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Effects of Glean, a Sulfonylurea Herbicide, on the Reproductive Biology and Fruit Set in Cherry Trees

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INTRODUCTION

Approximately 85% of the 475 million pounds of pesticides used annually in the U.S. are herbicides (Waddell and Bower, 1988; USDA, 1990). These phytotoxic compounds are applied to an estimated 270 million acres of cropland. There has always been a concern for damage to nontarget vegetation brought about by herbicide drift, believed by some to be as great as 25 to 50% of the applied chemical (Waddell and Bower, 1988). The concern for drift-damage has intensified over the last 10 years with the introduction of new classes of herbicides (sulfonylureas and imidazolinones) which are 100 times more toxic than formerly used compounds such as 2,4-D and atrazine (Beyer et al., 1987). Approximately 10 sulfonylurea and imidazolinone herbicides are currently being sold and many more will no doubt be registered in the future. The sulfonylurea compounds alone had over 230 U.S. patents approved by 1987. (Beyer et al., 1987).

Local problems in North Dakota, Colorado, and Washington suggest that the sulfonylurea herbicides have caused unexpected nontarget damage (Callihan and Lass, 1991; Westra et al., 1991; Mink and Howell, 1990). This is after the herbicides have been reviewed in the registration process for nontarget plant damage (Lewis and Petrie, 1991). The total number and nature of nontarget plant losses associated with these newly introduced herbicides are uncertain because there is no tabulation of nontarget plant damage at the national level.

The current pesticide registration process requires plant testing. However, it is limited to germination, emergence and vegetative vigor tests (Table 1). The process only tests a limited portion of a plants life cycle (Figure 1) and only with a few agricultural species (i.e., no herbaceous perennials or woody plants). The tests only involve germination and early vegetative growth of a plants life cycle (Figure 1). The registration process requires no reproductive or life cycle test data and therefore, nontarget plant reproductive processes may be at risk from the new herbicides.

The complaints of orchard growers in south-central Washington are an excellent example of farmer accusations regarding crop damage due to sulfonylurea drift from dry land wheat fields. Some growers claim that drifting sulfonylurea herbicides caused flower and fruit abortion on their cherry, apricot, and plum trees in 1988 and 1990, whereby greater than 80% of their crops were lost. The total dollar loss of all crops in this area due to herbicide drift has been estimated by local growers to be $40 million. Although these allegations have been investigated by local and federal representatives, including the Washington State Department of Agriculture and the U.S. Environmental Protection Agency, the issue remains unresolved for several reasons: 1) No data has ever been published pertaining to the influence of the herbicides in question ('Glean' and 'Harmony') on reproduction (flowering and/or fruit set) of orchard trees; 2) No plant reproduction data is currently required by OPP (Office of Pesticide Programs) for herbicide registration (Table 1; Figure 1); and 3) It is conceivable that new herbicides which are very toxic to vegetative growth may be even more toxic towards reproductive events. If so, concentrations affecting reproduction may be below the level of detection by conventional chemical analyses. Thus, the claims of orchard growers in Washington may be accurate, and if so, the current registration process may not be adequate with respect to these new chemicals.

EPA, Corvallis Environmental Research Laboratory undertook this study for Region X to help in their efforts to understand the issue of non-target plant damage in south-central Washington from sulfonylurea herbicides. Specifically, the study was designed to determine if low levels of 'Glean' interfere with the normal reproductive events in mature cherry trees. This was accomplished by measuring the reproductive response (flower and fruit set) of individual cherry trees to different exposure regimes in which both the amount and time of exposure (bud development and expansion) have been measured. This is intended to be a multi-year study; therefore, it is important to recognize that the results reported herein are preliminary and must not be misconstrued to be conclusive.

METHODS

Fifteen different twenty year old Royal Anne Cherry trees (Prunus Avium L.) grown and maintained by the Oregon State University Department of Horticulture, were used for each spring and summer/fall exposure. The trees are located at the Lewis-Brown Horticulture Farm in the Oregon Willamette Valley approximately five miles southeast of Corvallis, Oregon. The trees received the standard management practices used on all cherry trees under the care of the Department of Horticulture, including insecticide and fungicide treatments, pruning, fertilizing and fruit harvesting (Fisher et al., 1990)

For each exposure, fifteen uniform trees (i.e., similar in height and diameter) were selected from a row of twenty, in a block of 140 trees. Six branches on the same side of each tree were selected for treatment. The branches were on the perimeter of the canopy and were approximately equal distance from the ground. The six branches were each randomly assigned one of six different treatments; no treatment (control), water treatment (carrier control), 0.1, 0.01, 0.002 and 0.001 of the field application rate of 'Glean'. The field application rate used was 1/3 of an ounce per acre (2.34×10^{-6} Kg/m^2). The surfactant 'Unifilm 707' was used at a concentration of .05 percent of the spray solution. The carrier control not only tested for carrier effect, but also the effect of enclosing the treated branches overnight. Observations of treatment effects were recorded both as notes and photographs.

Bags made of 'Tyvek' were placed over the branches, except the control treatment, to prevent cross contamination of treatments. These were supported by a light weight frame made of sheet metal and fastened to the branch by tape. The bag and frame covered 90 cm of the branch of which the middle 50 cm was observed. The frames were placed on the branch the morning of the treatment application. The bags were placed on the frames late in the afternoon just prior to spraying the treatments. The branches were sprayed with the treatments for 15 seconds at 25 lbs of pressure using a two gallon stainless steel hand pump sprayer with a pressure gauge and a brass extension fitted with dual nozzles. The spray nozzles consisted of a nylon core and a disc with an orifice diameter of .61 mm (Spraying Systems Co., Wheaton, IL). The spray apparatus was calibrated and verified to deliver 60 ml of solution per spray event. This procedure ensured complete coverage of the 50 cm treatment segment on each branch to the point that excess liquid dripped from the branches. The bags were taped shut immediately following the spray treatment and were not removed until the next morning when the bag and the frame were also removed. The

bags remained on the tree over night to prevent cross contamination by volatilization of the chemical and to also provide uniform exposure conditions at each stage of application (bud, flower, fruit) in the event of rain. The treatment and bagging were conducted late in the day to avoid any potential heat damage to the treated branch.

Fifty centimeters on each branch were treated and monitored. The boundaries of the treatment zone were marked on each branch with a permanent marker. The treated area started 15 cm from the branch tip and extended toward the trunk for 50 cm. This area was chosen due to its high concentration of flower buds. At different times during the spring the numbers of buds, flowers and fruits present on the treatment segment of each designated branch were counted. During the course of the study the foliage was periodically examined and descriptive assessments recorded.

This investigation has examined herbicide exposure at two different stages of perennial reproduction (Figure 2). One set of fifteen trees was exposed in the spring during flower and fruit set, and a different row of trees was exposed in the late summer and early fall, a time when the next year's reproductive buds were developing (Tuffs and Marrow, 1925).

Spring Exposure

The treatments were applied once on each of the fifteen trees. Five randomly selected trees had the treatments applied during the green tip stage of floral bud expansion (March 15, 1991). An additional five trees were treated at the full bloom (April 2) and another five at the post bloom stage (April 26).

Cherry fruits were harvested on June 13. They were harvested three and a half weeks prior to full ripening to prevent possible loss to birds. The fruit were bagged, placed on ice and transported to the laboratory and refrigerated. The fruit were counted and weighed to determine a mean cherry weight per tree for each treatment. Cherries seeds were split to determine seed viability. Seeds were considered nonviable if the embryo was aborted and the endosperm was not developed. Nonviable cherries would have fallen off prior to the normal harvesting date; therefore seed viability was used as the criteria for determining fruit set.

The response variates (average cherry weight per tree and number of viable cherries) were analyzed assuming a randomized complete block design with trees as blocks and concentration levels randomly assigned to branches (experimental units) with a block (tree). Prior to the ANOVA and post-ANOVA analyses, the control treatments (carrier-control and no treatment) were compared for each response variable within each time period (green tip, full bloom, post bloom) with no significant difference detected (paired t-test, $p > 0.2$). The carrier-control was therefore removed from further consideration. Post-ANOVA analyses to determine specific pair-wise differences was accomplished using Tukey's studentized range (HSD) test at an alpha level of 0.05.

Prior to the overall test for treatment effects, the number of viable cherries response was transformed using the square root transformation to correct for non-constant variance.

Transformation of average cherry weight was not deemed necessary.

Summer / Fall Exposure

Because the loss of cherry fruit may be the result of injury during bud development in late summer, and early fall rather than during bud expansion in the spring, another series of experiments was initiated to investigate the effect 'Glean' has when it is applied during the differentiation of flower tissues in buds (Figure 2). These experiments were performed in exactly the same manner as described for the spring experiments except that the spray applications occurred on August 15, September 12 and October 10, and a different row of trees in the same block of cherry trees was used. The treatments were approximately one month apart to cover a broad spectrum of bud development. Data from this experiment (cherry fruit) will not be collected until late spring of 1992.

Pesticide incident reports submitted to the Washington State Department of Agriculture show that herbicides are being applied throughout the growing season (State of Washington, 1987; 1988); therefore nontarget plants may be subjected to multiple exposures from herbicide drift. Two experiments were set up to determine if repeated exposures to low levels of 'Glean' had an effect on bud development. Again, the same methodology was used with the exception that only the 0.002 and both control treatments were applied. In one experiment, branches from five trees were treated twice, one month apart on September 26 and October 24. In the other experiment, branches from five trees were treated three times, one week apart on September 19, 26, and October 3 and another set of branches from an additional five trees were similarly treated on October 10, 17 and 24. Again, data from these experiments will not be collected until late spring of 1992 when the cherries are harvested.

RESULTS (Spring exposure 1991)

Cherry Weight Data

When 'Glean' was applied at the green tip stage of bud development, no significant differences ($p = 0.19$) were found between any of the treatments and the controls in mean cherry weight (Figure 3). In contrast to this, when 'Glean' was applied at the full bloom stage, the 0.1 application rate significantly reduced the mean cherry weight ($p = 0.002$) to approximately one third of the control weight (Table 2). When the treatment was applied at the post bloom stage the reduction in mean cherry weight was significant at both the 0.1 and 0.01 treatment levels ($p = 0.0001$) (Figure 3). The 0.002 treatment level reduced mean cherry weight by 17 percent, but was not significantly different from the controls (Table 2).

Seed Viability/Fruit Set

Data on the number of viable cherries supports the results found from the mean cherry weight data. At the green tip stage none of the treatments caused a significant difference in comparison to the controls ($p = 0.68$) (Figure 4). Treatments at full bloom resulted in a significant difference at the 0.1 concentration ($p = 0.001$). However, this result is confounded because the 0.1 treatment was not significantly different from the 0.002

treatment at an alpha level of 0.05 despite the fact that there was a 77 percent reduction in the number of viable cherries between the 0.002 and the 0.1 treatments (Table 2). At the post bloom stage there were no viable cherries at either the 0.1 or 0.01 treatments (Table 2). Although the 0.002 treatment at the post bloom stage reduced the number of viable cherries by 19 percent in comparison to the control, this was not significantly different (alpha = 0.05). This is consistent with the analysis of the mean cherry weight data (Table 2).

Foliage Response

Chemical application at the green tip stage had no observable influence on leaf development or appearance. In contrast, treatments at either the full bloom or post bloom stage, when leaves were partially expanded, caused abnormalities in leaf morphology. The abnormality symptoms consisted of yellowing, cupping and reduced leaf expansion. The intensity and time at which these symptoms appeared varied, depending on the stage of treatment and the concentration of applied chemical. When the 0.1 'Glean' treatment was applied at the full bloom stage, symptoms were first observed after four weeks; and by ten weeks the leaves had withered and appeared dead. None of the other concentrations applied at the full bloom stage altered leaf development. At the post bloom stage, the 0.1, 0.01, and 0.002 treatments affected leaf morphology. The 0.1 and 0.01 treatments caused leaf symptoms to appear two and four weeks, respectively, after application. The 0.1 treated leaves appeared dead six weeks following exposure; whereas the 0.01 treated leaves remained alive, but permanently dwarfed. A few leaves on branches treated with the 0.002 application also showed the abnormal symptoms.

The only visible symptoms on branches treated in the fall were the yellowing of leaves. This was only apparent on branches receiving the 0.1 treatment on August 15th. No clear signs of injury were present on the other treatments made on September 12th and October 10th. The apparent absence of leaf response to 'Glean' in late fall coincided with the general yellowing of all leaves as fall progressed.

DISCUSSION

The data suggests that the impact of 'Glean' on mean cherry weight and viability is related to the time of application, with increasing sensitivity from the bud stage to the post bloom stage. Not only are the full bloom and post bloom stages more responsive to 'Glean', but there is also an increased sensitivity to lower concentrations of the chemical. Significant differences were found at the 0.01 treatment level at the post bloom stage, whereas the only significant difference was at the 0.1 treatment during the full bloom stage.

The increased sensitivity of the cherry trees to 'Glean' was associated with leaf expansion. At the green tip stage the leaves were compressed within the swollen vegetative buds, by full bloom they were beginning expansion and by post bloom they were one third to one half expanded. The preliminary results suggest that the sensitivity of fruit development and set on cherry trees exposed to 'Glean' is proportional to the extent of leaf expansion. The more fully expanded the new leaves are, the more susceptible the developing cherries are to damage. It is conceivable that the most sensitive stage would be when new leaves are

fully expanded and the fruits are still partially developed. This suggests that concentrations that produced no significant effects when applied early in leaf expansion may produce significant effects when applied at later stages. Our intention is to examine this possibility during the spring of 1992.

The positive correlation between leaf expansion and cherry loss (fruit abortion or reduced weight) to 'Glean' indicates that the leaf, not the bud or flower, may be the primary receptor surface for that portion of the applied chemical which is detrimental to fruit development. We propose that as the young leaves expand, their increased surface area permits greater absorption of the applied chemical leading to elevated levels of 'Glean' in the leaves. The action of 'Glean' may be explained by two different hypotheses. The chemical may be transported through the phloem to the developing fruit, where it reaches a concentration which proves to be inhibitory to cell division, one of the reported modes of action for 'Glean' (Beyer et al., 1987). A second possibility is that 'Glean' stays in the leaf where it prevents newly fixed carbon from entering the translocation stream, as reported by Bestman et al. (1990). If that occurred the developing fruits could be subjected to carbohydrate starvation, leading to reduced growth and abortion.

The experimental work described in this report covers only a small portion of the time when cherry buds are undergoing development and expansion. As shown in Figure 2 bud development begins in the early summer and continues for many months (Tuffs and Morrow, 1925). According to pesticide incident reports submitted to the State of Washington, Department of Agriculture (1987; 1988) numerous spray events occur during this period of bud development. In view of the pronounced response of leafed-trees to sulfonylurea herbicides during spring exposures, it became clear that it was very important to conduct a summer-fall study. Several EPA experiments have been initiated to investigate the effects of 'Glean' when applied at different stages of bud development. In all of these experiments, damage to cherry fruit in the form of mean cherry weight decrease and number of viable cherries will be measured.

CONCLUSIONS

The research described in this report is part of a three-year project. The final data will be collected on the fruit harvested in the spring of 1993. By the conclusion of the project, all treatments will have been examined twice. Although we believe that it is worthwhile to make tentative conclusions at this time to help focus the remaining research on key questions and issues, we feel strongly that as soon as our experiments are completed and the results thoroughly examined, the regulatory process for new pesticides should be reexamined. This should occur no later than 1993.

Tentative conclusions

1. Application of 'Glean' directly to swollen floral buds in the spring had no detrimental influence on normal flower and fruit development of cherry trees.

2. Application of 'Glean' at 0.1 of the recommended field concentration to fully open flowers and partially expanded leaves (less than one third expanded) caused 98 percent of

the fruits to abort and the leaves to die.

3. Application of 'Glean' at 0.1 and 0.01 of the recommended field concentration (1/3 of an ounce per acre) to post bloom fruit and partially expanded leaves (one third to one half full size) caused 100 percent of the fruits to abort at both concentrations, leaf malformation at 0.01 and dead leaves at 0.1.

4. The adverse influence of 'Glean' on cherry fruit development appears to be proportional to the extent to which new leaves have expanded.

Unanswered Questions

1. Are the responses to 'Glean' observed in this study during the spring of 1991 typical, or will they vary with yearly fluctuations in weather conditions?

2. What is the most sensitive stage in bud, flower and fruit development to 'Glean' damage? Two important stages deserving attention are the developing floral bud during the summer-fall season and the developing fruit in the spring after leaves are fully expanded.

3. How does the response to a multiple exposure of 'Glean' at low levels over several weeks compare to the response noted in this study to 0.1 and 0.01 concentration administered at the post bloom stage?

4. How do the pesticide concentrations used in these experiments compare to actual drift scenarios? The goal of the present work is to determine if the sulfonylurea herbicides have an adverse reproductive effect at low concentrations. If this is established, then an effort should be made to correlate drift concentrations to the experimental dosages.

5. What is the mode of action by which 'Glean' reduces cherry fruit growth and promotes abortion?

FINAL PRODUCT

When this research project is finished, we will have established the influence of low levels of 'Glean', a sulfonylurea compound, on the reproduction (fruit set) of cherry trees. This information will be valuable in evaluating the effectiveness of OPP's registration process; specifically, the currently required plant toxicity tests which do not have reproductive evaluations (see Lewis an Petrie, 1991).

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Table 1. OPP test specifications for the Germination/Emergence and Vegetative Vigor Tests required for pesticide registration (Gorsuch, 1991).

REQUIREMENTS	Endpoint Measurements		
	Germination	Emergence	Vegetative Vigor
# Plant Species	10	10	10
Dicot Sp/Family	6/4	6/4	6/4
Monocot Sp/Family	4/2	4/2	4/2
Seeds/Replicate	10	10	5
No. Replicates	3	3	3
Length (Days)	5	14	>14 (14-Day Post Germination)
Observations	Day 5	Day 10 & 14 [can be extended to 28 days for soil vigor]	Day 7, 14, Weekly

Table 2. Statistical data on cherry weight and number of viable cherry fruit compared to controls.

Stage	Concentration	CV	Cherry weight Mean	SD	Decrease From Control (%)	Square root # of viable fruits Mean	SD	Decrease From Control (%)	CV
Bud	Control	8.77%	2.283	0.263		3.252	0.883		26.2%
	0.001		2.325	0.214	-1.84	3.978	1.31	-23.64	
	0.002		2.399	0.195	-5.05	4.661	0.588	-43.32	
	0.01		2.333	0.196	-2.18	4.049	1.120	-24.51	
	0.1		2.214	0.237	3.06	3.275	0.916	-1.50	
Flower	Control	25.6%	2.667	0.526		4.436	1.285		39.3%
	0.001		2.214	0.434	16.99	2.963	1.878	34.47	
	0.002		2.428	0.422	8.96	2.685	0.701	40.62	
	0.01		2.363	0.262	11.38	3.931	1.089	10.88	
	0.1		0.992	0.828	62.82	0.629	0.869	86.08	
Young	Control	17.3%	2.408	0.495		3.363	1.056		43.3%
Fruit	0.001		2.597	0.216	-7.86	4.081	1.197	-20.58	
	0.002		1.997	0.196	17.05	2.754	1.738	19.03	
	0.01		0.941	0.193	60.93	0.0	0.0	100.00	
	0.1		0.084	0.188	96.51	0.0	0.0	100.00	

CV = Coefficient of Variation
SD = Standard Deviation

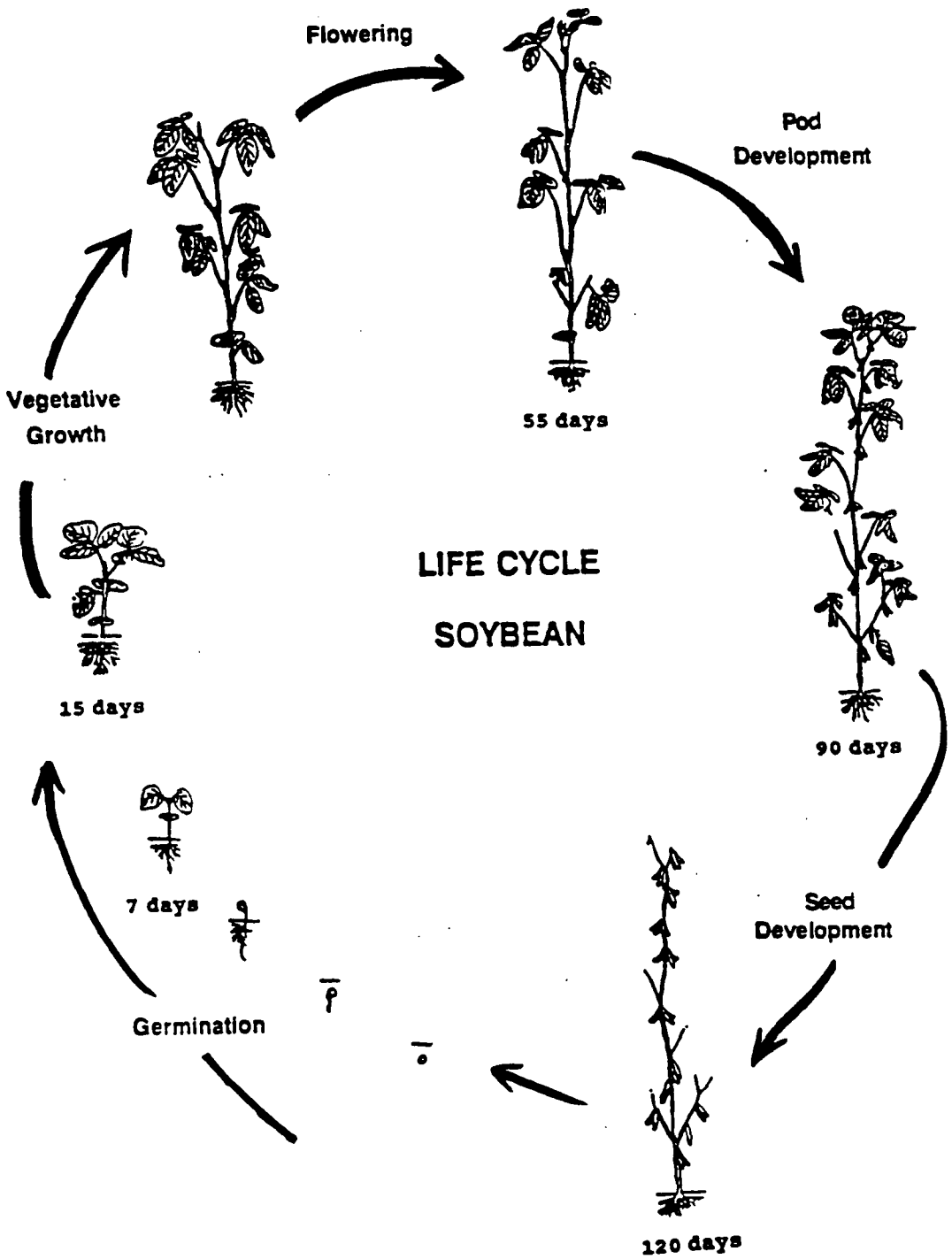

Figure 1. The life cycle of a soybean plant. Current test protocols are designed to protect only a small portion of the life cycle, seed germination and early seedling growth.

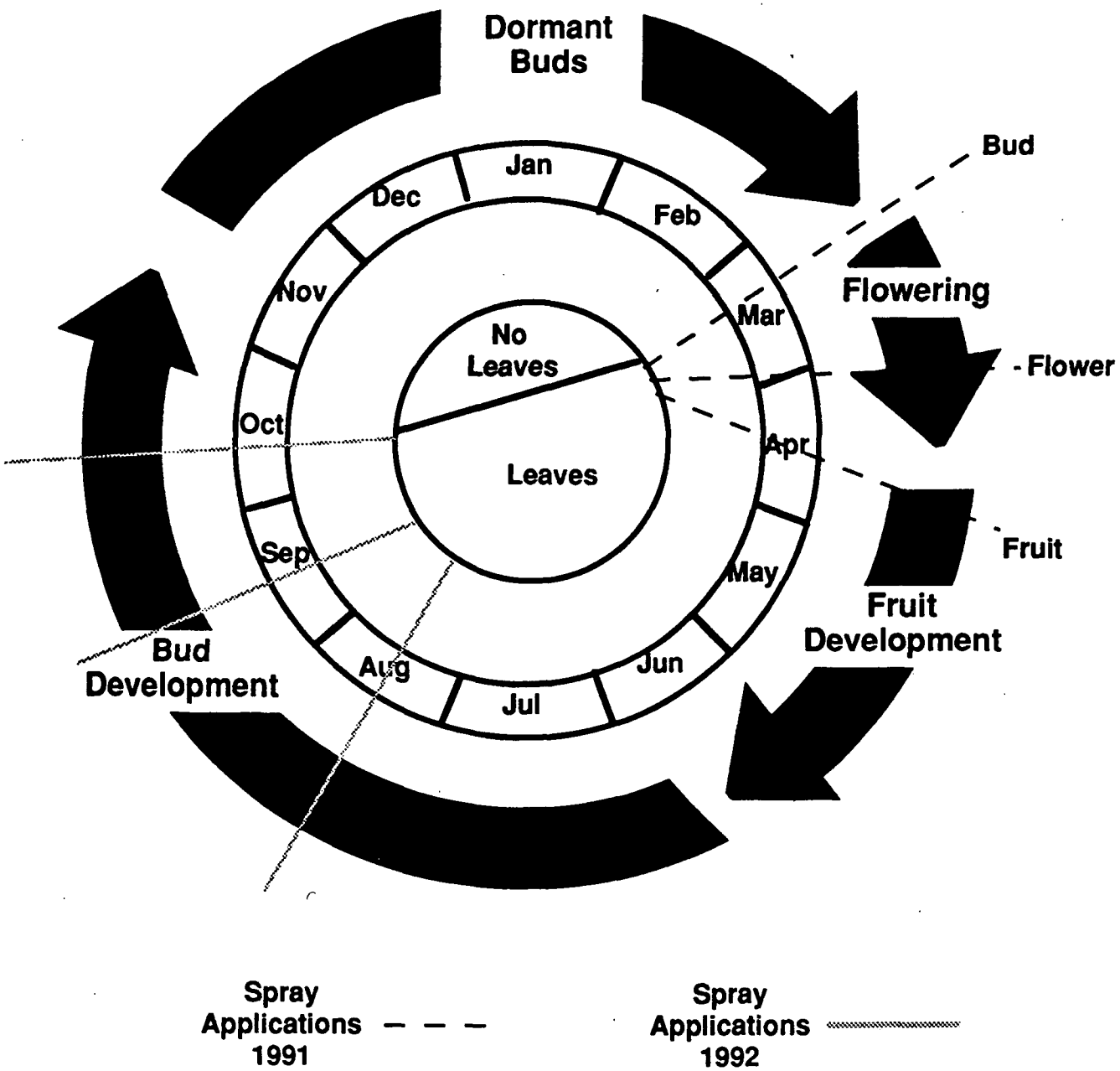

Figure 2. The annual reproductive cycle in cherry trees with the times of experimental treatments indicated. One set of fifteen trees was exposed in the spring during flower and fruit set, and a different row of trees was exposed in the late summer and early fall, a time when the next year's reproductive buds are developing (Tuffs and Marrow, 1925).

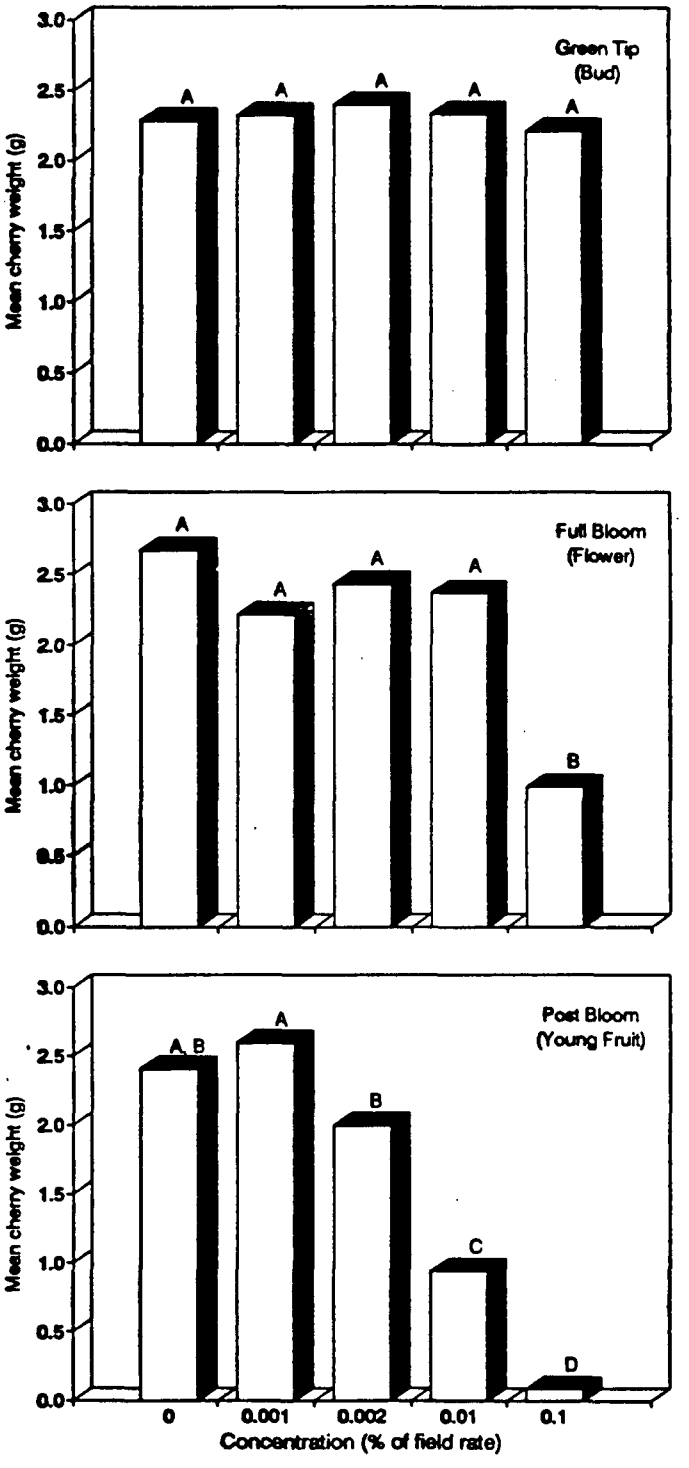

Figure 3. Differences in mean cherry fruit weight when applications of 'Glean' where sprayed on branches at different times of fruit bud expansion. Green tip stage (March 15, 1991). Full bloom stage (April 2, 1991). Post bloom stage (April 26, 1991). Different letters indicate significant differences between treatments at alpha = 0.05.

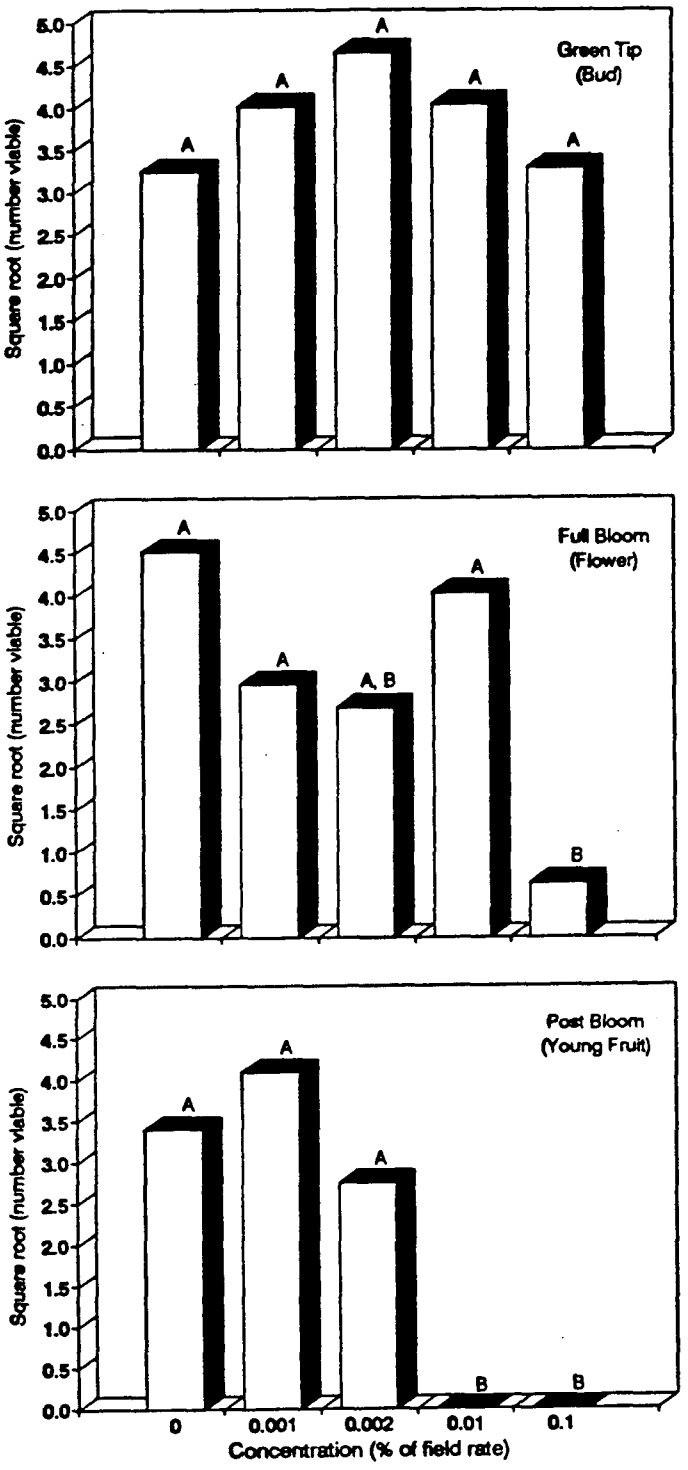

Figure 4. Differences in number of viable cherry fruit when applications of 'Glean' where sprayed on branches at different times of fruit bud expansion. Green tip stage (March 15, 1991). Full bloom stage (April 2, 1991). Post bloom stage (April 26, 1991). Different letters indicate significant differences between treatments at alpha = 0.05.