Reduced Sampling Frequency for Evaluating Fungicide Efficacy on Botrytis Fruit Rot of Strawberry

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ABSTRACT

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Evaluating fungicide efficacy in annual strawberry production systems can be labor intensive due to continuous harvesting over a relatively long season. The effect of reduced harvest number on the accuracy of least significant difference (LSD) separations for Botrytis fruit rot (*Botrytis cinerea*) incidence and marketable yield in fungicide efficacy studies was evaluated over three seasons. Fruit were harvested and graded twice a week for a total of 23 to 32 harvests each season. Data from each season were divided into different sample sets. Data from three different harvest periods (early, late, and whole season) and different harvesting frequencies (twice weekly, once weekly, every second, third, or fourth week) were compared with the complete data set (twice weekly for the whole season). Spearman's rank correlation and Pearson's product moment correlation coefficients were used to evaluate the correlation of the complete data sets with data sets from other sampling plans. Harvesting once a week for either the late- or whole-season periods accurately estimated LSD groupings for Botrytis fruit rot incidence among fungicide treatments. The precision of marketable yield estimates using once-a-week harvesting for the late or whole-season periods were relatively lower than for the incidence of Botrytis.

Additional keywords: chemical control, *Fragariae* × *ananassa*

Botrytis fruit rot (gray mold) caused by *Botrytis cinerea* is one of the major factors limiting production of strawberries in Florida. Data from fungicide efficacy trials (6,7,9,10) and informal surveys (D. E. Legard, unpublished data) suggest that marketable yields can be reduced by 10 to 15% on susceptible cultivars in Florida even with weekly applications of captan. Botrytis fruit rot is also an internationally important postharvest disease of strawberry (4,5).

Epidemics of Botrytis fruit rot in perennial strawberry production systems are initiated primarily by conidia produced in dead strawberry leaves within the field (1). Young, expanding strawberry leaves are asymptotically infected by the pathogen (2). When the leaf senesces, the pathogen quickly colonizes it and sporulates. Spores are either splash- or air-dispersed to infect different floral parts, including stamens and petals (3,11). Mycelia of *B. cinerea*

then invade the adjacent receptacle as it ripens and cause fruit rot. Direct infection of fruit by conidia is not considered very significant (13). The pathogen sporulates on diseased flowers and fruit, which become important sources of secondary inocula in annual production systems where there are multiple flowering and harvest cycles over several months. Because of the involvement of flowers in fruit infection, chemical control of Botrytis fruit rot focuses on protection of flowers (8,14).

In Florida, fungicides typically are applied weekly to commercial strawberries, and insecticides and miticides are applied as needed. Supplemental applications of fungicides are often made during disease epidemics, peak harvest periods, and rainy weather. Fruit are harvested by hand, typically every 3 days throughout the season (late November to early April). In research trials, fruit are harvested twice weekly to approximate commercial practices. Each harvest is graded into marketable and unmarketable fruit. Marketable fruit are counted and weighed; unmarketable fruit are separated into numbers of fruit with Botrytis rot and other unmarketable characteristics (i.e., other diseases, misshapen fruit, small fruit). During a normal season, fruit from 25 to 35 harvests are graded, weighed, and counted. Weekly fungicide applications and biweekly harvest and grading of fruit make fungicide efficacy

trials on annual strawberry labor intensive. Reducing plot size, the number of replicates, and the frequency or duration of harvest and grading operations can reduce the labor and time required for these studies. The purpose of this study was to investigate the effect of reduced sampling frequency and duration on estimates of treatment differences for Botrytis fruit rot incidence and marketable yield in fungicide efficacy trials on strawberry in the annual hill production system.

MATERIALS AND METHODS

Field experiments were conducted at the University of Florida, Gulf Coast Research and Education Center, Dover, during the 1995-96, 1996-97, and 1997-98 seasons to evaluate the efficacy of different fungicides, rates, and schedules for the control of Botrytis fruit rot. For all three seasons, nondefoliated, bareroot strawberry plants produced in Canada were transplanted into plastic-mulched, raised beds on 1.22-m centers (71 cm wide and 18 cm high at the center and 15 cm high at the edge) in soil fumigated with methyl bromide:chloropicrin (98:2). Plants were irrigated by overhead sprinklers for 10 to 14 days to facilitate the establishment of transplants, then irrigated and fertilized through drip tape. When necessary, overhead irrigation was used to provide frost and freeze protection. Fungicide treatments were applied with a CO₂ backpack sprayer (935 liters/ha). Most treatments consisted of weekly applications of a broad-spectrum protectant (captan or thiram) at different rates and schedules or in combination with other fungicides. Many treatments included applications at bloom of iprodione (Rovral), fenhexamide (Elevate), or fludioxonil + cyprodinil (Switch) to enhance Botrytis fruit rot control. Bloom treatments typically consisted of two applications made 1 week apart during both the first and second peak bloom periods when a majority of flowers were open. The number and composition of the treatments varied, but at least two treatments were evaluated each season. These treatments were unsprayed control and a grower standard of weekly applications of captan at 3.4 kg a.i./ha plus four bloom applications of iprodione at 0.6 kg a.i./ha. Fruit were harvested and graded twice weekly each season. Marketable fruit yield and count, unmarketable fruit count, and Botrytis fruit

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rot incidence (number of fruit with Botrytis rot divided by total number of marketable and unmarketable fruit) were determined for each harvest.

For the 1995–96 season, transplanting of cultivars Sweet Charlie and Oso Grande (in separate plots) was done on 18 October. Plots consisted of 16 plants set in two-row beds, 30 cm apart within and between rows. The experiment consisted of 22 fungicide treatments in a randomized complete block design with four replicates for each cultivar. Fungicide applications were typically made weekly from 1 November through 26 March. Bloom sprays for Botrytis fruit rot were applied on 6 and 13 December, 30 January, and 6 February for Oso Grande and on 29 November, 6 December, and 23 and 30 January for Sweet Charlie. Fruit were harvested and graded from 8 December through 25 March (32 harvests).

For the 1996–97 season, transplanting was done on 18 October, and only Sweet Charlie was used. Oso Grande was not used due to its relatively low level of susceptibility to Botrytis fruit rot. Plots consisted of 18 plants in two-row beds, 30 cm apart within and between rows. The experiment consisted of 24 treatments in a randomized complete block design with four replicates. Most fungicide treatments were applied weekly from 14 November through 4 March. Applications at bloom were made on 27 November, 4 December, and 14 and 21 January for the early spray programs and 14 and 21 January, and 18 and 25 February for the late programs. Fruit were harvested and graded from 20 December through 6 March (23 harvests).

For the 1997–98 season, 16 plants of Sweet Charlie per plot were set on 7 October in two-row beds, 30 cm apart within and between rows. The experiment consisted of 30 treatments in a randomized complete block design with four replicates. Fungicide treatments were applied weekly after either the first or second harvest each week, typically from 30 October through 10 March. Applications at bloom were timed to provide coverage during the first

two peak flowering periods. Fungicides for the first bloom were applied beginning on 18 November and for the second bloom beginning on 13 January. Fruit were harvested and graded from 1 December through 12 March (30 harvests).

To evaluate the effect of reducing the number of harvests used to rank Botrytis fruit rot incidence and marketable yield treatment differences, harvest data from each season were divided into several sample sets. Data from three different harvest periods were used to compare sampling plans. These included the whole season and the early (harvests before 1 February) and late (harvests after 31 January) periods. The division of the data sets into early and late periods corresponds with a natural division in fruiting patterns, with the early period encompassing the harvest of the first inflorescence of fruit and the late period the second inflorescence of fruit. Within each harvest period, harvest dates were numbered sequentially, beginning with 1 for the first harvest. Based on this numbering, samples were considered collected twice weekly, once weekly, and every second, third, and fourth week (Table 1). Two once-weekly sampling plans were included based on whether the harvest data number was odd or even.

Statistical analyses were performed using SAS (SAS Institute, Inc., Cary, NC). Prior to analysis, the incidence of Botrytis fruit rot data were transformed (arcsine square root). Analysis of variance was performed on each season's data set. Significant treatment effects were observed for all data sets. These treatment differences were then ranked using Fisher's protected least significant difference (LSD) test ($P \leq 0.05$). Correlation analysis was used to assess the effect of the sampling plans on the reliability of the estimates and the treatment rankings. Spearman's rank correlation coefficient (12) was used to evaluate the correspondence between treatment ranks in the different data sets. Pearson's product moment correlation coefficient was used to evaluate the correlation between the data sets from different

sampling plans. All the comparisons were made between the standard sampling plan (twice-weekly sampling for the whole season) and the corresponding data subsets each season.

RESULTS

Epidemics of Botrytis fruit rot developed to varying degrees each season (Fig. 1). Disease incidence was low during the 1995–96 season. During the 1996–97 and 1997–98 seasons, severe epidemics developed, with the highest Botrytis fruit rot incidence occurring in the 1997–98 season. The incidence of Botrytis was lower during the early period than the late period on Sweet Charlie for all three seasons (Table 2). During all three seasons, most of the fungicide spray treatments significantly reduced Botrytis fruit rot incidence when compared to the unsprayed control. The reduction in the incidence of Botrytis with the standard grower treatment (weekly captan applications plus iprodione bloom sprays) was typical of the level of control on commercial farms in Florida (Fig. 1).

There were two main harvests peaks each season, with a majority of marketable fruit harvested during the late period (Fig. 1). Botrytis fruit rot significantly reduced yields in the unsprayed control when compared to most fungicide treatments during the 1996–97 and 1997–98 seasons, but not during the 1995–96 season when incidence was low (Table 2). In the 1997–98 season, marketable yields were low due to the high incidence of Botrytis (Table 2).

Pearson's product moment (r) and Spearman's rank (r_s) correlations revealed differences in the ability of the various sampling plans to accurately predict the ranking and relative size of Botrytis fruit rot incidence means (Table 3). A strong positive correlation between data from the standard sampling plan (twice weekly for the whole season) and the reduced sampling plans are necessary for them to be worthwhile. We believe that a correlation of at least 0.925 ($r^2 = 86\%$) is indicative of an acceptable level of reliability. The coefficients for both Pearson's and Spearman's

Table 1. Number of harvests used to evaluate treatment differences for the different sampling plans for the whole-season, early-period, and late-period data sets each season

Frequency ^d	Whole season ^a			Early period ^b			Late period ^c		
	1995–96	1996–97	1997–98	1995–96	1996–97	1997–98	1995–96	1996–97	1997–98
Twice weekly	32	23	30	16	13	18	16	10	12
Once weekly (odd)	16	12	15	8	7	9	8	5	6
Once weekly (even)	16	11	15	8	6	9	8	5	6
Every second week	8	6	8	4	4	5	4	3	3
Every third week	6	4 ^e	5	3	3	3	3	2	2
Every fourth week	4	3	4	2	2	3	2	2	2

^a Number of harvests evaluated using whole-season data (all harvests).

^b Number of harvests evaluated using early-season data (harvests before 1 February).

^c Number of harvests evaluated using the late-season data (harvests after 31 January).

^d Sampling plan frequency relates to the time interval between harvests used in the sampling plan.

^e Number of samples evaluated for the early and late period may not add up to the number of samples for the whole season due to differences between the sample schedules for the whole season and the late period (i.e., February samples start on the first harvest after 31 January for the late-season period, but may occur several weeks later with the standard sampling plan).

Fig. 1. Average weekly Botrytis fruit rot incidence and marketable yield for cultivars Oso Grande (1995–96) and Sweet Charlie (1995–96, 1996–97, and 1997–98) for the unsprayed control and the grower standard treatment (weekly applications of captan at 3.4 kg a.i./ha) plus four bloom sprays of iprodione at 0.6 kg a.i./ha).

correlations were high every season for both odd ($r \geq 0.979$; $r_s \geq 0.964$) and even ($r \geq 0.975$; $r_s \geq 0.971$) harvests of the once-weekly samples for the whole-season data set with Sweet Charlie. The correlations for Oso Grande (1995–96 season) were also high for the once-weekly samples for the odd numbered harvests ($r = 0.970$; $r_s = 0.983$), but lower for the even numbered harvests ($r = 0.849$; $r_s = 0.679$) for the whole-season data set. Correlations between the standard sampling plan and the every second, third, or fourth week sampling plans were high for the 1997–98 season, but were lower in the 1995–96 and 1996–97 seasons.

Correlations between the standard and reduced sampling plans from the early-season data sets were too low to provide reliable estimates of Botrytis fruit rot inci-

dence (Table 3). Correlations for the twice-weekly sampling plans were as low as $r = 0.628$ and $r_s = 0.677$ for Sweet Charlie and only $r = 0.419$ and $r_s = 0.421$ for Oso Grande. The correlations for the late-season data sets were much higher than those for the early-season data. Correlations for Sweet Charlie from both the twice-weekly ($r \geq 0.992$; $r_s \geq 0.980$) and once-weekly ($r \geq 0.966$; $r_s \geq 0.952$) sampling plans were well above the acceptable limit of reliability. Correlations for Oso Grande were also high for the twice-weekly late-season sample ($r = 0.990$; $r_s = 0.989$), but varied between the odd ($r = 0.968$; $r_s = 0.966$) and even ($r = 0.819$; $r_s = 0.670$) harvests with the once-weekly sampling plan. Correlations were also low for the infrequent sampling plans (i.e., every second, third, and fourth week) for all the late-period data sets.

The reduced sampling plans did not predict the ranking and treatment means for marketable yield (Table 4) as accurately as for the incidence of Botrytis fruit rot. Correlations from both the once-weekly sample from the whole-season data sets and the twice-weekly sample from the late-season data sets were generally high. Correlations were also generally high for both cultivars for the once-weekly sample from the whole-season data sets ($r \geq 0.918$; $r_s \geq 0.895$), and with Sweet Charlie for the twice-weekly sample from the late-season data sets ($r \geq 0.944$; $r_s \geq 0.920$). The correlations for the early-season data sets were very low for both cultivars, even with the twice-weekly sample ($r \geq 0.430$; $r_s \geq 0.447$). The every second, third, and fourth week sampling plans were not consistently reliable for either cultivar.

Table 2. Summary of cumulative Botrytis fruit rot incidence and marketable yield each season for the unsprayed control and the grower standard (weekly captan applications plus bloom sprays of iprodione) treatment for the early, late, and whole season periods

Season, cultivar	Treatment ^c	Botrytis fruit rot incidence (%) ^a			Marketable yield (g) ^b		
		Early	Late	Whole	Early	Late	Whole
1995–96							
Oso Grande	Control	0.73	1.52	1.37	1853	5465	7317
	Grower standard	0.54	0.11	0.19	1553	6398	7951
Sweet Charlie	Control	2.22	5.90	5.33	2066	5214	7280
	Grower standard	0.20	1.06	0.90	2394	5645	8039
1996–97							
Sweet Charlie	Control	15.07	22.12	20.71	1456	3337	4793
	Grower standard	4.85	9.39	8.57	2216	5711	7927
1997–98							
Sweet Charlie	Control	25.71	39.78	35.39	1464	1406	2870
	Grower standard	12.57	14.07	13.66	2356	4109	6465

^a Cumulative incidence of fruit with Botrytis rot = (number of fruit with Botrytis rot/number of marketable and unmarketable fruit) $\times$ 100).

^b Cumulative marketable fruit (grams per plot).

^c The control treatment was not sprayed with any fungicides during the season. The grower standard was sprayed weekly throughout the season with captan (3.4 kg a.i./ha) and two applications of iprodione (0.6 kg a.i./ha) were applied during each of the first two peak flowering periods.

Table 3. Correlation coefficients between observations made from the standard sampling plan (twice weekly for the whole season) and those made by other sampling plans each season for Botrytis fruit rot incidence on cultivars Oso Grande (OG) and Sweet Charlie

Sampling plans ^c	Pearson's product moment correlation (r) ^a				Spearman's rank correlation (r_s) ^b			
	OG		Sweet Charlie		OG		Sweet Charlie	
	1995–96	1995–96	1996–97	1997–98	1995–96	1995–96	1996–97	1997–98
Whole season								
Once weekly (odd)	0.970	0.979	0.984	0.991	0.983	0.967	0.964	0.976
Once weekly (even)	0.849	0.982	0.975	0.990	0.679	0.973	0.971	0.975
Every second week	0.533	0.778	0.941	0.982	0.558	0.785	0.860	0.958
Every third week	0.456	0.802	0.950	0.948	0.432	0.755	0.926	0.935
Every fourth week	0.516	0.754	0.880	0.956	0.504	0.753	0.825	0.939
Early season								
Twice weekly	0.419	0.628	0.913	0.920	0.421	0.677	0.831	0.852
Once weekly (odd)	0.406	0.616	0.858	0.759	0.383	0.691	0.800	0.568
Once weekly (even)	0.268	0.500	0.810	0.932	0.246	0.534	0.751	0.869
Late season								
Twice weekly	0.990	0.998	0.997	0.992	0.989	0.988	0.995	0.980
Once weekly (odd)	0.968	0.980	0.966	0.980	0.966	0.963	0.956	0.962
Once weekly (even)	0.819	0.975	0.973	0.984	0.670	0.963	0.952	0.983
Every second week	0.466	0.782	0.920	0.907	0.520	0.773	0.898	0.902
Every third week	0.720	0.902	0.903	0.961	0.738	0.913	0.880	0.947
Every fourth week	0.376	0.762	0.852	0.920	0.300	0.768	0.856	0.886

^a Correlation for Botrytis fruit rot incidence between the standard sampling plan (twice weekly for the whole season) and the listed sampling plan. Correlation coefficients greater than 0.925 are large enough to suggested that estimates from those sampling plans produce reliable estimates ($r^2 \geq 0.86$).

^b Rank correlation between whole season Botrytis fruit rot incidence and the incidence estimated from the other sampling plans.

^c Frequency relates to the time interval between harvests used in the sampling plan. Weekly sampling plans used the first (odd) or second (even) harvest each week.

DISCUSSION

In annual strawberry, the number of samples necessary to estimate treatment differences and LSD groupings of the incidence of Botrytis fruit rot and marketable yield can be reduced to once-weekly sampling from either the whole season or the late season. Sampling plans utilizing early-season data or sampling frequencies of less than once a week were not as reliable.

It is preferable to collect the greatest number of samples possible to most precisely estimate experimental parameters such as the incidence of Botrytis fruit rot and marketable yield. However, labor and time constraints dictate that experiments on annual strawberry employ sampling plans that better manage these factors. Several modified sampling plans evaluated in this study would reduce the number of harvests that need to be evaluated. The standard sampling plan (twice-weekly sampling from the whole-season data set) included 23 to 32 harvests, while some of the reduced sampling plans had as few as 5 or 6 harvests, depending on the season. In general, the sampling plans that included only a few harvests were not suitable for estimating Botrytis incidence or marketable yield because of their lower accuracy in ranking treatments. However, the every third and every fourth week whole-season sampling plans for the 1997–98 season were highly correlated for the incidence of Botrytis (≥ 0.936), despite the reduction in sampling from 30 harvests to 4 or 5. This emphasizes the influence of season on estimates of treatment differences in strawberry with different sampling plans. Clearly, reducing the sampling of a crop such as annual strawberry to a couple of samples to predict treatment differences

over a 3- to 4-month-long season is not prudent, and we would not recommend using the every third or every fourth week sampling plans.

Sampling plans that used only early-season data were not reliable in estimating treatment differences in Botrytis incidence or marketable yield for the whole season. This was probably due to the characteristics of epidemics of Botrytis fruit rot and strawberry fruiting patterns. The disease progress curve for Botrytis fruit rot epidemics on annual strawberry is very complex, with several different peaks during the season. During the three seasons evaluated, a majority of the Botrytis fruit rot occurred during the late period. Marketable yield patterns also varied within each season. Normally, there are two peak harvest periods each season in Florida, and this occurred during the three seasons studied. A majority of the marketable yield comes from the second fruiting cycle, later in the season. For these reasons, the effect of fungicide treatments on Botrytis incidence and marketable yield during the early-season period does not reliably predict their effects for the whole season.

Estimates produced from the reduced sampling plans were not reliable for marketable yield (Oso Grande and Sweet Charlie) or the incidence of Botrytis fruit rot (Oso Grande) for the 1995–96 season. The incidence of Botrytis fruit rot was unusually low that season, especially on Oso Grande. The difficulty in using reduced sampling plans with Oso Grande was apparent when evaluating Spearman's rank correlations for the once-weekly sampling plans. Relatively large shifts in these correlation values occurred between the odd and even data sets (Table 3). We be-

lieve that the low Botrytis incidence that season contributed to the lack of precision of the subsampling plans for Oso Grande. Relatively higher Botrytis incidences were observed on Sweet Charlie that season and Botrytis incidence estimates from reduced sampling plans were more reliable for that cultivar.

Estimates of treatment differences for several of the sampling plans were very reliable for the incidence of Botrytis fruit rot on Sweet Charlie. However, the reliability of marketable yield estimates was not as good, especially when analyzed with Spearman's coefficient. Apparently, marketable yields from individual harvests are more variable than the incidence of Botrytis. This is expected because marketable yield can be influenced by many factors, including disease, that influence fruit number, size, and shape. Weather and other variables, such as harvesting and nutrition, may increase the variance of marketable yield.

The lower reliability of marketable yield compared to the incidence of Botrytis estimates obtained with the reduced sampling plans is not a serious problem in fungicide efficacy trials. Such studies are designed as preliminary evaluations of fungicides and are intended for general comparisons of the efficacy of a large number of fungicides; therefore, the key comparisons are differences in Botrytis incidence and their general rank grouping. However, marketable yield data are important because they can reveal treatment effects on factors not apparent on harvested fruit, such as growth promotion or inhibition, and phytotoxicity. More in-depth studies of labeled products are necessary to develop effective grower recommendations.

Table 4. Correlation coefficients between observations made using the standard sampling plan (twice weekly for the whole season) and those made by other sampling plans each season for marketable yield for cultivars Oso Grande (OG) and Sweet Charlie

Sampling plans ^c	Pearson's product moment correlation (r) ^a				Spearman's rank correlation (r_s) ^b			
	OG		Sweet Charlie		OG		Sweet Charlie	
	1995–96	1995–96	1996–97	1997–98	1995–96	1995–96	1996–97	1997–98
Whole season								
Once weekly (odd)	0.918	0.925	0.971	0.989	0.895	0.898	0.985	0.970
Once weekly (even)	0.949	0.961	0.974	0.982	0.936	0.954	0.916	0.962
Every second week	0.880	0.753	0.931	0.973	0.915	0.574	0.931	0.931
Every third week	0.753	0.716	0.876	0.914	0.741	0.579	0.928	0.914
Every fourth week	0.892	0.622	0.667	0.951	0.868	0.483	0.643	0.853
Early season								
Twice weekly	0.618	0.430	0.833	0.904	0.701	0.447	0.877	0.765
Once weekly (odd)	0.208	0.367	0.692	0.852	0.314	0.495	0.836	0.688
Once weekly (even)	0.719	0.410	0.702	0.849	0.715	0.493	0.635	0.705
Late season								
Twice weekly	0.960	0.944	0.985	0.972	0.884	0.920	0.970	0.934
Once weekly (odd)	0.856	0.858	0.959	0.961	0.754	0.779	0.912	0.919
Once weekly (even)	0.859	0.918	0.964	0.969	0.779	0.919	0.976	0.940
Every second week	0.850	0.676	0.881	0.944	0.821	0.526	0.795	0.850
Every third week	0.745	0.812	0.935	0.916	0.787	0.749	0.893	0.821
Every fourth week	0.804	0.591	0.733	0.929	0.727	0.485	0.682	0.881

^a Correlations for marketable yield between the standard sampling plan (twice weekly for the whole season) and those based on the listed sampling plan. Values greater than 0.925 are large enough to suggest that estimates from those sampling plans produce reliable estimates ($r^2 \geq 0.86$).

^b Rank correlation between whole season marketable yield and marketable yield based on the sampling plan.

^c Frequency relates to the time interval between harvests used in the sampling plan. Weekly sampling plans used the first (odd) or second (even) harvest each week.

The reduction in labor and costs incurred from using reduced sampling plans for fungicide efficacy trials can be significant. During the three seasons of the experiments reported in this study, over 241,000 fruit were hand harvested and graded, yielding 145,314 marketable fruit weighing 2,703 kg. Over 17,000 fruit with *Botrytis* rot were scored. Reducing grading to once a week for either the whole season or for the late period of the season will significantly reduce the labor necessary to harvest and grade fruit during the peak harvest period in Florida (February and March). A reduction in the amount of fruit being handled would increase the accuracy of fruit grading by reducing worker fatigue and reduce the time needed to collect data from these trials. There would only be a limited reduction in harvesting labor because fruit must be harvested twice weekly (and ungraded fruit discarded) regardless of the sampling plan used. This conforms best to the commercial harvesting schedule and prevents diseased fruit being left within the canopy and unduly influencing epidemics and treatment differences.

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