

SPATIAL PATTERNS AND SPREAD OF HERBICIDE RESISTANCE

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ABSTRACT

The spatial distribution of triallate resistant wild oats was examined at three (regional, field and patch) scales in a cereal cropping region of northern Montana. Regional patterns of resistance were investigated in 1992 and 1993 by making collections of seed from 237 fields. In 1994 seed was collected from three fields using a systematic grid method to select individual plants from 144 cells. The sampling of wild oats at the patch scale was conducted by placing eight random transects across a 25 m² grid and collecting all seed from within 17 quadrats located on each transect. All seed was tested for resistance to triallate and analysed using geostatistical techniques. The regional survey showed that 91% of fields were resistant to triallate in 1993, an increase of 28% over 1992. Semivariogram analysis indicated autocorrelation up to a distance of 1.5 km. A pattern was also detected at the field scale at a distance less than 15 m. At the patch scale autocorrelation was present below 1 m. The different patterns observed at each level are a result of the combined effects of biological and cultural practices. Land ownership may play an important role in seed dispersal at the regional level. The patterns at the field and patch level may be influenced by seed dispersal mechanisms and fitness.

Keywords: herbicide resistance, spatial distribution, triallate, wild oat

INTRODUCTION

Herbicide resistance is a major problem in the Fairfield Bench region of the state of Montana in the USA. This region has 18,130 ha of irrigated land that has been in continuous malt barley production for 24 years. Wild oat (*Avena fatua*) is an important grass weed in this region due to potential grain price reduction from contamination of the malt barley. Soil incorporated triallate applied prior to planting was the wild oat management option of choice in the malt barley production system, because it was effective and it was half the cost of post emergence wild oat herbicides. After 20 years of continuous use, triallate resistant wild oats were discovered in the Fairfield Bench region. The exact mechanism of resistance is unknown, however Collier *et al.* (1994) and Miller *et al.* (1995) provided evidence that primary metabolism of the triallate may be lacking in the resistant wild oat biotype. Experiments to determine the inheritance of triallate resistance are currently in progress. One other important aspect of the Fairfield Bench problem is the recent discovery of resistance to three out of the four remaining herbicide families registered for wild oat control.

In response to complaints of poor triallate performance on wild oats in 1991 on the Fairfield Bench, 44 fields were surveyed and 29% were determined to be resistant¹. The first objective of this study was to determine the frequency of triallate resistance in fields on the Fairfield Bench in 1992 and 1993, and to determine what relationships may exist between the occurrence of resistance and farming practices used in the resistant fields (results not reported here).

¹Wild Oat Resistance to Far Go in Montana, Report to Dr Doug Ryerson of Monsanto Corp. by Fay, Trunkle and Christianson, 1992.

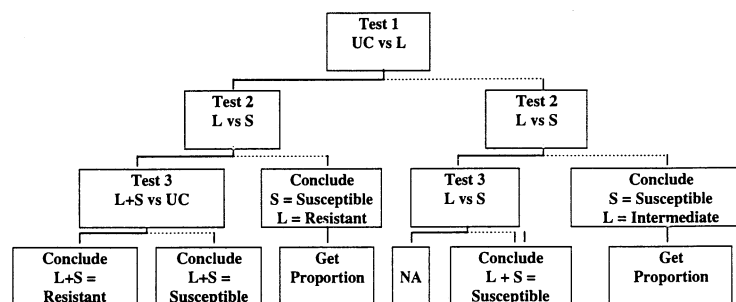
The regional spatial distribution of triazine resistant weeds was studied by Stephenson *et al.* (1990) and related to regional variation in farming practices. Spatial statistics were used to quantify the degree and scale of pattern in weed populations (Donald 1994; Mortensen *et al.* 1993; Cardina *et al.* 1995). There are no known studies that have quantified the distribution of herbicide resistant populations or individual plants at any scale. The spatial distribution of triallate resistance in the Fairfield Bench region, within fields, and within wild oat patches was the focus of this study. Aggregation of resistant fields may indicate specific introduction or resistance selection sites. The amount and/or pattern of aggregation may provide insight into the biological and/or agronomic processes that mediate gene flow at these different scales.

MATERIALS AND METHODS

Sampling of wild oat populations on the Fairfield Bench for triallate resistance began in 1992. The sampling region was divided into seventy 259 ha sampling areas along section lines. Four fields were randomly chosen from aerial photographs within each sampling area. A total of 280 fields were selected but, because some fields had been converted to pasture, a total of 237 fields were sampled for resistance.

Each field was sampled by a collector walking across the field in a "W" pattern, avoiding collection near field edges and in obvious herbicide skips. Every 12 m the seed from a single panicle was removed. Each field sample was considered a bulk composite sample. The 237 field samples were tested for response to triallate. Twenty-eight days after planting, wild oat seedlings were clipped at the soil surface. Seedlings that had not extended beyond the coleoptile (a typical susceptible response to triallate) were classified into a small size class and all other emerged seedlings were grouped into a large size class. Due to variability in seedling size in each size class, the difference between the small and large size class was determined using a t-test. Individual triallate treated plant fresh weights were measured for thirteen randomly chosen field subsamples that contained both large and small plants. The classes were determined to be different ($P < 0.01$).

Seedlings of each size class from a row within a flat were counted and weighed to determine mean fresh weight per plant for each size class. The mean of the three replicate mean fresh weights per plant was then calculated. The treated size class means were compared to each other and to the untreated mean fresh weight per plant of a field sample using t-tests at a 5% level of significance to determine if a sample was resistant or susceptible according to a series of decisions (Figure 1). F and t-tests (0.05 level of significance) were conducted using PROC TTEST in SAS.



Solid line represents no difference. Dashed line represents a significant difference.
L = large class; S = small class; UC = untreated control; NA = not applicable

FIGURE 1: The series of decisions used to determine if samples were resistant (R), susceptible (S), or a proportion of R and S relative to an untreated control (UC).

In 1993, 18 of the 237 fields sampled in 1992 were randomly selected and resampled for triallate resistance. Eleven fields had received triallate in 1992 and 1993, while seven had received triallate in 1992 and imazamethabenz in 1993.

Spatial autocorrelation, the likelihood that samples taken near each other are more similar than those taken at a further distance, was used to determine if there was aggregation of resistance frequency using GS+ in the Fairfield Bench region. The semivariance statistic was calculated to determine if pattern existed in the resistant wild oat population (Cardina *et al.* 1995).

In order to identify the frequency and patterns of resistance within fields a systematic grid sampling method was used in three fields in the Fairfield Bench region in 1993. Site 1 was in a portion of a continuous barley field suspected to contain resistance in 1992 so was not treated with triallate in 1993. Site 2 was a 1993 triallate treated (1.4 kg/ha) field. The third site was in a field which had received triallate at 1.4 kg/ha in 1992 and 1993 and was sampled in 1992 and the wild oats were found to be 100% triallate resistant. A 3600 m² grid containing 144, 25 m² cells, was placed at each site. Seed from an entire panicle was taken from a single wild oat plant nearest to the centre of each cell within the blocks. It is assumed that the individual plant sampled represents the resistance status of the population of wild oats within the grid cell and that the sampled wild oat plant's location was the centre of the cell.

For this experiment, because of the large sample sizes, seed samples were screened for triallate resistance using a petri dish bioassay developed by Colliver *et al.* (1994). Mean shoot growth for 7 days following treatment for the treated and untreated portions of each sample were compared using a t-test at 0.05 level of significance. Treated means significantly different than the untreated means were considered susceptible, and when no difference was detected the seedling was considered resistant. Results were mapped for each cell and the frequency of resistance determined for each site.

Spatial point pattern analysis was used to determine if the resistant or susceptible cells within a grid were aggregated, regular, or completely spatially random. If the processes governing the position of resistant or susceptible cells within a field are completely stochastic then they can be characterized as a homogeneous Poisson process and the process is concluded to be completely spatially random (CSR). Coordinate data sets for resistant and susceptible cell locations for the three sites were analyzed using a program developed by Hamilton *et al.* (1995). The procedure of spatial point pattern analysis follows the standard notation and terminology of Cressie (1991) and is demonstrated and outlined by Hamilton *et al.* (1995).

All three sites were also analysed using spatial autocorrelation in order to confirm the results of the spatial point pattern analysis method. The GS+ analysis package was used to analyse data sets for all three sites. Isotropic, or omni-directional, semivariograms were created for each site to determine if any spatial autocorrelation existed within the sites.

The frequency and pattern of resistance within patches of wild oats was assessed in two fields in 1994. A 25 m² grid was placed over a randomly selected patch within each field with eight transects crossing the area. Seventeen 0.063 m² quadrats were placed on each transect and all the seed within this area was collected. All seed was tested for resistance to triallate using the petri dish bioassay. Analysis was conducted using the same methods as used at the regional scale.

RESULTS AND DISCUSSION

Out of the 237 fields sampled in the Fairfield Bench region in 1992, 64% were found to contain triallate resistant seed. This was up from the estimate of 29% of the fields (44 sampled) identified as triallate resistant in 1991. In 1993, 10 (91%) out of 11 fields that had received continuous triallate, contained triallate resistant wild oat seed. The one field found to be 100% triallate susceptible in 1993, was measured to be 67% resistant in 1992. This result demonstrates potential problems in sampling methodology. However, in most cases, fields were found to increase in the proportion of resistant seed sampled (Table 1).

TABLE 1: Percent triallate resistant (R) and the change in percent resistant seed in samples from fields receiving triallate in 1992 and 1993 in the Fairfield Bench region.

Field	1992	1993	Change
93022	0	94	+ 94
93033	73	94	+ 21
93082	60	100	+ 40
93152	82	72	- 10
93274	61	98	+ 36
93332	67	100	+ 33
93463	95	84	- 11
93543	67	0	- 67
93563	69	100	+ 31
93194	100	100	0
93273	0	100	+100
Mean (%)	61	85	24
SD	32.9	29.7	47.4

Out of the 150 fields found to contain triallate resistance in 1992, 39% were 100% resistant and 61% had a mix of resistant and susceptible seeds in the sample. The proportion of resistance in samples containing resistant and susceptible seed ranged from 15% to 95% resistant seed. Susceptible seed in a sample from a field that had been treated with triallate indicates escapes from the effect of triallate are common. Susceptible escapes in these fields can result from skips in application, uneven incorporation, and/or germination after the triallate has volatilized from the soil. Those fields that were 100% resistant may have had more effective and thorough applications, or had evolved resistance in previous years allowing resistant plants to spread over the entire field.

Resistant seedlings from the 1992 sample, although delayed in growth, were equivalent to the untreated control by the end of a 28 day growth period. Susceptible wild oats either never emerged or never progressed past the coleoptile stage of emergence. The intermediate response class was made up of plants within a sample that were significantly smaller ($P < 0.05$) than the untreated control and significantly larger than the treated small class seedlings. Intermediate seedlings displayed some of the classic triallate symptoms: decreased height, darker green leaf colour, and leaves that were shorter and wider than the untreated plants, however, these plants continued to grow and appeared capable of reproduction. If triallate resistance is polygenic in wild oats, the plants with intermediate response may be the result of populations accumulating resistant alleles. The intermediate response could also be the result of a partially dominant single gene for resistance. The possibility also exists that many types of resistance have developed, some more fit and successful than others, and these intermediate plants may be a different form of resistance that is less fit than that found in the samples classified as resistant. Intermediate samples were considered to be resistant under field conditions due to the increased efficacy of triallate under greenhouse conditions which can improve herbicide uptake by increasing exposure of germinating wild oats to the triallate.

The seven fields that received triallate in 1992 and imazamethabenz in 1993 averaged a 51% increase in the proportion of triallate resistance in sampled seed. Two of the seven field samples were susceptible in 1992 and found to be resistant in 1993. Changing wild oat herbicide from triallate to imazamethabenz did not decrease the amount of triallate resistant seed sampled from fields. A significant increase in resistant seed took place, indicating that resistant types may be at least as fit or more fit than the susceptibles in the absence of triallate and presence of imazamethabenz.

The fact that resistant plants remained and may have increased in the system when they were not exposed to triallate suggests that the probability of selection for cross or multiple resistance to imazamethabenz may increase because imazamethabenz resistance may be selected from a population of wild oats that is maintaining its resistance to triallate.

The spatial distribution of fields containing triallate resistant wild oats was plotted on a map of the Fairfield Bench region. There was no obvious pattern to the regional resistance distribution. However, quantitative analysis of the distribution of resistance frequency data for each field indicated that patterns did exist. Anisotropic semivariogram analysis demonstrated no anisotropy. Anisotropy is measured along the major axis, at the angle of maximum variation (long axis of an ellipse), and the minor axis, perpendicular to the major axes. The range of influence along the major and minor axis were essentially identical for all the models indicating that autocorrelation has the same range of influence along all four axes (0°, 45°, 90°, 135°). Whatever patches that may exist will on average have a circular rather than an elliptic form.

Isotropic semivariogram analysis demonstrated that the spherical model best described the regional data with an r^2 of 0.637 and indicated that point pairs were autocorrelated up to a range of 14.7 grid units (1 unit = 0.1 km), at which point the model indicates a constant variance or asymptote. This may be interpreted as a 1.47 km zone of influence, where fields within 1.47 km of each other are likely to have similar proportions of resistance. An initial explanation may be that pattern of resistance up to 1.47 km may be the result of the clumping of land ownership or management approach. An alternative explanation could be that the slight patchiness of resistant fields may be attributed to the slow expansion of resistance from initial points of resistance selection.

In 1993, three fields were selected to measure the spatial distribution of triallate resistant wild oats within grids placed in each field. The frequency of triallate resistance in the first field grid (not treated with triallate in 1993) was 35%, whereas it was 64% in the triallate treated grid of the same field. In the third grid, within a field that had been determined to be 100% resistant in 1992 and was known to be treated with triallate in 1992 and 1993, 66% of the grid cells were resistant. The frequent occurrence of susceptible seed on plants that escaped triallate was a surprise. These escapes may be susceptible plants that escape due to heterogeneous herbicide distribution in the soil profile resulting from single pass incorporation (not recommended, but is common), or the escaped plants are resistant and are outcrossing at a much higher rate than expected. The fact that only one third of the population was resistant in the untreated grid may be evidence that resistant types are not as fit without the presence of the herbicide, or that the susceptible seed bank is just twice as large as that of the resistant types, because the susceptible plants were not exposed to triallate because of poor herbicide incorporation. If there is a fitness difference between resistant and susceptible types without the presence of a herbicide, the population may be shifted back to susceptible plants. Therefore, maintenance of susceptible wild oat populations could be exploited in a resistance recovery program in this region.

Spatial point pattern analysis was used to determine if there was a pattern in the resistant and susceptible wild oats at the field scale. The null hypothesis in this test was that resistance occurrence was completely spatially random (CSR). There was no evidence to reject the null hypothesis of spatial randomness for the resistant and susceptible cells within the field scale grid that did not receive triallate in 1993 and the susceptible grid cells on the two fields that were triallate treated in 1993. There was evidence, however, that aggregation of resistant wild oats may exist within triallate treated fields. The analysis further revealed that the aggregation of resistant plants occurred within patches that were 7 to 8 m wide and the distance between resistant patches was approximately 22 to 28 m.

Spatial point pattern analysis is based on the assumption that one determines all the occurrences of an event and their locations within a sampling area. In the systematic method of sampling used in this study, cells were established in a 5 m grid with the wild oat plant nearest the centre of each cell being sampled. The wild oat plants

were assumed to come from the centre of the cells and together represent the population of the sampling area. Isotropic semivariogram analysis of the three sites using the proportion of resistance on each plant sampled as the dependent variable resulted in no indication of spatial pattern in the resistant wild oats. Under the constraints of this experiment, it is difficult to interpret the semivariogram analysis. Spatial point pattern analysis may be more appropriate for detecting aggregation under the constraints of limited sampling and a categorical response variable.

The two patches that were examined using semivariogram analysis demonstrated that autocorrelation existed up to a distance of 1 m. This indicates that at a small scale there is a pattern associated with resistance in wild oats and that it does not occur at random. This was in agreement with our hypothesis that resistance developed from a point source and patches form under selection of triallate radiating from a central mother plant. The small size of influence (less than 1 m) can be explained in a number of ways. Firstly the level of dispersal will influence the size of resistance patches. Under high levels of dispersal such as occur under intensive cultivation the mixing of seed will be much greater leading to a decrease in the size of patches. Secondly if a fitness difference exist between the two biotypes in favour of the susceptible biotype then it will impose competitive pressure on the resistant biotype in the absence of triallate limiting the growth of patches.

If concluded that no spatial pattern exists for resistance, one may assume that resistance originated randomly and simultaneously throughout the fields sampled and has either not been present long enough to form patches or is not fit enough to form cohesive patches. Patchiness of resistance indicates that resistance occurred or was introduced at a lower frequency and has been present within the field for sufficient time to form cohesive patches in the presence of triallate. The conclusion that there is no pattern or aggregation to resistance in an untreated field may indicate that resistant wild oats are less fit than susceptible plants without the presence of triallate. Management strategies that remove triallate from the Fairfield agricultural system and replace it with a crop rotation and/or other non chemical wild oat management practices may be able to capitalise on fitness differences, encouraging the displacement of triallate resistant wild oats with susceptibles in the population so farmers can return to triallate within a few years.

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