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Spatially-Optimized Sequential Sampling Plan for Cabbage Aphids *Brevicoryne brassicae* L. (Homoptera: Aphididae) in Canola Fields

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Abstract

The cabbage aphid is a significant pest worldwide in brassica crops, including canola. This pest has shown considerable ability to develop resistance to insecticides, so these should only be applied on a “when and where needed” basis. Thus, optimized sampling plans to accurately assess cabbage aphid densities are critically important to determine the potential need for pesticide applications. In this study, we developed a spatially optimized binomial sequential sampling plan for cabbage aphids in canola fields. Based on five sampled canola fields, sampling plans were developed using 0.1, 0.2, and 0.3 proportions of plants infested as action thresholds. Average sample numbers required to make a decision ranged from 10 to 25 plants. Decreasing acceptable error from 10 to 5% was not considered practically feasible, as it substantially increased the number of samples required to reach a decision. We determined the relationship between the proportions of canola plants infested and cabbage aphid densities per plant, and proposed a spatially optimized sequential sampling plan for cabbage aphids in canola fields, in which spatial features (i.e., edge effects) and optimization of sampling effort (i.e., sequential sampling) are combined. Two forms of stratification were performed to reduce spatial variability caused by edge effects and large field sizes. Spatially optimized sampling, starting at the edge of fields, reduced spatial variability and therefore increased the accuracy of infested plant density estimates. The proposed spatially optimized sampling plan may be used to spatially target insecticide applications, resulting in cost savings, insecticide resistance mitigation, conservation of natural enemies, and reduced environmental impact.

Key words: spatial distribution, resampling software, edge effect, crop scouting, IPM

Stern et al. (1959) originally presented the concept of “integrated control,” now termed “integrated pest management” (IPM), wherein chemical control is used when and where it is needed, and pesticide applications should be as least disruptive to biological control and the environment as possible (Pedigo et al. 1986, Fitt and Wilson 2012). This concept was developed further (Brewer and Goodell 2012) and is now also being referred to as “threshold-based pest management” (Nansen and Ridsdill-Smith 2013). As part of developing threshold-based pest management, it is important to be able to estimate, by sampling, the population densities of arthropod pest species in a reliable and practically feasible manner. Pedigo

(1993) emphasized that the absence of cost-effective, user-friendly sampling plans for arthropod pests in food crops is a major impediment to the adoption of IPM. Several studies have demonstrated that deployment of reliable and practically feasible sampling plans may lead to reduction in pesticide applications and improved pest management (Sylvester and Cox 1961, Giles et al. 2000, Butler and Trumble 2012, Martini et al. 2012, Serra et al. 2013, Stubbins et al. 2014). Within a sampling plan, the sampling technique, unit, and pattern must be defined, and the accuracy and efficiency must be addressed, which are often competing needs (Landis 1993, Pedigo 1993). It is important that enough samples are acquired over a large

Table 1. Details of five sampled canola fields in Western Australia during 2013–2014

Year	Name	Latitude (S)	Longitude (W)	Field size (ha)	Variety	Date sown	Dates sampled	Field points	GS ^a range	Range of means ^b
2013	York	–31.9634	116.6802	60	Stingray	May-03	Aug. 17–21	59	3.0–5.5	0.000–0.250
2013	New Norcia	–31.0245	116.2102	92	Crusher	May-10	Aug. 28–31	100	3.0–5.5	0.168–0.700
2014	Northam	–31.6782	116.6038	13	TT50	April-29	Sept. 03–05	70	4.5–5.9	0.027–0.233
2014	Meckering	–31.6540	116.9527	67	43Y73	April-28	Sept. 05–08	100	4.5–5.8	0.029–0.346
2014	Grass Valley	–31.6471	116.7970	23	Stingray	April-26	Sept. 12–13	60	4.8–6.3	0.020–0.167

^aCanola growth stage range at time of sampling as by [Edwards and Hertel \(2011\)](#).

^bRange of means for proportion of plants infested for field quarter and field quarter edge strata.

enough area to obtain data that is representative of the entire population, while maintaining the practical feasibility of the sampling effort. Once a reliable estimate of the pest population density is made, an “action threshold” is used to decide whether intervention is warranted or not. An action threshold is the minimum number of insects (density or intensity) or proportion of plants infested that will cause economic damage and should therefore trigger management action ([Pedigo 1996](#), [New 2002](#)).

The major impediment to practically applying an action threshold for cabbage aphids in canola is that no sampling plan has been developed in which sampling effort and stratification have been validated using broad-scale empirical data. As a consequence, field inspectors may not be able to accurately estimate the cabbage aphid density in canola fields and may spend unnecessary amounts of time on sampling, both of which represent important impediments to successful and long-term sustainable pest management. Development of both practically feasible and accurate sampling plans is therefore critically important, and we argue this can be accomplished by modifying and combining conventional sequential and stratified approaches to sample plan development.

Sequential sampling optimizes and reduces the sampling effort relative to a fixed sample size. However, stratified sampling is commonly based on random sampling with a fixed sample size within a particular sampling universe, which has been subdivided to reduce sample variation and to ensure all areas of the habitat are sampled ([Buntin 1993](#)). Stratification is particularly important where arthropods are spatially aggregated and therefore display higher degrees of variation across space.

Many arthropod pests, including aphids, have exhibited aggregated spatial distributions ([Trumble 1982](#), [Feng and Nowierski 1992](#), [Powell et al. 2006](#)), which are caused by factors such as land topography ([Hill and Mayo 1980](#)), distance from crop edge ([Winder et al. 1999](#), [Nansen et al. 2005](#)), and plant phenology, including growth stage ([Ferguson et al. 2003](#)) and leaf age ([Kennedy and Booth 1951](#)). This information may be useful in reducing sampling effort and increasing sampling accuracy for emerging infestations of arthropods in broad-scale fields, wherein sampling is targeted toward plant parts and field regions where the arthropods are most likely to occur.

Here, we propose a spatially optimized sequential sampling plan for cabbage aphids in canola fields, in which spatial features (i.e., edge effects and large field sizes) and optimization of sampling effort (i.e., sequential sampling) are combined. We argue that a binomial sequential sampling plan may be used to decrease the time and labor required to inspect canola crops for arthropods, hence increasing the feasibility of insecticide applications targeted in both space and time, so that they are only applied when and where they are needed. As part of this, we determined the relationship between the proportions of canola plants infested and cabbage aphid densities per plant,

and proposed a stratified sampling approach to canola fields to account for large field sizes and edge effects.

Materials and Methods

Field Sampling

Five canola fields were sampled in Western Australia during 2013–2014, and details are presented in [Table 1](#). As in [Severtson et al. \(2015\)](#), sampling locations were geo-referenced (Garmin eTrex10; accuracy <3 m) along paths which were selected haphazardly to maximize scattering of sampling locations throughout fields and to include field edges and varying distances from field edges. “Field edges” here refer to areas of canola crop that fall within 20 m of the peripheral limits of the canola crop. It was shown by [Severtson et al. \(2015\)](#) that visual inspection of canola plants was more reliable than sweep netting, and that, from the onset of raceme production, cabbage aphids were distributed mostly on raceme terminals with much fewer numbers located on the underside of leaves. Therefore, visual inspection was employed as the sampling technique, and the racemes of individual canola plants as the sampling unit. Cabbage aphid colony length measurements on racemes were recorded for each of 10 plants per sampling location along the crop row. The ranges in growth stage within sampled fields were representative of growth stages in which cabbage aphids are most likely to cause economic damage to canola crops and therefore the most important time regarding crop scouting and for deciding on remedial action (see [Table 1](#); [Oilseeds Industry Association of Western Australia 2006](#), [Gu et al. 2007](#)).

Stratification of Fields Based on Size and Edge Effects

Stratification within sampling programs is the process of dividing a region into smaller regions, or strata, in order to reduce variability, hence improving sampling efficiency, and ensuring all areas within the region are sampled. In order to determine the degree of variability within fields, all five fields were stratified into two types of strata: “field quarters” and “field quarter edges.” Fields were divided into field quarters by drawing a north/south and an east/west line with intersects centered in each field. The correlation between the proportions of canola plants infested and cabbage aphid densities per plant was investigated for field strata, and the variability relative to the cabbage aphid action threshold of 20% of canola plants infested was assessed. Chi-square tests were performed (using Genstat for Windows v.15; VSN International Ltd., Hemel Hemstead, United Kingdom) for each of the five fields to test whether canola plants infested with cabbage aphids were distributed more along field edges, compared with uninfested plants, relative to regions further inwards. Areas which lay within 20 m of the field edge, for each field quarter, were considered to be field quarter edges. The distance of 20 m from the field edge was designated

because >99% of cabbage aphids were found within this distance in intensively sampled field edges in (Severtson et al. 2015).

Action Thresholds

Action thresholds of 10–20% of racemes infested in Canada and 20% of racemes infested in northern United States have been proposed for cabbage aphids in canola, with no sample number defined (Kandel and Berglund 2011, Anonymous 2015). During the widespread adoption of canola cultivation in Australia in the late 1990s (Salisbury 1999), an action threshold of 100% of sampled canola plants infested with at least 0.5 cm of cabbage aphid colony length on racemes was suggested as an action threshold, because economic damage by cabbage aphids was difficult to demonstrate in experimental field trials (Berlandier 2000, 2002; Berlandier and Valentine 2003). In 2004, and as a result of further research, an action threshold of 20% of plants infested with cabbage aphids (especially in low rainfall regions) was suggested and has since been recommended in canola-producing regions of Australia (Berlandier 2004, Edwards et al. 2008, Bellati et al. 2010, Berlandier et al. 2010). The current number of samples recommended to estimate aphid infestation levels in canola in Australia is a minimum of 20 plants selected at random and inspected at each of at least 5 points throughout a field (Bellati et al. 2010). Action thresholds of 50% of plants infested (with 25 mm or more aphid colony length on racemes), or four to five racemes infested per square meter (with 50 mm or more aphid colony length on racemes), have been proposed for New South Wales, Australia (Hertel et al. 2013). Here, the number of samples required is not defined. A threshold of 10% of plants infested with at least 25 mm of aphid colony length was also suggested in Australia (Berlandier and Baker 2007).

Development and Validation of the Binomial Sequential Sampling Plan

The following steps were used to develop and validate a binomial sequential sampling plan, as in Galvan et al. (2007) and Butler and Trumble (2012). Firstly, the stop lines were calculated for the action thresholds of 0.1, 0.2, and 0.3 proportion of plants infested using Wald's sequential probability ratio test within Resampling for Validation of Sample Plans (RVSP) software (Wald 1947, Naranjo and Hutchison 1997). These action thresholds were chosen because 0.2 proportion of plants infested with cabbage aphids is the current threshold used by industry in Australia, and sampling plans based on 0.1 and 0.3 action thresholds were developed to compare accuracy and efficiency (Berlandier 2004, Edwards et al. 2008, Bellati et al. 2010). Parameters in RVSP for development of stop lines were the same for all action thresholds: $\alpha = 0.1$ (i.e., 10% probability of type I error wherein the sampled lot is treated (e.g., the crop is sprayed) when the proportion of plants infested is below the action threshold), $\beta = 0.1$ (i.e., 10% probability of type II error wherein the sampled lot is not treated when the proportion of plants infested is above the action threshold), upper and lower boundaries (θ_1 and θ_2) = 0.1 above and below the action threshold, minimum sample size = 10 canola plants, and tally = 1 (i.e., plants with at least one cabbage aphid were considered infested). The above parameters were used again with only α and β parameters changed to 0.05 to compare the sample numbers required to make a decision when the error is decreased from 10 to 5%. Five hundred simulations were performed per action threshold.

Secondly, operating characteristic (OC) curves were plotted by using the OC functions calculated in RVSP for each action threshold, given three combinations of minimum sample size (10, 50, and

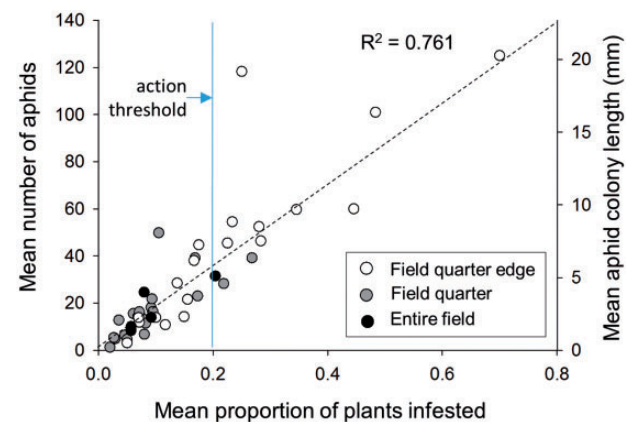


Fig. 1. Proportion of plants infested for field quarters ($N=20$), quarter edges ($N=20$), and entire fields ($N=5$) in relation to mean number of cabbage aphids on racemes (left y-axis) and equivalent mean cabbage aphid colony length on racemes (mm) (right y-axis).

100 canola plants) to validate and compare the accuracy of the sampling plans. The steepness of the OC curves at the 0.5 OC value provides an indication of the precision of the sampling plan, and the steeper the curve, the less probability of type I or type II error per given action threshold (Jones 1993).

Finally, in order to validate the sampling efficiency, the average sample number required to reach a decision using sampling plans based on each of the three action thresholds (0.1, 0.2, and 0.3 proportion of plants infested) was determined for each field stratum in RVSP (see Naranjo and Hutchison (1997)). A major advantage of using RVSP for developing and validating sample plan performance is that there are no *a priori* assumptions regarding the sampling distribution because directly resampling field data mimics the sampling process in the field and gives the ability to generate and quantify several different outcomes, as opposed to using a theoretical model based on an underlying sampling distribution (Naranjo and Hutchinson 1997).

Results

Stratification of Fields

Forty strata, which comprised 20 field quarters and 20 field quarter edges, ranged in proportion of plants infested with cabbage aphids from 0 to 70%, indicating significant variability between field strata (Table 1). A positive correlation existed between mean cabbage aphid colony length per plant and mean proportion of plants infested for field strata, accounting for 76.1% of the variance ($F=118.19$, $df=1,38$, $P<0.01$; Fig. 1). Interestingly, 9 out of 20 field quarter edges displayed above-threshold (i.e. 20% of plants infested) levels of plants infested, while only 2 out of 20 entire field quarters were above threshold. Analyses of edge effects resulted in infested plants distributed significantly more along field edges (i.e., < 20 m from edge) than further inwards for all five fields: York ($\chi^2=37.87$, $df=1$, $P<0.001$), New Norcia, ($\chi^2=208.40$, $df=1$, $P<0.001$), Meckering ($\chi^2=165.81$, $df=1$, $P<0.001$), Northam ($\chi^2=53.58$, $df=1$, $P<0.001$), and Grass Valley ($\chi^2=44.05$, $df=1$, $P<0.001$).

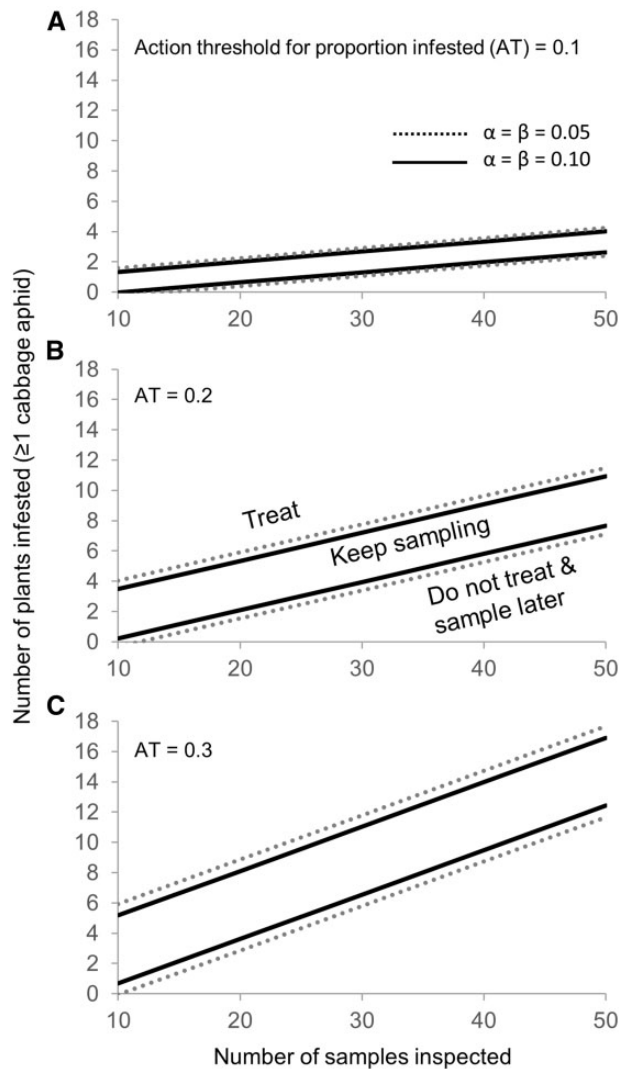


Fig. 2. Decision stop lines for the binomial sequential sampling plan for cabbage aphids on canola based on three action thresholds of (A) 0.1, (B) 0.2, and (C) 0.3 proportion of canola plants infested with at least one cabbage aphid and two α and β (error) parameters: 0.05 and 0.10. Average sample number (ASN) is the average number of samples required to reach a decision (treat or do not treat) given a minimum of 10 samples inspected and 500 resampling simulations.

Binomial Sequential Sampling Plan

The binomial sequential sampling plans for action thresholds of 0.1, 0.2, and 0.3 proportion of plants infested with cabbage aphids are shown in Fig. 2. Stop lines were calculated for action thresholds of 0.1 proportion of plants infested at 5% (slope = 0.040, y-intercepts ± 0.533) and 10% (slope = 0.066, y-intercepts ± 0.685) error, 0.2 proportion of plants infested at 5% (slope = 0.186, y-intercepts ± 2.181) and 10% (slope = 0.186, y-intercepts ± 1.628) error, and 0.3 proportion of plants infested at 5% (slope = 0.293, y-intercepts ± 3.002) and 10% (slope = 0.293, y-intercepts ± 2.240) error. As sampling continues, if the cumulative number of plants infested, relative to the cumulative number of plants inspected, increases to above the upper stop line, the sampled lot is considered to be above-threshold, and a decision is made to treat (i.e., insecticide application or other remedial action). Likewise, if the cumulative number of plants infested, relative to the cumulative number of plants inspected, falls below the lower stop line, the sampled lot is considered

to be below-threshold, and a decision is made to not treat and sample at a later date. If a decision has not been made after 50 canola plants have been inspected, a decision should be made to not treat and sample at a later date for that sampled region of interest.

Validation of Sampling Plan

Validation of the sequential sampling plans, using OC functions and average sample number calculations, revealed that the sampling effort required to reach a decision changed considerably when the minimum sample size and levels of acceptable error changed for each action threshold. The OC curves for action thresholds of 0.1, 0.2, and 0.3 proportion of plants infested showed varying levels of accuracy between thresholds, and increased OC curve steepness, and therefore increased accuracy, for all thresholds when minimum sample sizes were increased from 10 to 50 or 100 (Fig. 3). When the action threshold was set to 0.1 proportion of plants infested, the probability of not acting when the infestation level reached 0.1 proportion of plants infested was 45% with a minimum sample size of 10; this decreased to ~20% with a minimum sample size of 50, and ~15% when the minimum sample size was increased to 100 (Fig. 3A). For the action thresholds of 0.2 and 0.3 proportion of plants infested, the probability of not acting when their respective threshold levels were reached was ~45% for both (Fig. 3B and C). For the current cabbage aphid threshold of 0.2 proportion of canola plants infested, and given a minimum sample size of 10 and error rate of 10% (i.e., α and β parameters), the binomial sequential sampling plan displayed ~10% probability of not treating when infestation levels reach 0.3 proportion of plants infested and ~10% probability of treating when infestation levels reach 0.1 proportion of plants infested (Fig. 3B). Furthermore, given a minimum sample size of 10 and 10% error (i.e., α and β parameters), the overall average sample numbers required to make a decision across all fields and strata using the sampling plans were 13.4 ± 2.1 (SD), 17.2 ± 4.4 , and 16.6 ± 6.4 for 0.1, 0.2, and 0.3 proportion of plants infested, respectively. Reducing the error to 5% increased the average sample number required to make a decision to 15.9 ± 3.9 , 24.2 ± 8.7 , and 22.2 ± 11.8 for 0.1, 0.2, and 0.3 proportion of plants infested, respectively. The average sample number required to reach a decision for each action threshold and field stratum is displayed in Fig. 3D, E, and F. Here it is seen that considerably more sampling effort is required to reach a decision as 1) the action threshold increases from 0.1 to 0.2 and 0.3, 2) the proportion of plants infested within a given stratum reaches the action threshold, and 3) the level of acceptable error is decreased from 10 to 5%. The average sample number required to reach a decision decreases as the infestation levels within strata become further distanced, either higher or lower, from the action threshold employed.

Discussion

In this study, we demonstrated that taking spatial variability into account greatly reduced the sampling effort needed to obtain reliable estimates of cabbage aphid-infested plants in canola fields. Thus, we have demonstrated ways to generate more useful data and also make sampling for cabbage aphids in canola more practically feasible. Stratification according to field quarter edges and field quarters were equally important to ensure field regions which may contain above threshold levels of cabbage aphid-infested plants are not overlooked.

Nematollahi et al. (2014) presented a random sampling plan based on data collected from 500 m² size plots; this is the only

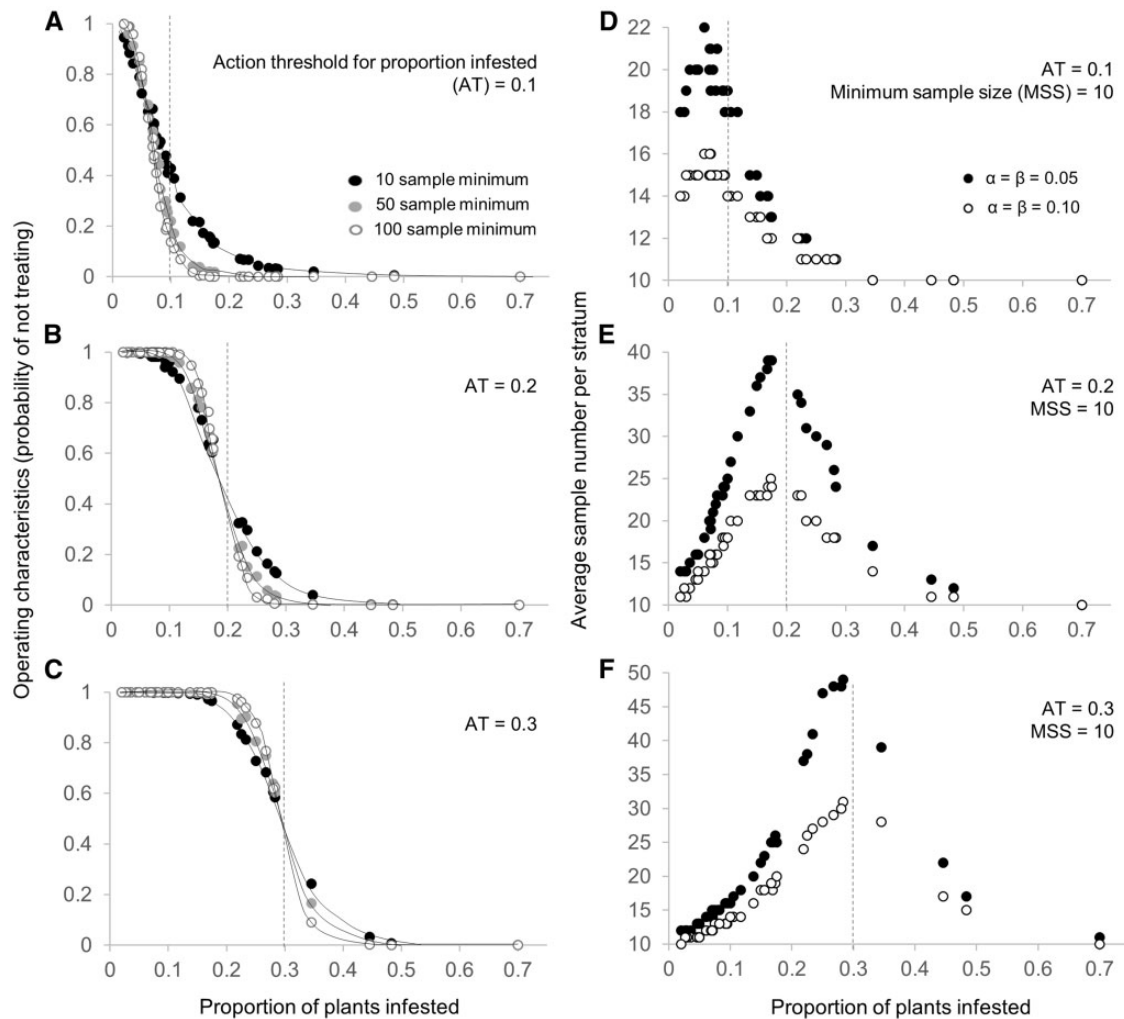


Fig. 3. Operating characteristic (OC) curves for binomial sequential sampling plan for cabbage aphids on canola based on action thresholds of (A) 0.1, (B) 0.2, and (C) 0.3 proportion of plants infested with at least one cabbage aphid per field stratum and a minimum sample size of (●) 10, (■) 50, and (○) 100 samples per action threshold. Average sample number required for each field stratum according to their average proportion of plants infested for the action thresholds of (D) 0.1, (E) 0.2, and (F) 0.3 proportion of canola plants infested with at least one cabbage aphid.

known published sampling procedure developed for cabbage aphids in canola. Here, the authors presented a sample size of 19–20 whole plants inspected at random throughout a field and all cabbage aphids counted. It may be argued that the field sizes presented are atypical of broad-scale canola production and insufficient to account for the distribution (including edge effects) in large fields. Nematollahi et al. (2014) did, however, recognize the need to stratify fields to reduce variability. Edge effect spatial patterns of arthropods in field crops and their importance within sampling plans has been identified in many arthropod species (Cusson et al. 1990, Winder et al. 1999, Ferguson et al. 2003). Edge effects in large fields typically result in zero-inflated data sets wherein most data points, which are positioned in the field interior, contain few or no detectable cabbage aphid populations. In this scenario, cabbage aphid populations are either overestimated (where the edge is sampled at the expense of the field interior) or underestimated (where the interior is sampled at the expense of the edge). It is therefore important to collect cabbage aphid distribution data from typical size fields in order to give a better representation of field situations.

Buntin (1993) emphasized how stratification of sampling regions improves sampling efficiency where there is variability between

strata, especially for spatially aggregated organisms. Spatial aggregation of cabbage aphids along field edges allows for stratification and targeted sampling because it is much easier to predict where they will occur compared to an organism which is distributed randomly or more uniformly. Care must be taken regarding where samples are positioned in the field, so as to not disregard areas which may contain below or above-threshold levels of infestation. Stratification of fields means growers will have the ability to assess where cabbage aphid infestation levels are above-threshold and, where possible, allow for targeted insecticide applications where they are required, such as that proposed by Fleischer et al. (1999), Blom et al. (2002) and Ferguson et al. (2003).

Sequential sampling provides a means of optimizing and reducing the sampling effort where less samples are required compared to fixed sample size methods, such as that reported by Stubbins et al. (2014). Of the sequential sampling methods, binomial (i.e., presence/absence) sequential sampling involves the least sampling effort because assessment of the presence of arthropod pests on plants is much less time consuming than counting arthropod pests on individual plants. The current action threshold for cabbage aphids in canola is a function of proportion of plants infested (i.e., 20%).

Furthermore, the spatial distribution of cabbage aphids in canola fields, relative to field edges, has been more accurately modeled using presence/absence of cabbage aphids on plants, rather than cabbage aphid counts or colony lengths on racemes; this further supports the use of binomial rather than count data in the development of a sampling plan (Edwards et al. 2008, Severtson et al. 2015).

Numerous sequential sampling plans developed for arthropods in field crops have used 10% as an acceptable level of error (i.e., α and β parameters; Cusson et al. 1990, Naranjo and Hutchison 1997, Cocco et al. 2015). In the present study, the upper and lower stop lines for 5% and 10% error (i.e. α and β parameters) were close together for all action thresholds. However, the average sample number required to reach a decision at 5% error was almost double the sample number required at 10% error when the infestation level reached the action threshold. When the action threshold for proportion of plants infested was 0.2, ~25 samples were required to reach a decision at 10% error, compared to ~40 samples required at 5% error. In addition, the accuracy of the sampling plan, based on the OC functions for the action threshold of 0.2 proportion of plants infested, may be considered appropriate with a minimum sample size of 10 when compared to increased minimum sample sizes. It may therefore be proposed that the optimal parameters for the sampling plan should be based on 10% error (i.e., α and β parameters) and a minimum sample size of 10.

For each canola field, the binomial sequential sampling plan, for the action threshold of 0.2 as the proportion of plants infested, should be applied firstly to a field edge stratum. This includes field perimeters as well as within-field regions of noncrop vegetation (e.g., remnant vegetation), as these regions are potential sources of cabbage aphids initiating on weeds (Severtson et al. 2015). Plants may be inspected haphazardly (e.g., every two paces) parallel to the crop edge within each stratum. If the sampling procedure shows above-threshold levels of infestation at the edge, then the sampling procedure should also be applied to a stratum greater than 20 m from the field edge to indicate whether above-threshold infestation levels are restricted to the edge or continue further inwards. This is important because cabbage aphids may, in some cases, be distributed further inwards. If the sampling procedure shows below-threshold levels of infestation at the edge, then sampling should not continue beyond the edge, as cabbage aphids are unlikely to be detected further inwards. After each stratum has been sampled according to the binomial sequential sampling plan, a decision can then be made, based on the sampling results, as to whether insecticide is warranted and, if so, on a field-wide scale or spatially targeted to the edge.

In this study, a significant need for stratification has been demonstrated in order to reduce spatial variability of infested canola plants between field quarter strata and their respective field quarter edge strata. However, this stratification of fields into eight strata is proposed only as a minimum, and further stratification is recommended to reduce variability, particularly in large fields, to avoid overlooking areas or patches which contain above-threshold levels of infested plants.

The distance of 20 m from the field edge was chosen because previous studies involving intensively sampled canola field edges showed that almost all cabbage aphids were restricted to this region in these fields (Severtson et al. 2015). According to this parameter, the field edges in this study showed significant differences in number of infested plants between the field edge and field interior for all five fields. However, it may be useful to modify field edge distance parameters to the width of spray equipment likely to be employed. Field edges stratified according to spray equipment widths would

allow for insecticide applications to be more accurately targeted. Spatially targeted insecticide applications in field crops would reduce the cost of insecticide inputs as well as provide environmental benefits, such as refuges for natural enemies that may maintain low levels of cabbage aphids in unsprayed areas at below-threshold levels and prevent secondary pest outbreaks (Midgarden et al. 1997).

Without reliable sampling plans, prophylactic and uniform applications of insecticides are often applied because of the risk of yield loss due to crop-damaging arthropods. Prophylactic and uniform applications of insecticides are likely to lead to increased insecticide resistance and secondary pest outbreaks due to a lack of beneficial arthropods (Dutcher 2007, Matthews 2008). Use of practically feasible and spatially optimized sampling plans are likely to reduce the risk of excessive use of insecticides to manage cabbage aphids in canola.

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