Evaluation and Analysis of Crop Inspection Procedures to Maintain Genetic Purity in Pedigreed *Triticum aestivum* L. Seed

BY

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Abstract

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Ensuring genetic purity of *Triticum aestivum* L. is important to wheat breeders, farmers, processors, and the end consumers. The pedigreed seed production system is designed to meet quality assurance standards and to ensure the traits developed by breeders are maintained from seed to end use. With privatization of crop inspection services, there has been considerable interest in improving crop inspection procedures to increase efficiencies, increase accuracy of the inspection, and protect the seed grower and the inspector. The primary objectives of the research were to compare, differentiate, and analyze current Canadian Seed Growers' Association (CSGA) procedures with inspection procedures from a comparable U.S. region to Manitoba and the United Kingdom, and to develop and evaluate potential improvements in the crop inspection process. A database of pedigreed wheat crop inspection reports from Manitoba between 2009 and 2012 was developed to determine the range and mean of field sizes inspected at the different pedigree levels and to evaluate the frequency of offtypes detected using existing inspection procedures. There was nearly zero correlation between field size and heterogeneity, and less than one percent of 2112 inspections had more than thirty offtypes in 60,000 sampled plants. A field validation experiment was conducted in 2014 showed Canadian inspection procedures consistently inspected the largest area of the field. A theoretical analysis of offtype pollen contamination in pedigreed wheat fields showed potential problems in subsequent generations of pedigreed seed production depending on the inheritance, contamination levels, and area contaminated. This research provided valuable information for improvement of crop inspection procedures and ensured that purity standards can be met within the pedigreed seed certification process.

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List of Abbreviations

AAFC	Agriculture & Agri-Food Canada
CFIA	Canadian Food Inspection Agency
CSGA	Canadian Seed Growers' Association
FERA	Food & Environment Research Agency
GM	Genetically Modified
LSCI	Licensed Seed Crop Inspector
NDSSD	North Dakota State Seed Department
OECD	Organization for Economic Co-operation & Development
UPOV	International Union for the Protection of New Varieties of Plants
WBVWG	Wheat and Barley Variety Working Group

1 General Introduction

Ensuring genetic purity of *Triticum aestivum* L. cultivars is important in today's modern agriculture setting. When a wheat breeder produces a new cultivar with specific traits, farmers and processors desire that the traits remain pure throughout the time it takes to generate certified seed. Pedigreed seed benefits farmers who purchase certified seed by guaranteeing genetic standards for the traits they desire for their crop. Pedigreed seed certification also ensures that the seed lot meets a purity standard (minimal weeds), a germination standard, and has minimal disease (Agriculture and Rural Development, 2007).

Crop inspection benefits breeders, pedigreed seed producers, farmers, processors, and the end use consumer. By ensuring the traits are not masked by pollen contamination, or volunteer wheat containing different traits, all growers and users of pedigreed seed receive the beneficial traits of the cultivar and a guarantee that unwanted or detrimental traits are kept out of the pedigreed seed at an acceptable level. Brûlé-Babel et al. (2006) modelled the incidence of genetically engineered genes into non-genetically engineered crops via pollen transfer and volunteer genetically engineered plants in non-genetically engineered self-pollinating crops such as wheat. This paper shows how the frequency of traits change in wheat populations with selectively advantageous or detrimental traits.

As the Canadian Seed Growers' Association (CSGA) worked with the Canadian Food Inspection Agency (CFIA) to privatize crop inspection, there was considerable interest in improving crop inspection procedures to reduce costs, increase the accuracy of the inspection, and protect the tester or inspector and the pedigreed seed grower. The primary objective of this study was to compare, differentiate, and analyze current crop inspection practices of the CSGA with crop inspection procedures in a similar region in the USA and the United Kingdom. A similar initiative

was taken by the United Kingdom in 2008 and has been observed in the USA and Canada (United Kingdom, 2009).

The Organization for Economic Co-operation and Development Review (United Kingdom, 2009) raised concerns about interspecific contamination in pedigreed crops when contaminants were not evenly distributed across the field. Recommendations were made to include tolerance of heterogeneity levels to be put in place due to the uneven distribution of heterogeneity. When examining intraspecific contaminations, the OECD found that a smaller quadrat size of $10m^2$ (opposed to $20m^2$) would suffice unless plant density was below average. By estimating the purity of the seed crop before beginning an assessment of the crop, a Licensed Seed Crop Inspector (LSCI) may decide to use a smaller quadrat size to save time. This needs to be examined further as this could lead to crops which do not meet the CSGA requirements, but are passed due to the smaller sample size. After evaluating intraspecific impurities using a $10m^2$ quadrat, the tester could save time by using the $10m^2$ quadrat as part of the $20m^2$ quadrat to measure interspecific impurities. The OECD recommended an analysis of whether larger samples or more samples should be taken in a pedigreed seed field given a lower plant density (United Kingdom, 2009).

Although the OECD report indicated that there was no correlation between impurities in the field and the size of the field, careful consideration should be taken as the larger the field is, the higher the value of the pedigreed crop (United Kingdom, 2009).

Intraspecific contaminants could have two possible distributions: uneven or even. If the seed were contaminated, the intraspecific contamination would be more likely to show an even distribution in the field. If the intraspecific contaminants are a result of volunteers from a previous crop of the same species, it will most likely show an uneven, patchy distribution in the field.

The scientific standard Type I error (alpha value) is 0.05. Since the scientific standard may not be ideal for pedigreed seed crop testing, it may be worthwhile to determine whether the value should be changed or remain the same. By altering the alpha value, the confidence interval would also be altered.

The objectives of this study were to: 1) Compare crop inspection procedures in North Dakota, Manitoba, and The United Kingdom and develop recommendations to improve Canadian pedigreed crop inspection processes; 2) Determine whether field size and the different sampling methods affect the potential error in granting or denying a crop inspection certificate; 3) Determine how effective sequential sampling schemes are at reducing the time spent by an inspector in a seed plot; 4) Examine whether a sampling scheme could utilize $10m^2$ quadrats to detect intraspecific contaminants as opposed to the standard $20m^2$ quadrats used to detect interspecific contaminants and; 5) Develop acceptable confidence intervals for which a seed crop would be passed or failed based on the relative abundance of contaminants.

The benefits of a more efficient way to inspect pedigreed seed could lower the cost of pedigreed seed and encourage more farmers to use certified seed rather than their own "common seed".

2 Literature Review

In Canada, pedigreed seed is regulated at the national level by various federal agencies such as the Canadian Food Inspection Agency (CFIA) and the Canadian Seed Growers Association (CSGA) through their Circular 6 document (Canadian Seed Growers' Association [CSGA], 2017). In contrast, in the United States of America, each state has its own pedigreed seed standards. For example, the North Dakota government regulates pedigreed seed through the state seed department from the North Dakota Century Code (North Dakota State Seed Department [NDSSD], 2012a). The North Dakota State Seed Department dictates specific regulations for the production of pedigreed seed such as previous land requirements, field inspections, and standards such as noxious weeds, varietal contaminants, and germination. North Dakota has the following hierarchy of pedigreed wheat seed: Breeder followed by Foundation, Registered, and Certified in this order (NDSSD, 2012a).

Pedigreed seed regulations in Canada's Circular 6 document put forth by the CSGA under the CFIA is similar to North Dakota with some minor differences (CSGA, 2017). The Circular 6 defines the way in which Canadian pedigreed seed growers shall produce seed with specific land requirements for different classes of seed, field inspections, germination, and impurity standards such as weed seed tolerances. Pedigreed wheat seed in Canada has a hierarchy: Breeder followed by Select, Foundation, Registered, and Certified in this order (CSGA, 2017). The Select seed class is not listed in the North Dakota regulations. As a result, Canadian pedigreed seed regulations include one additional level of pedigreed seed for wheat compared to North Dakota.

In England and Wales (United Kingdom), FERA (The Food & Environment Research Agency) acts as the Certifying Authority for pedigreed seed as stated by The Plant Varieties and Seeds Act of 1964 and The England Seed Marketing Regulations of 2011 (Food & Environment Research Agency [FERA], 2013). The United Kingdom has a pedigreed wheat seed hierarchy of

Breeder seed producing Pre-basic (select) seed followed by Basic (foundation), Certified Generation 1 (C1), and Certified Generation 2 (C2) (FERA, 2013). The hierarchy of seed between North Dakota, Canada, and the United Kingdom are shown in Table 2.1. Although the levels of pedigreed seed are named differently in the United Kingdom, the number of levels of pedigreed wheat seed is the same as in Canada.

In North Dakota, a pedigreed wheat field will not be granted certification status if another wheat field of a different variety was grown in the previous year. Furthermore, if the pedigreed wheat field of a given variety was not inspected in the previous year, the same variety grown this year would not be given certification (NDSSD, 2012b).

Likewise in Canada, pedigreed seed crops should be planted on areas where previous crops will not contaminate the pedigreed seed crop. The Circular 6 regulations recommend avoiding planting on land previously producing: uninspected crops of the same variety, other varieties of the same species, and crops whose seeds are of similar size to the pedigreed seed crop grown. Land which has grown a different variety of wheat or non-pedigreed wheat in the past two years is not suitable for registered or certified wheat production and wheat cannot have been grown for three years prior to foundation wheat production (CSGA, 2017).

In the United Kingdom, two classes are described for previous crops on a pedigreed wheat seed field: good practise and minimum compatibility. Good practice for previous land use dictates no other variety of wheat or other cereal in the previous two years may have been grown on a pedigreed wheat seed field. Minimum compatibility states no other variety of wheat can be grown in the previous year (FERA, 2013).

Table 2.1. Names of the first to fifth generation of pedigreed wheat seed for Canada, North Dakota, and the United Kingdom (Van Gastel et al., 2002).

Definition of Generation	Canada	North Dakota	United Kingdom
1st Generation	Breeder	Breeder	Breeder
2nd Generation	Select	Foundation	Pre-Basic
3rd Generation	Foundation	Registered	Basic
4th Generation	Registered	Certified	Certified 1
5th Generation	Certified	----------	Certified 2

2.1 Canadian Pedigreed Wheat Inspection Procedures

The CSGA Circular 6 document dictates that wheat and other cereals must be past the Zadoks 59 stage to be inspected (CSGA, 2017). The isolation distance in Canada differs depending on the reproductive biology of neighbouring plant species and cultivars. If two pedigreed wheat fields are both of the same variety, only 1m isolation needs to be between them. If another cereal, different wheat variety, or non-pedigreed wheat is beside a pedigreed wheat field, a 3m isolation distance is required between the two fields for Foundation, Registered, and Certified seed. Select and Breeder seed require a 10m isolation. While North Dakota regulations only proclaim impurity acceptance levels for "inseparable other crops", Canadian regulations break down the crops of similar size and give different acceptance levels for different similar sized seeds. Since wheat is a mainly self-pollinating crop, the isolation distance is mostly for mechanical separation. The document also indicates maximum impurity levels for Foundation, Registered, and Certified pedigreed wheat in Table 2.2 (CSGA, 2017). The level of purity among Foundation (9,999:10,000) and Certified (1,999:2,000) seed is 99.99% and 99.95% respectively.

Table 2.2. The maximum impurity acceptance levels for Foundation, Registered, and Certified *Triticum aestivum* L. during infield inspection conducted at the heading stage (Impurity: Pure Pedigreed Seed) (CSGA, 2017).

Impurity	Foundation	Registered	Certified
Different Cultivars & Offtypes	1:10,000	1:10,000	1:2,000
Barley	1:5,000	1:5,000	1:2,500
Buckwheat & Rye	1:10,000	1:10,000	3:10,000
Durum & Triticale	1:10,000	1:10,000	1:2,000
Oats	1:2,500	1:2,500	1:1,250
Prohibited Noxious Weeds	0	0	0

Canadian wheat field inspection takes place after the wheat has headed (Z59) (Zadoks et al., 1974). Before the inspector enters a pedigreed seed field, they must first confirm with the seed grower and need to check to see if the acreage is correct (Canadian Food Inspection Agency [CFIA], 2014). Inspectors must also look at stubble to both ensure they are in the correct field and guarantee the crop meets the previous land requirements. During the inspection, the inspector must walk to each side of the field to get the North, South, East, and West isolation distances. They must also note whether the crop is next to another cereal, other crop, ditch, or a tree line. Inspectors must also note the type of isolation used to separate crops (mowed, cultivated), the width of the isolation (0m, 1m, or 3m), and the condition of the isolation (good, fair, or poor). While walking the field, inspectors give a rough estimate of the yield (good, average, or poor), the stand uniformity, and the general appearance. Inspectors report objectionable weeds by frequency, but offtypes and other cereals are reported by counts. The inspector is vigilant throughout the entire inspection for weeds, offtypes, and other crops and will record these in their field notes. The inspector will make six counts of 10,000 plants for each inspected field. These counts are randomly spaced throughout the field and the inspector records any offtypes or other varieties in counts and other cereals in counts. The counts are arranged diagonally across rows in order to get a more representative and unbiased sample. Canadian inspectors are not required to

report any difficult to separate weeds in a pedigreed wheat seed crop in counts, only objectionable weeds by frequency.

After the pedigreed seed is cleaned in Canada, impurity standards must be met. Canada's grade tables show a Number 1 and Number 2 based on germination percentage. Number 1 pedigreed seed must meet an 85% germination standard while Number 2 must meet a 75% germination standard. Comparing Table 2.3 with Table 2.5 shows that North Dakota tends to allow more impurities in their pedigreed seed. Both Canada and North Dakota do not allow prohibited noxious weeds in either of their respective cleaned samples for any level of pedigreed seed.

Table 2.3. The impurity acceptance level of Foundation, Registered, and Certified *Triticum aestium* L. cultivars in harvested and cleaned seed lots of pedigreed seed in Canada (CSGA, 2017).

Impurity	Foundation (per kilogram) & 85% Germination	Registered (per kilogram) & 85% Germination	Certified (per kilogram) & 85% Germination
Total Weed Seeds	2	3	3
Other varieties	0	0	5
Other crop seeds	0	1	2
Prohibited Noxious Weeds	0	0	0

2.1.1 Canadian Pedigreed Seed Inspection Patterns

Crop inspection patterns in Canada vary, but patterns must comply with the Canadian pedigreed crop inspection protocols. Figure 2.1 shows six different crop inspection patterns. The diamond, zig zag, and circular methods are commonly used in Canadian pedigreed crop inspections. Different crop inspection patterns can be used depending on the opinion of the inspector and the field shape.

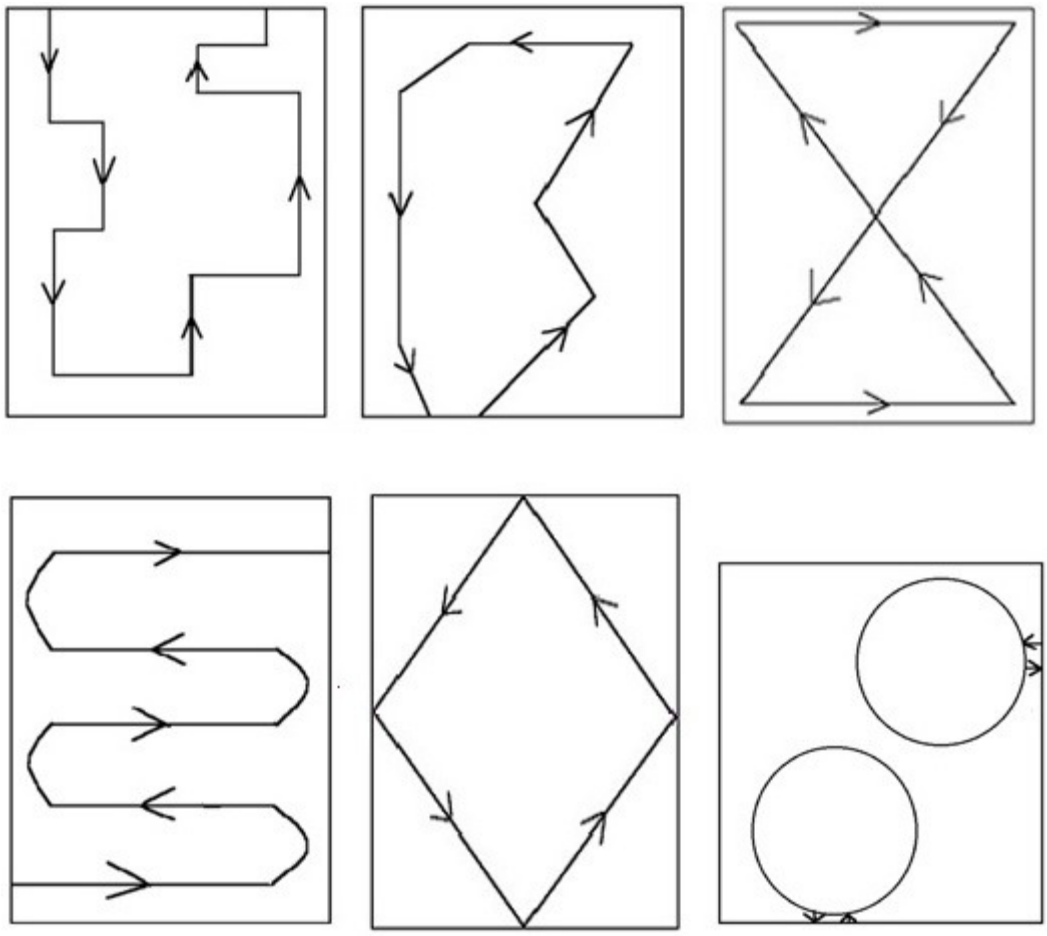

Figure 2.1. Crop inspection patterns used to inspect rectangular and square fields (CFIA 2014).

2.2 North Dakota Pedigreed Wheat Inspection Procedures

Pedigreed seed crop inspection in North Dakota takes place when the wheat has completed the heading stage (Z59) in accordance to Zadoks et al., (1974). Zadoks et al. (1974) identified several distinguishing characteristics common to cereals including wheat from seedling to maturity. This is important for testers or inspectors as it assigns certain characteristics at various levels of the wheat's development stage (tillering, heading, or maturity) (Zadoks et al., 1974). Since a tester may examine pedigreed seed fields more than once in a season a few weeks of growth can occur between inspection times. This means the inspector may have to use different identifying characteristics to see if there are varietal contaminations in the lots.

In North Dakota, the pedigreed seed grower must isolate their crop 1.52m away from other crops of the same species for Foundation, Registered, and Certified seed. The seed sector of North Dakota sets specific standards for various impurities in the different levels of pedigreed seed in Table 2.4 (NDSSD, 2012a).

Table 2.4. The North Dakota impurity acceptance level of Foundation, Registered, and Certified *Triticum aestivum* L. cultivars for field inspection during the heading stage (Spike Impurities: Pure Pedigreed Seed Spikes) (NDSSD, 2012b).

Impurity	Foundation	Registered	Certified
Different Cultivars	1:10,000	1:5,000	1:2,000
Inseparable Other Species	1:30,000	1:10,000	1:5,000
Prohibited Noxious Weeds[1]	0	0	0

[1]North Dakota inspectors can judge the prohibited or objectionable weeds acceptance level.

Wheat inspections in North Dakota occur after the spike is fully extended and has reached its final height (important in distinguishing tall offtypes) after Zadoks 59. Prior to entering the pedigreed seed field, the inspector will contact the grower to determine whether the field had been sprayed recently, check to ensure isolation distance meets the requirement (1.52m), the stage of the crop, and to see if the grower would like to accompany the inspector. The inspector should make note of the distinguishing traits and the variants on their North Dakota State Seed Department (NDSSD, 2012a) field notes. As the inspector begins the inspection, they are instructed to best determine the location in the field when the seeder was first put in the ground and seeding began. The beginning point is a high priority area to check because seeding implements may still have remnants of a previous crop or variety which would be cleaned out at the beginning of a new field. North Dakota inspectors are required to look for bin sites, rock piles, waterways, and along roads or railways for volunteer grain (blown off from rail car, truck, or grain trailer) or problematic weeds. The inspector then verifies the varietal identity with the variety description. The area to be inspected for counts is determined by the following estimation process. When the inspector is ready to start making counts, the inspector will take one step in an average, representative area of

the field, and count the number of spikes (or tillers) within that step in a single row. The inspector will repeat this process twice more and obtain the average number of spikes in three different steps. The inspector will multiply the average number of spikes per step by four rows to get the average number of spikes in four rows by one step. The inspector will divide the target number of spikes (10,000) by the average number of spikes in one step to determine the number of steps which need to be taken to determine the impurities in a count of 10,000 spikes. The inspector will go to representative areas of the field and pick four rows and walk the required number of steps to evaluate the 10,000 spikes. If the inspector finds offtypes or variants, the inspector will pull the entire plant with all tillers and count the number of spikes that each offtype or variant has. The inspector will make ten counts of ten thousand spikes totalling 100,000 spikes per field (NDSSD, 2012a). Figure 2.2 shows how different wheat plants would give different levels of spike offtypes based on North Dakota's seed inspection practices.

Figure 2.2. A picture showing five different wheat plants from the same cultivar ranging from one to five tillers. Photo taken in Crystal, ND by Randy Preater of CSGA.

After the harvesting and cleaning of the pedigreed seed lot, sampling is required to determine the purity of the seed, as well as the quantities of impurities, germination, and inert matter (foreign material other than broken seeds). At least 85% of the seeds must germinate, and no prohibited noxious weeds are allowed in the sample. The sample must contain a minimum of 99% of the variety being certified and no more than 1% inert matter is allowed in the seed lot.

Table 2.5 shows the relative amounts of impurities allowed in North Dakota pedigreed seed (NDSSD, 2012a). The original North Dakota document used the impurity measurement of number of seeds per two pounds, so a conversion to kilograms was needed to compare to Canada and the United Kingdom. Basing allowable impurities relative to weight means that a higher proportion of impurities may be allowed in large seeded varieties than for small seeded varieties.

Table 2.5. The North Dakota impurity acceptance level of Foundation, Registered, and Certified *Triticum aestivum* L. cultivars in a harvested and cleaned seed lot. North Dakota uses the imperial system so both the metric conversion (kilograms) and the original imperial measurement (pounds) are shown (North Dakota, 2012b).

Impurity	Foundation 85% Germination	Registered 85% Germination	Certified 85% Germination
Total Weed Seeds	4.4	11	22
Other varieties	1.1	4.4	6.6
Other crop seeds	1.1	4.4	6.6
Prohibited Noxious Weeds	0	0	0
Objectionable weed seeds	1.1	1.1	2.2

2.3 United Kingdom Pedigreed Wheat Inspection Procedures

A pedigreed wheat seed inspector from the United Kingdom will inspect a pedigreed seed wheat field after the spike has emerged, preferably one week after emergence. Prior to the inspection, the inspector must check the CERT 3 Crop Inspection Report to check area, previous land use, location, variety, and pedigreed seed level. A 2m isolation distance is recommended to prevent contamination during harvest. This must be checked during an inspection. The inspector will examine evenly dispersed quadrats throughout the field while running across the drill width (perpendicular or diagonally across rows). Depending on the field size, different numbers of quadrats may be used. For a pedigreed wheat field less than or equal to three hectares (7.41 acres) in area, a minimum of five quadrats are required. Pedigreed wheat fields greater than three hectares in area require a minimum of ten quadrats to be counted for varietal and species contaminants. A

quadrat in the United Kingdom is defined as 1m by 20m running perpendicular to the seed rows. Spikes are selected randomly and examined with great detail to confirm the variety identity. In addition to checking for variety identification, inspectors are also looking for the disease Loose Smut. Inspectors then proceed to fill out the CERT 3C Crop Inspection Report which is the standard form for cereal crop inspection. The form contains boxes where varietal contaminants, species contaminants, isolation distance, neighbouring crops, wild oat counts, and lodging percentage are reported.

The United Kingdom recommends a minimum of one inspection at least a week after the spike emerges. The isolation distance must be measured and detail on the surroundings must be reported. If the pedigreed wheat is next to a different variety of wheat or crop which could contaminate the seed field, the grower must have a minimum isolation distance of 2m.

Table 2.6. Purity standards for wheat where the crop is verified as Higher Voluntary Standards[1] (HVS) in the United Kingdom (Animal and Plant Health Agency, 2016).

Impurity	Maximum Tolerance[2]		
	Basic	C1 Seed	C2 Seed
Other varieties	1:2,000	1:1,000	3:1000
Other species	1:10,000	1:10,000	1:10,000
Prohibited noxious weeds[3]	n/a	n/a	n/a

[1] Regulations identify Minimum standards and Higher Voluntary Standards (HVS). To provide the most similar comparison the HVS are reported here.
[2] The stated regulation indicates purity standards of 99.95%, 99.9% and 99.7% for Basic, C1 Seed and C2 Seed, respectively. For consistency of comparison, these numbers have been converted to number of impurities per total number of plants.
[3] Weed tolerances are specified by weed species.

2.4 Wheat Ecology and Reproduction

Offtypes are a main concern for the Canadian pedigreed seed industry. Great effort is put in by seed growers to limit the amount of offtypes in their fields. Sometimes contamination can occur through the growth of volunteers from previous seed crops, unsanitary equipment, improper pedigreed seed production techniques, or through pollen mediated gene flow. If contamination

occurs through seed spread in the previous fall, several factors can influence the survivability of the seed and whether or not the seed will germinate to produce a healthy plant (Willenborg and Van Acker, 2008).

Viable wheat seeds from shattering, harvest losses, wild life or livestock activities such as grazing, or scattering from harrowing may not germinate the following year which poses a risk to pedigreed seed fields in the future (Willenborg and Van Acker, 2008). Previous land use regulations in seed standards from Canada, North Dakota, and the United Kingdom prohibit pedigreed wheat from being grown on the same land on which wheat was grown in the previous year. Studies on the survivability of wheat seeds have shown that wheat seeds can germinate up to five years after going dormant (Beckie, 2001). Willenborg and Van Acker (2008) outlined several factors leading to higher dormancy rates of wheat seeds including: different cultivars (Komatsuzaki and Endo, 2008), environmental conditions during maturation (Pickett, 1993), and spring environmental conditions (Nyachiro et al., 2008). However, very few wheat kernels overwinter and still retain the ability to germinate in the following year due to predation, disease presence, or harsh environmental conditions (Anderson and Soper, 2003; Willenborg and Van Acker, 2008).

The difference among wheat cultivars reproduction varies considerably with regards to amount of pollen produced, the amount of pollen shed, the outcrossing rate, and the length of time the stamen remains receptive to pollen (Willenborg and Van Acker, 2008). These factors can play an important role in determining if foreign pollen contaminates a pedigreed wheat field, although there are other important influences to consider. In order for cross-pollination to occur between two different cultivars, the pollen from one cultivar must be viable to fertilize an ovum of another cultivar. The two cultivars must flower nearly simultaneously, coupled with appropriate

environmental conditions, and without genetic incompatibility between the pollen and ovum (Willenborg and Van Acker, 2008).

2.5 OECD Review of Crop Inspection Procedures

Ideally, sampling schemes which can detect both inter- and intraspecific contaminants would be preferable as this method would save time and money for the inspector and the CSGA. One of the concerns raised in this report is that intraspecific contamination of pedigreed crops can be unevenly distributed across the field. This can threaten the pedigreed status of a seed grower's field which would normally meet the purity requirements for the crop. The Talbot sampling method uses a sequential based procedure and is based on crop size (David et al., 1996). The Talbot method allows the tester to save time by reducing the number of counts required, provided the impurities are below a certain threshold. This method is more complex and places more pressure on the tester. Sequential sampling methods may differ for inter- and intraspecific impurities which could make it harder for the CSGA to keep track of the inspection reports (United Kingdom, 2009).

Rouse (2009), a statistician, reviewed the revised guidelines for control plot tests and field inspection brought forth by the OECD, and defined some variables:

- α= P(rejecting a lot when it is acceptable) → Type I error (grower risk)
- β= P(accepting a seed lot when it should be rejected) → Type II error (tester risk)
- Power = 1-β

Rouse (2009) proposed to revisit the alpha value of 0.05 and proposed a higher alpha value of 0.2-0.4 after reviewing Carmer (1976) which examined the relative cost of Type I and II errors. The statistician also went on to evaluate the post control plot, a sample of the seed lot to determine

purity. Within the post control, the goal for the tester is to evaluate purity and uniformity of the seed plot. Rouse (2009) suggested either taking larger samples or more samples. The later of the two suggestions is desirable as the samples are more likely to provide a better representation of the plot. Another suggestion from Rouse (2009) for evaluating the purity standard of the pedigreed seed plot is to use fewer samples. This would save time as fewer samples are needed to evaluate whether a seed lot is pure or impure.

2.6 Sampling for Low Probability Events

The ability to detect rare events is important in pathology (Brown and Hovmøller, 2002), entomology (Roitberg and Prokopy, 1987), veterinary medicine (Doherr and Audige, 2001), meteorology (Frei and Schär, 2001), ecology (Higgins et al., 2003), and genetics (Stewart et al., 2003), among various other fields. The problem with sampling rare biological events is that different sampling methods are often needed based on the type of distribution of a particular organism (Venette et al., 2002). Choosing a sampling strategy is important in situations involving low probability events. A flawed sampling strategy can lead to misleading or biased results. Nested sampling, cluster sampling, stratified random sampling, and simple random sampling are examples of probability-based sampling strategies (Venette et al., 2002). Another type of rare event sampling is targeted sampling which is a non-probability based sampling method where certain criteria are used to select a sample of a particular group. Targeted sampling has been used in hidden-population Acquired Immune Deficiency Syndrome (AIDS) research (Magnani et al., 2005), soil sampling (Taylor et al., 2003), and pathogen sampling (Ward, 1994). After choosing a sampling scheme it is important to manage the Type I and Type II errors. Table 2.7 shows the potential outcomes sampling can yield.

Table 2.7. A table representing four possible outcomes from sampling rare events modified from Venette et al., 2002, where A is the number of offtypes correctly identified as offtypes, B is the number of incorrectly identified offtypes, C is the number of offtypes which missed detection, and D is the number of plants correctly identified as not offtypes.

	True Results	
Sampling Results	Contamination	No Contamination
Contamination	Correct (A)	Incorrect (B)
No Contamination	Incorrect (C)	Correct (D)

Sensitivity (*Se*) is the conditional probability that an offtype will be classified as detected and is defined as *Se* = A/(A+C) (Venette et al., 2002). Specificity (*Sp*) is the conditional probability that an offtype will not be classified as detected and is defined as *Sp* = D/(D+B) (Venette et al., 2002). Accuracy (*Acc*) is the ability of an inspection to produce correct results and is defined as *Acc* = (A+D)/(A+B+C+D). Precision (*PPV*) is the ability of the inspector to produce consistent results and is defined as A/(A+B) (Greiner and Gardner, 2000). If sensitivity is less than one, there will be undetected offtypes not being counted, and if specificity is less than one, then false offtypes will be detected and make the offtype count higher than it should be (Venette et al., 2002). If the offtype is rare, a smaller specificity is usually more detrimental than a smaller sensitivity since a few plants mistakenly determined to be offtypes will make the calculated offtype frequency incorrectly higher than the actual offtype frequency (Venette et al., 2002).

Different sensitivity and specificity hierarchies exist and Venette et al. (2002) uses higher-level sensitivity (*HSe*) and higher-level specificity (*HSp*) when dealing with large numbers of organisms such as when inspecting the very large number of plants in a pedigreed seed field. Higher-level sensitivity is the probability that an inspection will accurately determine if a pedigreed seed field is pure enough to meet pedigreed seed standards. Higher-level specificity is the probability that an inspection will accurately determine that a pedigreed seed field does not meet pedigreed seed standards and will be justly rejected. These two measures of accuracy are

dependent on the number of offtypes in a sample size of n plants exceeding a cut-off number for sample units, T such that $1 \leq T \leq n$ (Venette et al., 2002). In cattle disease testing, the inspector can sample fewer cattle if the test has a sensitivity close to one (Donald et al., 1994). If specificity is less than one, sampling too many cattle can lead to false positives (Donald et al., 1994). For example, in one Canadian sample of 10,000 plants, an inspector might conclude that there were ten tall offtypes and 9,990 plants that were not offtypes. However, the inspector may have wrongly called one of the plants an offtype and missed two offtypes which were not included in the inspection findings. In this case, A = (10-1) = 9; B = 1; C = 2; D = (9,990-2) = 9,988.

Example Calculations:

Sensitivity = Power = 9/(9+2) = 9/11 and specificity = 9,988/(9,988+1) = 9,988/9,989

False positive rate (α = Type I error) = 1 – specificity = 1/9,989 ≈ 0.0001

False negative rate (β = Type II error) = 1 – sensitivity = 2/11 ≈ 0.182

Accuracy = (9 + 9,988) + (9+1+2+9,988) = 9,997/10,000

Precision = 9/(9+1) = 9/10

2.7 Offtype Distribution in Different Sample sizes and Contamination Rates

Offtype distributions are useful for predicting the number of offtypes found in different sample sizes and contamination rates. Figure 2.3 shows the probability (P) of detecting one offtype at varying sample sizes (n) and contamination rates (f) using the formula $P = 1-(1-f)^n$.

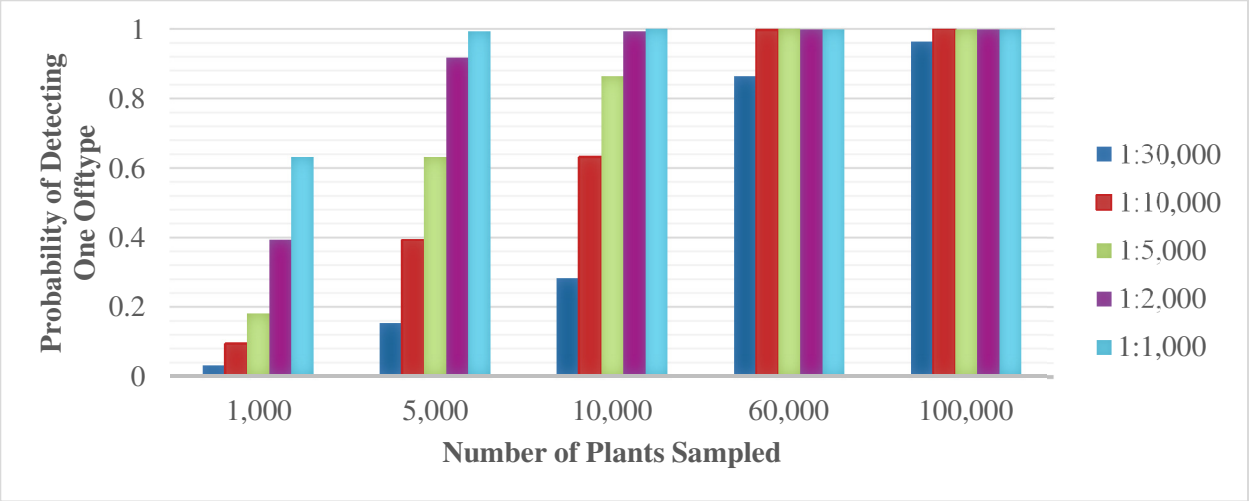

Figure 2.3. A probability chart developed from Brûlé-Babel et al., 2006 showing the probability of detecting one offtype when various plant counts are sampled in different levels of contamination ranging from one offtype in 30,000 plants to as high as one offtype in 1,000 plants.

Being able to predict one offtype in differing contamination levels and sample sizes are useful, but inspectors would also like to be able to predict the number of offtypes in a set sample size with varying levels of contamination. Binomial distributions, Poisson distributions, and hypergeometric distributions are examples of discrete distributions which have the potential to determine offtype distributions which could help inspectors determine possible contamination rates based on how many offtypes they find in an inspection. Each of these distributions has specific conditions which need to be met in order to accurately predict the level of offtypes in different contamination rates. Figures 2.4, 2.5, and 2.6 show the predicted number of offtypes in a sample size of 60,000 in contamination rates ranging from 1:30,000 to 1:1,000 in a binomial distribution, Poisson distribution, and hypergeometric distribution, respectively.

Conditions for Binomial Distribution (Gagnon, 2014)

1. Test consists of a sequence of *n* trials, where *n* is fixed prior to experiment
2. Test has two possible outcomes per trial (success *p* and failure)
3. Trials are independent with *k* successes = 0, 1, 2,...,n
4. Probability of a success *p* or failure *(1-p)* is constant among trials

$$P(X=k) = \frac{n!p^k\,(1-p)^{n-k}}{(n-k)!k!}$$

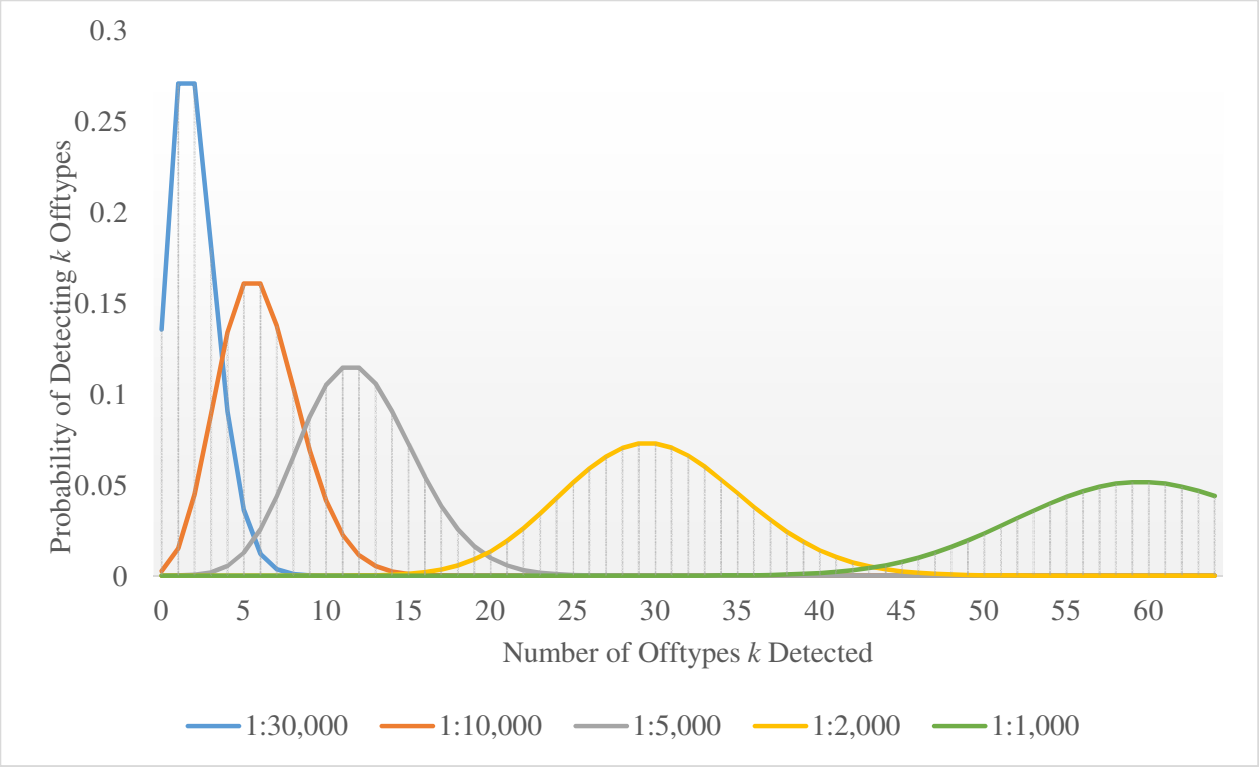

Figure 2.4. A probability chart showing the probability of detecting different numbers of offtypes using the binomial distribution if 60,000 plants were sampled in fields with offtype contamination ranging from 1:30,000 offtypes to 1:1,000 offtypes.

Conditions for Poisson Distribution (Gagnon, 2015)

1. The variable represents the number of occurrences of some event over a certain area
2. The probability of the occurrence of the event in a certain area is constant
3. Number of events that occur in a specific space are independent of other areas

$P(X=k) = \frac{\lambda^k e^{-\lambda}}{k!}$ for k successes = 1, 2, 3, ...

λ = average number of offtypes in a sample size

k = number of offtypes

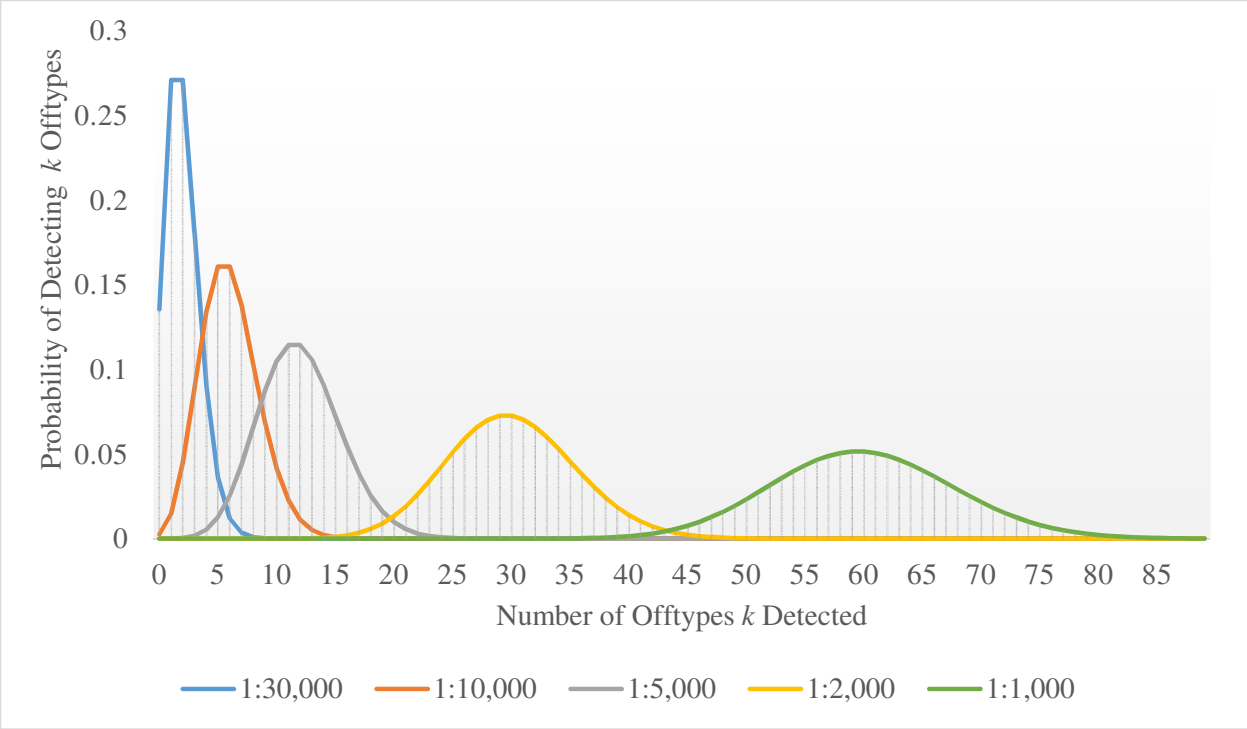

Figure 2.5. A probability chart showing the probability of detecting different numbers of offtypes using the Poisson Distribution if 60,000 plants were sampled in fields with offtype contamination ranging from 1:30,000 offtypes to 1:1,000 offtypes.

Conditions for Hypergeometric Distribution

1. Only two outcomes are possible
2. The sample must be random
3. Selections are not replaced

$$P(X=i) = \left(\frac{\binom{X}{i}\binom{N-X}{n-i}}{\binom{N}{n}} \right)$$

i = number of offtypes
N = population size
X = number of offtypes in finite population
n = number of plants sampled

The recommended seeding rate of Canada Western Red Spring wheat is 94-135kg/ha (84-120 lbs/acre) to produce between 247-301 plants/m^2 (23-28 plants/ft^2) (Manitoba Agriculture, 2016). Using this target plant density, there should be between 2.47 x 10^6 and 2.96 x 10^6 plants/hectare (1.0 x 10^6-1.2 x 10^6 plants/acre). As the number of acres increases, the number of wheat plants (N) increases, and the hypergeometric distribution changes as well. Donald et al.

(1994) claims the hypergeometric distribution is close to the binomial distribution when N is greater than 30.

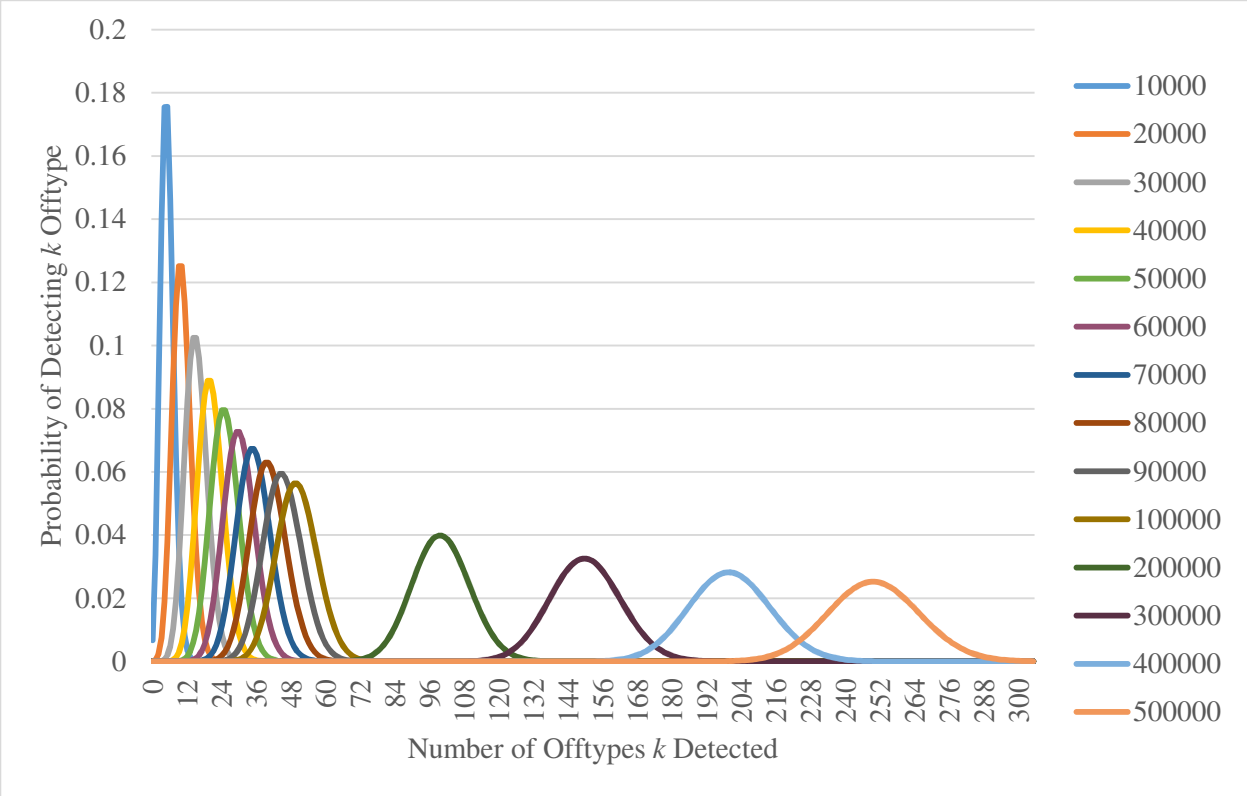

Figure 2.6: A hypergeometric probability distribution chart showing the effect of different sample sizes taken in a large field with 250,000 offtypes in 500,000,000 total plants or a 1:2,000 contamination level.

As sample size increases, the standard deviation about the mean of the hypergeometric distribution increases as well and becomes more like the Gaussian normal distribution as seen in Figure 2.6. This is similar to Figure 2.4 and Figure 2.5 when comparing the probability functions of the number of offtypes predicted when comparing the contamination rate. As contamination rate increases, so does the standard deviation of the probability distribution and becomes more normalized.

2.7.1 Sampling Methods for Large Areas Too Excessive to Inventory

Inventorying (measuring entire field) is the most accurate way to find out what level of contamination a field has. The problem with taking an inventory of an entire pedigreed seed field is that it takes much more time and money to sample the whole field than it does to sample a smaller portion of the field, especially as field size increases. When national, provincial, or state parks compile a list of species, destroy foreign plant species, or examine distribution of species, the type of sampling method can change depending on the task at hand (Rew et al., 2006). If the objective of the survey is to destroy foreign plants, survey efforts should be focused on areas known to have more foreign plants, but if the objective is to simply determine frequency or distribution, a non-biased approach should be used (Rew et al., 2006). With previous knowledge of the frequency or distribution, a stratified sampling strategy could be implemented (Rew et al., 2006). Previous literature describes higher frequencies of foreign plants near roads, railways, and trails. These areas are often disturbed, and foreign plants are more likely to grow in these areas (Rew et al., 2006). This could be why the North Dakota State Seed Department requires their inspectors to examine roadways, railways, rock piles, and other disturbed sites where offtypes are more likely to be located (NDSSD, 2012a).

Rew et al (2006) evaluated different survey methods for reliability, accuracy, and survey effort to determine which method gives a representative sample of foreign plants from the area being surveyed using weighted distributions of non-indigenous plant species to mimic patches. Grid sampling takes place at the grid intersections, while the random sampling method used completely random walking, and random sampling points. The transect methods always started on a man-made right-of-way such as a road or railway. The transects ran perpendicular to the right-of-way and random sampling points were taken (Rew et al., 2006). Grid-style sampling and

random sampling detected more foreign plant patches than the transect sampling methods in random and weighted foreign plant distributions (Rew et al., 2006).

Although grid and random sampling detected more foreign plants, they are not preferable to transect sampling since transect sampling is more practical and wastes less time and effort. Previous work shows the most important factor when designing the survey to be the number of samples (Hirzel and Guisan, 2002). If two different populations of the same size with the same number of foreign plants, but with different foreign plant patch sizes were sampled, there is a higher probability of detecting the smaller patches than larger patches because there are fewer larger patches than smaller patches (Rew et al., 2006).

2.7.2 Sampling for Qualitative Offtypes

Qualitative offtypes can be easier to detect than quantitative offtypes. An offtype is considered a qualitative offtype if the trait or traits which are different from the traits of the cultivar of the pedigreed seed crop are different by discrete classes (Brûlé-Babel, 2015). An example of qualitative offtypes in wheat are an awned offtype in an awnless pedigreed seed crop. Guidelines from the International Union for the Protection of New Varieties of Plants (UPOV) directs inspectors to use visual assessment to determine whether a plant is an offtype or not (Anonymous, 2008). Visual assessment is often used because it is both quicker and cheaper than measuring, which is often the case with quantitative offtypes (Anonymous, 2008). Although molecular fingerprints could be used to detect offtypes, this is not practical in the field.

2.7.3 Sampling for Quantitative Offtypes

Quantitative offtypes are often more difficult to detect than qualitative offtypes. A quantitative offtype is an offtype with traits which vary by degree and have a continuous phenotypic distribution (Brûlé-Babel, 2015). Quantitative offtypes are often the product of an

environmental interaction or a trait controlled by many genes (Brûlé-Babel, 2015). An example of a difficult to detect offtype is a taller or shorter wheat plant in a pedigreed wheat field. Plant height is a trait which is influenced by the environment and plant density which makes determining whether a plant is an offtype or not very difficult. This is also a common type of offtype detected in wheat, along with many other species. The main stem of a cereal plant is usually taller than its tillers which can make a field of cereal plants appear to be uneven in height. This can even be seen in doubled haploid populations of cereals where the traits at all loci are identical (Brûlé-Babel, Personal Communication). Since visual assessment by different inspectors are often subjective, physically measuring data is often needed to determine if a plant is an offtype (Anonymous, 2008). In Canada, plant breeders determine the limit between what can be considered the norm and what is outside the norm for a cultivar. For instance, in AAC Brandon, plants greater than 15cm above the norm are considered offtypes where plants between the norm and 15cm are considered variants.

2.7.4 Factors Influencing Offtype Detection

The ability to find offtypes in a field can be influenced in many ways. Environmental and inspector factors can influence whether an offtype is counted or missed (Buckland et al., 1993). Offtypes can be difficult to find depending on its phenotype (Bibby and Buckland, 1987). Certain offtypes and variants are more easily detectable than others. Awned offtypes in awnless wheat are easily detectable, while it can be difficult to differentiate shades of green of different offtypes. Weather conditions (rain, clouds, or wind), sun position, and time of day are also important considerations when conducting an inspection or survey (Buckland et al., 1993). In addition, the factors associated with the inspector can influence the detection of offtypes. The level of experience, vision, height, and alertness can affect the accuracy of the inspection (Barnett et al., 2007). The topography of the field can also influence the detectability of offtypes during an

inspection (Moore et al., 2011). It is also more difficult to find offtypes as distance from the observer increases (Buckland et al., 1993). The consequences of failing to detect offtypes can lead to pedigreed seed fields being approved when contamination is above acceptable levels.

The detection of rare or elusive organisms have been documented in different animal surveying studies such as monitoring invasive insect pests (Venette et al., 2002), surveying fresh water fish (McManamay et al., 2014), habitat conservation for endangered amphibians (Canessa et al., 2012), and bird migration studies (Sanderlin et al., 2014). Since animals are not static like plants, most survey methods to detect animals rely on recurrent visits to sample sites (Moore et al., 2011). While animal scientists can measure efficiency of sampling based on the number of recurrent visits, plant detection is dependent on total sampling time in one visit (Moore et al., 2011). Since plants and offtypes are static, the goal of the inspection should be to maximize detectability in as little time as possible in one visit (Moore et al., 2011). One issue plaguing pedigreed seed inspections is that different traits are detectable at different stages in the crops lifecycle. An example of this is that most pedigreed wheat inspections take place after Zadoks 59, but the ability to determine chaff colour is only possible after ripening (Zadoks 90) (Brûlé-Babel, Personal Communication).

In areas with rare, and unevenly distributed offtypes, detectability can be difficult. Factors influencing detectability of patches of unevenly distributed offtypes include the size and shape of the patch (Chen et al., 2009) and the patch phenotype (Garrard et al., 2008).

Sometimes, a plant can appear to be an offtype when it is not and it is important the inspector does not tally these plants as offtypes. Environmental factors, disease presence, and different cultural practises can result in atypical plants. In a field with varying topography, false offtypes can develop from different light levels, temperatures, and water levels can produce

atypical plants with different colours from varying anthocyanin concentrations (Anonymous, 2008). Diseased plants can also appear to be offtypes and careful examination should be taken to determine whether a plant is diseased or if it is an offtype. Damage from insects, hail, sun, wind, excessive moisture, herbicide damage, or frost damage can produce plants which may appear to be offtypes (Anonymous, 2008). In other plant species such as fruit-bearing trees, lack of pollination may produce phenotypes which are different from the norm (Anonymous, 2008).

2.8 Statistical Methods Used to Analyze Low Probability Events

Determining the confidence level of an inspection procedure is important for comparing different jurisdictions pedigreed seed inspection methods. Inspectors trying to stop incoming foreign organisms have used the formula $f_{max} = 1 - [1\text{-}P(x > 0)]^{1/n}$ to find the maximum frequency (f_{max}) of the foreign organism to find one or more foreign organisms in a sample of n individuals (Venette et al., 2002). Rearranging the formula above to determine the sample size n, with a certain degree of confidence, the formula then becomes $n = \frac{\ln[1-P(x>0)]}{\ln(1-f_{max})}$ (Venette et al., 2002). This method assumes that samples are independent of one another, but may not be useful if the distribution of offtypes is uneven (Venette et al., 2002).

2.9 Risk Analysis Methodology

If offtypes are evenly distributed, a Poisson or binomial distribution can be used to predict the amount of offtypes found in a sample of plants (Wiles et al., 1992). When offtypes are unevenly distributed into patches, a negative binomial distribution may be more appropriate as negative binomial distributions are often used to reflect weed spatial distributions (Wiles et al., 1992). The probability function for a negative binomial distribution is $P(X=k) = \binom{k-1}{r-1}(1-p)^{k-r}p^r$ with a mean of $\mu = \frac{r}{p}$ and a variance of $\sigma^2 = \frac{r(1-p)}{p^2}$, where r is the number of offtypes detected, and p is the probability of finding r offtypes in k plants sampled.

2.10 Survey Effort

The goal of an efficient crop inspection is to provide accurate results, while minimizing inspection costs. Survey effort or search effort compares the time required to achieve different probabilities of detection (Moore et al., 2011). Wintle et al. (2005) uses the single-visit detectability, *d*, to determine the probability of a false absence in the equation P(false absence) = 1-*d* (Garrard et al., 2008).

As mentioned earlier, plant detectability increases with amount of time spent surveying (Moore et al., 2011). Garrard et al. (2008) proposed a method to estimate survey effort in plant studies utilizing time spent surveying. The probability that a plant is detected *D* after *t* time spent searching with a detection rate of λ can be calculated as follows: $D = 1\text{-}e^{-\lambda t}$, where $e \sim 2.71828$ (Garrard et al., 2008).

Due to budget and time constraints, the inspector may choose to expend energy in different areas depending on the distribution and rarity of the offtypes. A suggestion put forth by MacKenzie and Royle (2005) claims that if there is a high frequency of offtypes, it is more efficient to sample a smaller area more intensely, however if offtypes are rare in a field, a larger area should be sampled less intensively (Garrard et al., 2008).

3 Evaluation of Canadian Pedigreed Wheat Inspection Reports

3.1 Abstract

The Canadian pedigreed seed industry is important to Canada's farmers, food processors, and consumers as well as Canada's economy and reputation as a major player in the global agriculture industry. It is useful to revisit whether or not heterogeneity (number of offtypes) is associated with field size as pedigreed seed fields can range in size from less than 0.4 hectares (one acre) to larger than 259 hectares (640 acres). By comparing the number of offtypes with field size from 3,782 Manitoba pedigreed *Triticum aestivum* L. wheat inspection reports from 2009 to 2012, this research examines the relationship between the number of offtypes and the acreage of different pedigree levels. All pedigree levels showed very little correlation between field size and heterogeneity. There were zero offtypes found in 54.9% of the inspections examined in the 3,782 fields. Only 0.8% of the inspections had more than thirty offtypes (the maximum number of offtypes allowed per inspection of fields producing Certified seed) in the entire inspection.

3.2 Introduction

Plant breeders, seed growers, seed processors, farmers, and consumers desire high-quality, genetically pure seed for multiple reasons. It is therefore useful to review current pedigreed seed inspection procedures to ensure current protocols for field inspections are protecting the reputation of the quality standards that the Canadian Seed Growers' Association (CSGA) has earned on a global scale.

The Canadian seed industry is important to Canada's economy contributing about $5.61 billion, implying an economic turnover of 57,420 jobs, $1.67 billion in wages, and an estimated $81 million in taxes based on the multiplier from Statistics Canada (Agriculture and Agri-Food Canada [AAFC], 2014). Over 3500 pedigreed seed growers produced about 50 different crops and over 2000 different varieties on 1.2 million acres in Canada in 2013 (AAFC, 2014). In 2007,

investment into public and private sector plant breeding research was about $56 million Canada-wide. By 2017, that investment is expected to top $116 million (AAFC, 2014). Canada exports 88% of its pedigreed seed to the United States (75%), the Netherlands (4%), China (3%), Germany (3%), and Japan (3%). In recent years, Canadian seed exports to developed nations have decreased and have increased greatly in developing countries (AAFC, 2014).

Most wheat and barley cultivar development is payed for by the public sector (72%) with producer groups and private sector investing in the remainder of the variety development (Wheat and Barley Variety Working Group [WBVWG], 2015). Wheat and barley producer groups such as the Manitoba Wheat and Barley Growers Association or the Western Grains Research Foundation provide funding to public institutions such as universities or to government run research facilities to create better cultivars with respect to disease resistance, yield, and the final use of the cultivar (WBVWG, 2015).

Prior to 2014, the Canadian Food Inspection Agency (CFIA), a department of the Government of Canada, conducted pedigreed seed inspections for pedigreed seed growers and for the CSGA. Starting in 2014, pedigreed seed inspections were contracted out to private agricultural companies with auditing by CFIA conducted on between 10-15% of pedigreed seed fields.

In Canada, pedigreed seed production is highest in the Prairie Provinces, Ontario, and Quebec. In 2013 and 2014, Manitoba had the highest amount of pedigreed seed acres in Canada (330,648 and 311,190 acres, respectively) in part due to a growing demand for pedigreed soybean seed (Dawson, 2014). In 2014, Manitoba farmers dedicated nearly 3% of their cropland towards pedigreed seed production compared to only 1.2% in Alberta and 0.73% in Saskatchewan (Dawson, 2014). In 2014, 31% of Manitoba pedigreed seed acres (97,359 acres) were dedicated to pedigreed wheat, second only to soybean acres (123,061 acres). Alberta seeded 68,003

pedigreed wheat acres and Saskatchewan was the top pedigreed wheat seed producer in Canada at 114,616 acres. Ryegrass (14,815 acres) and alfalfa (13,089 acres) had the third and fourth highest pedigreed seed acres, respectively (Dawson, 2014).

In 2015, Manitoba was once again the largest producer of pedigreed seed in Canada planting 380,131 acres, an increase of 68,941 acres from 2014. Manitoba also had the largest increase in pedigreed seed acres in Canada between 2014 and 2015. The largest pedigreed seed acreages in Manitoba were soybeans (132,861) and wheat (132,217). Ryegrass (22,251) and timothy (20,390) had the third and fourth highest pedigreed seed acreage in Manitoba (Jennifer Stow, Personal Communication; Canadian Seed Growers' Association, 2015). Saskatchewan was the largest pedigreed wheat producer in Canada in 2015, planting 144,410 acres. Alberta was the third largest producer of pedigreed wheat seed in Canada in 2015, growing 78,516 acres. Ontario, Manitoba, Alberta, Quebec, and Saskatchewan had the greatest number of pedigreed seed growers in Canada at 853, 718, 693, 635, and 534 growers respectively (Jennifer Stow, Personal Communication; Canadian Seed Growers' Association, 2015).

The objectives of this study was to: 1) determine whether field size and the number of offtypes are related and 2) evaluate how past wheat inspections fared with current offtype thresholds.

3.3 Materials and Methods

CSGA provided access to data containing 3,782 pedigreed wheat inspection reports in Manitoba between 2009 and 2012. These inspections were conducted by CFIA employees prior to the switch to private inspections in 2014. In each of the reports, the acreage, offtype counts, cultivar, and pedigree were recorded. In these reports, the level of pedigree is recorded as the

pedigree of the seed used to plant that crop and not the pedigree level the crop will produce for sale. These data were collated to generate a database that could be analysed in more detail.

After compiling the Manitoba pedigreed wheat inspection reports, the total offtypes (including any false positives mistakenly identified as offtypes) were compared with the acreage after sorting them by the pedigree level seeded. In addition, the standard deviation and standard error from the different inspection reports using each of the six individual counts were calculated in order to evaluate the presence of offtype patches. Statistical parameters including mean, median, mode, standard deviation, skewness, and kurtosis were also developed using Microsoft Excel to understand the relationship of the pedigree level to both field size and heterogeneity (number of offtypes). The linear regression equations and R-square values were developed using Microsoft Excel.

3.4 Results and Discussion

The largest number of pedigreed wheat fields was in the Registered class in all four years with the greatest number of fields occurring in 2012 (Table 3.1). Tables 3.2 and 3.3 show calculated statistical parameters to describe the size of pedigreed fields and number of offtypes for the different pedigree levels of Manitoba pedigreed wheat fields from inspection reports collected between 2009 and 2012. As pedigree level decreased from Breeder through to Registered and Certified, the field size and number of offtypes increased as well.

Table 3.1. The number of pedigreed wheat fields inspected by Canadian Food Inspection Agency representatives in Manitoba between 2009 and 2012.

Pedigree	2009 Fields	2010 Fields	2011 Fields	2012 Fields	Total Fields
Breeder	49	47	31	77	204
Select	117	118	106	156	497
Foundation	299	240	181	244	964
Registered	548	485	524	555	2,112
Certified	1	2	1	1	5

The field size distribution of all pedigree levels showed a positively skewed acreage due to the large outlying fields in each pedigree as seen in Table 3.2. Table 3.3 shows that all pedigree levels show a positive skew for the amount of offtypes except for the Certified seeded wheat fields which showed no skew. Table 3.2 shows that all pedigree levels have a leptokurtic distribution (greater than three). Table 3.3 shows that all pedigree levels except for the Certified seed wheat fields also have a leptokurtic distribution for number of offtypes. The Certified seeded wheat fields heterogeneity showed a platykurtic distribution. Table 3.2 shows as field size and the number of fields increase, skewness and kurtosis decrease.

Table 3.2. Statistical parameters summarized on data for field size in acres of pedigreed wheat inspection reports in Manitoba between 2009 and 2012.

	N	Mean Acres	Median Acres	Mode Acres	Standard Deviation	Skewness	Kurtosis
Breeder	204	1.94	1.93	0.25	2.44	7.01	57.35
Select	497	27.56	5.00	60.00	42.55	2.59	8.78
Foundation	964	107.60	90.00	160.00	85.19	1.70	4.60
Registered	2,112	132.21	125.00	160.00	80.36	1.64	5.20
Certified	5	132.00	108.00	N/A	109.54	1.78	3.67

[1] N is defined as the number of fields inspected in each pedigreed class between 2009 and 2012.

Table 3.3. Statistical parameters summarized on data for the total number of offtypes in pedigreed wheat inspection reports in Manitoba between 2009 and 2012.

	N	Mean Offtypes	Median Offtypes	Mode Offtypes	Standard Deviation	Skewness	Kurtosis
Breeder	204	1.53	0.00	0.00	4.92	8.23	83.52
Select	497	1.61	0.00	0.00	3.28	4.26	24.83
Foundation	964	2.39	0.00	0.00	4.94	5.01	38.67
Registered	2,112	3.49	0.00	0.00	13.02	19.58	573.30
Certified	5	1.00	1.00	2.00	1.00	0.00	-3.00

[1] N is defined as the number of fields inspected in each pedigreed class between 2009 and 2012.

Figure 3.1 plots the total number of offtypes of the Registered wheat fields with their respective acreage. There is very little linear association ($R^2 = 0.0047$) between number of offtypes

and field size. Table 3.4 shows similar results for pedigreed seed fields planted with Breeder (R^2 = 0.0082), Select (R^2 = 0.0028), Foundation (R^2 = 0.0102), and Certified (R^2 = 0.1384). There were only five fields seeded with Certified seed which could be why the R^2 is greater than for the other pedigree levels with many more fields. A line of best fit was generated to compare the association between number of offtypes and field size. A summary of the equations created from the lines of best fit are in Table 3.4. All slopes (m) were positive except the slope of the Certified seeded fields, which likely stems from only having five fields. The slopes ranged from -0.0034 to 0.1829, all of which are close to zero.

Table 3.4. Association of number of total offtypes and field size in Manitoba pedigreed wheat fields between 2009 and 2012.

Seed Used	Number of Fields 2009-2012	Average Field Size 2009-2012 (Std. Err)[1]	Average Offtypes 2009-2012 (Std. Err)[1]	$Y_{OFFTYPES} = mx_{ACRES} + b$	R^2 (p-value)
Breeder	204	1.94 (0.17)	1.53 (0.34)	Y = 0.1829x + 1.18	0.0082 (0.1980)
Select	497	27.56 (1.91)	1.61 (0.15)	Y = 0.0041 + 1.4986	0.0028 (0.2361)
Foundation	964	107.60 (2.74)	2.39 (0.16)	Y = 0.0058x + 1.7579	0.0102 (0.0017)
Registered	2,112	132.21 (1.75)	3.49 (0.28)	Y = 0.0111x + 2.0205	0.0047 (0.0016)
Certified	5	132.00 (48.99)	1.00 (0.45)	Y = -0.0034x + 1.4483	0.1384 (0.5375)

[1] Standard Error = standard deviation/$\sqrt{n}$

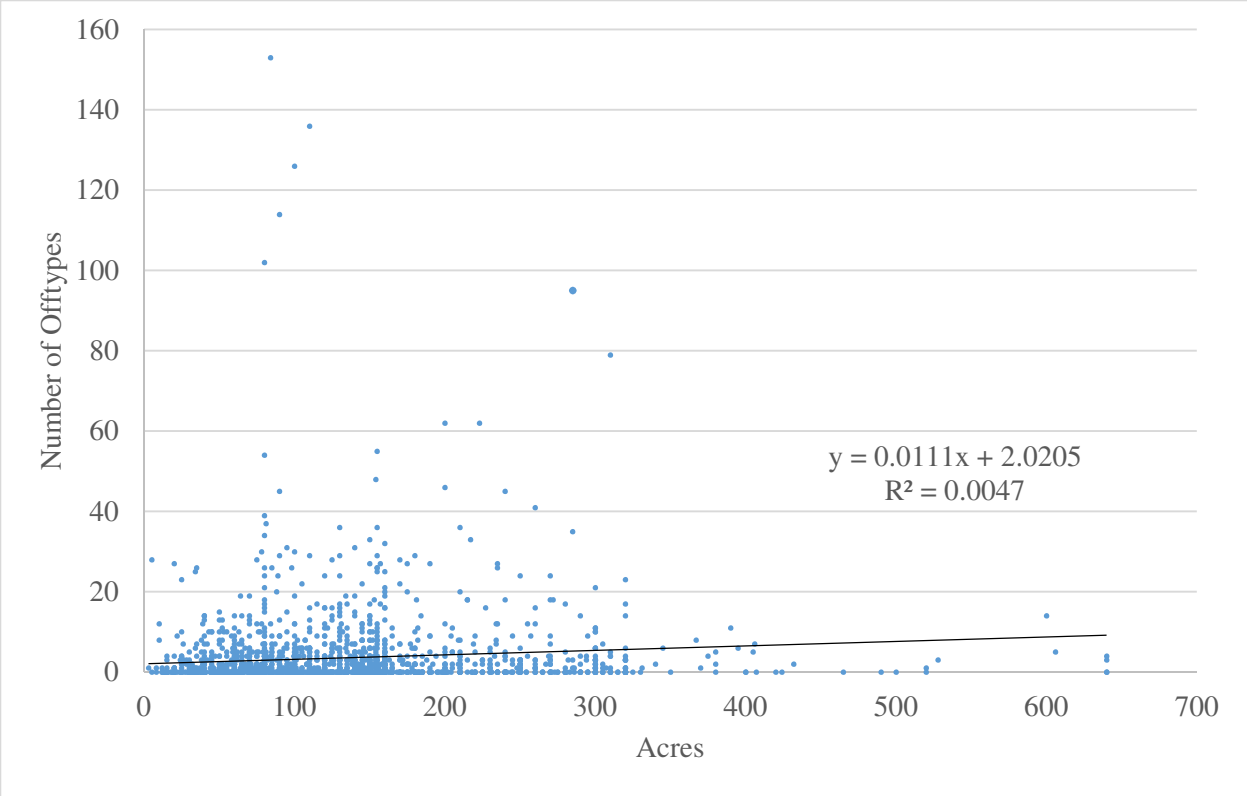

Figure 3.1. The relationship between number of offtypes and field size for Registered seeded pedigreed wheat fields from the CSGA database between 2009 and 2012.

Table 3.4 shows that as pedigree level decreases both the offtypes and the acreage increase. The small R-square value between acreage and offtypes could be explained because all 3,782 fields were grouped together rather than separated based on their pedigree. Table 3.4 shows when the 3,782 fields are broken down into their pedigree their respective R^2 values are close to zero.

The largest standard deviation (69.6 offtypes) for a Manitoba wheat inspection report between 2009 and 2012 was a Registered Falcon winter wheat field which averaged 71.83 offtypes per count and totalled 431 offtypes in the inspection. This particular field also had the largest difference in offtypes found among counts which would be expected given the large standard deviation. The highest count of 10,000 plants showed 200 offtypes, while the lowest count showed 18 offtypes. This field showed an uneven distribution of offtypes with offtype counts of 18, 28, 25, 62, 98, and 200 in the six 10,000 plant sample. There were over ten times as many offtypes in

the sixth count as there were in the first count. If the seed was contaminated, the distribution of offtypes would most likely have been more evenly dispersed. With such a varying distribution of offtypes, the contaminated offtype seed could have been spread unevenly through harrowing a neighbouring wheat field, a harvester allowing too many seeds to escape from a previous harvest, or perhaps the use of improperly cleaned equipment such as planting or harvesting equipment.

The largest standard error of the number of offtypes from an inspection of the six counts was 2.45 and was shared by 397 data points encompassing all four years, all five pedigree levels, several different cultivars, and field size ranging from 0.08 acres to 520 acres. Of the 3,782 wheat fields inspected, 54.9% (2075) had zero total offtypes detected in their inspections. Only 0.8% (32) of wheat fields inspected out of 3,782 had more than thirty offtypes in the entire inspection.

In total, 85 fields out of the 3,782 wheat fields had a difference of five or more offtypes between the maximum offtype count and the minimum offtype count. Nineteen fields out of the 3,782 wheat fields had a difference of ten or more offtypes between the minimum offtype count and the maximum offtype count. Only seven fields out of the 3,782 wheat fields had a difference of 20 offtypes between the minimum offtype count and the maximum offtype count. Appendix 8.1 shows a breakdown of Manitoba wheat inspection reports based on the minimum and maximum number of offtypes found in the counts in each inspection. This is useful for examining patches of offtypes in the field. By comparing the difference between the minimum and maximum number of offtypes with the average number of offtypes in a pedigreed seed inspection, you can determine whether a field has an even or uneven distribution of offtypes throughout the field.

As pedigreed seed is produced from Breeder seed down to Certified seed, there is a higher potential for genetic contamination with each generation of seed production. Breeder and Select seed are often the purest level of seed as vigorous roguing of any offtypes and variants takes place

on small pieces of land. As the pedigree level decreases towards Registered and Certified, the field sizes are often too large to rogue by hand and this is one of the reasons why an increased frequency of offtypes is seen in the lower pedigree levels. In addition, offtypes that were not removed in previous generations of pedigreed seed production will contribute to offtypes in the next generation and accumulate as the seed is moved from Breeder seed to Certified seed.

Another reason why lower pedigree levels have higher rates of offtypes is that after every level of pedigree, there is another chance of contamination from seeding equipment, harvest operations, and from the seed cleaning process. By the time a variety is being produced at the Certified level, the seed could have been handled by the breeder, select grower, foundation grower, and registered grower and have been subject to many different fields, seeders, combines, and seed conditioning equipment, all of which can introduce contaminants if specific precautions are not taken (Joe Wallace, Personal Communication).

Under special circumstances, CSGA will allow Certified seed to reproduce Certified seed. If there is a lack of Registered and Foundation seed, possibly due to extreme environmental conditions, CSGA will allow Certified seed to be grown and produce Certified seed (Randy Preater, Personal Communication). Only five such pedigreed wheat fields were grown in Manitoba in a four year period as seen in Table 3.5 out of a total of 3,782 fields showing how rare this occurrence is.

Table 3.5. Number of Manitoba pedigreed wheat fields in each pedigree class exceeding contamination rejection thresholds and the proportion of fields exceeding the offtype threshold between 2009 and 2012.

Pedigree Seeded	Total Fields	Number of fields exceeding rejection threshold	Rejection Threshold[1]	Proportion of Fields Exceeding threshold
Breeder	204	5	6	0.02451
Select	497	20	6	0.040241
Foundation	964	77	6	0.079876
Registered	2,112	29	30	0.013731
Certified	5	0	30	0

[1] Number of offtypes not including additional variants allowed in variety description

Very few Manitoba Registered pedigreed wheat fields exceeded the 1:2,000 offtype threshold (Figure 3.1 and Table 3.5). Twenty-nine fields exceeded the rejection threshold between 2009 and 2012, but this does not take into account the additional variant tolerances set in the variety description. Variants are periodically occurring, abnormal wheat plants in a cultivar at certain frequencies. Some cultivars may have a genetic mutation which produces variants at higher frequencies than others such as the wheat cultivar Roblin (Brûlé-Babel, Personal Communication). Variants can also arise from cultivars being bred using fewer generations of selections, meaning more variation within that line and that not all loci may be homozygous (Brûlé-Babel, Personal Communication). AAC Brandon, a Hard Red Spring Wheat is an example of a cultivar with two variants: "1) plants that are 15cm taller than the norm and 2) plants with awnless or apically awned spikes may occur at a frequency of up to 0/20,000 (none) in Breeder Seed and Select, 1/10,000 in Foundation; 2/10,000 in Registered and 3/10,000 in Certified Seed" (CFIA, 2015). These variants in AAC Brandon would be in addition to the tolerances set in each pedigree level of spring wheats. Cardale is another spring wheat with higher levels of additional tolerances of variants. The variety description states that "Awnless plants that otherwise conform to the norm of the variety may occur at a frequency of up to 4/20,000 in Breeder, 1/10,000 in Select and Foundation; 2/10,000 in Registered and 4/10,000 in Certified seed. Tall variant plants (10-15cm taller) may occur at a

frequency of up to 10/10,000 in Breeder and Select, up to 15/10,000 in Foundation and Registered and up to 25/10,000 in Certified seed. Talls greater than 15cm are not variants; they are offtypes" (CFIA, 2017). As a result, in a pedigreed Cardale field seeded with Registered seed, the rejection threshold would be pushed to 55 offtypes in 10,000 sampled plants for tall variant plants (10-15cm), but the rejection threshold would remain at 30 offtypes in 10,000 sampled plants for offtypes not listed as variants.

The current standards set by the CSGA seem to be ensuring pedigreed seed growers and breeders are taking the proper precautions to prevent contamination in their fields. The number of offtypes in a field seems to be relatively unrelated to field size, which indicates increased sampling on larger fields is unnecessary which agrees with a past study examining the number of barley offtypes and field size (United Kingdom, 2009). Pedigreed seed fields with contamination exceeding recommended thresholds seem to be a rarity which also agrees with a study in pedigreed barley fields (United Kingdom, 2009). In addition, high frequency patches of offtypes appear to be uncommon based on data from past Manitoba wheat inspection reports.

4 Experimental Modelling of Offtypes in Different Sampling Practices

4.1 Abstract

Minimizing contamination in pedigreed seed fields is important for protecting the traits breeders have bred into cultivars that are desired by farmers, processors, and consumers. Since many different procedures exist to inspect pedigreed seed for offtypes, variants, other crops, and troublesome weeds, it is useful to compare procedures used by the Canadian Seed Growers' Association (CSGA) and other jurisdictions. This comparison is necessary to devise inspection protocols able to minimize falsely accepting or rejecting a pedigreed seed field, while providing a cost and time efficient inspection process. In order to compare current CSGA inspection procedures, with inspection procedures used in North Dakota, and the United Kingdom, mock inspections were conducted on a validation experiment with varying contamination levels, as well as on forty-three Manitoba pedigreed *Triticum aestium* L. wheat fields. One way inspections could become more efficient is by reducing the number of counts (plant, spike, or quadrat) per inspection, so permutations were developed using different counts of each inspection from the validation experiment and were compared using their coefficients of variation. Relative to other jurisdictions, the Canadian inspection procedures consistently inspected the largest area of the field. Plant measured offtypes had similar results to spike measured offtypes and had little effect on the time needed to determine plant density. The results of the permutation analysis showed that as the number of counts is decreased, variability increases, raising the likelihood of falsely accepting or rejecting a pedigreed seed field.

4.2 Introduction

Pedigreed seed inspection procedures are important for maintaining purity for cultivars with specific traits desired by processors and consumers. Different countries have differing inspection procedures and different standards. Each jurisdiction has advantages and disadvantages

to their procedures with regards to accuracy and efficiency and it is useful to compare these methods. Evaluation of pedigreed seed inspection procedures is important to find the appropriate inspection practices for the desired outcomes of each jurisdiction.

North Dakota farmers grew over 2.5 million hectares (6.3 million acres) of spring wheat in 2015 (United States Department of Agriculture, 2015), compared to only 1.2 million hectares (3.0 million acres) in Manitoba in the same year (Statistics Canada, 2015). North Dakota is the largest spring wheat producer in the United States (United States Department of Agriculture, 2015). In the United Kingdom, farmers grow just under 2.0 million hectares (5.0 million acres) annually (United Kingdom, 2014). These large acreages of wheat rely on a sound pedigreed seed production system to ensure that cultivars being grown have the desired characteristics that were incorporated by plant breeders. This is particularly important for farmers who must declare the variety type or class when delivering to grain buyers. Recent privatization of pedigreed crop inspection has prompted an assessment of the inspection process and standards.

Counts of offtypes are made in pedigreed fields and must meet certain standards in order to grant pedigreed seed certification. In both North Dakota and the United Kingdom, offtype counts are based on the number of offtypes per total number of spikes examined in a pedigreed wheat field. In Canada, offtypes are recorded based on the entire plant, rather than spikes. The number of counts made in the field also varies from one jurisdiction to the other. For example, North Dakota inspectors take more counts than Canada, while inspectors in the United Kingdom use sequential sampling as opposed to one sampling scheme for every field size.

Seeding rates, row spacings, and environmental conditions can contribute to differing numbers of spikes per plant in fields (Chen et al., 2008). As the number of viable, seed-producing spikes of a plant increases, the number of seeds that the plant contributes to the next generation

increases (Fischer et al., 1976). Quantifying the number of offtypes on a plant basis, versus a spike basis, may affect the total number of contaminants in the seed if there are differences in number of spikes per plant or seeds per spike between the offtypes and the cultivar being inspected. Fischer et al., (1976) examined the effect of increasing the seeding density on yield components of wheat. Using four different seeding rates ranging from 50kg/ha to 300kg/ha, Fischer et al (1976) found that as seeding rates increased, plant density increased, number of spikes per plant decreased, spike density increased, and the number of spikelets per spike decreased. As seed density increased from 50kg/ha to 300kg/ha, plant density increased from 120 plants/m^2 to 480 plants/m^2 (a 300% increase) while spike density increased from 403 spikes/m^2 to 458 spikes/m^2 (a 13.6% increase).

At the higher pedigree levels, seeding rates are usually lower to maximize the number of seeds produced per plant, while on conventional farms planting Certified or farm-saved seed, seeding rates are usually greater around 120lbs/acre (134.5kg/ha) to help suppress weeds and increase uniformity. If offtypes respond differently to seeding rate than the cultivar being inspected, their actual frequency of offtypes in subsequent generations of pedigreed seed may also differ.

In addition to methods of counting offtypes, different walking patterns or sampling strategies have the potential to detect different patches of offtypes, other difficult to separate species, and weeds (Rew et al., 2006). If offtypes are distributed evenly and the field is weed-free, the type of walking pattern would be irrelevant. However, if the offtypes are distributed unevenly, different walking patterns have the potential to detect or not detect the patches of varying size and shape.

Most efforts to examine unevenly distributed plant patches have been in weed science studies and ecological studies examining the effects of foreign invading plant species on native

plant species. Studying weed patches is important as it could lead to reduced herbicide needs and less harm to the environment (Rew and Cousens, 2000). Methods used in weed patch studies have the potential to be adapted to pedigreed seed inspection when inspecting for interspecific contamination such as estimating the frequency of weeds, looking for prohibited noxious weeds, or when looking for difficult to separate species. When examining intraspecific contamination such as counting variants or offtypes of one species, it is more difficult to identify patches.

As indicated in chapter 3, levels of offtypes in pedigreed seed fields inspected from 2009 to 2012 in Manitoba were often much lower than the maximum tolerated number. Field simulations and validation studies are necessary to assess the potential for detection of offtypes using different inspection methods. The objectives of this study were to: 1) to compare numbers of offtypes detected in fields with known numbers of contaminants and patches of contamination using different inspection procedures and walking patterns, and 2) to compare the amount of time and distance walked of inspection methods in real farm fields.

4.3 Materials and Methods

4.3.1 Farmers Edge Data Collection

In 2014 and 2015, pedigreed winter and spring wheat inspections were performed in conjunction with Farmers Edge, a company that provided pedigreed seed inspections. Farmers Edge located pedigreed wheat fields of various pedigree levels and field sizes. In total, thirteen fields seeded with Foundation seed and thirty fields seeded with Registered seed were inspected. Of the forty-three pedigreed wheat fields inspected, ten fields were seeded to AAC Brandon, two were to AAC Elie, two were to AAC Gateway, two were to AAC Iceberg, one was to AAC Penhold, five were to Carberry, five were to Cardale, five were to Emerson, five were to Faller, one was to Harvest, two were to Pasteur, and three were to Prosper. The fields were inspected using three inspection procedures while offtypes, field size, count number, and distance walked

were recorded. The time required to count spikes vs. plants in a $1m^2$ quadrat was recorded in a representative area for each field. The Garmin 64S used in the mock inspection experiment was used in the wheat inspections. Each field was walked three times (diamond, zigzag, and circular pattern) using the Canadian inspection method. The first inspection included the inspection methods of both the United Kingdom and North Dakota. The walking pattern order was randomly decided for each field.

4.3.2 Validation Experiment

Mock field inspections were performed on a 57 acre field near Pilot Mound, MB using different evenly distributed and unevenly distributed offtypes. The experiment field location was NW 25-4-11W and is owned and farmed by Sheffield Farms Ltd. The previous cropping history was as follows: 2013 was Liberty-Link canola (5440), 2012 was farm-saved Harvest wheat seed, 2011 was Liberty-Link canola (5440), and 2010 was farm-saved Harvest wheat seed. Prior to 2010, Sheffield farms rotated canola with AC Domain wheat, an apically awnletted cultivar. In 2013, soil samples were taken to determine nutrient levels in the soil. Residual nitrogen levels were 50 lbs/acre and residual phosphorus levels were 21 parts per million. When seeding took place on May 3, 2014, fertilizer and seed were placed with a 15.25m (50') John Deere 1835 air drill with 25.4cm (10") row spacing, 50.8cm (20") mid-row banders, and 10cm (4") openers. The nitrogen was applied in urea form (46-0-0) at a rate of 70 lbs/acre. The phosphorus was applied in the form of mono-ammonium phosphate (11-52-0) at a rate of 15 lbs/acre. All fertilizer was applied through the mid-row banders between the seed rows.

In order to simulate different levels of contamination, an awned wheat variety, Carberry was introduced at known contamination levels (1:30,000, 1:10,000, 1:5,000, 1:2,000, and 1:1,000) into the awnless variety, Harvest, prior to seeding. The experimental design was a randomized

complete block design with two blocks and five treatments per block representing an even distribution of contaminants (Table 4.1). The treatments were planted in strips running east to west and the blocks were separated by a tree line (Figure 4.1). In addition, 15.25m by 15.25m (50' by 50') patches were superimposed on the eastern half of each main treatment to simulate uneven distributions of offtypes (1:1,000 and 1:500) within the field. For every pass made by the air drill, one patch was spread overtop and incorporated via harrowing. Table 4.1 shows the treatment recipes for two drill passes per block totalling four drill passes per two blocks. The estimated acreage per treatment was 6.4 acres, but enough seed was produced for 2.8 hectares (seven acres) in case the drill was metering seed out too fast.

Table 4.1. The targeted contamination level, and mixing rate for the respective treatments planned for seed to be planted in seven acres.

Treatment	Contamination Level	Carberry:Harvest Ratio	Carberry (g)/2.8 hectares	Harvest (g)/2.8 hectares
1	1:30,000	0.03786:1307.8215	11.03	381,006.7
2	1:10,000	0.03786:435.9115	33.09	380,984.6
3	1:5,000	0.03786:217.9339	66.18	380,951.5
4	1:2,000	0.03786:87.3720	165.03	380,852.7
5	1:1,000	0.03786:43.6642	330.08	380,687.6

To ensure the treatment recipes were calculated correctly, germination tests and thousand kernel weights of the two cultivars used in the experiment were determined. The thousand kernel weights of each cultivar were measured using an OHAUS balance scale and the germination tests were conducted by 20/20 Seed Labs. Certified Carberry seed with 98% germination was used as the contaminant in Certified Harvest seed with 89% germination. Carberry is awned and Harvest is awnless, making Carberry easily detectable within a field of Harvest. To ensure the Harvest seed was weighed accurately, a five gallon pail was filled to a specific marked level and weighed (15,710.8 grams). This pail was used as the standard measuring pail and the required seed for each treatment was measured out and distributed amongst other pails until the desired amount of seed

was measured. To complete the requirements for the different recipes, the contaminants were evenly divided up among the different pails and placed near the top of each pail. There was always about 24.25 pails worth of Harvest to divide up the contaminants. Rather than using the conveyor to move seed into the seed tank on the drill, it was decided that the pails would be carried manually into the seed tank to avoid loss of seed or contaminate the different treatments in case of leftover seed in the conveyor. When dumping the treatment pails, we tried to make the pile at the centre of the metering system to avoid running the metering system out of seed on one side.

The tractor pulling the air drill used a GPS auto-steer system to guarantee no overlap between treatments and to make straight headlands and rectangular experiment plots. The seeding route followed the experiment design and proceeded from low contamination (1:30,000) to high contamination (1:1,000). After the drill seeded each treatment, flags were placed on the four corners of each treatment to mark the area for each treatment. Figure 4.1 shows the experiment design of the 57 acre field.

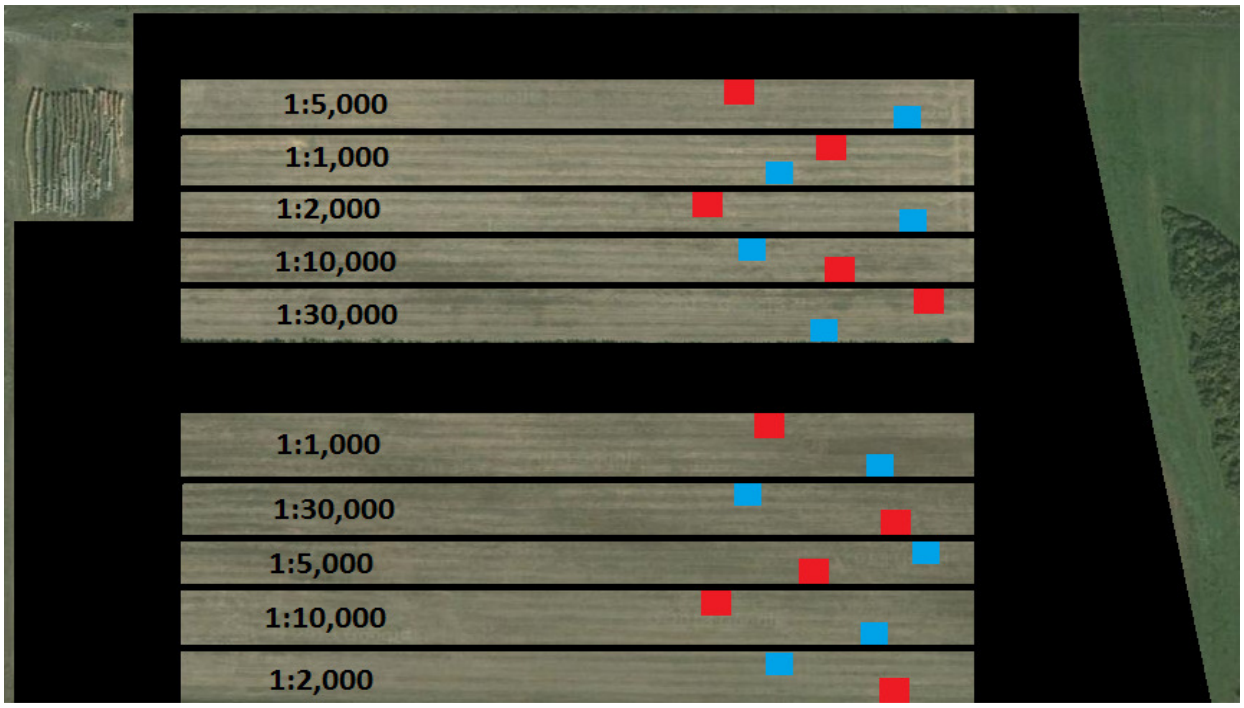

Figure 4.1. Experimental design in the 57 acre field (NW25-4-11W) in southwestern Manitoba. The frequency of the offtype is listed on the left hand side. These treatments were randomized and the black area separated the two replicates. The blue and red squares represent the patches of either 1:500 or 1:1,000 offtypes. The black area was used as the control to evaluate the purity of the Certified Harvest seed. The top of the map is North, the right side is East, the bottom is South, and the left side is West.

Seeding the patches took place on May 4, 2014. A 15.25m (50') rope was used to measure the edges of the patch. To find the location of the patches, pacing from the east side of the treatment was used after randomly assigned distances from the east side of the patch. The distance of pacing was randomly allotted and was defined as the distance from the eastern part of the treatment to the east side of the patch. Flags were placed in the corners of each square patch, marked either 1:1,000 or 1:500. After the patches were flagged, either 76 Carberry seeds on the 1:1,000 designated patches or 146 Carberry seeds on the 1:500 assigned patches were broadcast by hand by walking around the patch and dropping the contaminants randomly throughout the patch. The patches were then harrowed individually using a 1.8m (6') diamond harrow pulled behind an all-terrain vehicle. After each patch was incorporated, the harrow bar was lifted to

ensure the trash built up from within the patch did not leave the patch in case some contaminants were caught in the trash. The pile of trash was then spread out within the patch by hand.

On June 3, 2014, plant densities were measured for each experiment plot. A 1m by 1m square was made using flexible, light-weight tubing divided into four quadrats, each 0.25m^2 in area. Fifteen plant counts were taken per block with one taken on the east, west, and middle of each of the five treatments. At the time of the plant counts, the wheat was between the Zadoks 12 and Zadoks 13 stages.

In July 2014, each treatment of each block was used as a mock field inspection. Each treatment was walked three times and counts of offtypes were recorded using inspection procedures from Canada, North Dakota, and the United Kingdom. The entire field was walked in different patterns (circular, diamond, and zig zag). Using a Garmin 64S GPS unit for guidance, all boundaries of the patches and the treatments were marked and borders were made. The GPS unit was used to ensure the inspection was kept within a specific treatment and mapped the walking pattern as well as the distance travelled.

4.3.3 Permutation Generation and Analysis

In order to evaluate the effectiveness of sequential sampling, permutations of data were developed from both the validation experiment and the Farmers Edge data. Different combinations of both plant and spike offtypes in both sets of data were generated. Since the Canadian inspection procedures require six counts of 10,000 plants per inspection, the different combinations of counts include using: one, two, three, four, five, or six counts with six different combinations of one count (6C1), fifteen different combinations of two counts (6C2), twenty different combinations of three counts (6C3), fifteen different combinations of four counts (6C4), six different combinations of five counts (6C5), and one combination of six counts (6C6) respectively. Using Microsoft Excel,

each of the forty-three Farmers Edge inspections and validation inspections developed a complete set of permutations. A complete set of permutations was developed for the United Kingdom inspections with five total counts per inspection. There were ten counts for both North Dakota inspections and larger United Kingdom fields. The number of different combinations which can be made is very large, therefore the amount of combinations developed was capped at twenty. Choosing which combinations to use was done randomly.

By examining permutations it can be determined whether more or less sampling is required. In order to evaluate the permutations, their respective coefficients of variation (std. deviation/average) were calculated and graphed. The coefficients of variation were compared with one another and the results summarized. The method used to compare different coefficients of variation was developed from Zvomuya et al. (2008) in order to determine whether different coefficients of variation are significantly different. Since each of the different contamination levels were inspected six times using Canadian inspection procedures, five degrees of freedom were used to calculate the significance of each of the permutations. When ten counts took place such as in the North Dakota inspection procedures or when the field size is greater than three hectares in the United Kingdom inspection procedures, nine degrees of freedom was used. Four degrees of freedom were used in the United Kingdom inspections when the field size was less than or equal to three hectares. Thirty-nine fields with one or more offtypes were left and were split into three groups of thirteen fields based on their acreage. These groups used twelve degrees of freedom. An alpha value of 0.05 was used to evaluate the p-values for both the Farmers Edge data and the validation experiment data, mainly because the alpha value of 0.05 is the scientific standard. By altering the alpha value higher or lower, the significance of the permutations can change.

4.3.4 Statistical Analysis

Correlations of different parameters in the data collected in the forty-three pedigreed *Triticum aestivum* L. Farmers Edge fields and the validation experiment were generated using the "PROC CORR" function in SAS statistical analysis software (Version 9.3). Analysis of variance (ANOVA) for plant and spike detected offtypes were conducted using the "PROC GLM" function for the validation experiment and the Farmers Edge fields to evaluate the effects that different walking patterns and jurisdictions inspection protocols have on offtype detection. The model statement used to evaluate the variables: detected plant and spike offtypes were as follows for the different PROC GLM procedures:

Farmers Edge jurisdiction analysis (Table 4.5)

Plant/Spike Offtypes = Cultivar Pattern Jurisdiction Cultivar*Pattern Pattern*Jurisdiction and Pattern* Jurisdiction*Cultivar

Validation experiment jurisdiction analysis (Table 4.8)

Plant/Spike Offtypes = Block Treatment Jurisdiction Block*Treatment Treatment*Jurisdiction Jurisdiction*Block Treatment*Jurisdiction*Block

Farmers Edge walking pattern analysis (Table 4.11)

Plant/Spike Offtypes = Pattern Cultivar Pedigree FieldSize FieldSize*Pattern FieldSize*Cultivar Pedigree*Cultivar Pattern*Cultivar Pattern*Pedigree

Time required to measure plant and spike density analysis (Table 4.13)

Time to count $1m^2$ plants/spikes = Cultivar Pedigree Pedigree*Cultivar

All four ANOVA analyses assumed completely fixed effects and used Type III Sums of Squares which is why "PROC GLM" was appropriate for these tests.

4.4 Results and Discussion

The plant densities in Table 4.2 show that different areas of the field (NW 25-4-11W) had different plant densities although the seeding rate remained constant. This could be because the field was zero-tilled, or that the soil was variable with slight topographical differences. The Manitoba government recommends a targeted plant density of 247 to 302 m^{-2} for commercial wheat fields. Only two treatments met this recommendation, while the rest fell short. Pedigreed seed growers often target lower plant densities than commercial wheat growers so they can increase the number of seeds produced relative to the number of seeds seeded. By lowering the plant density, the field can appear to be more uneven in height from an increase in the amount of tillers a wheat plant has and can also be less competitive to weeds.

Table 4.2. A summary of plant densities taken on June 3, 2014 when wheat plants were at the 3-leaf stage (Zadoks 13).

Block	Frequency	Average plant count per $1m^2$	Meters long by 1m wide to 10,000 plants
North	1:30,000	225.7	44.3
North	1:10,000	247.0	40.5
North	1:5,000	236.3	42.3
North	1:2,000	246.3	40.6
North	1:1,000	230.0	43.5
South	1:30,000	205.7	48.6
South	1:10,000	214.7	46.6
South	1:5,000	250.7	39.9
South	1:2,000	219.7	45.5
South	1:1,000	220.7	45.3

4.4.1 Different Jurisdiction Comparison

4.4.1.1 Farmers Edge Data

After completing inspections from forty-three different pedigreed wheat fields in southern Manitoba, a number of recorded parameters (field size, plant and spike offtypes, plant and spike density, and total inspected area) showed both strong and weak correlations. Field size showed a

correlation coefficient of 0.25 (p-value of 0.0042) and 0.21 (p-value of 0.0163) with total plant and spike offtypes respectively. Plant and spike offtypes had the strongest correlation of 0.99 (p-value of <0.0001). Plant and spike density also had a strong correlation of 0.66 (p-value of <0.0001) and both parameters had a correlation of approximately -0.20 with p-values of 0.0219 and 0.0201 respectively with the total inspected area. Plant density was not correlated with either the plant offtypes or the spike offtypes, but spike density showed a correlation of 0.24 (p-value of 0.0053) and 0.25 (p-value of 0.0048) with plant offtypes and spike offtypes respectively when the entire inspection (all counts) was analyzed. However, if the individual counts were analyzed, both plant density and spike density were correlated with plant offtypes and spike offtypes.

After sampling the forty-three fields using inspection procedures from Canada, North Dakota, and the United Kingdom, Tables 4.3 and 4.4 show that Canadian inspection procedures find more offtypes and sample a larger area of the field on average. Since the United Kingdom bases their inspection procedures on field size, the same area was sampled for each field (ten counts of $20m^2$). A commonality among the three jurisdictions is that as the total sampled area increased, the total plant and spike offtypes detected increased as well. The proportion of offtypes changed similarly among the three jurisdictions as the number of sampled plants changed.

Table 4.3. A summary of the plant and spike offtypes detected per inspection from the three different jurisdictions inspection procedures from forty-three Manitoba pedigreed wheat fields in 2014 and 2015.

Jurisdiction	Number of Inspections	Minimum Offtypes Detected in an Inspection		Maximum Offtypes Detected in an Inspection		Average Offtypes per Inspection (Std. Error)	
		Plants	Spikes	Plants	Spikes	Plants	Spikes
Canada	43	0	0	56	181	16.77 (1.89)	55.35 (6.17)
North Dakota	43	0	0	32	112	8.42 (1.03)	28.14 (3.50)
United Kingdom	43	0	0	33	103	9.88 (1.18)	33.19 (3.84)

Table 4.4. A summary of the average distance per inspection using the three different jurisdictions inspection procedures after inspecting forty-three pedigreed wheat fields in Manitoba in 2014 and 2015.

Jurisdiction	Number of Inspections	Minimum Area Sampled (m^2) During an Inspection	Maximum Area Sampled (m^2) During an Inspection	Average Area Sampled (m^2) per Inspection (Std. Error)
Canada	43	314.1	526.3	336.2 (6.0)
North Dakota	43	163.1	336.7	178.5 (3.8)
United Kingdom	43	200	200	200 (0.00)

After inspecting the forty-three pedigreed wheat fields using Canadian inspection procedures, Canadian inspection procedures evaluated the largest area of the field, followed by the United Kingdom and North Dakota respectively. Table 4.5 shows the expected number of offtypes and the actual number of offtypes found in the validation experiment using the Canadian inspection system. The difference between blocks is minimal, but the difference between the actual and expected is large in some contamination treatments. For instance, four times as many offtypes were found in the actual inspection in the 1:30,000 contamination treatment than were expected, which could indicate patches accounted for the increased number of offtypes found. Alternatively, the largest contamination treatment (1:1,000) showed that roughly half of the number of offtypes were detected out of the total number of offtypes expected. This could be due to offtypes going undetected. The patches of offtypes could have been detected in some of the treatments, but not in others. Also, since Carberry is slightly shorter in height, this could have resulted in less offtypes being detected.

Table 4.5: The average expected (not including patches) and actual average number of offtypes for each of the contamination treatments in the validation experiment using the Canadian inspection procedures.

Block	Contamination Rate	Actual	Expected
North	1:30,000	8	2
North	1:10,000	11	6
North	1:5,000	16.3	12
North	1:2,000	23	30
North	1:1,000	32	60
South	1:30,000	8.3	2
South	1:10,000	10.3	6
South	1:5,000	16.3	12
South	1:2,000	24.3	30
South	1:1,000	31	60

When the data were analysed using PROC GLM, the cultivar, walking pattern, and jurisdictions showed significant differences. The interactions among the three sources of variation showed insignificant differences when both spike offtypes and plant offtypes were analysed. Table 4.6 shows the analysis of variation for both spike and plant offtypes. Results show that the cultivar and the type of inspection procedures show significantly different results. The walking pattern also showed a significant difference when using the jurisdiction data. The effect of the cultivar could be due to some cultivars being bred without complete homozygosity and having some variants. For instance, Cardale and AAC Brandon consistently showed many tall offtypes, while other cultivars had very low heterogeneity and were much more uniform. The jurisdiction effect was also significantly different, which is expected as Canada consistently sampled more area than either the North Dakota or United Kingdom inspection methods.

Table 4.6. Analysis of variance of number of plant and spike measured offtypes from data collected from forty-three pedigreed seed fields in southern Manitoba in 2014 and 2015.

Source	Degrees of Freedom	Mean Square		Pr>F	
		Plant	Spike	Plant	Spike
Cultivar	11	408.47	4,820.49	<0.0001	<0.0001
Pattern	2	497.27	5,236.89	0.0024	0.0017
Jurisdiction	2	463.86	4,876.49	0.0034	0.0025
Cultivar x Pattern	12	133.46	1,324.91	0.0680	0.0674
Pattern x Jurisdiction	4	27.16	305.14	0.8273	0.7920
Cultivar x Pattern x Jurisdiction	46	15.95	196.15	1.0000	1.0000
Error	51	72.97	723.00		

4.4.1.2 Validation Results

After the validation experiment treatments were sampled three times using inspection procedures from North Dakota, Canada, and the United Kingdom, the data were analysed to compare the ability of the different procedures to find efficiencies and measure the ability to detect offtypes. Table 4.7 shows the sample area results where Canadian inspection procedures are dependent on plant density, North Dakotan inspection procedures are dependent on spike density, and United Kingdom sampling is dependent on field size. The Canadian inspection sampled the most area, and highest percentage of the plots, while North Dakota sampled the second highest area and percentage of the plots. The United Kingdom inspection sampled the least area and percentage of the plot and had no standard error because each field was less than 7.5 acres as the United Kingdom inspection procedures are not based on plant or spike density, but on the field size. When the field size is less than 7.5 acres, inspectors take five counts of $20m^2$ area regardless of the plant density.

Table 4.8 shows the resulting heterogeneity found after using Canadian, North Dakotan, and United Kingdom sampling schemes on the validation experiment. The Canadian inspection procedures detected the most plant and spike offtypes on average, followed by North Dakotan

inspection procedures, and United Kingdom procedures. When comparing both Tables 4.7 and 4.8, the average plant and spike offtypes increased as the average sampled area increases as well. The average plant offtypes/m^2 for the Canadian, North Dakotan, and United Kingdom inspections were 0.0700 plants/m^2, 0.0877 plants/m^2, and 0.0860 plants/m^2, respectively. The average spike offtypes/m^2 for the Canadian, North Dakotan, and United Kingdom inspections were 0.2218 spikes/m^2, 0.2655 spikes/m^2, and 0.2653 spikes/m^2, respectively. There was a 0.0177 plant offtypes/m^2 difference between the Canadian and North Dakotan detections. There was a 0.0437 spike offtypes/m^2 difference between the Canadian and North Dakotan detections. The standard error also increased in both plant and spike measured offtypes as the average sampled area increased. This was the same trend as seen in Tables 4.3 and 4.4 where both the standard error and number of plant and spike measured offtypes increased as the sampled area increased.

Table 4.7. A summary of the area inspected per inspection from the five different treatments in two replications (north and south block) in the validation experiment on NW 25-4-11W in 2014.

Jurisdiction	Number of Inspections	Minimum Area Sampled (m^2) per Inspection	Maximum Area Sampled (m^2) per Inspection	Average Area Sampled (m^2) per Inspection (Std. Error)
Canada	30	239.4	291.6	262.26 (3.05)
North Dakota	30	145	172	156.30 (1.68)
United Kingdom	30	100	100	100.00 (0.00)

Table 4.8. A summary of the number of offtypes per inspection from the five different treatments in two replications (north and south block) in the validation experiment on NW 25-4-11W in 2014.

Jurisdiction	Number of Inspections	Minimum Offtypes per Inspection		Maximum Offtypes per Inspection		Average Offtypes per Inspection (Std. Error)	
		Plants	Spikes	Plants	Spikes	Plants	Spikes
Canada	30	7	17	34	117	18.37 (1.63)	58.17 (5.34)
North Dakota	30	6	19	30	95	13.70 (1.30)	41.50 (3.95)
United Kingdom	30	5	10	19	63	8.60 (0.80)	26.53 (2.73)

The validation experiment was analysed to see whether different jurisdictions produced different results. Several significant differences were revealed (Table 4.9). As expected, the different contamination treatments showed a significant difference of detected spike and plant offtypes, as did the different jurisdictions procedures. As predicted, the north and south blocks (replicates) showed an insignificant difference when either plant or spike offtypes were measured. The treatment x jurisdiction interaction also showed a significant difference. With different levels of contamination, the different jurisdictions had different levels of detectability. This could be due to the differing amounts of sampling each jurisdiction conducts. It could also be explained through the different placement of the patches in each treatment. Since the patches were only 50' by 50', the inspection could bypass the patch, or go through part of, or the entire patch leading to potential differences among the inspections. The results are seen in Table 4.9.

Table 4.9. Analysis of variance of the number of plant and spike offtypes detected from the jurisdiction results in the validation experiment.

Source	Degrees of Freedom	Mean Square		Pr>F	
		Plant	Spike	Plant	Spike
Block	1	21.5	51.4	0.0864	0.5262
Treatment	4	856.3	7,933.3	<0.0001	<0.0001
Jurisdiction	2	715.9	7,512.2	<0.0001	<0.0001
Block x Treatment	4	13.4	69.0	0.1226	0.7025
Treatment x Jurisdiction	8	46.0	409.0	<0.0001	0.0040
Block x Jurisdiction	2	15.4	499.1	0.1222	0.0245
Block x Treatment x Jurisdiction	8	3.8	100.7	0.8211	0.6078
Error	60	7.0	126.4		

4.4.2 Comparing Walking Pattern

The forty-three pedigreed seed fields were inspected using a circular, diamond, and a zig-zag sampling pattern with only the Canadian inspection procedures to see if different sampling patterns led to any differences in the amount of offtypes detected. The number of spike offtypes and plant offtypes detected were found to be comparable between the different sampling patterns.

Table 4.10 shows the number of offtypes detected using different walking patterns. Where the walking patterns differ, is in the total distance required to complete the inspection. The average distance required to complete a diamond walking pattern was smaller than the circular and zig-zag pattern.

Table 4.10. A summary of the total number of plant and spike offtypes from the three different inspections for the different walking patterns performed on each of the forty-three pedigreed wheat fields from Farmers Edge in 2014 and 2015 using the Canadian inspection system.

Walking Pattern	Minimum Offtypes per Inspection		Maximum Offtypes per Inspection		Average Offtypes per Inspection (Std. Error)	
	Plants	Spikes	Plants	Spikes	Plants	Spikes
Circular	0	0	60	193	16.44 (1.81)	53.16 (5.89)
Diamond	0	0	59	186	16.53 (1.82)	52.63 (5.70)
ZigZag	0	0	56	181	16.39 (1.87)	53.72 (5.93)

Table 4.11. A summary of the total distance of the three different inspections for the different walking patterns performed in each of the forty-three pedigreed wheat fields from Farmers Edge in 2014 and 2015 using the Canadian inspection system.

Walking Pattern	Minimum Distance per Inspection (m)	Maximum Distance per Inspection (m)	Average Distance per Inspection (Std. Error)
Circular	1,334.37	5,265.98	2,313.52 (82.12)
Diamond	1,389.03	4,811.6	2,194.38 (71.91)
ZigZag	1,633.13	5,066.68	2,401.63 (74.08)

The walking pattern data were analyzed using PROC GLM, and only cultivar and field size showed significant differences for both the plant and spike measured offtypes (Table 4.12). Certain cultivars have more variants than others which are counted as offtypes. The field size was partitioned into five classes based on the number of acres. The classes were field sizes less than eighty acres, fields between eighty and 160 acres, fields between 160 and 240 acres, fields between 240 and 320 acres, and fields larger than 320 acres. The walking pattern, pedigree, and the interactions showed insignificant differences for both the plant and spike measured offtypes.

Table 4.12. Analysis of variance showing the effect that walking pattern had on the detection of the number of plant and spike offtypes detected from the forty-three pedigreed wheat fields from Farmers Edge in 2014 and 2015.

Source	Degrees of Freedom	Mean Square		Pr>F	
		Plant	Spike	Plant	Spike
Pattern	2	26.0	251.0	0.7888	0.7929
Cultivar	11	427.4	4,537.2	0.0002	<0.0001
Pedigree	1	53.9	560.1	0.4845	0.4734
Field Size	4	550.5	4,546.6	0.0012	0.0040
Pattern x Field Size	8	12.7	117.5	0.9985	0.9988
Cultivar x Field Size	5	113.5	1,239.6	0.4013	0.3426
Cultivar x Pedigree	2	199.9	1,559.3	0.1678	0.2423
Pattern x Cultivar	22	3.0	51.5	1.0000	1.0000
Pattern x Pedigree	2	1.6	6.8	0.9859	0.9938
Error	71	109.2	1,078.3		

Even though the diamond walking pattern has many advantages including time savings and reducing inspector fatigue, there are often more problem areas on the outskirts of the field such as prohibited noxious weed invasions, isolation violations, and other species or cultivar contamination from roadways, railways, or from neighbouring fields. Since the diamond inspection reaches the approximate centre of each of the sides of the field, many parts of the isolation remain uninspected. In theory, the zig-zag inspection is the most thorough of the walking patterns and would be better than the other inspection patterns at detecting the potential problem areas on the outer boundaries of the pedigreed seed field, but due to the nature of the fields inspected, these differences were not apparent. The time required to conduct an inspection could be influenced by the cultivar because cultivars with higher offtype levels could take longer to inspect than cultivars with low levels of heterogeneity.

4.4.3 Comparing Number of Spike Offtypes with Number of Plant Offtypes

The time required to count the number of spikes versus the number of plants using a $1m^2$ quadrat is presented in Figure 4.2.

Table 4.13. Pearson correlation coefficients and p-values in parenthesis from measuring the time it took in seconds to count $1m^2$ of plants and spikes in forty-three different pedigreed wheat fields from Farmers Edge in Manitoba in 2014 and 2015.

	Spike Density	Plant Density	Spike Time	Plant Time
Acres	0.00232	0.09334	-0.03616	0.15724
	(0.9882)	(0.5516)	(0.8179)	(0.3139)
Spike Density		0.65772	0.75364	0.74152
		(<0.0001)	(<0.0001)	(<0.0001)
Plant Density			0.72460	0.63991
			(<0.0001)	(<0.0001)
Spike Time				0.85240
				(<0.0001)

One observation was omitted from the graph as that field suffered significant hail damage which decreased the number of spikes and plants and the amount of time to count the spikes and plants in a $1m^2$ quadrat. As expected, Table 4.13 shows that the time required to count either plants or spikes increased as plant or spike density increased. There was also a high association between the time required to measure plant density and spike density which was likely because there was a high association between plant and spike density. There did not seem to be an association between field size and plant density, spike density, and the time required to measure plant or spike density. As field size increased, the pedigree levels tended to decrease, which would intuitively lead to higher plant densities. This was not seen (Table 4.13), but an economical explanation is possible to explain the lack of association: some growers put less popular cultivars on smaller fields and put the most desirable cultivars on their larger fields. For example, in 2015, AAC Brandon was replacing AC Carberry, and many commercial wheat growers were improving their genetics. Pedigreed seed growers were growing AAC Brandon on more area than AC Carberry as AC Carberry was being outperformed by AAC Brandon.

Table 4.14. Analysis of variance of the time required to measure plant and spike density in forty-three pedigreed wheat fields from Farmers Edge in Manitoba in 2014 and 2015.

Source	Degrees of Freedom	Mean Square		Pr>F	
		Plant Time	Spike Time	Plant Time	Spike Time
Cultivar	11	1,266.7	1,048.6	0.0111	0.0057
Pedigree	1	1,371.6	2,751.5	0.0870	0.0068
Cultivar x Pedigree	2	392.5	499.8	0.4178	0.2296
Error	28	435.9	322.2		

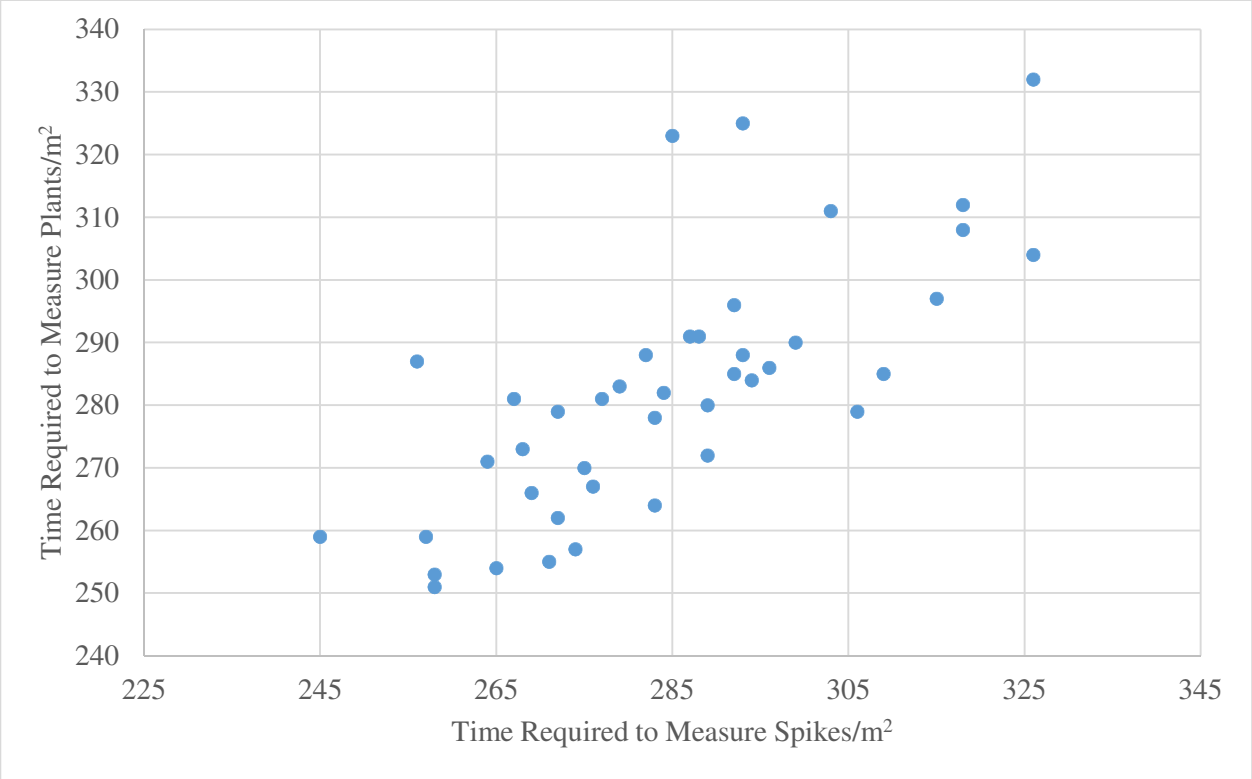

Figure 4.2. A comparison of the time spent to measure the plant and spike density in $1m^2$. The Pearson Correlation Coefficient was 0.8524 with a p-value of <0.0001, N=42. One outlying observation was omitted due to hail damage.

The time taken to measure spike density or plant density can be influenced by several factors. The time to measure plant density can be influenced by the pedigree level as higher pedigree levels tend to have lower seeding rates producing fewer plants, and quickening the time needed to measure the plant density. Some fields can be badly lodged from too much fertilizer,

environmental conditions, or because of the type of cultivar. Lodged fields increase the time required to measure the plant density. The type of seeder used can also influence the time needed to measure plant density as certain seeders disperse plants more evenly than others. Plants that are seeded more evenly require less time to differentiate between plants, decreasing the time required to measure plant density. The time needed to measure spike density can also be influenced by lodging, which increases as lodging increases. As plant density increases, the average number of spikes per wheat plant increases so the type of seeding apparatus only has a small effect on the time required to measure spike density.

4.4.4 Coefficient of Variation Permutation Comparisons

Tables 4.15-4.17 along with Figures 4.3-4.5 were generated from permutations developed from data collected from the validation experiment on NW25-4-11W in 2014. The goal of using permutations was to examine the effects of sequential sampling and to see whether less sampling could produce as accurate results as the complete inspection. The North Dakota inspection offered the greatest comparison of permutations, since there were ten counts per inspection, followed by the Canadian inspection with six counts per inspection, and the United Kingdom inspection with five counts per inspection. The coefficients of variation from the different permutations developed from the North Dakota inspections in the validation experiment were compared. When comparing 90% (10C9) of the inspection with 80% (10C8) of the inspection, there was an insignificant difference in all contamination levels with either plant or spike offtypes.

In Tables 4.15-4.20 when there was no significance between permutations, it meant that there was no significant difference between sampling one permutation to another, but if there was a significant difference, the higher permutation should be used to reduce the coefficient of variation, minimizing the probability of falsely rejecting or approving a seed field. Many counts

of different inspections showed zero offtypes. If most of the counts of the complete inspection were zero, but one count showed a higher number of offtypes, the standard deviation would be higher than the mean, which could lead to a coefficient of variation being greater than one. In addition, as more offtypes were detected, the average offtypes would increase, but the standard deviation may not necessarily increase, unless the inspector detects a patch of offtypes. This was seen in Figure 4.3 where the coefficient of variation of the 1:5,000 treatment was greater than the coefficient of variation of the 1:10,000 treatment because a patch was detected in the 1:5,000 treatment which increased the standard deviation.

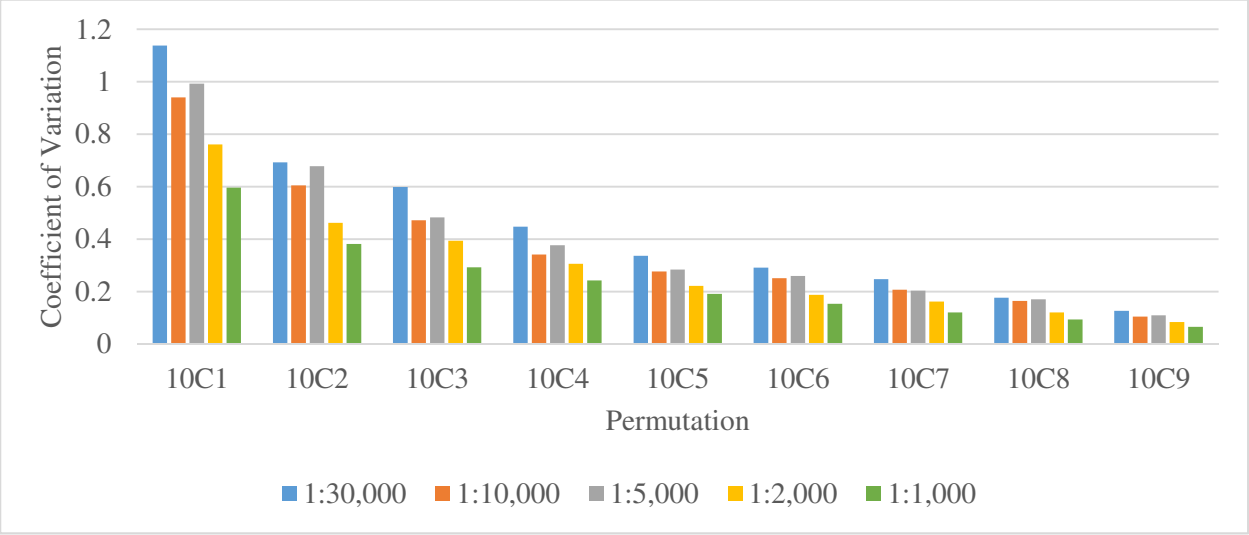

Figure 4.3. A comparison of the coefficients of variation of permutations generated from the validation experiment using North Dakota inspection procedures and calculating coefficients of variation based on plant offtypes. The permutations nCr were developed using 1, 2, 3, ..., or 9 counts (r) out of a possible ten counts (n).

Table 4.15. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the validation experiment using North Dakota inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. An alpha value of 0.05 was used to determine if the permutations were statistically significant (Yes) or not statistically significant (No) with five degrees of freedom with contamination treatment rates ranging from 1:30,000 to 1:1,000.

		Treatment									
Permutation[1] being compared		1:30,000		1:10,000		1:5,000		1:2,000		1:1,000	
		PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
10C1	10C2	No	No	No	No	No	No	No	No	No	No
10C1	10C3	No	No	No	No	No	No	No	No	Yes	No
10C1	10C4	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C3	No	No	No	No	No	No	No	No	No	No
10C2	10C4	No	No	No	No	No	No	No	No	No	No
10C2	10C5	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C4	No	No	No	No	No	No	No	No	No	No
10C3	10C5	No	No	No	No	No	No	No	No	No	No
10C3	10C6	Yes	No	No	No	No	No	Yes	Yes	Yes	No
10C3	10C7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C5	No	No	No	No	No	No	No	No	No	No
10C4	10C6	No	No	No	No	No	No	No	No	No	No
10C4	10C7	No	No	No	No	No	No	Yes	Yes	Yes	Yes
10C4	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C5	10C6	No	No	No	No	No	No	No	No	No	No
10C5	10C7	No	No	No	No	No	No	No	No	No	No
10C5	10C8	Yes	No	No	No	No	No	No	No	Yes	Yes
10C5	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C6	10C7	No	No	No	No	No	No	No	No	No	No
10C6	10C8	No	No	No	No	No	No	No	No	No	No
10C6	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

10C7	10C8	No	No	No	No	No	No	No	No	No	No
10C7	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C8	10C9	No	No	No	No	No	No	No	No	No	No

[1] Permutation refers to subsamples of complete inspections; 10C1 = one count of ten counts (complete inspection), 10C2 = two counts (subsample) of ten counts (complete inspection)... 10C9 = nine counts (subsample) of ten counts (complete inspection)

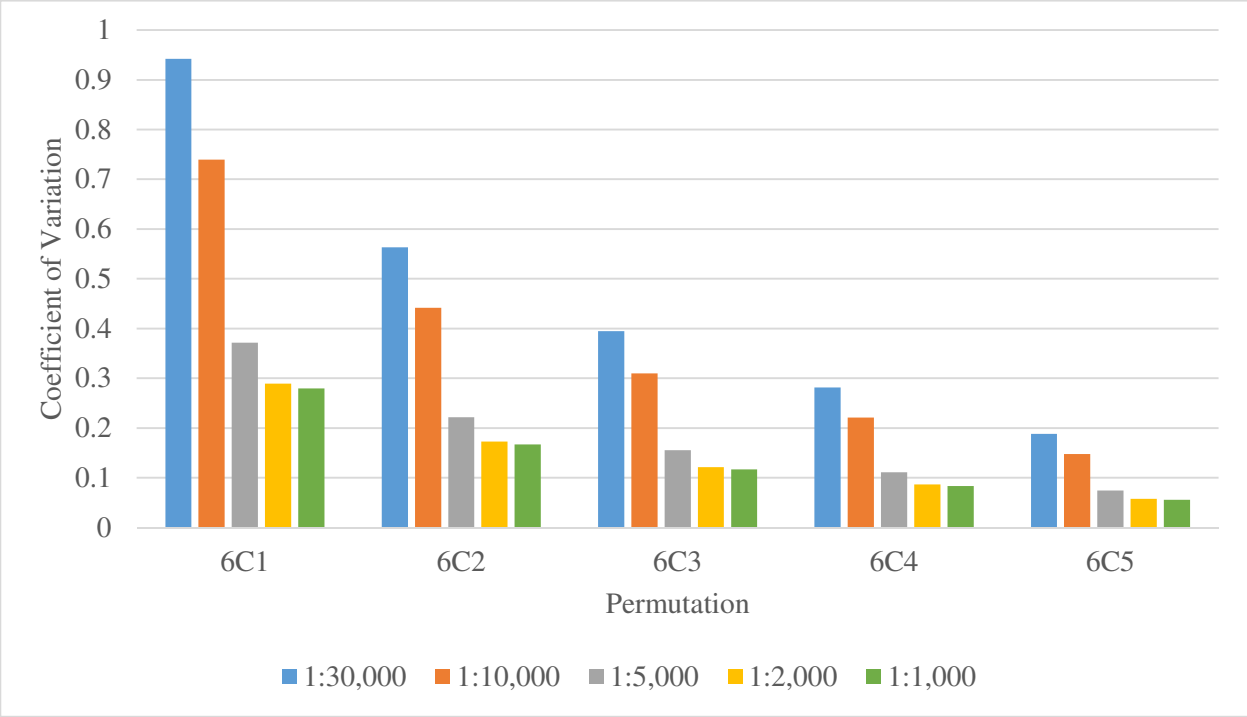

Figure 4.4. A comparison of the coefficients of variation of permutations generated from the validation experiment using Canadian inspection procedures and calculating coefficients of variation based on plant offtypes. The permutations nCr were developed using 1, 2, 3, 4, or 5 counts (r) out of a possible six counts (n).

Tables 4.15 and 4.16 illustrate similar results, with the exception that Canadian inspection procedures were used in place of North Dakotan inspections. Table 4.16 shows that when 83.3% (6C5) of inspections were compared with 66.6% (6C4) of inspections, there were insignificant differences across all levels of contamination for both plant and spike offtypes. When comparing 83.3% (6C5) of the inspection with 50% (6C3) of the inspection, there were significant differences throughout all contamination levels for both spike and plant offtypes. This compares with Table 4.15 when comparing 90% (10C9) of the inspection with 70% of the inspection in that all

contamination levels using both plant and spike offtypes showed significant differences when using an alpha value of 0.05.

Table 4.16. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the validation experiment using Canadian inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. An alpha value of 0.05 was used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with five degrees of freedom.

		Treatment									
Permutations[1] being compared		1:30,000		1:10,000		1:5,000		1:2,000		1:1,000	
		PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
6C1	6C2	No	No	No	No	No	No	No	No	No	No
6C1	6C3	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C1	6C4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C1	6C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C2	6C3	No	No	No	No	No	No	No	No	No	No
6C2	6C4	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C2	6C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C3	6C4	No	No	No	No	No	No	No	No	No	No
6C3	6C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C4	6C5	No	No	No	No	No	No	No	No	No	No

[1] Permutation refers to subsamples of complete inspections; 6C1 = one count of six counts (complete inspection), 6C2 = two counts (subsample) of six counts (complete inspection)... 6C5 = five counts (subsample) of six counts (complete inspection)

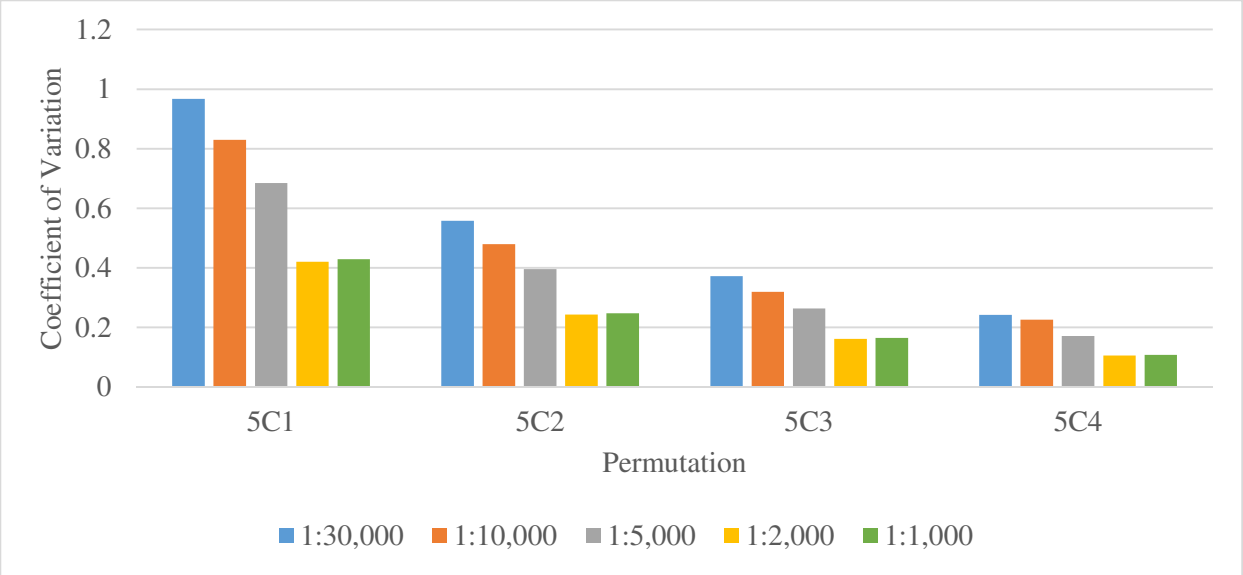

Figure 4.5. A comparison of the coefficients of variation of permutations generated from the validation experiment using United Kingdom inspection procedures and calculating coefficients of variation based on plant offtypes. The permutations nCr were developed using 1, 2, 3, or 4 counts (r) out of a possible five counts (n).

Tables 4.15 and 4.16 were compared with Table 4.17, further similarities were found when compared the coefficients of variations developed from permutations from the three different jurisdictions inspection procedures. When comparing 80% (5C4) of the inspection with 60% (5C3) of the inspection, no significant differences were detected in coefficients of variation existed between all contamination levels for both plant and spike measured offtypes. After comparing 80% (5C4) of the inspection with 40% (5C2) of the inspection, all differences were significant at all contamination levels for both spike and plant offtypes.

When comparing the North Dakota validation data with the United Kingdom data, there were some similarities. At an alpha-value of 0.05, comparing 80% of the inspection with 60% of the inspection for both jurisdictions showed an insignificant difference. Likewise, comparing 80% of the inspection for both jurisdictions with 40% showed a significant difference at an alpha value of 0.05. The Canadian permutations did not align exactly with either the North Dakota or United Kingdom permutations, but were still useful in comparing significant differences.

Examining the North Dakota data showed that regardless of contamination level, when one permutation was compared with another permutation that had a difference of two or three samples, they were not significantly different. Alternatively, if a permutation was compared with another permutation with a difference of three or more samples, they were significantly different at an alpha value of 0.05. As the permutation level increased toward the maximum sampling level, the variation was reduced. As expected, this showed that as sampling increased towards the complete inspection, the risk of falsely accepting or rejecting a pedigreed seed field diminished.

Table 4.17. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the validation experiment using United Kingdom inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. An alpha value of 0.05 was used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with five degrees of freedom.

		Treatments									
Permutations[1] being compared		1:30,000		1:10,000		1:5,000		1:2,000		1:1,000	
		PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
5C1	5C2	No	No	No	No	No	No	No	No	No	No
5C1	5C3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5C1	5C4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5C2	5C3	No	No	No	No	No	No	No	No	No	No
5C2	5C4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5C3	5C4	No	No	No	No	No	No	No	No	No	No

[1] Permutation refers to subsamples of complete inspections; 5C1 = one count of five counts (complete inspection), 5C2 = two counts (subsample) of five counts (complete inspection)... 5C4 = four counts (subsample) of five counts (complete inspection)

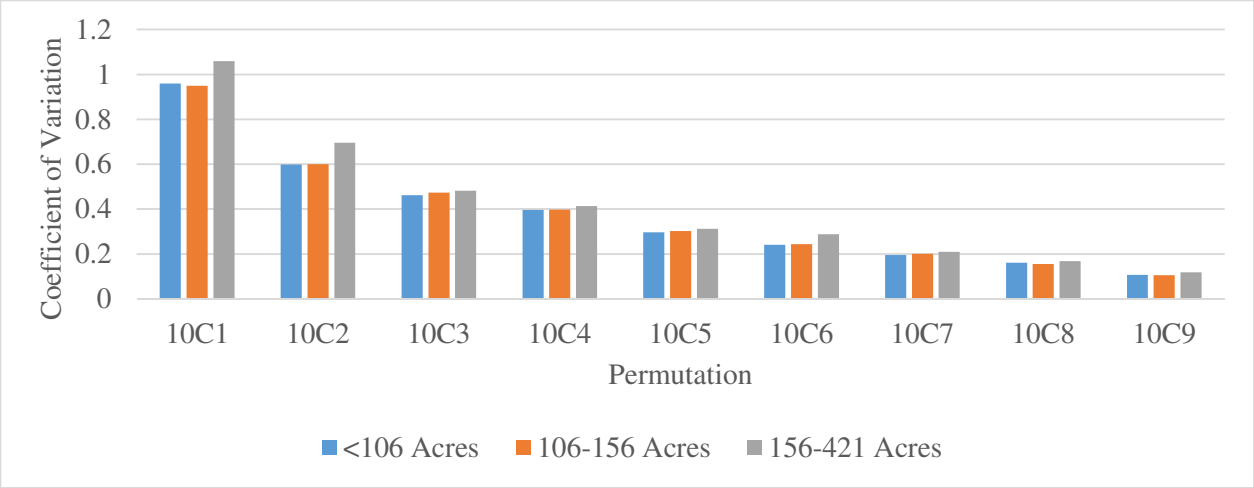

Figure 4.6. A comparison of the coefficients of variation of permutations generated from the Farmers Edge inspections using North Dakota inspection procedures and calculating coefficients of variation based on plant offtypes. The permutations nCr were developed using 1, 2, 3, ..., or 9 counts (r) out of a possible ten counts (n).

Table 4.15 and Table 4.18 represent data collected from the validation experiment and from pedigreed seed fields from Farmers Edge, respectively, using ten counts as recommended by the North Dakota inspection process. The most important component of the tables to examine was whether the 10C9 was significantly different with the other permutations. Comparing 10C9 with 10C8 for both Tables 4.15 and 4.18, all values for the validation experiment were significantly different, while in the Farmers Edge permutation data, half of the values were not significantly different. The largest acreage class and the spike measured offtypes for the middle acreage class showed an insignificant difference, but the smaller acreage class, as well as the plant measured offtypes for the middle acreage class, showed a significant difference. One reason for the difference is that different degrees of freedom were used for both tables. Table 4.15 used five degrees of freedom because there were six inspections completed for each contamination level and Table 4.18 used twelve degrees of freedom since each acreage class had thirteen different fields inspected.

Table 4.18: A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the Farmers Edge inspections using North Dakota inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. An alpha value of 0.05 was used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with twelve degrees of freedom.

Permutations[1] being compared		<106 Acres		106-156 Acres		156-421 Acres	
		PLT	SPK	PLT	SPK	PLT	SPK
10C1	10C2	No	No	No	No	No	No
10C1	10C3	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C4	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C5	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C6	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C7	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C8	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C3	No	No	No	No	No	No
10C2	10C4	No	No	No	No	Yes	Yes
10C2	10C5	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C6	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C7	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C8	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C4	No	No	No	No	No	No
10C3	10C5	Yes	Yes	Yes	No	Yes	Yes
10C3	10C6	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C7	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C8	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C5	No	No	No	No	No	No
10C4	10C6	Yes	Yes	Yes	Yes	No	No
10C4	10C7	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C8	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C5	10C6	No	No	No	No	No	No
10C5	10C7	Yes	No	Yes	No	Yes	Yes
10C5	10C8	Yes	Yes	Yes	Yes	Yes	Yes
10C5	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C6	10C7	No	No	No	No	No	No
10C6	10C8	Yes	No	Yes	Yes	Yes	Yes
10C6	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C7	10C8	No	No	No	No	No	No
10C7	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C8	10C9	Yes	Yes	Yes	No	No	No

[1] Permutation refers to subsamples of complete inspections; 10C1 = one count of ten counts (complete inspection), 10C2 = two counts (subsample) of ten counts (complete inspection)... 10C9 = nine counts (subsample) of ten counts (complete inspection)

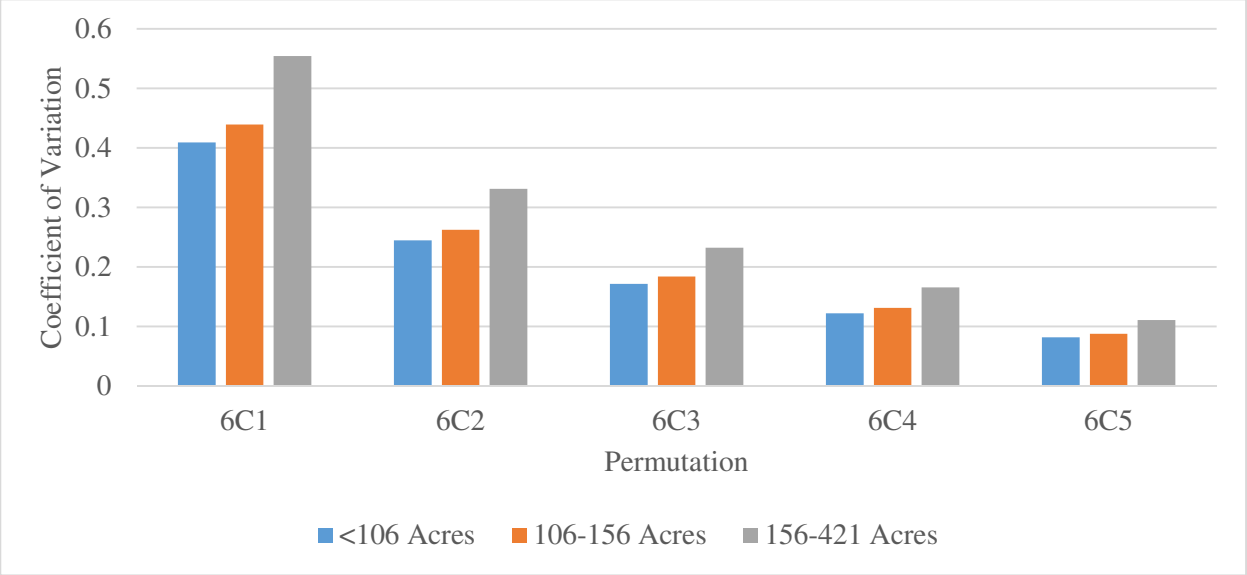

Figure 4.7. A comparison of the coefficients of variation of permutations generated from the Farmers Edge inspections using Canadian inspection procedures and calculating coefficients of variation based on plant offtypes. The permutations nCr were developed using 1, 2, 3, 4, or 5 counts (r) out of a possible six counts (n).

When comparing Tables 4.16 with Table 4.19, there were a few differences similar to comparing Tables 4.15 and 4.18. Table 4.19 showed all but the spike measured offtypes in the small acreage class of fields had significant differences when 6C4 and 6C5 were compared. This differed from the results from Table 4.16 which showed all insignificant differences. Having an increased number of degrees of freedom in Table 4.19 likely contributed to the differences seen between Tables 4.16 and 4.19.

Table 4.19. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the Farmers Edge inspections using Canadian inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. An alpha value of 0.05 was used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with twelve degrees of freedom.

Permutations[1] being compared		<106 Acres		106-156 Acres		156-421 Acres	
		PLT	SPK	PLT	SPK	PLT	SPK
6C1	6C2	Yes	Yes	Yes	Yes	Yes	Yes
6C1	6C3	Yes	Yes	Yes	Yes	Yes	Yes
6C1	6C4	Yes	Yes	Yes	Yes	Yes	Yes
6C1	6C5	Yes	Yes	Yes	Yes	Yes	Yes
6C2	6C3	No	No	No	No	No	No
6C2	6C4	Yes	Yes	Yes	Yes	Yes	Yes
6C2	6C5	Yes	Yes	Yes	Yes	Yes	Yes
6C3	6C4	No	No	No	No	No	No
6C3	6C5	Yes	Yes	Yes	Yes	Yes	Yes
6C4	6C5	Yes	No	Yes	Yes	Yes	Yes

[1] Permutation refers to subsamples of complete inspections; 6C1 = one count of six counts (complete inspection), 6C2 = two counts (subsample) of six counts (complete inspection)... 6C5 = five counts (subsample) of six counts (complete inspection)

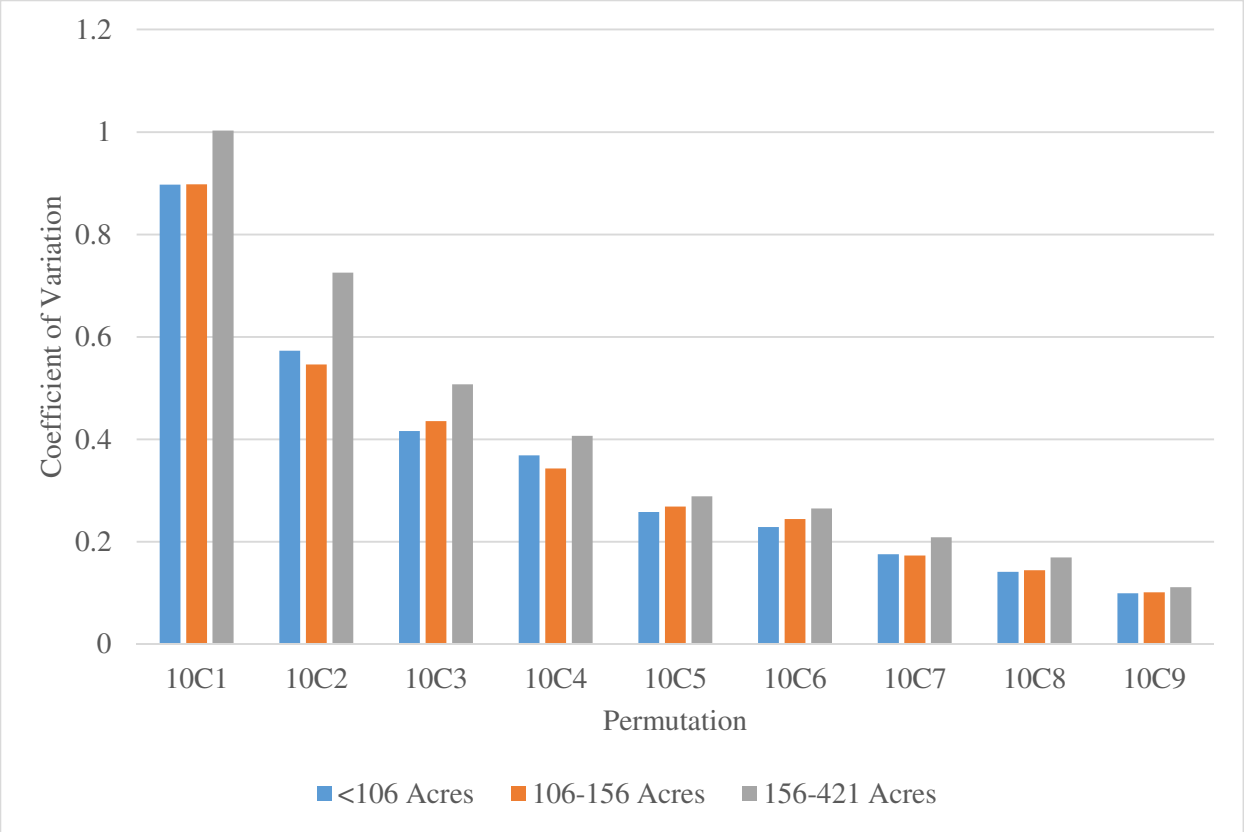

Figure 4.8. A comparison of the coefficients of variation of permutations generated from the Farmers Edge inspections using United Kingdom inspection procedures and calculating coefficients of variation based on spike offtypes. The permutations nCr were developed using 1, 2, 3, ..., or 9 counts (r) out of a possible ten counts (n).

Tables 4.17 and 4.20 have different permutations because the United Kingdom uses sequential sampling differentiating their number of counts when field size becomes larger than three hectares (7.41 acres). Fields less than three hectares require five counts of $20m^2$, while fields greater than three hectares require ten counts of $20m^2$. All inspections on the validation experiment were less than three hectares, while the smallest inspection completed for Farmers Edge was 19.4 hectares (48 acres).

Table 4.20 is better compared to Tables 4.15 and 4.18 because each of those tables were generated from ten counts, rather than five counts. After comparing 10C8 with 10C9, both the plant and spike measured offtypes for the smaller and middle acreage classes showed insignificant differences at an alpha value of 0.05, while the larger acreage class showed significant differences

for both the plant and spike measured offtypes. This was different from Table 4.18 where both the plant and spike measured offtypes in the largest acreage class was not significant, along with the spike measured offtypes in the middle acreage class when comparing 10C9 with 10C8.

When Tables 4.18-4.20 are compared, there were some unique trends when the highest percentage of the inspection (6C5 or 10C9) was compared with the second highest percentage of the inspection (6C4 or 10C8). At an alpha value of 0.05 some tables showed different significance results at different acreage sizes.

At the smallest acreage class, the North Dakota (Table 4.18) procedures showed a significant difference when 10C9 and 10C8 were compared, while the United Kingdom procedures showed no significant difference when 10C9 and 10C8 were compared. The Canadian procedures results showed that plant measured offtypes were significantly different when 6C5 and 6C4 were compared.

At the middle acreage class, the United Kingdom (Table 4.20) procedures resulted in insignificant differences, while the North Dakota (Table 4.18) procedures showed that plant measured offtypes were significantly different and spike measured offtypes were not significantly different when 10C9 and 10C8 were compared. The Canadian (Table 4.19) procedures showed significantly different results when 6C5 and 6C4 were compared in the middle acreage class.

For the largest acreage class, both the Canadian (Table 4.19) and United Kingdom (Table 4.20) inspection procedures showed significant differences when the highest and second highest percentage of the inspection were compared. The North Dakota (Table 4.18) inspection procedures showed the opposite where 10C9 and 10C8 were not significantly different.

For some of the fields with low levels of contamination, high coefficients of variation were observed. For instance, an inspection of a field of Pasteur wheat had a low level of contamination

with a higher coefficient of variation. For the Canadian inspection of the Pasteur field, the counts yielded zero, one, zero, two, zero, and zero plant offtypes in the six counts which yielded a coefficient of variation of 1.6. Both the North Dakota and United Kingdom inspections of the Pasteur field yielded two counts with one plant each and eight counts of zero detected plant offtypes. When the majority of counts were zero with one counts showing one or more offtypes with a specific standard deviation, a higher coefficient of variation will occur than if the majority of the counts have the same standard deviation, but have a higher mean. This could be why the higher acreage class showed significant differences between the most complete permutations (10C9 and 10C8 or 6C5 and 6C4) as some of these inspections had very low average offtypes per inspection, increasing the coefficient of variation for those inspections.

Table 4.20. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the Farmers Edge inspections using United Kingdom inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. An alpha value of 0.05 was used to determine if the permutations were statistically significant (Yes) or not statistically significant (No) with twelve degrees of freedom.

Permutations[1] being compared		<106 Acres		106-156 Acres		156-421 Acres	
		PLT	SPK	PLT	SPK	PLT	SPK
10C1	10C2	No	No	No	No	No	No
10C1	10C3	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C4	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C5	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C6	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C7	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C8	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C3	No	No	No	No	No	No
10C2	10C4	Yes	No	Yes	Yes	Yes	Yes
10C2	10C5	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C6	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C7	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C8	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C4	No	No	No	No	No	No
10C3	10C5	Yes	Yes	Yes	Yes	Yes	Yes

10C3	10C6	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C7	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C8	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C5	No	No	No	No	No	No
10C4	10C6	Yes	Yes	No	No	Yes	Yes
10C4	10C7	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C8	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C5	10C6	No	No	No	No	No	No
10C5	10C7	No	No	Yes	Yes	No	No
10C5	10C8	Yes	Yes	Yes	Yes	Yes	Yes
10C5	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C6	10C7	No	No	No	No	No	No
10C6	10C8	Yes	Yes	Yes	Yes	Yes	Yes
10C6	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C7	10C8	No	No	No	No	No	No
10C7	10C9	Yes	Yes	Yes	Yes	Yes	Yes
10C8	10C9	No	No	No	No	Yes	Yes

[1] Permutation refers to subsamples of complete inspections; 10C1 = one count of ten counts (complete inspection), 10C2 = two counts (subsample) of ten counts (complete inspection)... 10C9 = nine counts (subsample) of ten counts (complete inspection)

Table 4.21. A summary of the statistical significance of when two coefficients of variations calculated using permutations[1] developed from Appendixes 8.2-8.7. Using alpha values of 0.01, 0.05, and 0.1, the least complete permutations[1] not significantly different from the most complete permutation[1] are listed below to compare the effects that different alpha values have on permutation[1] significance.

Offtype Frequency	Canadian Validation - Comparing with 6C5 (Most Complete Inspection)						North Dakota Validation - Comparing with 10C9 (Most Complete Inspection)						United Kingdom Validation - Comparing With 5C4 (Most Complete Inspection)					
	α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01	
	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
1:30,000	6C4	6C4	6C4	6C4	6C2	6C2	10C8	10C8	10C8	10C8	10C5	10C5	5C4	5C4	5C3	5C3	5C1	5C1
1:10,000	6C5	6C5	6C4	6C4	6C3	6C2	10C9	10C9	10C8	10C8	10C5	10C5	5C3	5C3	5C3	5C3	5C2	5C1
1:5,000	6C5	6C5	6C4	6C4	6C3	6C3	10C9	10C9	10C8	10C8	10C5	10C5	5C4	5C4	5C3	5C3	5C2	5C2
1:2,000	6C5	6C5	6C4	6C4	6C3	6C3	10C8	10C8	10C8	10C8	10C5	10C5	5C4	5C4	5C3	5C3	5C2	5C2
1:1,000	6C5	6C5	6C4	6C4	6C3	6C3	10C8	10C8	10C8	10C8	10C6	10C6	5C4	5C4	5C3	5C3	5C2	5C2

[1] Permutation refers to subsamples of complete inspections; 6C1 = one count of six counts (complete inspection), 6C2 = two counts (subsample) of six counts (complete inspection)... 6C5 = five counts (subsample) of six counts (complete inspection)

Table 4.22. A summary of the statistical significance of when two coefficients of variations calculated using permutations developed from Appendixes 8.8-8.10. Using alpha values of 0.01, 0.05, and 0.1, the least complete permutations[1] not significantly different from the most complete permutation[1] are listed below to compare the effects that different alpha values have on permutation[1] significance.

Field Size (Acres)	Canadian Farmers Edge – Comparing With 6C5 (Most Complete Inspection)						North Dakota Farmers Edge – Comparing with 10C9 (Most Complete Inspection)						United Kingdom Farmers Edge – Comparing With 10C9 (Most Complete Inspection)					
	α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01	
	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
<106	6C5	6C5	6C5	6C5	6C4	6C4	10C9	10C9	10C9	10C9	10C7	10C8	10C9	10C9	10C8	10C8	10C7	10C7
106-156	6C5	6C5	6C5	6C5	6C4	6C4	10C9	10C9	10C9	10C8	10C8	10C8	10C9	10C9	10C8	10C8	10C7	10C7
156-421	6C5	6C5	6C5	6C5	6C4	6C4	10C9	10C9	10C8	10C8	10C7	10C7	10C9	10C9	10C9	10C9	10C8	10C8

[1] Permutation refers to subsamples of complete inspections; 6C1 = one count of six counts (complete inspection), 6C2 = two counts (subsample) of six counts (complete inspection)... 6C5 = five counts (subsample) of six counts (complete inspection)

The results of the permutation analysis can vary based on different alpha values. When alpha values are lowered from the scientific standard of 0.05 to 0.01, the probability of making a Type I error (probability of falsely rejecting a pedigreed seed field) are decreased, but the power of the test is decreased as well. When the alpha value is lowered to 0.01, the probability of making a Type II (probability of falsely accepting a pedigreed seed field) error is increased. If the alpha value is increased from the scientific standard of 0.05 to 0.1, the probability of making a Type I error and the power of the test increases. When the alpha value increases to 0.1, the probability of making a Type II error decreases. In Appendixes 8.2-8.10, the Type I error refers to the probability of falsely concluding that two permutations coefficients of variation are significantly different. The Type II error in Appendixes 8.2-8.10 refers to the probability of falsely concluding that the coefficients of variation of two permutations are not significantly different.

Tables 4.21 and 4.22 summarize the results of Appendixes 8.2-8.10 showing the lowest permutations coefficient of variation that is insignificantly different with the highest permutations coefficient of variation using different alpha values and jurisdictions sampling protocols. When the alpha value is 0.1, significant differences in offtype detection are seen with only slightly less sampling. However, when the alpha value is lowered to 0.01, much larger reductions in sampling are needed to declare significant differences from the complete inspection.

If a jurisdiction was going to change the amount of sampling it does in a pedigreed seed field, it must decide whether it is better to balance Type I and Type II errors or if they want to minimize either Type I or Type II errors, while simultaneously raising the other type of error (Type I or Type II). When determining whether pedigreed seed inspection procedures need to be changed, the jurisdiction should decide if they want to minimize the chance of falsely concluding that two permutations are significantly different or not different, agreeing with past literature

(Rouse, 2009). If the jurisdiction decided their goal is to increase the efficiency of the inspection and are willing to sacrifice accuracy by reducing the amount of sampling, they may consider using a lower alpha value (0.01). In this scenario, the lower alpha value decreases the risk of incorrectly concluding that two permutations are different. With a higher Type II error, this jurisdiction will have a greater risk of falsely concluding two permutation coefficients of variations are the same. This jurisdiction favours less sampling locations per field meaning inspectors will spend less time per field, potentially saving money for either the pedigreed seed grower or the inspection association affiliated with that jurisdiction.

Alternatively, if a jurisdiction chooses to place accuracy as its highest priority and is willing to sacrifice decreased sampling, a reduction in the Type II error is desirable, which means increasing the alpha value from 0.01 to 0.05 or 0.1. In this scenario, the jurisdiction is electing for inspection accuracy and potentially increased inspection in order to truthfully determine whether a field should be accepted or rejected. This jurisdiction could face larger inspection fees as more time will be needed to inspect a given pedigreed seed field.

If a jurisdiction opts for a more balanced approach using the scientific standard alpha value of 0.05, they likely could reduce sampling by one permutation, but may want to keep their existing standards to maintain the current coefficient of variation. Tables 4.21 and 4.22 show a decrease by one permutation level from the highest permutation level to be insignificantly different in most contamination levels and field sizes at an alpha value of 0.05.

All of the forty-three fields that were sampled showed that the offtypes were evenly distributed. No varietal patches were found, however there were patches of weeds and other species detected. These were all found within 100m of the outer boundaries of the fields. There were some fields that had Nodding Thistle, a prohibited noxious weed, within 2m of the outskirts

of the pedigreed seed field. One field had a very high wild oat population along one entire side of a square 160 acre quarter section due to a neighbouring farmer dragging their harrows from their field into the pedigreed seed field during the previous fall. Another field had awned wheat at one end of a pedigreed barley field due to a neighbouring farmer dragging wheat straw onto the pedigreed seed field from the previous fall and contaminating the barley field. Previous research agrees with these results and has shown more problems near right-of-way's or field edges (Rew et al., 2006).

The inspections performed in the validation experiment and in the forty-three Manitoba seed fields showed some interesting conclusions. The Canadian inspection procedures inspected the most area of the field in both the Farmers Edge inspections and in the validation experiment, demonstrating the Canadian standards have a higher probability of accurately evaluating a pedigreed seed field over North Dakotan or United Kingdom inspection procedures. The results were similar when plant offtypes were compared to spike offtypes and this had little effect on the time required to measure plant density. Previous literature suggests Canadian inspections are the most thorough because the Canadian inspection consistently sampled the greatest proportion of the field (Rew et al. (2007). The permutation analysis showed that as you reduce the number of counts, the accuracy of the inspection is diminished for the three jurisdictions as well as different contamination levels.

5 Theoretical Modelling of Genetic Purity in Subsequent Pedigreed Seed Generations

5.1 Abstract

Predicting the frequency of *Triticum aestivum* L. offtypes in the current and future generations is important for breeders, pedigreed seed growers and inspectors, commercial wheat growers, processors, and consumers. Pollen migration into pedigreed wheat fields can contaminate seed fields in future generations and can push the offtype frequency beyond the rejection threshold based on current Canadian Seed Growers' Association pedigreed seed standards. Additionally, if the contaminant has a selective advantage, the frequency of offtypes may increase in subsequent generations, unless roguing takes place to remove the offtypes. With many factors influencing the contamination rate, this paper compared the effects of different selective advantages, outcrossing rates, and the relationship between the field edge, where contamination can occur and the size of the field. Moreover, the probabilities of detecting higher or lower levels of offtypes were calculated and graphed to determine the likelihood of accurately rejecting or accepting pedigreed seed fields at varying contamination levels. As the length of the field edge between two different cultivars increased relative to the area of the field, the potential damage caused by foreign pollen migration increases. With different inheritances, recessive offtype alleles will be masked in the next generation after contamination occurs, but would show up in later generations, potentially causing pedigreed seed fields to miss purity standard targets depending on the initial contamination rate.

5.2 Introduction

The rules governing seed inspection may differ depending on the mode of reproduction and morphology of the crop species. This is seen when comparing cross-pollinated species with self-pollinated species. In Canada, self-pollinated crops such as wheat, barley, and soybeans have five pedigreed seed levels (Breeder, Select, Foundation, Registered, and Certified) where cross-

pollinated species such as canola, rye, corn, and hemp often only use three levels (Breeder, Foundation, and Certified). Self-pollinated species exhibit lower levels of outcrossing than cross-pollinated species which is why the Circular 6 document specifies different isolation distances for different crops (CSGA, 2017).

Genetic purity is very important to a large portion of consumers who do not want to consume genetically modified organisms. Genetic purity is also important to breeders, seed growers, farmers, and processors for non-GM crops or organic crops. Ensuring genetic purity is more difficult when working with cross-pollinated crops as opposed to self-pollinated crops. Many cross-pollinated crops like corn have herbicide-resistant genes as a result of genetic modification of their genomes. Consumer resistance to genetically modified organisms continues to delay the widespread use of genetically-engineered wheat cultivars, but the study of gene-flow among wheat cultivars is useful for predicting the detection of offtypes in pedigreed wheat (Brûlé-Babel et al., 2006).

Outcrossing is determined by several factors including the genotype of the pollen donor and the pollen receiver, environmental conditions such as humidity, temperature, and wind speed and direction, and the distance viable pollen grain can travel, which is why outcrossing rates are different between different species. Outcrossing is one way foreign genetics can contaminate pedigreed seed. One issue with outcrossing is that genetically modified traits can infiltrate non-genetically modified crops (Salisbury, 2002; Gilbert, 2010). Outcrossing is also a concern for pedigreed seed production. Minimizing outcrossing for pedigreed seed production is important to retain genetic purity in cultivars bred by breeders to have specific traits. Outcrossing can be a result from wind-blown or insect-carried foreign pollen. Outcrossing is undesirable for the pedigreed seed industry as many issues can arise from contaminated seed. Intellectual property

infringements are one issue with outcrossing of genetically modified crops. The investment in genetically modified crops by companies is large and they want to see their investment protected.

Volunteer grain from previous crops, railways, roadways, rock piles, and even waterways can contaminate pedigreed seed fields (North Dakota, 2012; Hucl et al., 2004). Volunteer contamination usually produces an uneven distribution of offtypes in pedigreed seed fields. Volunteer contamination can be costly to seed growers as they can have their fields rejected if contamination is above the threshold for pedigree status. In addition, contamination can be introduced to a pedigreed seed field via combines, augers, trucks, seeders, and seed cleaning equipment. The effect of gene flow from genetically modified (GM) wheat into non-GM wheat was modelled by Brûlé-Babel et al (2006). Using Hardy-Weinberg equations, the effect of herbicide tolerant gene transfer was evaluated in multiple generations of non-gm wheat using different outcrossing rates and a selective advantage of 95% to estimate the effect of a herbicide tolerant wheat plant in a non-herbicide tolerant wheat field.

Genetic purity is of great importance, especially in the context of genetically modified foods. Canada has already seen markets close due to contamination of genetically modified crops. For example, Europeans were turning away Canadian flax shipments due to small amounts of a genetically modified flax called CDC Triffid. CDC Triffid was found in minute quantities (1:10,000) and was enough to surpass Europe's zero tolerance policy (Flax Council of Canada, 2014). CDC Triffid was legal to grow in Canada for just a few years before it was banned in 2000. Nine years later, CDC Triffid was still found in Canadian flax which put the $320 million industry in jeopardy (Franz-Warkentin, 2012). One of the sources of contamination of CDC Triffid was the result of contaminated breeder seed at the University of Saskatchewan's Crop Development Centre (Dawson, 2010).

As predicted by Beckie et al (2003), volunteer herbicide resistant canola has become problematic on the Canadian Prairies (Beckie et al., 2003). There are three major types of herbicide resistant canola on the market. Herbicide resistant canola cultivars dominate the canola acres in western Canada and provide resistance to either glyphosate, glufosinate, or imazamox. Gilbert (2010) took 288 volunteer canola plants from the side of a North Dakota road and tested for herbicide resistance. From this study, 41% of the plants were resistant to glyphosate and 40% of the plants were resistant to glufosinate. The study also found that two out of the 288 tested plants were resistant to both glyphosate and glufosinate which meant contamination from a neighbouring field provided the dual resistance genes as the cultivars were not bred this way. This could be problematic in a field setting if the plants were resistant to both herbicides as farmers rely on both herbicides to control weeds and spray other herbicide resistant crops like corn or soybeans. It is very important to farmers that pedigreed canola producers ensure herbicide resistant stacking does not occur in their cultivars (Owen and Zelaya, 2005).

The objectives of this study were to: 1) Model the effect offtype gene flow has on future generations of pedigreed wheat, and 2) to examine the effect selection of superior offtype genetics can have on future generations of pedigreed wheat.

5.3 Materials and Methods

To evaluate the effect offtypes may have on future generations through higher migration rates (pollen-flow from neighbouring wheat fields) or different selection pressures, gene frequencies were calculated over several generations to predict the effect on phenotypic expression using different initial offtype frequencies, migration rates, selection rates, and offtype gene expressions (dominant or recessive).

5.3.1 Migration of Foreign Pollen in Pedigreed Wheat Fields

In order to follow the generational effect of offtype pollen migration rate on a pedigreed wheat field, equations were used from Brûlé-Babel et al (2006). Models were developed to predict the effect gene flow from genetically modified, herbicide-tolerant wheat has on non-herbicide-tolerant wheat gene frequencies (Brûlé-Babel et al., 2006). The frequency of wheat pollinations from the migrant population (m) ranged from 0.25% to 4% were used to evaluate the effect offtype pollen would have after entering pedigreed wheat fields. Within the non-migrant population, the selfing rate (S) was 98%. P_t, H_t, and Q_t are the genotypic frequency of generation *t* of A_1A_1, A_1A_2, and A_2A_2 respectively, while P_{t+1}, H_{t+1}, and Q_{t+1} are the frequencies of A_1A_1, A_1A_2, and A_2A_2 in generation (*t+1*). P_m, H_m, and Q_m are the frequencies of A_1A_1, A_1A_2, and A_2A_2 in the migrant population. Initial offtype frequency ranged from 1:30,000 to 1:1,000, which mimicked the treatment contaminations from the validation experiment. These frequencies were based on Canada's acceptance levels in *Triticum aestivum* L. pedigreed seed inspections. Equations defining P_{t+1}, H_{t+1}, and Q_{t+1} for migration mediated gene flow are listed below.

Equation 1: The genotypic frequency of A_1A_1 in generation (t+1).

$$P_{t+1} = m(P_m + 0.5H_m)(P_t + 0.5H_t) + (1 - m)[S(P_t + 0.25H_t) + (1 - S)(P_t + 0.5H_t)^2]$$

Equation 2: The genotypic frequency of A_1A_2 in generation (t+1).

$$H_{t+1} = m[(P_m + 0.5H_m)(Q_t + 0.5H_t)] + m[(Q_m + 0.5H_m)(P_t + 0.5H_t)] + (1 - m)\{S(0.5H_t) + 2(1 - S)[(P_t + 0.5H_t)(Q_t + 0.5H_t)]\}$$

Equation 3: The genotypic frequency of A_2A_2 in generation (t+1).

$$Q_{t+1} = m(Q_m + 0.5H_m)(Q_t + 0.5H_t) + (1 - m)[S(Q_t + 0.25H_t) + (1 - S)(Q_t + 0.5H_t)^2]$$

5.3.2 Selection for Offtype Phenotypes

Brûlé-Babel et al (2006) modelled the effect selection pressure of a herbicide such as glyphosate would place on volunteer glyphosate tolerant wheat in a non-herbicide tolerant field of

wheat. The study examined the effect a 95% selection pressure would place on the non-herbicide tolerant wheat field (Brûlé-Babel et al., 2006). In the case of non-herbicide tolerant wheat offtypes in non-herbicide tolerant wheat, the main concern is whether the offtype is more fit and produces more seeds per plant than the pedigreed seed wheat plants. Two relative fitness levels of 0.85 (pedigree seed crop produces 15% less seed than the offtype) and 0.7 were used as examples of common differences between cultivars within wheat classes such as within the Hard Red Spring Wheat Class (ex. Glenn wheat vs. AAC Cameron) or differences seen between two different cultivars in different wheat classes such as Canada Hard Red Spring wheat and Canada Northern Hard Wheat (ex. Glenn and Prosper, respectively). The effect of a superior (higher seeds/plant) offtype was modelled against an inferior pedigreed seed plant (lower seeds/plant). The relative fitness of the genotypes of A_1A_1 (*P*), A_1A_2 (*H*), *and* A_2A_2 (*Q*) are W_{11}, W_{12}, and W_{22} respectively. Equations defining the frequency of the A_1 allele $f(A_1)$ and A_2 allele $f(A_2)$ for selection mediated gene flow are listed below.

Equation 4: Frequency of A1 allele.

$$f(A_1) = (PW_{11} + 0.5(HW_{12}))/(PW_{11} + HW_{12} + QW_{22})$$

Equation 5: Frequency of A2 allele.

$$f(A_2) = (QW_{11} + 0.5(HW_{12}))/(PW_{11} + HW_{12} + QW_{22})$$

If we assume we have three genotypes: A_1A_1, A_1A_2, and A_2A_2, their frequencies in any given generation can be calculated as follows:

$$f(A_1A_1) = S\left[\frac{PW_{11} + 0.25(H)W_{12}}{\overline{W}}\right] + (1-S)\left[\frac{PW_{11} + 0.5(H)W_{12}}{\overline{W}}\right]^2$$

$$f(A_1A_2) = S\left[\frac{0.5(H)W_{12}}{\overline{W}}\right] + 2(1-S)\left[\frac{PW_{11} + 0.5(H)W_{12}}{\overline{W}}\right]\left[\frac{0.5(H)W_{12} + QW_{22}}{\overline{W}}\right]$$

$$f(A_2A_2) = S\left[\frac{QW_{22} + 0.25(H)W_{12}}{\overline{W}}\right] + (1-S)\left[\frac{QW_{22} + 0.5(H)W_{12}}{\overline{W}}\right]^2$$

Where:

S = The percentage selfing in the population.

1-S = The percentage of random mating in the population.

P = The frequency of A_1A_1 in the previous generation.

H = The frequency of A_1A_2 in the previous generation.

Q = The frequency of A_2A_2 in the previous generation.

$\overline{W}$ = The average fitness of the population.

W_{11} = The relative fitness of the A_1A_1 genotype.

W_{12} = The relative fitness of the A_1A_2 genotype.

W_{22} = The relative fitness of the A_2A_2 genotype.

5.3.3 Offtype Predictability

Determining the probability of correctly and incorrectly rejecting or accepting a pedigreed seed inspection is important to quantify in order to manage risk. Using the same data used to develop the Poisson distribution showing the probability of detecting k offtypes in Figure 2.5, a chart was developed to determine the probability of detecting more than k offtypes. All probabilities greater than k offtypes were added together to find the probability that the number of offtypes was greater than k.

5.4 Results and Discussion

The importance of managing risk in pedigreed wheat inspections is evident in its potential consequences of improperly accepting or rejecting pedigreed seed fields. Based on Figure 5.1, different contamination rates show different probabilities of falsely accepting a field at different k

thresholds. Since different pedigree levels will have different rejection thresholds, it is important to examine the different probabilities at these different thresholds. Tables 2.2 and 2.4 show the different contamination acceptance levels for offtypes, other crops, and weeds. The contamination rate of 1:30,000 is the North Dakota acceptance level for difficult to separate other crops such as barley or rye. The 1:10,000 contamination rate is the maximum acceptance rate for offtypes in fields producing Foundation seed in North Dakota and for fields producing Foundation or Registered seed in Canada. The 1:2,000 contamination rate is the maximum acceptance level for fields producing certified seed in both North Dakota and Canada. With six counts of 10,000 plants in Canada, the rejection thresholds are seven or more offtypes and thirty one or more offtypes in seed producing Registered seed or Certified seed respectively. If the rejection threshold is ten offtypes in 60,000 sampled plants, the probability of detecting more than ten offtypes at a contamination frequency of less than 1:30,000 is near zero. When the contamination frequency increases to 1:10,000, the probability of detecting ten offtypes in 60,000 sampled plants is less than 5%, but at a contamination frequency of 1:5,000 the probability of detecting more than ten offtypes is 65.3%. At higher contamination rates of 1:2,000 and 1:1,000, the probabilities of detecting greater than ten offtypes in 60,000 sampled plants are both greater than 99.99%.

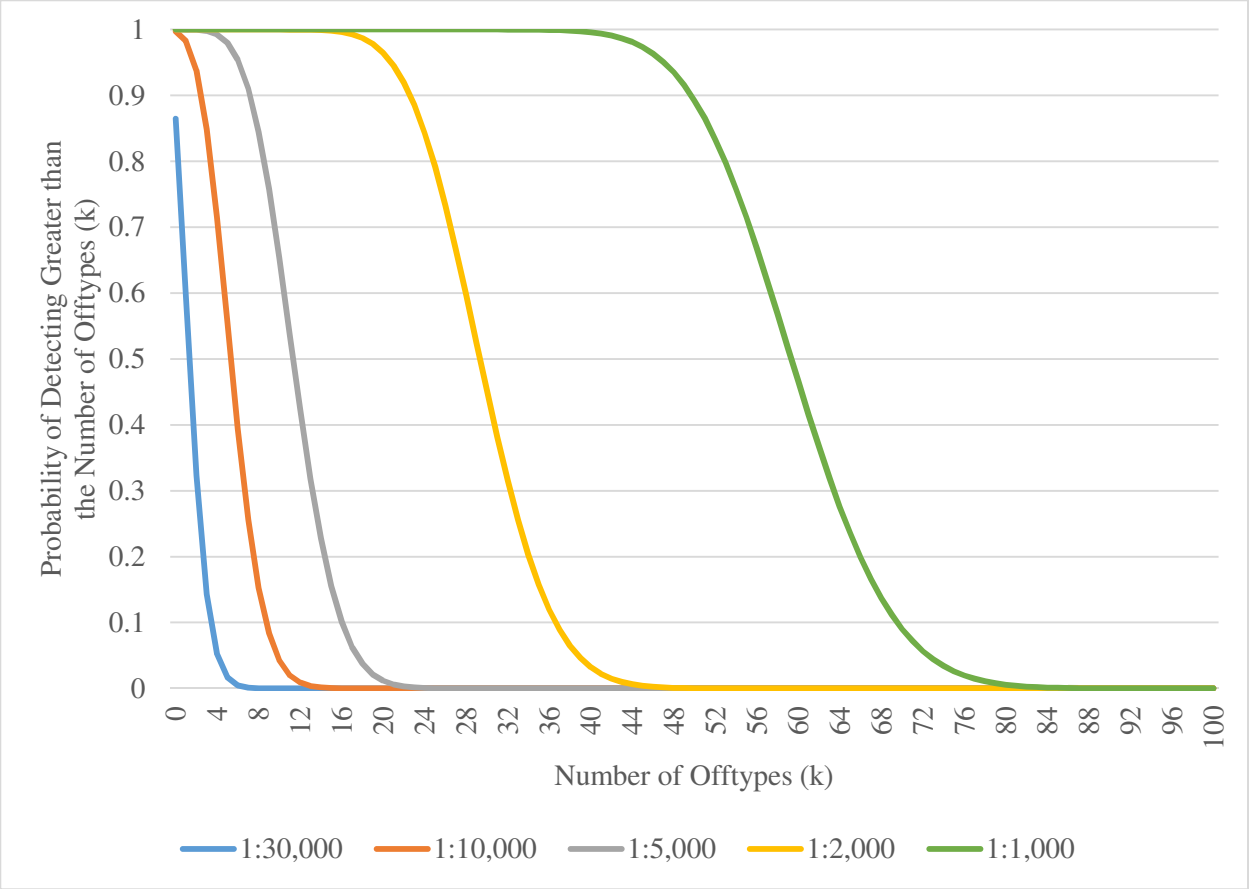

Figure 5.1. The probability of detecting more than k offtypes at five different offtype frequencies after sampling 60,000 plants.

Using Figure 5.1 to calculate the probability of detecting more offtypes than a specific offtype rejection threshold can lead to a field being either falsely or correctly rejected. Figure 5.1 is very important for evaluating risk and is also useful to evaluate detection levels over multiple generations since offtype frequencies will most likely not be static. Different pedigree classes and jurisdictions have varying offtype thresholds which is why it is important to compare different contamination frequencies with different offtype rejection thresholds. Many cultivars include additional variants to increase the rejection threshold which shows the usefulness of knowing the probability for all levels of *k* offtypes.

Figure 5.1 shows that when contamination rates are high, the probability of detecting more than the offtype threshold is high when 60,000 plants are sampled. If the offtype threshold is thirty-

one offtypes in 60,000 sampled plants, an inspector would have about a 50% chance of detecting thirty-one or more offtypes and about a 50% chance of detecting less than thirty-one offtypes if the contamination rate was 1:2,000.

Table 5.1. Possible contamination scenarios assuming a 3m contaminated area along the adjacent pollen donor side of different sizes of square or rectangular pedigreed seed fields.

Seed Field Length (m)	Seed Field Width (m)	Pollen Donor Adjacent Length (m)[1]	Contaminated Area (m^2)	Field Area (m^2)	% Area Contaminated
800	400	400	1200	320,000	0.00375
800	400	800	2,400	320,000	0.0075
800	800	400	1200	640,000	0.001875
800	800	800	2,400	640,000	0.00375
800	1,600	400	1200	1,280,000	0.000938
800	1,600	800	2,400	1,280,000	0.001875
1,600	400	400	1200	640,000	0.001875
1,600	400	800	2,400	640,000	0.00375
1,600	400	1,600	4,800	640,000	0.0075
1,600	800	400	1,200	1,280,000	0.000938
1,600	800	800	2,400	1,280,000	0.001875
1,600	800	1,600	4,800	1,280,000	0.00375
1,600	1,600	400	1,200	2,560,000	0.000469
1,600	1,600	800	2,400	2,560,000	0.000938
1,600	1,600	1,600	4,800	2,560,000	0.001875

[1] The distance where the pollen donor field runs parallel to the pedigreed seed field

Contamination from pollen-mediated gene flow can occur from fields of a different cultivar in close proximity. Table 5.1 shows the different field sizes and field dimensions with different adjacent contamination source field lengths. As the area of the pedigreed seed field increases relative to the length of the side of the field providing the foreign pollen, the contaminated area decreases. Lower levels of pedigreed seed tend to be produced on larger fields. If higher pedigree levels are grown on smaller fields, a small amount of contamination can have a larger impact on the genetic purity of the pedigreed seed field which can influence whether the next generations can meet the purity standards if that field is next to a field growing a different cultivar.

Depending on the cultivar providing the pollen contamination, different outcrossing rates and selective advantages are possible. Wind direction, speed, humidity, and temperature can also influence how much of a neighbouring field becomes contaminated. With outcrossing rates ranging from 0.2% to as high as 10% in different *Triticum aestivum* L. genotypes (Hucl and Matus-Cádiz, 2001), this is a major factor determining the frequency of contamination being spread to pedigreed seed fields. The distance the pollen travels is also important. Although a three meter isolation is required between pedigreed wheat fields of different varieties, foreign pollen can travel beyond the isolation distance (Hucl and Matus-Cádiz, 2001). Depending on the genetic traits in the foreign pollen, the heterozygotes in the next generation may or may not express the foreign pollens traits. If the contaminating pollen has alleles dominant to the pedigreed seed field it should show up in the next generation. Alternatively, if the alleles in the foreign pollen are recessive to the pedigreed seed field alleles, they will be expressed in the second generation as the traits will be masked in the heterozygote.

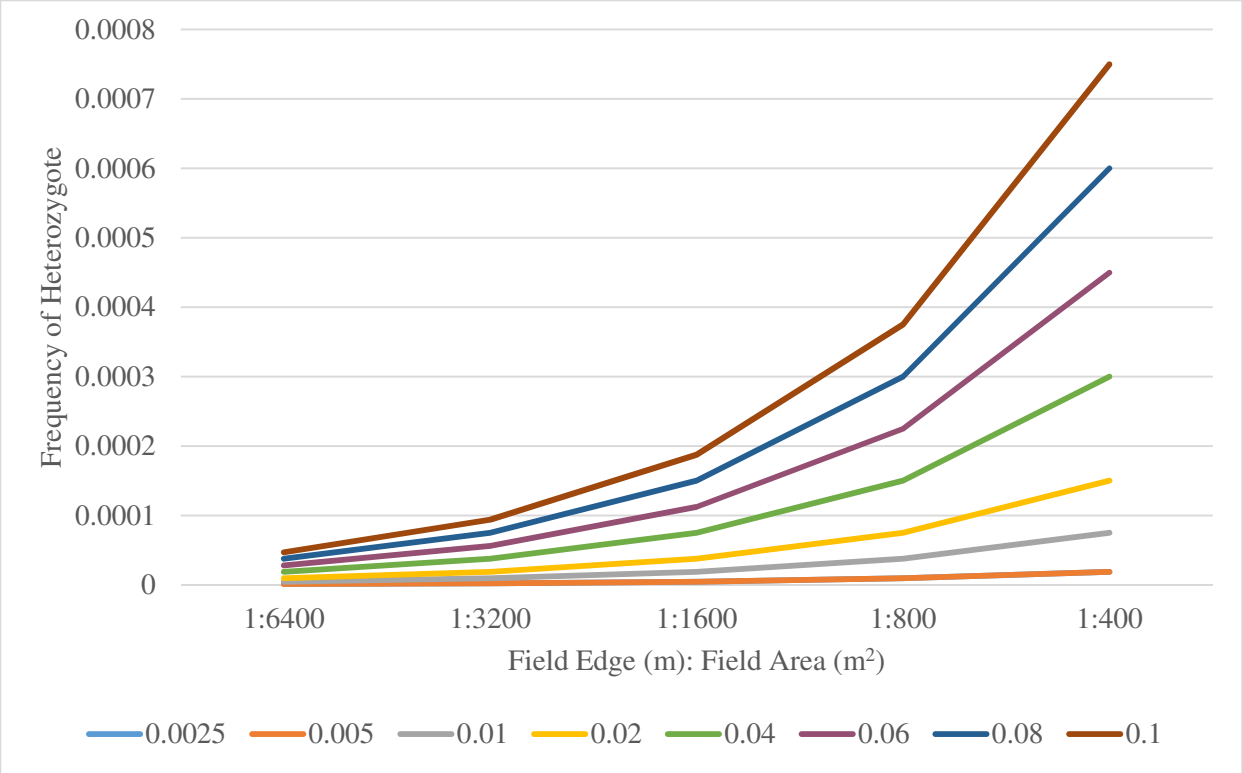

Figure 5.2. The frequency of heterozygotes in the next generation after contamination within 3m of one side of a pedigreed seed field from a different cultivar with outcrossing rates range from 0.25% to 10%.

As the edge of two fields of different cultivars grows relative to the field area, the chance of outcrossing from one cultivar into another grows. Figure 5.2 shows common field edge to field area ratios in Manitoba pedigreed seed fields. The chart assumes a pollen flow distance of 3m beyond the isolation distance with different outcrossing rates. The frequency of heterozygotes increases as the field edge increases. If an outcrossing event occurs from a neighbouring wheat field, there will be less of an effect if the field size is large and if the edge of the neighbouring field is small. In contrast, if the field size is small with a larger neighbouring field edge with another wheat cultivar, the frequency of heterozygotes in the next generation will be greater. Figure 5.2 uses examples of square or rectangular fields with one linear field edge. In reality, pedigreed seed fields have many different shapes and sizes, and both non-linear and linear field edges of varying lengths apart with at least 3m of space between two differing cultivars. In addition, the orientation

of a field edge may have an effect on outcrossing. For instance, if the field edge of a pollen donor is North or West of a pedigreed seed field, there could be more foreign pollen transferred into the pedigreed seed field than if the field edge was south or east of the pedigreed seed field due to the dominant direction of the prevailing winds. Higher pedigree levels of wheat (Breeder and Select) have a 10m isolation distance requirement to other wheat fields and have a restricted field size which would help reduce contamination from foreign pollen. In addition, field size is restricted in higher pedigreed wheat fields which helps ensure thorough roguing of offtypes and variants.

Depending on the allelic interaction of the contaminated heterozygote, the contaminant may not be detected until future generations. If the detectable traits of the contamination were dominant over the pedigreed cultivar, the heterozygote would be detectable in the next generation after the contamination event. If the heterozygote exhibited codominance, it should still be detectable in the pedigreed seed field. If the detectable traits from the contaminated wheat are recessive to the pedigreed cultivars traits, the heterozygote would have the same phenotype as the pedigreed seed field in the generation after the contamination event. However, homozygous recessive wheat plants will show the contaminated phenotype after seeding the first generation of wheat seed since the contamination. The recessive homozygotes would be detectable in the pedigreed seed field.

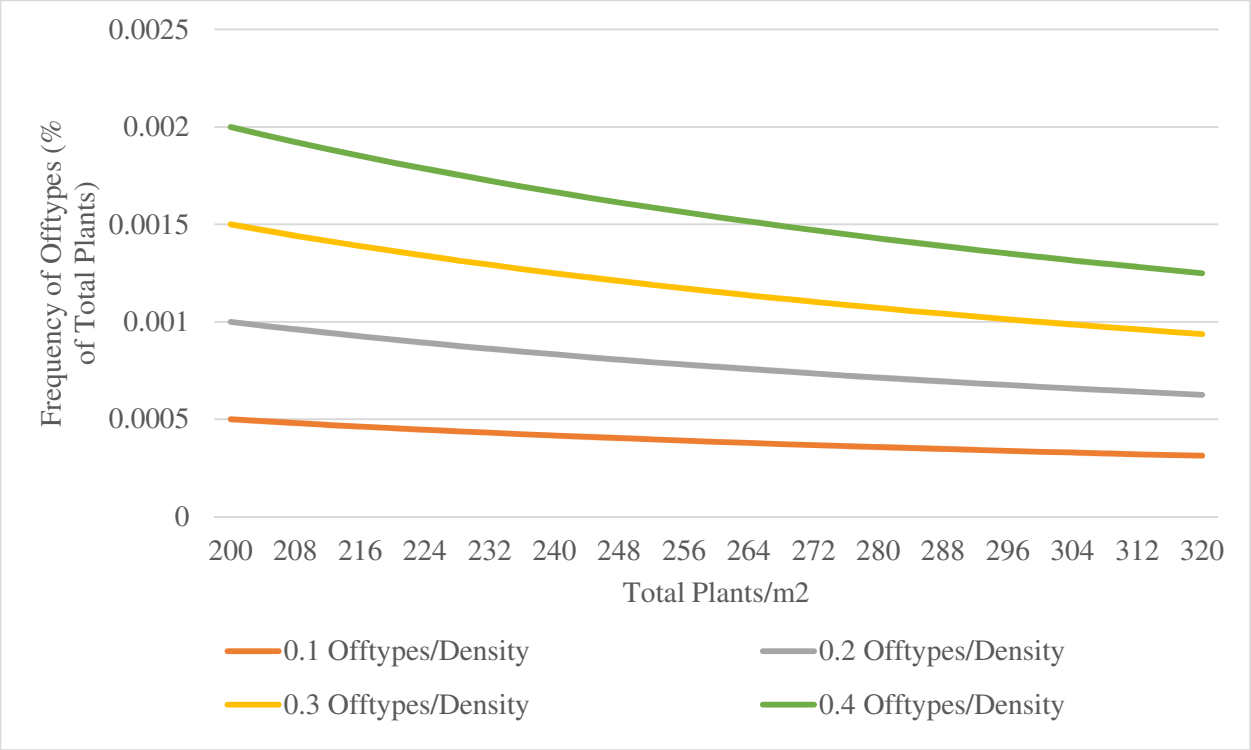

Figure 5.3. The frequency of offtypes germinated from dormant seed ranging in 0.1 to 0.4 offtypes/m^2 with total plant densities including offtypes and pedigreed plants ranging from 200 plants/m^2 to 320 plants/m^2.

If wheat seed from a previous crop germinates in a pedigreed seed field, it can contaminate the pedigreed seed field and lead to rejection if the offtype frequency surpasses the rejection threshold. Figure 5.3 shows the effect dormant wheat seed in the soil has on pedigreed wheat fields of varying plant densities commonly seeded in Manitoba (Manitoba, 2016). As plant density increases, the frequency of offtypes decreases as a percentage of the total plants. As pedigree level is decreased from breeder to Certified, plant density tends to increase, and often increases further when wheat is grown commercially. If Breeder seed is planted on a contaminated field, the frequency of offtypes relative to the pedigreed seed field would be much higher than if the same amount of contaminated seed was in a Registered seeded field as the plant density would be higher in the Registered seeded field.

MCVET trials show significant yield differences among cultivars in wheat classes, and larger differences between classes. For instance in Neepawa, MB, Glenn wheat, yielded 77 bushels/acre, AAC Cameron yielded 94 bushels/acre, and Prosper wheat yielded 110 bushels/acre (Seed Manitoba, 2016). Glenn and AAC Cameron wheat are both Canada Hard Red Spring wheat, while Prosper is a Canada Northern Hard Wheat. In Rosebank, AAC Cameron yielded 76 bushels/acre, Glenn yielded 72 bushels/acre, and Prosper yielded 92 bushels/acre (Seed Manitoba, 2016). This shows that a contamination of these different cultivars may have different effects depending on the location and that location's environmental conditions. It is important to predict the effect of contamination from a cultivar which produces more seeds per plant than the pedigreed seed field has on future generations.

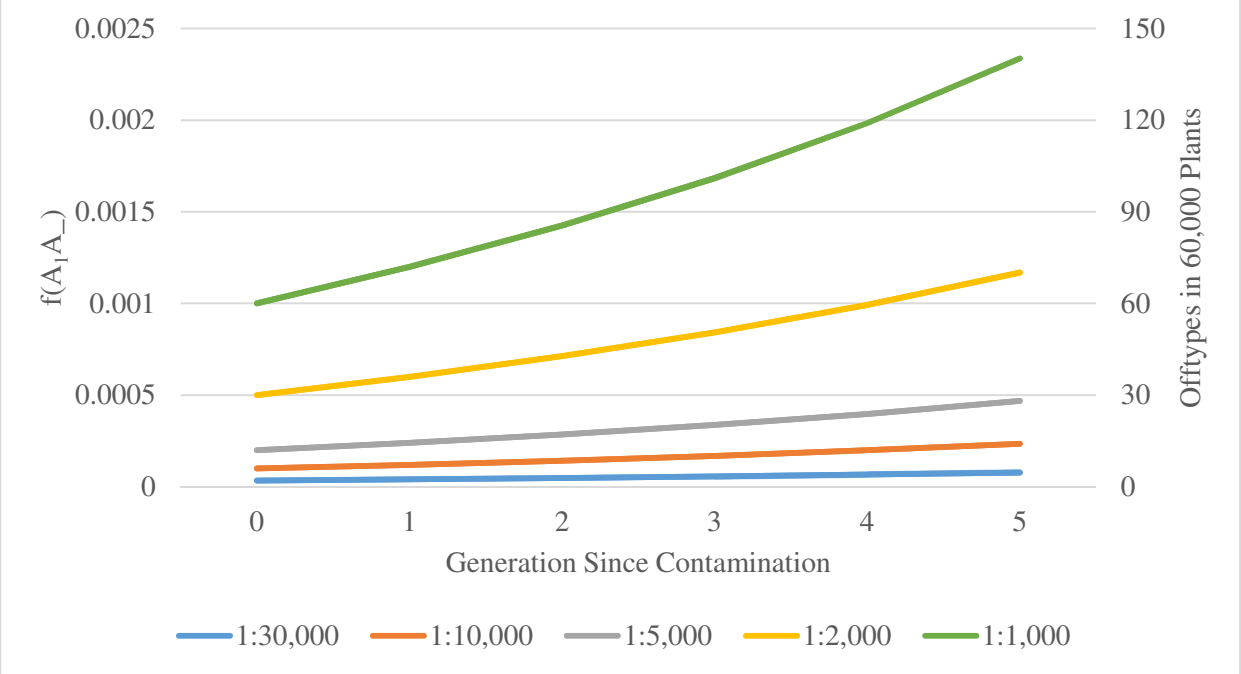

Figure 5.4. A chart used to examine the effect of selection for A_1A_1 or A_1A_2 offtypes over an A_2A_2 field when the relative fitness of A_1A_1 and A_1A_2 is 1.0 and A_2A_2 is 0.85 when initial contamination rates range from 1:30,000 to 1:1,000 A_1A_1 plants and an outcrossing rate of 2%.

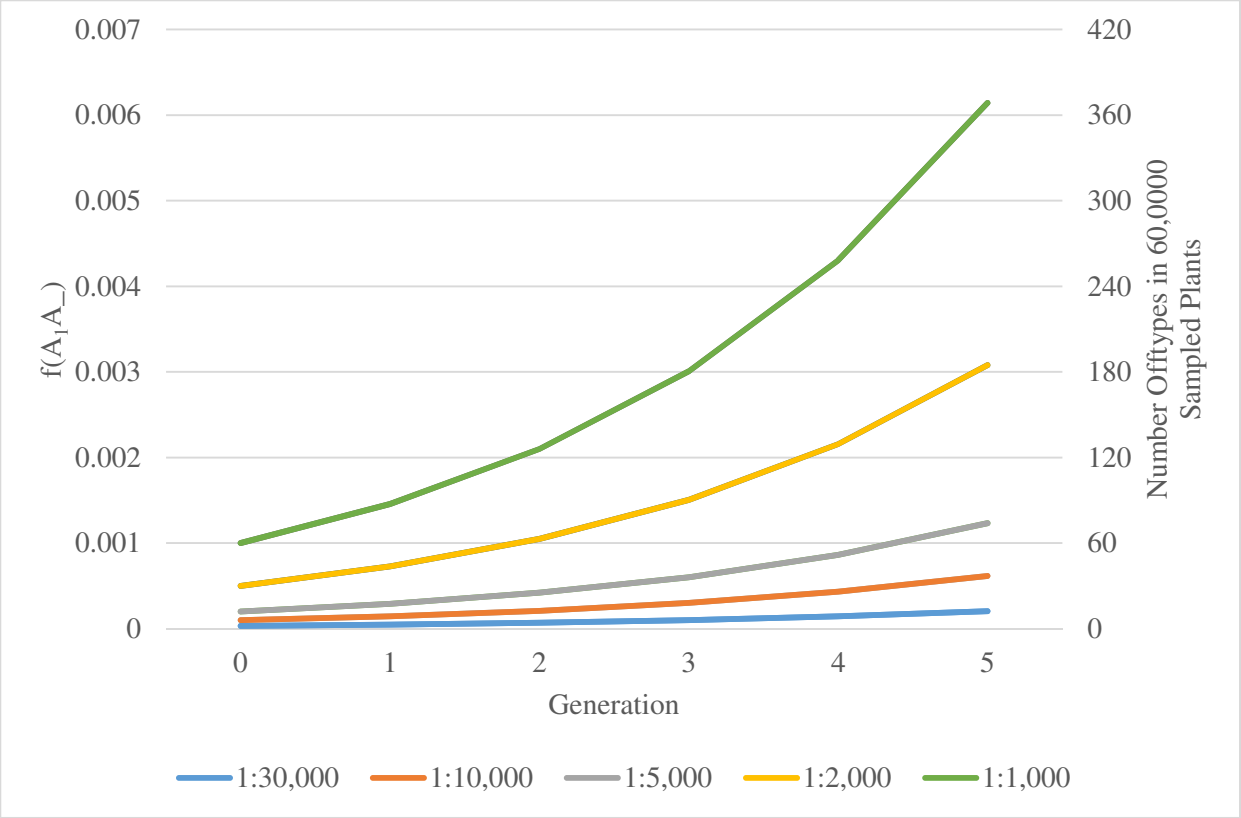

Figure 5.5. A chart used to examine the effect of selection for A_1A_1 or A_1A_2 offtypes over an A_2A_2 field when the relative fitness of A_1A_1 and A_1A_2 is 1.0 and A_2A_2 is 0.7 when initial contamination rates range from 1:30,000 to 1:1,000 A_1A_1 plants and an outcrossing rate of 2%.

The effect selection plays on future generations of offtypes are different than the effects of migration. With regards to offtype contamination frequency, migration is mainly from the spread of offtype pollen into a pedigreed seed field or volunteers from a previous crop. The selection for offtype genotypes over pedigreed seed offtypes is affected more by the performance of the offtype usually with respect to yield. If an offtype produces more seeds per plant than a pedigreed seed plant, the offtype has a selective advantage over the pedigreed seed crop. The selective advantage of different offtypes when the offtype allele A_1 is dominant to A_2 is important to examine, as over several generations the offtype frequency (A_1A_1 or A_1A_2) will increase. Figures 5.4 and 5.5 illustrate the differences between relative fitnesses. When the homozygous recessive pedigreed wheat plant (A_2A_2) has a relative fitness of 0.85, the frequency of offtypes does not increase nearly

as quickly as when the recessive homozyote has a relative fitness of 0.7. Both Figures 5.4 and 5.5 also show how different initial contamination rates can influence the offtype frequency in future generations. As expected, lower initial contamination rates lead to slower accumulation of contaminants due to selective advantage. A low initial contamination in a Select field may not exceed rejection levels for Foundation seed, but in the following year, it could exceed the rejection threshold for Registered seed since the rejection threshold for both Foundation and Registered is 1:10,000. This could be possible if the offtype has a selective advantage, which would increase the frequency of offtypes beyond the allowable limit for a given pedigree level.

If there is a selective advantage of an offtype over a pedigreed seed field, that field may meet Canadian pedigreed wheat standards for that year. Depending on the extent of the selective advantage of the offtype, the resulting contaminated seed may not meet pedigreed seed standards in future years as the proportion of offtypes to pedigreed wheat increases. The contamination threshold in both pedigreed wheat producing Foundation and Registered seed is 1:10,000. This meant a Foundation seeded field with an offtypes containing a selective advantage at a frequency of 1:10,000 may not meet the requirements to produce Registered wheat (Figures 5.4-5.6).

When the phenotype of the heterozygote and the offtype are the same, thorough roguing could eliminate the offtype alleles in one generation. If the situation were reversed and the offtype allele was recessive, the recessive allele would persist in the undetectable heterozygote if the recessive offtypes were removed from the field. The frequency of the offtype allele would gradually decrease and approach zero over many generations if only the homozygous recessive offtypes were rogued.

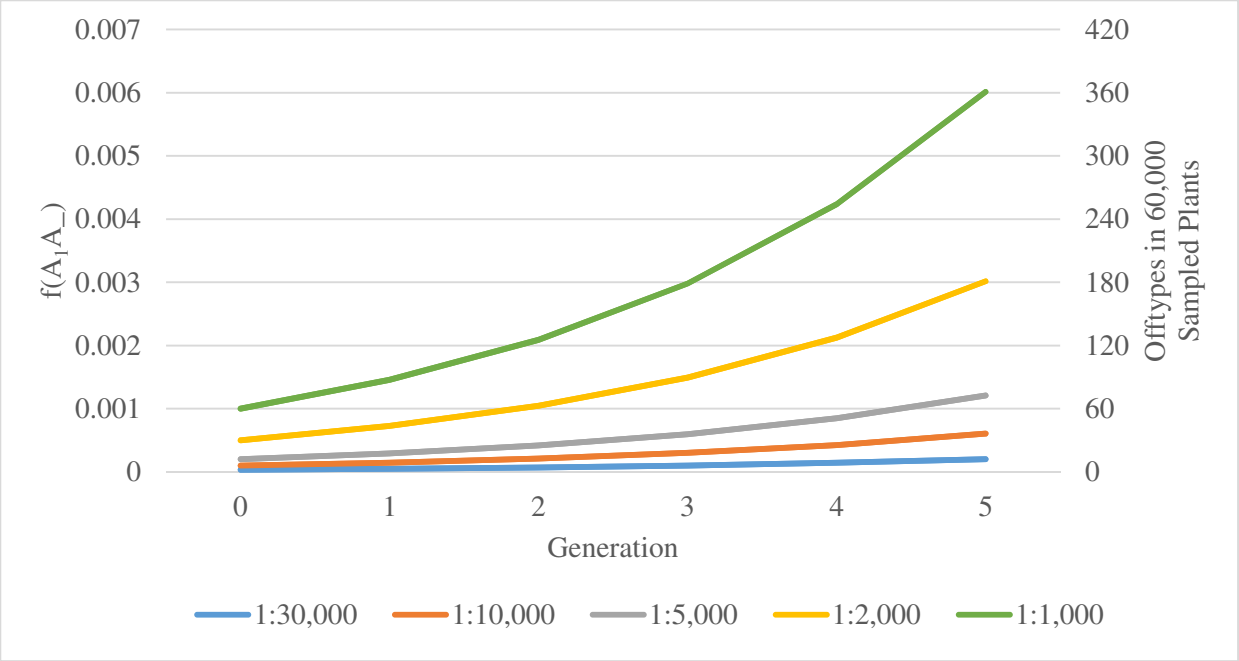

Figure 5.6. A chart used to examine the effect of selection for A_1A_1 or A_1A_2 offtypes over an A_2A_2 field when the relative fitness of A_1A_1 is 1.0, A_1A_2 is 0.85, and A_2A_2 is 0.7 when initial contamination rates range from 1:30,000 to 1:1,000 A_1A_1 plants and an outcrossing rate of 2%.

If the scenario was changed so that the relative selective advantage of the dominant homozygote (A_1A_1) was different than the heterozygote (A_1A_2), the frequency of offtypes will be different than the scenarios in Figures 5.4 and 5.5. This situation in Figure 5.6, in which the relative fitness of the heterozygote is between the recessive and dominant homozygotes, could better portray the quantitatively inherited yield traits.

5.4.1 Summary

Figure 5.1 is useful in evaluating the risk of properly or improperly accepting or rejecting a pedigreed seed field. This chart assumes that all offtypes will be detected and may not be entirely useful for more difficult to detect offtypes. Figure 5.1 is less useful in predicting the number of offtypes in 60,000 sampled plants if the contamination is not evenly spread throughout the field. If a patch escapes detection, the fields seeded the following year using the contaminated fields seed will show more of an even distribution due to mechanical mixing during harvest, seed transport, seed cleaning, and then seeding.

The severity of contamination via outcrossing from a neighbouring field is influenced by the level of outcrossing, the length that a neighbouring field is adjacent to the pedigreed seed field, and the size of the field. Figure 5.2 shows that pedigreed seed fields with a low adjacent field edge with a different variety relative to its field size would have lower contamination from outcrossing in the next generation.

If offtypes persist from previous generations in the soil of a pedigreed seed field, a higher plant density is beneficial to reduce the offtype frequency if those dormant seeds germinate. Figure 5.3 demonstrates the effect dormant offtypes can have on different plant densities which is useful as plant density decreases as pedigree level increases. A dormant offtype seed would have negative effects if left in a sparsely populated pedigreed field, meant to increase the number of seeds per plant of rare seed such as from a new cultivar. In addition, if this offtype seed has a selective advantage, it could cause future generations to be rejected for too many offtypes.

The importance of studying multiple generations of offtypes are exhibited in Figures 5.4-5.6. Different scenarios were given using dominant offtypes comparing different outcrossing rates and relative fitness pressures assuming a common 2% outcrossing rate. If the offtype is dominant to the pedigreed seed variety and the heterozygote has the same phenotype as the dominant homozygote, thorough roguing can be used to remove all offtype alleles. If the offtype is recessive to the pedigreed seed cultivar, but the heterozyote has the same phenotype as the pedigreed seed variety, it would take several generations to remove the offtype allele from the pedigreed seed field. Offtypes would continue to be generated by the undetected heterozygotes selfing or mating with other heterozygotes. The offtype frequency would eventually approach zero if the recessive offtypes were continually rogued every year it was grown.

If the forces of migration or selection are severe enough, one generation of migration or selection for an offtype phenotype could lead to a rejection of the field in the next generation depending on the parameters and initial offtype contamination rate. Figures 5.4-5.6 illustrate the compounding effect that higher contamination rates, larger outcrossing rates, and greater selection pressures place on pedigreed seed fields. The situations in Figures 5.4-5.6 show only either selection or migration. In reality, most offtypes will differ in both the outcrossing rate and selection pressure. The magnitude of difference will be the determining factor in how much the offtype frequency will change over time.

Previous work by Brûlé-Babel et al. (2006) examined the effect of foreign genetically engineered pollen in a non-GM crop with a 95% selective advantage. This study modelled the effect higher yielding wheat offtypes had on lower yielding pedigreed wheat fields. The two are both useful to examine how the purity of a pedigreed seed field can change quickly or slowly over time with other genotypes with a selective advantage. Prior work from Hucl and Matus-Cátiz (2001) showed outcrossing between 0.2% and 10% in wheat cultivars which was useful in modelling the effect of neighbouring fields with different outcrossing rates. Higher outcrossing cultivars have the potential to produce greater heterozygotes in the following generation if outcrossing does occur into a pedigreed seed field.

6 General Discussion and Conclusions

6.1 Conclusions

The accuracy of a pedigreed *Triticum aestivum* L. wheat inspection is important for breeders, seed growers, commercial farmers, food processors, and consumers. Falsely accepting or rejecting a pedigreed seed field has negative ramifications extending beyond the potential financial loss to the seed grower or commercial farmer. Since different wheat cultivars have different end uses and different pest management traits, it is important to maintain a high standard of genetic purity of a given pedigreed seed field so the buyer of the pedigreed seed is getting the traits they paid for. Industries such as food processors, feed formulators, or ethanol plants purchasing commercial wheat want specific seed traits and desire high purity of the cultivars they purchase to give consistent and predictable products for consumers purchasing their goods.

This study examined the Canadian, North Dakotan, and United Kingdom wheat inspection procedures to analyze risk, find efficiencies, and protect the seed grower or inspector. After analyzing Manitoba pedigreed wheat inspection reports from 2009-2012, the number of offtypes were compared to the pedigree level, field size, and cultivar. At all pedigree levels, heterogeneity had a very low correlation with field size. This was an important finding as it shows increased sampling as field size increases may not be necessary. The Manitoba wheat inspection reports showed that a high proportion of the inspected fields were well below current wheat standards suggesting that the current Canadian standards seem to be working.

After comparing the North Dakotan, United Kingdom, and Canadian jurisdictions inspection procedures in the validation experiment and the pedigreed wheat fields throughout southern Manitoba, the Canadian inspection procedures always inspected the highest percentage of the field. The different walking patterns showed insignificant differences in offtype detection but when deciding on a walking pattern, the inspector should take into account higher weed

distributions on the perimeter of the field and any other roads, railways, or waterways. Even though the diamond pattern was the fastest walking pattern on average to inspect a pedigreed seed field, the zig-zag pattern inspected a higher percentage of the outside of the field, which would more likely experience higher offtype and weed patches.

A large component of this study was the examination of whether or not inspecting entire plants or individual spikes as offtypes made a difference in the outcome of the inspection. Since, wheat is able to produce more spikes if it has enough space, it was important to examine the potential implications of counting plant or spike offtypes. If an offtype wheat plant happened to germinate in an area with a higher plant density, it will not contribute as many seeds to the next generation as a germinated wheat plant in a lower density. If an offtype seed grows between seed rows, or in a plugged seed row with ample space, it would have more space on average than the pedigreed wheat plants and would likely contribute more offtypes to the next generation and increase the frequency of offtypes relative to the pedigreed wheat seed.

In order to evaluate the effect that decreased sampling has on the accuracy of a pedigreed wheat field inspection, permutations of complete field inspections were developed. Using the scientific standard alpha value of 0.05 along with comparing the coefficients of variation of different permutations, the need to continue sampling at current levels was evident after the analysis. However, by lowering the alpha value, decreased from 0.1 to 0.05 or 0.01, decreased sampling could be seen as a viable option. When the alpha value is reduced from 0.1 to 0.05 or 0.01, the inspection can become more efficient, although a sacrifice is made due to an increased Type II error, and jurisdictions may not elect to add that additional risk to their inspection process.

A theoretical analysis of offtype frequency changes over several generations due to gene movement or selection was created. It is evident that offtypes which have much greater gene

migration or a higher selection pressure over the pedigreed wheat field will be problematic in future generations. The most problematic scenario would be if foreign pollen infiltrated a pedigreed wheat field which had characteristics leading to higher selection for new wheat offtypes. This could potentially raise the frequency of offtypes to levels where a pedigreed seed field could be rejected depending on the migration rate and selection pressure. If the migration rate or selection pressure is smaller, pedigreed seed fields are still at risk of being rejected as offtype frequency can increase over multiple generations.

6.2 Implications

This research has potential consequences for committees considering modification pedigreed field inspection procedures. There are a number of factors to consider when implementing new inspection procedures. A jurisdiction needs to decide whether inspectors should be using individual spikes or entire plants to count offtypes as well as measure plant or spike density. In addition, a decision could be made on the type of walking pattern, amount of sampling points, and number of plants or spikes evaluated per sample point.

If a jurisdiction decides to change the way it inspects pedigreed seed fields, there could be some competitive advantages including quicker and more affordable inspections for seed growers if they decrease the amount of sampling required for an inspection. Alternatively, if a jurisdiction opts for increased sampling the inspection would have a higher probability of being an accurate representation of the field. Altering inspection procedures could also influence the detection of unevenly distributed offtypes. With more sampling and more sampling locations in an inspection, the probability of detecting a patch would increase.

Furthermore, there could be implications for pedigreed seed growers depending on the type of inspection the jurisdiction imposes. For instance, if a jurisdiction elects to count spike offtypes,

a pedigreed seed grower would be wise to diligently rogue any offtypes with ample space which could greatly increase the amount of spikes on those plants, possibly failing their fields.

6.3 Limitations

Since spring and winter wheat cultivars were examined in this study, it is useful to determine which other crops could potentially benefit from this research, and recognize that different crops may need different inspection procedures to measure the varietal purity of those different species. This research is likely applicable to other cereals such as barley, oats, and rye. This research may not apply to crops which exhibit different plant characteristics than wheat such as corn, canola, soybeans, flax, and even bi-annual or perennial crops.

Although the permutation analysis examined different scenarios for decreased sampling relative to a complete inspection, the study never took into account the effect of increasing sampling beyond what is a complete inspection in either the North Dakotan, Canadian, or United Kingdom inspection.

The current field inspection procedures rely heavily on visible offtypes such as differences in colour, height, shape, and other physical characteristics to distinguish offtypes and variants from the pedigreed wheat field. Since there are many cultivars which may share similar physical characteristics, offtypes may go undetected in pedigreed wheat fields when they are nearly undetectable. This would not be as important if the offtype and the pedigreed wheat fields have the same end uses, but would be important if the offtype would have negative effects on the end use of the pedigreed seed field.

6.4 Suggestions For Future Research

This research into the inspection process of pedigreed wheat fields will help jurisdictions review and modify their existing field inspection procedures. However, there are other research

aspects relating to pedigreed seed inspection that could be explored. Future research could expand the number of sample points beyond what either the North Dakota or United Kingdom inspections permit. For instance, a permutation study similar to the one in this study could be conducted on fifteen or as high as twenty sample points to conclusively confirm whether or not increased sampling could provide an even more accurate inspection. This future study could also examine the consequences of decreasing or increasing the amount of plants or spikes in a sample point. For example, a study could conduct simulated inspections using twelve counts of 5,000 plants and compare it to a Canadian inspection. Both the simulated inspection and the Canadian inspection would have surveyed a total of 60,000 plants, but vary in the size and amount of their individual samples. Upcoming research could also include a validation experiment where varying patch sizes and shapes could be planted and inspected using different count sizes and different total amount of inspections.

Other pedigreed seed species may be subject to field inspection studies in the future. Many crops do not behave in a similar manner as wheat does which means these crops may need their own modified field inspection procedures. Canada's Circular 6 document already takes into account these different physiological characteristics of the different species. These modified inspection procedures could apply to row crops such as corn or soybeans, pedigreed seed potato inspections, and forage seed production.

Future research could involve further theoretical analyses on selection and migration over multiple generations. The next study could look at the combinational effects of migration and selection of offtype genotypes over multiple generations. Since there are many different potential combinations of mutation rates and selection pressures, it could be useful to track different

groupings of these parameters. The numerous scenarios have different consequences and outcomes which would be useful for both inspectors and pedigreed seed growers to know.

7 References

Agriculture and Agri-Food Canada. "Canadian Seed Sector Profile". *Government of Canada.* July 2014. Web. 2 November 2015. <http://www.agr.gc.ca/eng/industry-markets-and-trade/statistics-and-market-information/agriculture-and-food-market-information-by-region/canada/canadian-seed-sector-profile/?id=1405967530785>

Agriculture and Rural Development. "Seed for cereal crops." *Government of Alberta*. 1 Nov. 2007. Web. 16 May 2013.

Anderson R.L. and Soper G. 2003. Review of volunteer wheat (*Triticum aestivum*) seedling emergence and seed longevity in the soil. *Weed Technology*. 17: 774-749.

Animal and Plant Health Agency. 2016. Technical Standard Supplement. *Government of the United Kingdom.* December 2016. Web 2 July 2017.

Anonymous. "Document TGP/10: Examining Uniformity". *International Union for the Protection of New Varieties of Plants.* October 2008. Print.

Barnett D.T., Stohlgren T.J., Jarnevich C.S., Chong G.W., Ericson J.A., Davern T.R. and Simonson S.E. 2007. The art and science of weed mapping. *Environmental Monitoring and Assessment.* 132: 235-252.

Beckie H. 2001. Impact of herbicide resistant crops as weeds in Canada. Proceedings of the Brighton Crop Protection Conference – Weeds: 135 – 142. Brighton, UK; British Crop Protection Council.

Beckie H.J., Warwick S.I., Nair H. and Séguin-Swartz G. 2003. Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). *Ecological Applications*. 13: 1276-1294.

Bibby C.J. and Buckland S.T. 1987. Bias of bird census results due to detectability varying with habitat. *Acta Ecologica Sinica*. 8: 103-112.

Brown J.K.M. and Hovmøller. 2002. Aerial Dispersal of Pathogens on the Global and Continental Scales and Its Impact on Plant Disease. *Science Magazine.* 297:537-541.

Brûlé-Babel A.L. 2017. *University of Manitoba.* Personal Communication.

Brûlé-Babel A.L., Willenborg C.J., Friesen L.F. and Van Acker R.C. 2006. Modeling the influence of gene flow and selection pressure on the frequency of GE herbicide-tolerant trait in non-GE wheat and wheat volunteers. *Crop Science*. 46: 1704-1710.

Buckland S.T., Anderson D.R., Burnham K.P. and Laake J.L. 1993. *Distance Sampling: Estimating Abundance of Biological Populations*. Chapman and Hall, London. 446pp.

Canadian Food Inspection Agency. "Seed Program Specific Work Instructions (SWI 142.1.1) – Pedigreed Seed Crop Inspection". *Government of Canada*. June 2014. Web. 3 July 2014. <http://www.inspection.gc.ca/plants/seeds/seed-inspection-procedures/pedigreed-seed-crop-inspection/eng/1379613380842/1379613485624>

Canadian Seed Growers' Association. "2015 Canadian Seed Growers' Association (CSGA) November Acreage and Membership Report". October 2015. Web. 10 November 2015. <http://seedgrowers.ca/csga-releases-the-2015-acreage-and-membership-report>

Canadian Seed Growers' Association. 2017. "Canadian Regulations and Procedures for Pedigreed Seed Crop Production (Circular 6)". February 2017. Web. 2 July 2017.< http://seedgrowers.ca/wp-content/uploads/Circ6-ALL_ENGLISH_Rev01.12-2017_20170323_Complete-1.pdf>

Canessa S., Heard G.W., Parris K.M. and McCarthy M.A. 2012. Integrating variability in detection probabilities when designing wildlife surveys: a case study of amphibians from south-eastern Australia. *Biodiversity Conservation.* 21: 729-744.

Carmer S.G. 1976. Optimal significance levels for applications of the least significant difference in crop performance trials. *Crop Science.* 16: 95-99.

Chen C., Neill K., Wichman D. and Westcott M. 2008. Hard red spring wheat response to row spacing, seeding rate, and nitrogen. *American Society of Agronomy.* 100: 1296-1302.

Chen G., Kéry M., Zhang J. and Ma K. 2009. Factors affecting detection probability in plant distribution studies. *Journal of Ecology.* 97: 1383-1389.

David O., Kempton R.A. and Nevison I.M. 1996. Designs for controlling interplot contamination in variety trials. *Journal of Agricultural Science.* 127: 285-288.

Dawson A. 2010. "GM-free certified seed part of plant to regain flax exports to EU". *Alberta Farm Express.* Retrieved from http://www.albertafarmexpress.ca on 2 December 2016.

Dawson A. 2014. "Manitoba No. 1 in pedigreed seed acres". *Manitoba Co-operator.* Retrieved from http://www.manitobacooperator.com on 28 October 2015.

Doheer M.G. and Audige L. 2001. Monitoring and surveillance for rare health-related events: a review from the veterinary perspective. *Philosophical Transactions of the Royal Society.* 356: 1097-1106.

Donald A.W., Gardner I.A. and Wiggins A.D. 1994. Cut-off for aggregate herd testing in the presence of disease clustering and correlation of test errors. *Preventative Veterinary Medicine*. 19: 167-187.

Fischer R.A., Aguilar I., Maurer R. and Rivas S. 1976. Density and row spacing effects on irrigated short wheat at low latitude. *The Journal of Agricultural Science.* 87: 137-147.

Food & Environment Research Agency. "Guide to Seed Certification Procedures in England and Wales". *Government of the United Kingdom*. June 2013. Print.

Franz-Warkentin P. "Flax sector slowly recovers from Day of the Triffids". *AGCanada.com.* 10 Oct 2012. Web 5 April 2015. <https://www.agcanada.com/daily/flax-sector-slowly-recovers-from-day-of-the-triffids>

Frei C. and Schär C. 2001. Detection Probability of Trends in Rare Events: Theory and Application to Heavy Precipitation in the Alpine Region. *Journal of Climate.* 14: 1568-1584.

Gagnon R. 2015. STAT 2000: *Basic Statistical Analysis 2.* [Class Handout]. Department of Statistics, University of Manitoba, Winnipeg, MB, Canada.

Garrard G.E., Bekessy S.A., McCarthy M.A. and Wintle B.A. 2008. When have we looked hard enough? A novel method for setting minimum survey effort protocols for flora surveys. *Austral Ecology.* 33: 986-998.

Gilbert N. 2010. GM crop escapes into the American wild. *Nature News*. 6 August 2010. Web 6 April 2015. < http://www.nature.com/news/2010/100806/full/news.2010.393.html>

Greiner M. and Gardner I.A. 2000. Epidemiologic issues in the validation of veterinary diagnostic tests. *Preventative Veterinary Medicine*. 45: 3-22.

Higgins S.I., Nathan R. and Cain M.L. 2003. Are Long-Distance Dispersal Events in Plants Usually Caused by Nonstandard Means of Dispersal? *Ecological Society of America*. 84: 1945-1956.

Hirzel A. and Guisan A. 2002. Which is the optimal sampling stragegy for habitat suitability modelling. *Ecological Modelling.* 157: 331-341.

Hucl P., Matus-Cádiz M. 2001. Isolation distances for minimizing out-crossing in spring wheat. *Crop Science.* 41: 1348-1351.

Hucl P., Matus-Cádiz M., Sahota A.S., Middleton A., Mooney D. and Maruschak J.L. 2004. Sources of off-types in pedigreed seed of common spring wheat. *Canadian Journal of Plant Science*. 84: 519-523.

Komatsuzuki M. and Endo O. 1996. Volunteer wheat in upland fields. *Weed Resistance*: 41: 197-204.

Magnani R., Keith S., Saidel T. and Heckathorn D. 2005. Review of sampling hard-to-reach and hidden populations for HIV surveillance. *Official Journal of the International AIDS Society.* 19: S67-S72.

MacKenzie D.I. and Royle J.A. 2005. Designing occupancy studies: general advice and allocating survey effort. *Journal of Applied Ecology.* 42: 1105-1114.

Manitoba. Manitoba Agriculture. "Aiming for Higher Wheat Yields". *Manitoba Agriculture.* Web. 19 Nov 2016. <http://www.gov.mb.ca/agriculture/crops/production/print,aiming-for-higher-wheat-yields.html>

McManamay R.A., Orth D.J. and Jager H.I. 2014. Accounting for variation in species detection in fish community monitoring. *Fisheries Management and Ecology.* 21: 96-112.

Moore J.L., Hauser C.E., Bear J.L., Williams N.S.G. and McCarthy M.A. 2011. Estimating detection-effort curves for plants using search experiments. *Ecological Society of America.* 21: 601-607.

North Dakota State Seed Department. 2012a. "General Seed Certification Requirements". *Government of North Dakota.* December 2012. Web. 14 July 2013. < https://www.nd.gov/seed/news/GenCropReq.pdf>

North Dakota State Seed Department. 2012b. "Specific Crop Requirements – Small Grains and Flax". October 2012. Web. 2 July 2017. < https://www.nd.gov/seed/news/SeedStandards.pdf>

Nyachiro J.M., Clarke F.R., DePauw R.M., Knox R.E. and Armstrong K.C. 2002. Temperature effects on seed germination and expression of seed dormancy in wheat. *Euphytica*. 126: 123-127.

Owen M.D.K and Zelaya I.A. 2005. Herbicide-resistant crops and weed resistance to herbicides. *Pest Management Science*. 61: 301-311.

Pickett A.A. 1993. Cereals: seed shedding, dormancy, and longevity. *Aspects of Applied Biology*. 35: 17-28.

Preater R. 2015. *Canadian Seed Growers' Association*. Personal Communication.

Rew L.J. and Cousens R.D. 2000. Spatial distribution of weeds in arable crops: are current sampling and analytical methods appropriate? *Weed Research.* 41: 1-18.

Rew L.J., Maxwell B.D., Dougher F.L. and Aspinall R. 2006. Searching for a needle in a haystack: evaluating survey methods for non-indigenous plant species. *Biological Invasions*. 8: 523-529.

Roitberg B.D. and Prokopy R.J. 1987. Insects that Mark Host Plants. *BioScience.* 37: 400-406.

Rouse J. "Annual meeting of representatives of the National Designated Authorities for the implementation of the OECD schemes for the varietal certification of seed moving in international trade – United States response to the revised guidelines for control plot tests and field inspection." *Organisation for Economic Co-operation and Development.* Dec. 2009. Print.

Salisbury P. 2002. Pollen movement in canola (*Brassica napus*) and outcrossing between *B. napus* crops. *University of Melbourne*.

Sanderlin J.S., Block W.M. and Ganey J.L. 2014. Optimizing study design for multi-species avian monitoring programmes. *Journal of Applied Ecology.* 51: 860-870.

Seed Manitoba. 2016. 2017 Variety and Selection & Growers Source Guide. *Seed Manitoba.* December 2016. Web. 11 December 2016. <http://www.seedmb.ca/wp-content/uploads/2016/12/seed_mb_2012.pdf>

Statistics Canada. "Principal field crop areas, June 2015". *Government of Canada*. June 2015. Web. 5 November 2015. < http://www.statcan.gc.ca/daily-quotidien/150630/dq150630b-eng.htm>

Stewart C.N., Halfhill M.D. and Warwick S.I. 2003. Genetic Modification: Transgene Introgression From Genetically Modified Crops to Their Wild Relatives. *Nature Reviews Genetics.* 4: 806-817.

Stow J. 2015. *Manitoba Seed Growers' Association.* Personal Communication.

Taylor J.C., Wood G.A., Earl R. and Godwin R.J. 2003. Soil factors and their influence on within-field crop variability, Part II: Spatial analysis and determination of management zones. *Biosystems Engineering.* 84: 441-453.

United Kingdom. "Annual meeting of representatives of the National Designated Authorities for the implementation of the OECD schemes for the varietal certification of seed moving in international trade – Choosing a sampling scheme for cereal crop sampling". *Organisation for Economic Co-operation and Development.* June 2009. Print.

United Kingdom. "Annual meeting of representatives of the National Designated Authorities for the implementation of the OECD schemes for the varietal certification of seed moving in international trade – Draft revised guidelines for control plot tests and field inspection of seed crops – Part IV (characteristics for assessing varietal identity and varietal purity." *Organisation for Economic Co-operation and Development.* June. 2008. Print.

United Kingdom. "Farming Statistics: Provisional crop areas, yields and livestock populations at June 2014 – United Kingdom". *Department for Environment, Food & Rural Affairs*. October 2014. Web. 5 November 2015. <https://www.gov.uk/government/statistics/farming-statistics-provisional-crop-areas-yields-and-livestock-populations-at-1-june-2014-uk>

United States Department of Agriculture. 2015. "2015 Wheat Varieties". *North Dakota Wheat Commission.* July 2015. Web. 5 November 2015. <https://www.nass.usda.gov/Statistics_by_State/North_Dakota/Publications/Crop_Varieties/pub/Wheat/whtvr15.pdf>

Venette R.C., Moon R.D. and Hutchison W.D. 2002. Strategies and statistics of sampling for rare individuals. *Annual Review of Entomology.* 47: 143-174.

Wallace J. 2016. *Wallace Seed Service.* Personal Communication.

Ward M.G. 1994. Sampling to make decisions in the Plant Health and Seeds Inspectorate. *Aspects of Applied Biology.* 37: 217-224.

Wheat and Barley Variety Working Group. "Exploring options for producer involvement in wheat and barley variety development. November 2015. Web 3 July 2017. <http://westerngrains.com/wp-content/uploads/2017/01/JRG-Consulting-Wheat-Barley-Breeding-Executive-Summary-for-Public-Distribution1.pdf>

Wiles L.J., Oliver G.W., York A.C., Gold H.J. and Wilkerson G.G. 1992. Spatial distribution of broadleaf weeds in North Carolina soybean (*Glycine max*) fields. *Weed Science Society of America.* 40: 554-557.

Willenborg C.J. and Van Acker R.C. 2008. The biology and ecology of hexaploid wheat (*Triticum aestivum* L.) and its implications for trait confinement. *Canadian Journal of Plant Science*. 88: 997-1013.

Wintle B.A., Kavanagh R.P., McCarthy M.A. and Burgman M.A. 2005. Estimating and dealing with detectability in occupancy surveys for forest owls and arboreal mammals. *Journal of Wildlife Management*. 69: 905-917.

Zakoks J.C., Chang T.T. and Konzak C.F. 1974. A decimal code for the growth stages of cereals. *Weed Research.* 14: 415-421.

Zvomuya F., Janzen H.H., Larney F.J. and Olson B.M. A long-term field bioassay of soil quality indicators in a semiarid environment. *Soil Science Society of America Journal*. 72: 683-692.

8 Appendices

Appendix 8.1. Partitioning of 3,782 Manitoba pedigreed wheat inspection reports into the total offtypes of an inspection with the maximum and minimum number of offtypes found in the individual counts.

Difference Between Maximum and Minimum Number of Offtypes in an Inspection Report	Number of Fields														
	Breeder Total Offtypes			Select Total Offtypes			Foundation Total Offtypes			Registered Total Offtypes			Certified Total Offtypes		
	<10	10-20	>20	<10	10-20	>20	<10	10-20	>20	<10	10-20	>20	<10	10-20	>20
0-5	199	2	0	483	8	4	915	28	13	1917	114	360	5	0	0
6-10	0	0	3	0	0	1	0	4	1	3	8	23	0	0	0
11-20	0	0	0	0	1	0	0	1	2	0	0	5	0	0	0
21-30	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
31-40	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
>40	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0

Appendix 8.2. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the validation experiment using Canadian inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. Alpha values of 0.01, 0.05, and 0.1 were used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with five degrees of freedom.

Permutation [1]		1:30,000						1:10,000						1:5,000					
		$\alpha = 0.1$		$\alpha = 0.05$		$\alpha = 0.01$		$\alpha = 0.1$		$\alpha = 0.05$		$\alpha = 0.01$		$\alpha = 0.1$		$\alpha = 0.05$		$\alpha = 0.01$	
1	2	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
6C1	6C2	No	No	No	No	No	No	Yes	No	No	No	No	No	Yes	Yes	No	No	No	No
6C1	6C3	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No

6C1	6C4	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
6C1	6C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C2	6C3	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
6C2	6C4	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
6C2	6C5	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
6C3	6C4	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
6C3	6C5	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
6C4	6C5	No	No	No	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No

[1] Permutation refers to subsamples of complete inspections; 6C1 = one count of six counts (complete inspection), 6C2 = two counts (subsample) of six counts (complete inspection)... 6C5 = five counts (subsample) of six counts (complete inspection)

Appendix 8.3. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the validation experiment using Canadian inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. Alpha values of 0.01, 0.05, and 0.1 were used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with five degrees of freedom.

Permutation [1]		1:2,000						1:1,000					
		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01	
1	2	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
6C1	6C2	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
6C1	6C3	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
6C1	6C4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C1	6C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C2	6C3	No	No	No	No	No	No	No	No	No	No	No	No
6C2	6C4	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
6C2	6C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C3	6C4	No	No	No	No	No	No	No	No	No	No	No	No
6C3	6C5	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
6C4	6C5	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No

[1] Permutation refers to subsamples of complete inspections; 6C1 = one count of six counts (complete inspection), 6C2 = two counts (subsample) of six counts (complete inspection)... 6C5 = five counts (subsample) of six counts (complete inspection)

Appendix 8.4. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the validation experiment using North Dakotan inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. Alpha values of 0.01, 0.05, and 0.1 were used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with five degrees of freedom.

Permutation [1]		1:30,000						1:10,000						1:5,000					
		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01	
1	2	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
10C1	10C2	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C1	10C3	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
10C1	10C4	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C1	10C5	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C1	10C6	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C1	10C7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C3	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C2	10C4	No	No	No	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
10C2	10C5	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C2	10C6	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C2	10C7	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C2	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C4	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C3	10C5	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
10C3	10C6	Yes	Yes	Yes	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
10C3	10C7	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C3	10C8	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C3	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C5	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C4	10C6	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C4	10C7	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No

10C4	10C8	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C4	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C5	10C6	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C5	10C7	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C5	10C8	Yes	Yes	Yes	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
10C5	10C9	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C6	10C7	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C6	10C8	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	Yes	No	No	No	No	No
10C6	10C9	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C7	10C8	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C7	10C9	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C8	10C9	No	No	No	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No

[1] Permutation refers to subsamples of complete inspections; 10C1 = one count of ten counts (complete inspection), 10C2 = two counts (subsample) of ten counts (complete inspection)... 10C9 = nine counts (subsample) of ten counts (complete inspection)

Appendix 8.5. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the validation experiment using North Dakotan inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. Alpha values of 0.01, 0.05, and 0.1 were used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with five degrees of freedom.

Permutation [1]		1:2,000						1:1,000					
		$\alpha = 0.1$		$\alpha = 0.05$		$\alpha = 0.01$		$\alpha = 0.1$		$\alpha = 0.05$		$\alpha = 0.01$	
1	2	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
10C1	10C2	No	No	No	No	No	No	No	No	No	No	No	No
10C1	10C3	Yes	Yes	No	No	No	No	Yes	Yes	Yes	No	No	No
10C1	10C4	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C1	10C5	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C1	10C6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

10C1	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C3	No	No	No	No	No	No	No	No	No	No	No	No
10C2	10C4	No	No	No	No	No	No	Yes	Yes	No	No	No	No
10C2	10C5	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C2	10C6	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C2	10C7	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C4	No	No	No	No	No	No	No	No	No	No	No	No
10C3	10C5	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
10C3	10C6	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	No	No	No
10C3	10C7	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C3	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C5	No	No	No	No	No	No	No	No	No	No	No	No
10C4	10C6	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
10C4	10C7	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C4	10C8	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C4	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C5	10C6	No	No	No	No	No	No	No	No	No	No	No	No
10C5	10C7	No	No	No	No	No	No	Yes	Yes	No	No	No	No
10C5	10C8	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	No	No
10C5	10C9	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
10C6	10C7	No	No	No	No	No	No	No	No	No	No	No	No
10C6	10C8	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
10C6	10C9	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C7	10C8	No	No	No	No	No	No	No	No	No	No	No	No
10C7	10C9	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C8	10C9	No	No	No	No	No	No	No	No	No	No	No	No

[1] Permutation refers to subsamples of complete inspections; 10C1 = one count of ten counts (complete inspection), 10C2 = two counts (subsample) of ten counts (complete inspection)... 10C9 = nine counts (subsample) of ten counts (complete inspection)

Appendix 8.6. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the validation experiment using United Kingdom inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. Alpha values of 0.01, 0.05, and 0.1 were used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with five degrees of freedom.

Permutation [1]		1:30,000						1:10,000						1:5,000					
		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01	
1	2	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
5C1	5C2	No	No	No	No	No	No	Yes	No	No	No	No	No	Yes	Yes	No	No	No	No
5C1	5C3	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
5C1	5C4	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
5C2	5C3	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
5C2	5C4	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
5C3	5C4	Yes	Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes	No	No	No	No

[1] Permutation refers to subsamples of complete inspections; 5C1 = one count of five counts (complete inspection), 5C2 = two counts (subsample) of five counts (complete inspection)... 5C4 = four counts (subsample) of five counts (complete inspection)

Appendix 8.7. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the validation experiment using United Kingdom inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. Alpha values of 0.01, 0.05, and 0.1 were used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with five degrees of freedom.

Permutation [1]		1:2,000						1:1,000					
		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01	
1	2	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
5C1	5C2	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
5C1	5C3	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
5C1	5C4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5C2	5C3	Yes	No	No	No	No	No	Yes	Yes	No	No	No	No
5C2	5C4	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
5C3	5C4	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No

[1] Permutation refers to subsamples of complete inspections; 5C1 = one count of five counts (complete inspection), 5C2 = two counts (subsample) of five counts (complete inspection)... 5C4 = four counts (subsample) of five counts (complete inspection)

Appendix 8.8. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the Farmers Edge inspections using Canadian inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. Alpha values of 0.01, 0.05, and 0.1 were used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with twelve degrees of freedom.

Permutation [1]		<106 Acres						106-156 Acres						156-421 Acres					
		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01	
1	2	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
6C1	6C2	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
6C1	6C3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C1	6C4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C1	6C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C2	6C3	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
6C2	6C4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C2	6C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C3	6C4	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
6C3	6C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6C4	6C5	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No

[1] Permutation refers to subsamples of complete inspections; 6C1 = one count of six counts (complete inspection), 6C2 = two counts (subsample) of six counts (complete inspection)... 6C5 = five counts (subsample) of six counts (complete inspection)

Appendix 8.9. A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the Farmers Edge inspections using North Dakotan inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. Alpha values of 0.01, 0.05, and 0.1 were used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with twelve degrees of freedom.

Permutation [1]		<106 Acres						106-156 Acres						156-421 Acres					
		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01	
1	2	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
10C1	10C2	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No
10C1	10C3	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C1	10C4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No

10C1	10C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C3	No	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes	No	No	No	No
10C2	10C4	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	No	No
10C2	10C5	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C4	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C3	10C5	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No
10C3	10C6	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C3	10C7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C5	Yes	Yes	No	No	No	No	Yes	No	No	No	No	No	Yes	Yes	No	No	No	No
10C4	10C6	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No	No	No
10C4	10C7	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C5	10C6	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C5	10C7	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No
10C5	10C8	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
10C5	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C6	10C7	No	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes	No	No	No	No
10C6	10C8	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C6	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C7	10C8	No	No	No	No	No	No	Yes	Yes	No	No	No	No	No	No	No	No	No	No

10C7	10C9	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
10C8	10C9	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	No	No	No	Yes	Yes	No	No	No	No

[1] Permutation refers to subsamples of complete inspections; 10C1 = one count of ten counts (complete inspection), 10C2 = two counts (subsample) of ten counts (complete inspection)... 10C9 = nine counts (subsample) of ten counts (complete inspection)

Appendix 8.10: A summary of the statistical significance when two coefficients of variations calculated using permutations developed from inspecting the Farmers Edge inspections using United Kingdom inspection procedures and measured both plant (PLT) and spike (SPK) offtypes. Alpha values of 0.01, 0.05, and 0.1 were used to determine if the permutations are statistically significant (Yes) or not statistically significant (No) with twelve degrees of freedom.

Permutation [1]		<106 Acres						106-156 Acres						156-421 Acres					
		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01		α = 0.1		α = 0.05		α = 0.01	
1	2	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK	PLT	SPK
10C1	10C2	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	No	No	No	No	No	No
10C1	10C3	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C1	10C4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C1	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C3	Yes	Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes	No	No	No	No
10C2	10C4	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C2	10C5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C2	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C4	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C3	10C5	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No

10C3	10C6	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C3	10C7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C3	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C5	Yes	Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes	No	No	No	No
10C4	10C6	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	No	No
10C4	10C7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
10C4	10C8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C4	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C5	10C6	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C5	10C7	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No	No	No
10C5	10C8	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No
10C5	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C6	10C7	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	No	No	No	No	No	No
10C6	10C8	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No
10C6	10C9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10C7	10C8	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10C7	10C9	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
10C8	10C9	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	No	No

[1] Permutation refers to subsamples of complete inspections; 10C1 = one count of ten counts (complete inspection), 10C2 = two counts (subsample) of ten counts (complete inspection)... 10C9 = nine counts (subsample) of ten counts (complete inspection)