Figure 3).

Once a random non-gorse, undisturbed spot was found, the samples were taken systematically. A three meter square was outlined, and soil and bulk densities samples were alternated at every meter distance (Figure 1).

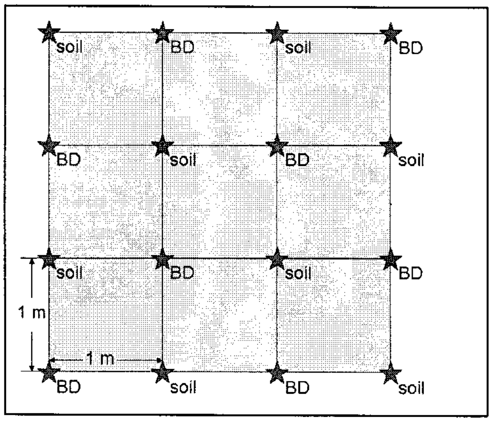

Figure 1 - Systematic control sampling design

Sampling Technique

When the random spot was located, then the gorse plant with the nearest main stem was chosen. Because gorse can spread vegetatively, it sometimes formed dense thickets, and it was difficult to tell if an original plant was being sampled or if this was an old rooted lateral branch. Significant effort was taken to try and find the mother plant. However, even in the case where this may not have happened, the sampled plant and soil should still effectively represent the time length of infestation.

Notes were taken about other plants that are growing near the sampled plant, as well as any noteworthy observations. The litter was removed from around the base of the plant. Care was taken not to compact the soil before sampling was done. The top 10 cms of soil was sampled.

Soil samples were taken at four different areas around the plant, about 20 cms from the base. The four different soil samples were pooled, and put in a plastic bag. These samples were kept cool until they could be air dried for one to two weeks.

Then two bulk densities were taken, also around 20 cms from the base. These were tested separately to increase accuracy of the measurement. Because of the sand, a 10 cm deep hole was dug, and water and plastic wrap were used to measure volume. The soil was stored in plastic bags, but the tests were not necessarily run near the time of collection so the percent moisture is not meaningful.

For each plant, three foliage samples were taken. If possible, these were taken on different branches. The samples were always taken on the newest growth, receiving the most light. Because gorse doesn't really have leaves, just the tip 2-3 inches were taken, with no attempt to separate stem from leaves. These samples were pooled, stored in paper bags, and air dried within a couple of weeks of collection.

After the soil and foliage collection, the plant was cut down and stem sampling was done. Gorse often has multiple stems, so often multiple stems were collected in order to make sure to get the oldest stem. In a few cases, a plant was so rotten that the rings could not be counted. When this happened, this plant was discarded, and the next random number spot was used. Stem samples were stored in paper bags, and then air dried within a couple of weeks.

Analysis

The soil and foliage samples were sent to Department of Plant and Soil Sciences, Soil and Plant Tissue Testing Laboratory at the University of Massachusetts.

Except for bulk density, the soil tests performed were the lab's standard soil test with organic matter. In addition the soluble salts and total nitrogen were added on to the standard tests. This included pH, buffer pH, extractable nutrients (K, Ca, Mg, Fe, Mn, Zn, Cu, B), extractable heavy metals (Pb, Cd, Ni, Cr), and extractable Al, cation exchange capacity, percent base saturation, percent organic matter.

Bulk densities is the dry weight of the soil per unit volume, and was determined by drying the soil at 105° C for 24 hours. Although soil moisture content was calculated, it is not meaningful because the soil may have dried out while being stored.

For each foliage sample, a determination of the Total Tissue P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B, Mo, Pb, Cd, Ni, and Cr, plus Total Nitrogen.

For counting the rings, the gorse stems were sanded using a belt sander with 50, 80 and then 120 grit. The rings are easily visible by eye or hand lens, and were counted by two people.

Results:

Description of Community and Vegetation:

The gorse grew in thick populations in the sand dunes. There was a definite line where the gorse stopped, and the broom and grass continued towards the beach. The was not any apparent reason for this.

It tended to grow with many stems, either straight up or laterally along the ground, making a tangled weave. The study area was lined by wetlands on the east, and my personal observation was that the gorse tended to grow more matted and lower, the closer to the wetland.

The gorse rarely had plants directly underneath, and if there were plants, they were usually small and unhealthy. The plants that could sometimes survive were the native rose, sword fern, and carex.

The top 10 cms of soil varied from sand, to clay, all the way to a slightly mottled wetland soil. Despite using nested ANOVAs, there did not seem to be any significant effect on the gorse and what kind of soil it was growing in.

The stems collected ranged in ages from 4 to 26, which is consistent with what is known of gorse's biology. The older samples tended to be closer to the beach.

Gorse's Effects on Soil Parameters

ANOVAs were done, using the means of each population for the gorse and control sites. Highlighted rows are not significant at $\alpha = .10$.

	p value	Control ± SE	Samples ± SE	Total ± SE
BD (g/cm^3)	0.11	0.89 ± 0.04	0.80 ± 0.03	0.85 ± 0.03
Total N (%)	0.10	0.199 ± 0.022	0.249 ± 0.019	0.224 ± 0.015
pH	0.00	5.4 ± 0.1	4.8 ± 0.1	5.1 ± 0.1
P (ppm[3])	0.36	5.6 ± 0.3	6.0 ± 0.4	5.8 ± 0.2
CEC (meq/100g)	0.23	8.39 ± 1.61	10.91± 1.21	9.65 ± 1.03
K (ppm)	0.90	57.5 ± 7.6	58.7 ± 4.7	58.1 ± 4.3
Ca (ppm)	0.45	386.3 ± 72.6	306.5 ± 71.4	346.4 ± 50.2
Mg (ppm)	0.79	306.9 ± 69.7	281.8 ± 63.2	294.3 ± 45.5
NH4 (ppm)	0.01	3.6 ± 0.6	7.0 ± 1.0	5.3 ± 0.7
NO3 (ppm)	0.01	8.8 ± 0.4	18.6 ± 3.1	13.7 ± 1.9
Al (ppm)	0.89	40.7 ± 8.5	42.2 ± 6.0	41.4 ± 5.0
OM (%)	0.03	4.9 ± 0.5	6.6 ± 0.5	5.7 ± 0.4
Soluble Salts[4]	0.00	0.132 ± 0.004	0.259 ± 0.023	0.196 ± 0.020
B (ppm)	0.32	0.15 ± 0.02	0.17 ± 0.01	0.16 ± 0.01
Mn (ppm)	0.00	3.54 ± 0.62	8.65 ± 1.33	6.10 ± 0.97
Zn (ppm)	0.94	1.13 ± 0.06	1.22 ± 0.10	1.13 ± 0.06
Cu (ppm)	0.72	0.41 ± 0.08	0.45 ± 0.07	0.43 ± 0.05
Fe (ppm)	0.10	24.39 ± 3.80	34.77 ± 4.52	29.58 ± 3.15
Ext. Pb (ppm)	0.01	0.40 ± 0.12	1.70 ± 0.38	1.05 ± 0.26
Ext. Cd (ppm)	0.88	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.01
Ext. Ni (ppm)	0.00	0.10 ± 0.03	0.27 ± 0.04	0.19 ± 0.03
Ext. Cr (ppm)	0.01	0.05 ± 0.01	0.10 ± 0.01	0.07 ± 0.01

Table 1 - ANOVA results for soil samples, gorse vs. control. Highlighted rows are not significant.

Another idea for further analysis on these data would be to use MANOVA on variable groups that are biologically linked in some way. For example, if a MANOVA is done on P, K, Mg and Ca, the results are significant. The remaining question is; if looking at those variables simultaneously is a biologically meaningful thing to do.

[3] ppm = mg/kg
[4] Deciseimens/meter = mmhos/cm

Gorse's Effects on Soil Variables Over Time

Linear regressions were done using each sample for all populations of gorse. . Each soil variable was regressed against ring count. Highlighted rows are not significant at $\alpha = .10$.

	p value	r^2	trend
BD (g/cm^3)	0.017	0.043	decreasing
Total N (%)	0.000	0.344	increasing
pH	0.000	0.200	decreasing
CEC (meq/100g)	0.026	0.074	increasing
P (ppm)	0.655		
K (ppm)	0.706		
Ca (ppm)	0.061	0.053	decreasing
Mg (ppm)	0.155		
NH4 (ppm)	0.264		
NO3 (ppm)	0.219		
Al (ppm)	0.580		
OM (%)	0.000	0.323	increasing
Soluble Salts	0.143		
B (ppm)	0.346		
Mn (ppm)	0.086	0.045	decreasing
Zn (ppm)	0.708		
Cu (ppm)	0.319		
Fe (ppm)	0.755		
Ext. Pb (ppm)	0.008	0.102	increasing
Ext. Cd (ppm)	0.629		
Ext. Ni (ppm)	0.764		
Ext. Cr (ppm)	0.186		

Table 2 - Linear regression results for ring count vs. soil variable. Highlighted rows are not significant.

Significance with a linear regression means that there is a significant effect of time on the soil variable. The r^2 value simply says how much of the variation is explained by that variable. In such a complex system, and only having one independent variable, these r^2 are reasonable.

There are several ideas for further analysis on these data. One would be to use multivariate multiple regression on variable groups that are biologically linked in some way. For example, note that although total N was significant, NO_3 and NH_4 were not. Since NO_3 and NH_4 are related variables, multivariate multiple regression would be able to use both of them as dependent variables.

Also, factor analysis may be able to group the variables thereby decreasing the number of variables that are meaningful. This type of procedure may allow for a more simplistic, but still useful model.

The data are not necessarily varying in a linear fashion. However, even using more complex curves, the r^2 values do not increase by much.

Gorse's Effects on Foliar Variables Over Time

Linear regressions were done using each sample for all populations of gorse. . Each foliar variable was regressed against ring count. Highlighted rows are not significant at $\alpha = .10$.

	p value	r^2	trend
N	0.121		
P (ppm)	0.002	0.136	decreasing
K (ppm)	0.004	0.123	decreasing
Ca (ppm)	0.825		
Mg (ppm)	0.108		
B (ppm)	0.716		
Mn (ppm)	0.175		
Zn (ppm)	0.015	0.087	decreasing
Cu (ppm)	0.301		
Fe (ppm)	0.277		

Table 3 - Linear regression results for ring count vs. foliar variable. Highlighted rows are not significant.

All of the same types of analysis can be done on the foliar vs. time, can be done on the soil vs. time.

Discussion:

It is clear that there are interesting results from this experiment. Every part came up with significance. There are still many types of analysis that can be done to help clarify what the results might mean.

One of the first noticeable results is that counting the rings of gorse in this particular area seemed to work as a way of judging time. Not only were the rings clear, but also reflect what is known about gorse's life cycle. It is not a technique that can necessarily be transferred to other areas or plants. Complicating issues might be; not clear seasons, fire history, extensive use of poisons, and age of infestation longer then plant age.

There was a choice that had to be made about how to look at the soil. Because this experiment was tied to research done by Ruth Mitchell in England, the researcher chose to look at the top 10 cm of soil, and not the top 10 cm of mineral soil. This looks at the changes of the top 10 cm, but does not look at soil nutrient pools. This could explain apparent discrepancies in the data, such as total N increasing in the top 10 cm, but not significant in the foliage. Gorse appears to be quickly creating OM that is high in N, but it is not necessarily getting to the new plant tissue.

A cursory comparison of Prof. Mitchell's results to mine show that in her research, gorse is increasing pH in a very acidic environment. On the Washington coast, it is lowering pH. It appears that gorse may have an ideal pH level, and that it is capable of changing the soil pH to approach that level.

There were some other odd results, like the highly significant increase of Pb in the top 10 cms of the soil. This seems to be happening only for that heavy metal, so the argument that maybe more things are just accumulating doesn't really work.

This research was only part of what would have to be done to have a whole picture of what is happening both above and below ground when invasion happens. Two things that would need to be added are looking at the biota of the soil, and also documenting and testing different soil layers so that nutrient pools can be estimated.

This would cost about triple the amount of this grant, but may still be a small amount of money if information is sought about sensitive areas that are to be restored. If it is not needed or possible to look at the situation over time, then the sample sizes could probably be lowered.

The primary researcher is working on her masters' thesis with regards to this research. She and Prof. Reichard have not started working on paper(s) to publish, but will keep CIPM notified of any papers accepted. She is planning on going on for a PhD in quantitative invasive species ecology with Prof. Reichard. From her point of view, it is too soon to see any type of benefit from the research, although she's very sure there will be.

Budget:

Foliar Quantity	Foliar Price	Soil Quantity	Soil Price	Total
0	$20.00	19	$26.00	$494.00
20	$20.00	0	$26.00	$400.00
7	$20.00	7	$26.00	$322.00
41	$20.00	0	$26.00	$820.00
0	$20.00	40	$26.00	$1,040.00
0	$20.00	33	$26.00	$858.00
0	$20.00	13	$26.00	$338.00
68		112		$4,272.00
				$4,275.00

Appendix A: Figures

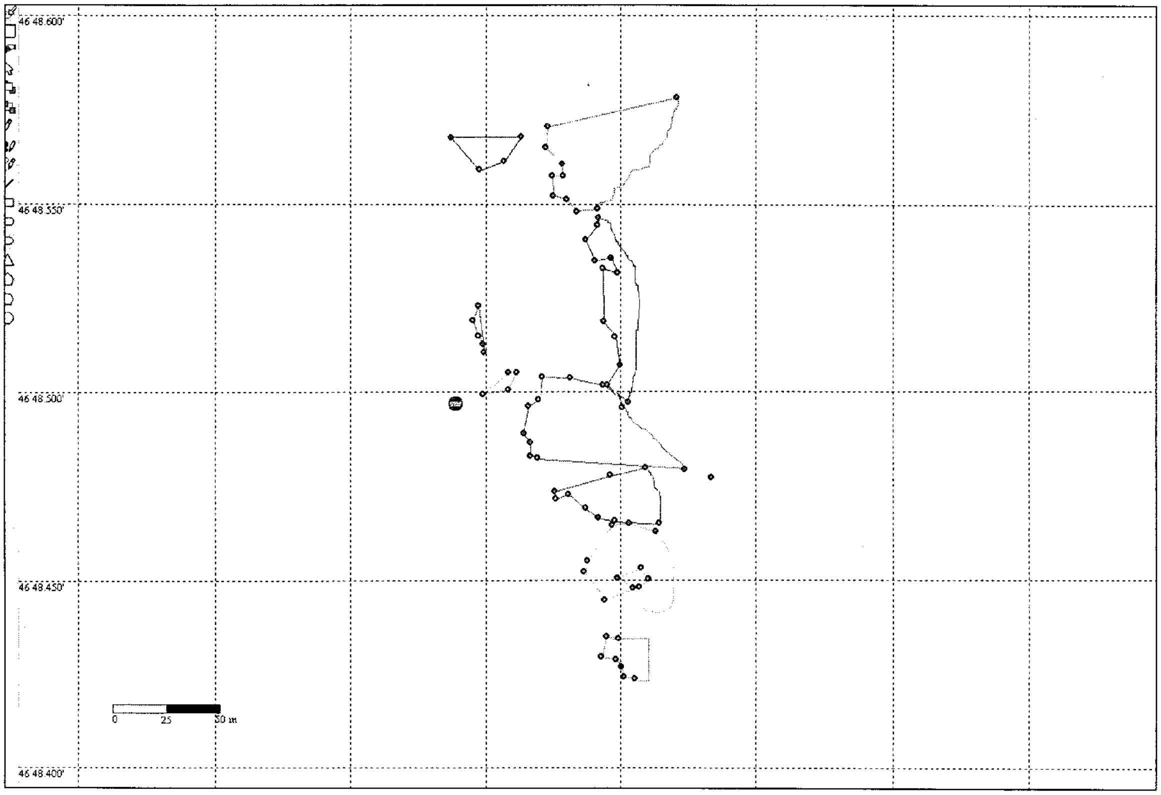

Figure 2 - Rough map of populations

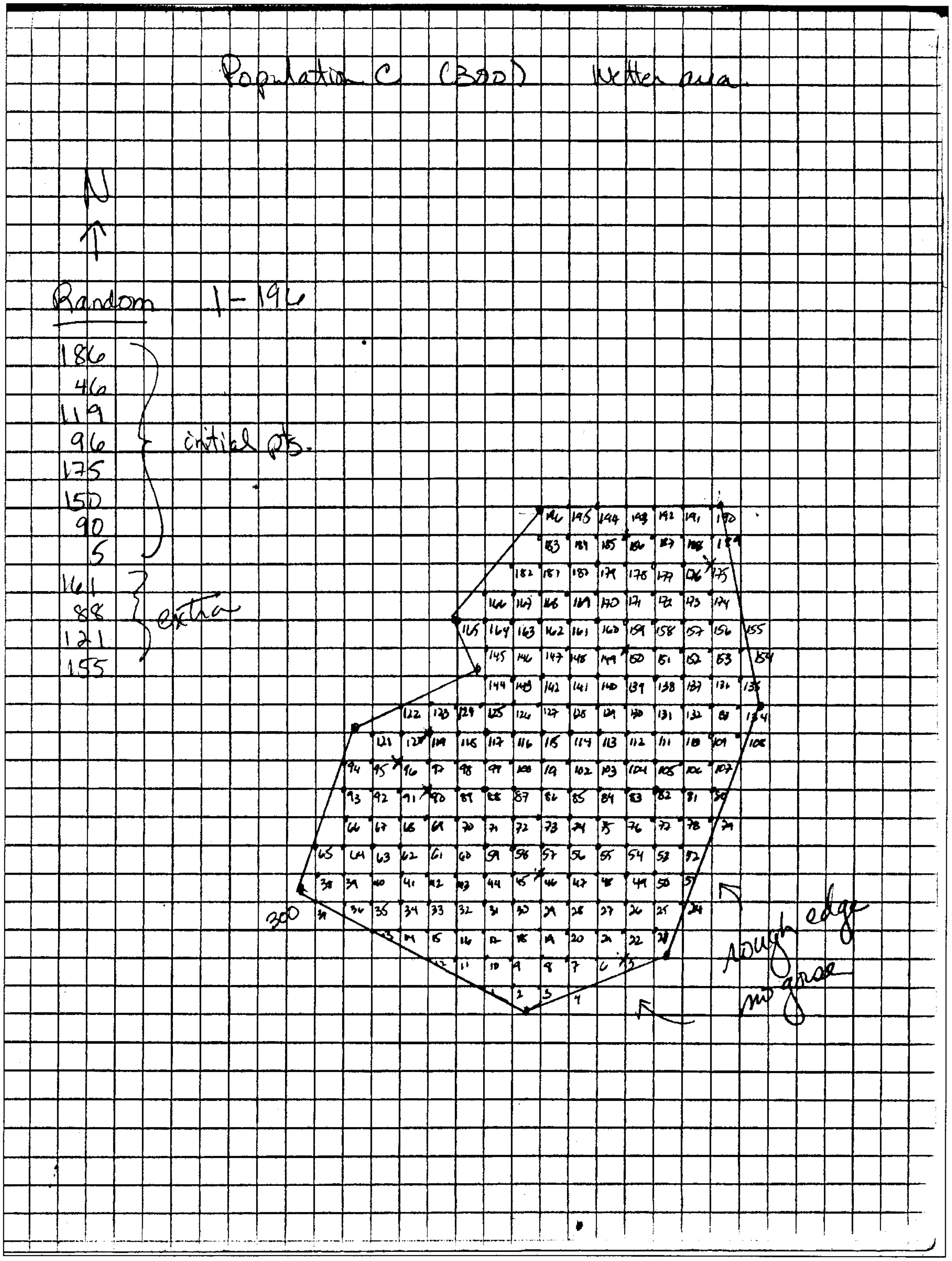

Figure 3 - Example of a single population mapped to square meter scale

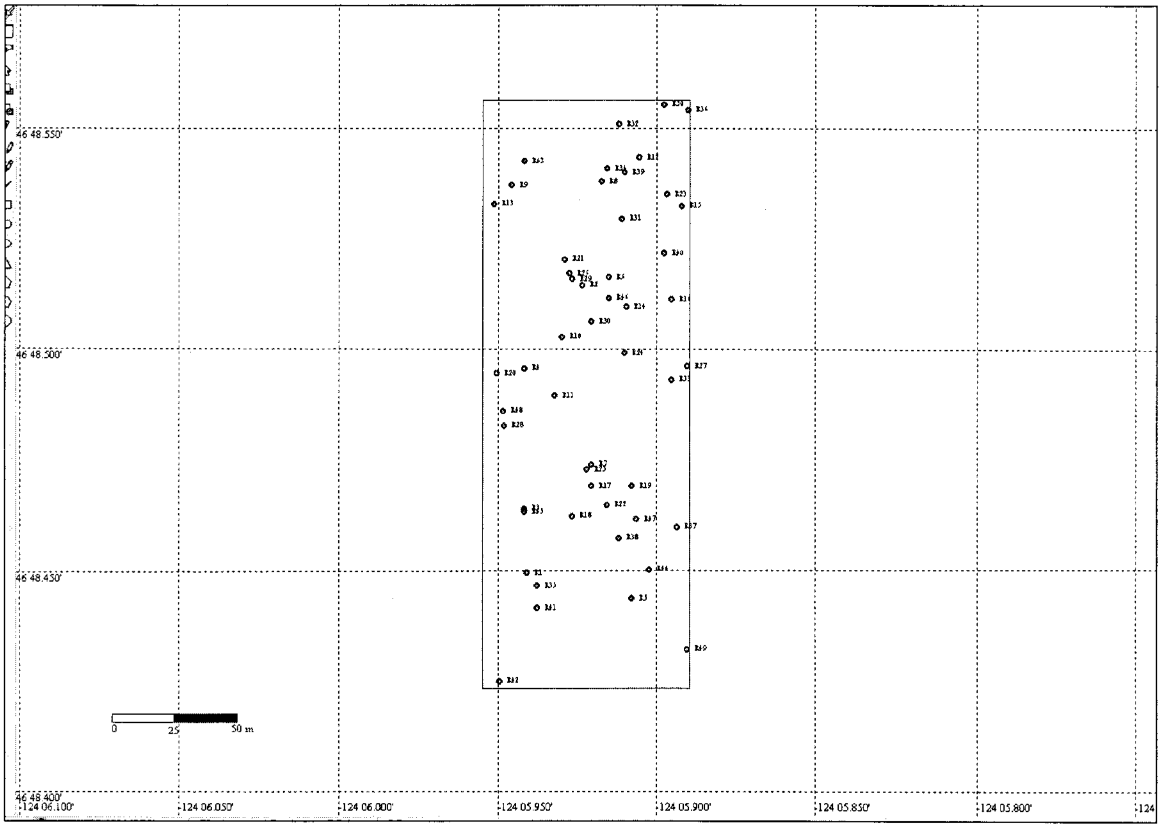

Figure 4 - Random control numbers picked in study site.