WORKING PLAN

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ICE NUCLEATING BACTERIA AND CONELET ABORTION IN DOUGLAS-FIR ORCHARDS

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MINISTRY OF FORESTS
COASTAL SEED ORCHARDS
DUNCAN, B.C.

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1.0 INTRODUCTION

The recently completed Quantitative Seed Loss (QSL) study, Sx 82609Q, indicates that, in Douglas-fir seed orchards, a large loss of potential seed can occur through conelet abortion (Table 1). Depending on the year and the orchard site, 9 to 90% of cones initiated in coastal orchards never mature. The magnitude of these losses and the lack of information on specific causes of abortion were the reasons for initiating this study.

Frost damage has been assumed to be the major cause of abortion but losses often occur during damp springs when heavy frosts are not recorded.

Colangeli et al. (1989) have implicated ice nucleating bacteria in the abortion of Fdc conelets. These bacteria induce freezing damage in plant tissue at temperatures 5-8°C higher than it would normally occur.

In angiosperms, these bacteria may be controlled by spray applications of fixed copper solutions. These are cheap and easy to apply and may have the beneficial side effect of improving the chronically low Cu status of orchard trees.

A trial will be carried out in three parts:

Study 1 A Geographical Survey to determine the populations of ice nucleating bacteria in various orchards as well as determining weather conditions and abortion levels. This survey may aid in future orchard management and location decisions.

Study 2 An Operational Trial of Fixed Copper Application will evaluate the effectiveness of spraying a fixed copper solution on the orchard by examining both bacterial populations and abortion levels on sprayed and control branches. The operational feasibility of this technique will also be examined.

Study 3 Timing of Fixed Copper Application will test the effectiveness of 1, 2 or 3 times of application of a Fixed Copper solution. Damage to seeds and cones caused by application after seed cones have emerged will be assessed.

The studies will each be conducted over 3 flowering seasons in order to allow for the variation between years.
### TABLE 1

**QSL STUDY SUMMARY**

**STATISTICS**

(Contributors: C. Bartram, C. Leadem, G. Miller, H. Rooke)

<table>
<thead>
<tr>
<th>Seed Orchard</th>
<th>Cone Efficiency</th>
<th>Seed Efficiency</th>
<th>Extraction Efficiency</th>
<th>Germination Efficiency</th>
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<tbody>
<tr>
<td>Dewdney</td>
<td>.81</td>
<td>.58</td>
<td>.95</td>
<td>.94</td>
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<tr>
<td>Koksinlah</td>
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<td>.98</td>
<td>.94</td>
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<tr>
<td>Quinsam</td>
<td>.88</td>
<td>.55</td>
<td>.97</td>
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</tr>
<tr>
<td>Snowdon</td>
<td>.91</td>
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</tr>
<tr>
<td>means</td>
<td>.84</td>
<td>.53</td>
<td>.96</td>
<td>.95</td>
</tr>
</tbody>
</table>

**Year 1983**

| Dewdney      | .62             | .52             | .97                   | .96                   |
| Koksinlah    | .70             | .37             | .93                   | .94                   |
| Quinsam      | .87             | .57             | .96                   | .97                   |
| Snowdon      | .77             | .41             | .94                   | .97                   |
| means        | .74             | .47             | .96                   | .96                   |

**Year 1984**

| Dewdney      | .10             | .22             |                      | NO HARVEST            |
| Koksinlah    | .34             | .06             | .63                   | .79                   |
| Quinsam      | .22             | .14             | --                    | .93                   |
| Snowdon      | .16             | .08             | --                    | .99                   |
| means        | .21             | .13             | .63                   | .87                   |

**Year 1985**

| Dewdney      | .47             | .31             | --                    | --                    |
| Koksinlah    | .76             | .33             | --                    | --                    |
| Quinsam      | .52             | .40             | --                    | --                    |
| Snowdon      | .20             | .34             | --                    | --                    |
| means        | .49             | .35             | --                    | --                    |

---

* Seed potential in 1982 assumed equal to orchard mean during 1983-85.

** GE in 1984 based on filled and empty seed germination; 1985 results not yet available.
2.0 BACKGROUND

2.1 Conelet Abortion Levels

A recent survey of Ministry of Forests Fdc seed orchards has shown a wide variation in cone efficiency* between orchards and especially between years (Table 1). It is not unusual for 20-30% of initiated cones to abort before they mature. These losses have a direct impact on the cost of seed orchard seed by reducing seed production.

Cone losses during the summer appear to be mainly due to insects (Nelson et al. 1986), especially in a poor crop year (i.e. 1984), while pathogenic fungi appear to have little impact on seed losses. Seed losses to insects are currently monitored annually in Ministry of Forests orchards.

Early abortion of conelets is usually attributed to frost damage because when hard frosts occur late in the season, an increase in conelet losses is noted. However, it appears that conelet abortion also occurs when hard frosts are not recorded in the area.

These observations are often very subjective as neither accurate abortion studies or on-site weather data may be available.

In laboratory trials, the LT50 (critical temperature for 50% death) for Douglas-fir conelets throughout the spring was -4.5°C (Timmis 1977). The LT10 was about -3°C. The safety zone for frost protection was about -1°C (at this temperature, 83% of clones have less than 10% damage).

In Study 1, monitoring of basic weather data and conelet abortion levels in coastal seed orchards will be established.

2.2 Ice Nucleating Bacteria (INB)

Certain bacteria, when present on plant parts, have been shown to increase frost damage by providing nuclei for ice formation within plant tissues. In the absence of these bacteria, many angiosperm species can supercool frost sensitive plant parts well below 0°C without ice forming in tissues, therefore minimal damage occurs. The INB disrupt this protective mechanism resulting in more freezing injury (Lindow, 1982).

* Cone efficiency defined as number of cones surviving to harvest divided by the number of cones initiated.
Colangeli et al. (1989) have demonstrated that an INB, pseudomonas syringae, may be present on conelets of Douglas-fir during the time of frost susceptibility. Population dynamics of the bacteria varied between years. The results of the study suggest that there may be a causal relationship between the bacteria and conelet abortion. A survey of populations of p. syringae will be included in Study 1 to estimate the extent of these bacteria in Fdc orchards and the weather conditions which promote their presence.

There are a number of methods available for controlling INB (Lindow 1983). Chemicals which inhibit ice nucleation may be applied just previous to an expected frost. Populations of antagonistic bacteria or antibacterial agents (i.e. copper-containing fungicides or antibiotics) can reduce the population of INB, thus reducing frost damage.

In two Fdc seed orchards with past histories of frost damage (Snowdon and Sechelt) we have decided to spray Fixed Copper fungicide in an attempt to prevent conelet abortion. This treatment was chosen because it is inexpensive, and is recommended for use on Douglas-fir for certain foliar diseases (B.C. Min. of Agriculture and Fisheries 1987).

In Studies 2 and 3, we examine the effect of these sprays on bacterial populations and abortion will be evaluated. Ice nucleating activity may be present for several days after bacteria are destroyed (Lindow 1983) therefore spray treatment will be carried out early in the season. Residual activity of the fungicide should be effective throughout the pollination season when conelets are most susceptible to frost, however, multiple fungicide applications will be used at Snowdon to evaluate their effectiveness.
3.0 STUDY 1 GEOGRAPHICAL SURVEY

3.1 Objective

To evaluate currently available survey methods for conelet abortion, ice nucleating bacteria and weather for their usefulness in an operational monitoring program in Fdc orchards.

To determine in which, if any, of the coastal Fdc orchards ice nucleating bacteria may contribute to conelet abortion levels.

3.2 Location

The geographic survey of abortion levels and bacterial populations will be carried out at the following NDF orchards:
- Saanich (SE quarter)
- Koksilah
- Quinsam
- Snowdon
- Surrey

and in the following cooperative orchards if approved by the appropriate company:
- Mt Newton (Fletcher Challenge)
- Nootka (CPFP)
- Harmac (MacMillan Bloedel)
- Sechelt (CanFor)

If orchards are sprayed with Fixed Copper solutions (Study 2, 3), estimates will be made for the untreated portions of the orchard.

3.3 Duration

Because of the annual variation in abortion levels (see Table 1) the study will be replicated over three years to get a reasonable comparison between the sites included. At that time recommendations will be made as to what routine monitoring should be continued.

3.4 Methods

3.4.1 Conelet Abortion Estimates

An estimate for each orchard of the proportion of cones which abort soon after pollination will be obtained using a 3 stage sampling technique described by Bartram and Millar (1988). Forty trees will be sampled (with replacement) proportional to crop size (from the March bud estimates). Only trees carrying a medium to heavy crop will be included.

One whorl and one branch in that whorl will then be chosen on each of the 40 trees using equal probabilities. If less than 10 female buds are present on a branch a new one will be chosen. If a tree or whorl is chosen twice, a second branch on that tree will be counted. If a branch is chosen twice, it will be recorded twice.
Conelet abortion will be assessed in early June, after conelets have become pendant. The number of aborted (either upright or pendant) and developing healthy cones will be counted on the entire branch selected, up to 100 cones total per branch.

3.42 Bacterial Populations

Sampling to estimate the ice-nucleating bacterial population will be done just before reproductive bud burst and again about 2 weeks later. The period just before bud burst is defined as the time when reproductive buds on 2 clones or families in the orchard have flushed. The second survey should be carried out on a day which coincides with a phenological survey (to define the phenological status of the orchard).

Sampling will be similar to foliar sampling methods. The 40 trees selected for abortion studies will be used. Two shoots will be collected from the 5th whorl of each tree, bulked in a labelled plastic bag and either frozen immediately or forwarded to the University of Victoria.

P. syringae population size and the presence or absence of ice-nucleating activity will be assessed at the University of Victoria, Botany Dept.

3.43 Temperature

Temperatures in April and May will be recorded in all orchards. A Stevenson screen containing either a hydrothermograph or a data logger will be set up in each orchard for this purpose. Where data loggers are available, temperatures below the crowns, at mid crown and in the upper crown will be recorded.

3.44 Productivity

The time required for the following activities will be recorded.

Weather Monitoring
- set up of system
- data transfer

Abortion Counts
- tree selection
- field work
- data transfer

Bacterial Analysis
- field work
- specimen storage and transfer

Down time will be recorded separately with an explanation of the problem.
3.5 Report

The average proportion of cones surviving to maturity will be calculated for each orchard using the method described by Bartram and Millar (1988).

Abortion levels and bacterial population will each be plotted on graphs of location (x axis) and year (y axis) to visually evaluate any trends.

The cone efficiency, bacterial population, and days below 0 or below 2°C over the time of cone emergence will be presented in tabular form.

4.0 STUDY 2 OPERATIONAL TRIAL OF FIXED COPPER APPLICATION

4.1 Objective

Evaluate the effect of a Fixed Copper spray on a) abortion levels, and b) the activity of ice-nucleating bacteria within orchards.

To assess the operational feasibility of fixed copper spray through productivity records.

4.2 Experimental Design

The study will be carried out at Sechelt Seed Orchard #116. As there is no effective method to estimate conelet abortion on individual trees, the effect of Fixed Copper spray will be assessed on pairs of branches on sprayed trees.

Within the spray area, thirty trees having a good crop will be selected randomly for evaluation. Four pairs of branches will be evaluated on each tree - 2 on the North side and 2 on the South side. Branches within pairs will be close together and of similar appearance. Each branch will carry at least 10 female buds. Branches in pairs will be assigned to treated (T1) or untreated (To) using a coin toss. Branches will be labelled with a Tree #, Branch # and Treatment about 0.5 m from the end of branch.

Branches in To will be covered with a garbage bag (up to tag) just before spraying and uncovered afterwards.

4.3 Methods

4.3.1 Fixed Copper Application

An operational spraying of Fixed Copper will be carried out on about 2/3 of the crop sector of the Sechelt Seed Orchard (#116). A Hardi Mistblower will be used to apply a 400 g/100 L solution to trees in the trial sector just before seed cone emergence. Trees will be thoroughly wetted with the spray to the point of runoff.

To document the effectiveness of the spray in reducing bacterial population, a sample of foliage from 2 groups of 15 trees in the test area will be taken prior to spraying and 2 days after spraying. Bacterial counts will be done on these samples to estimate the effect of the spray on populations within the orchard.
4.32 Productivity

Data for productivity of Fixed Copper application on an operational basis will be kept including:

1. Preparation time - preparing solutions
2. Clean-up time
3. Down time
4. Time to spray
5. Number of trees sprayed
6. Volume of spray used.

To evaluate the effectiveness of Fixed Copper spray coverage, cards will be hung in the upper crown near sample branches, retrieved immediately after spray and coverage recorded.

4.33 Conelet Abortion Counts

Counts for conelet abortion will be carried out in early June on all sample branches after conelets have become pendant. The number of healthy developing conelets and the number of aborted conelets will be recorded from the tip of the branch to the tag.

4.34 Seed Set

In August, 3 cones from each sample branch will be collected, put in SEPARATE paper bags (1 cone per bag) for evaluation of seed set in treated and untreated cones. Bags will be labelled and taken to CLRS (with a list of samples) for extraction and counting of filled seed.

4.35 Phenology Records

Phenology records will be kept for female and male flowers on the sample branches twice a week from bud burst to the time the cones become pendant.

4.36 Ice-Nucleating Bacteria

To evaluate the relationship between bacterial population and abortion on a single branch, foliar samples will be taken from all sample branches, 4 days after bud burst. Samples consist of 2 branch tips 15 cm long (5 g fresh wet). Samples will be labelled with Tree #, Branch # and Treatment, placed in plastic bags and frozen immediately. Because of the cost of analysis only certain samples will be analyzed. After abortion counts are in, pairs of branches (treated and untreated) for evaluation will be selected to represent:

a) a range of abortion levels (2 low, 2 high, 4 medium)
b) a range of phenology E, M, L (2 of each)

Analysis of samples for ice-nucleating activity and for P. syringae population size will be carried out at University of Victoria.
4.4 Analysis

4.41 Productivity

The time to carry out Fixed Copper spraying will be estimated for a whole orchard. If abortion is lowered by this technique, the costs will be calculated per extra cone produced. Volume of spray required and costs per tree will be calculated.

4.42 Conelet Abortion

The proportion of conelets (arcsine transformed) surviving will be subject to ANOVA.

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</thead>
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<td>Trees x North/South</td>
</tr>
<tr>
<td>Trees x North/South</td>
<td>29</td>
<td>--</td>
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<tr>
<td>Treatment x North/South</td>
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<td>Trees x Treatment x North/South</td>
</tr>
<tr>
<td>Trees x Treatment x North/South</td>
<td>58</td>
<td>--</td>
</tr>
</tbody>
</table>

Total: 119

This is a split-plot design which includes tests for differences between the North and South sides of the trees. Trees act like blocks so that tree interactions provide the appropriate error terms.

4.43 Bacterial Population

The relationship between abortion levels and bacterial populations will be plotted and a linear or curvilinear regression developed if applicable for early, intermediate and late phenology groups. Treated and untreated branches will be analyzed separately.

4.44 Seed Set

Filled seed per cone and % filled seed (arcsine transformed) will be subjected to ANOVA to determine if spraying with Fixed Copper causes any reduction in seed set.

<table>
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<th>SOURCE OF VARIATION</th>
<th>df</th>
<th>ERROR TERM</th>
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<td>North/South</td>
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<tr>
<td>Trees x North/South</td>
<td>29</td>
<td>Cones</td>
</tr>
<tr>
<td>Treatment</td>
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<td>Trees x Treatment x North/South</td>
</tr>
<tr>
<td>Treatment x North/South</td>
<td>29</td>
<td>Trees x Treatment x North/South</td>
</tr>
<tr>
<td>Trees x Treatment x North/South</td>
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<td>Cones</td>
</tr>
<tr>
<td>Cones(Trees x Treatment x North/South)</td>
<td>120</td>
<td>--</td>
</tr>
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</table>

Total: 239
5.0 STUDY 3. TIMING OF FIXED COPPER APPLICATION

5.1 Objective

To assess damage caused to open flowers by Fixed Copper at different phenological stages.

To assess the effect on ice-nucleating bacteria of different timing of Fixed Copper solution application.

5.2 Experimental Design

This study will be carried out at Snowdon Seed Orchard. It will involve spraying trees at different times, monitoring any effects on bacterial populations and conelet abortion, and evaluating any damage to conelets from spraying the solution on opened buds.

Four Treatments will be evaluated:

To - control
T1 - Fixed Copper Spray - just before budburst
T2 - Fixed Copper Spray - just before budburst & 2 weeks later
T3 - Fixed Copper Spray - just before budburst & 2 & 4 weeks later.

Fifteen trees having a good crop will be selected randomly for evaluation. Groups of four branches will be evaluated on each tree - 1 group on the North side and 1 on the South side. Branches within groups will be close together and of similar appearance. Each branch will carry at least 10 female buds. Branches in groups will be assigned to treatments using random number tables. Branches will be labelled with a Tree #, Branch # and Treatment on a tag placed over the distal 0.5 m of the branch.

To - bagged for all treatments
T1 - bagged for second and third treatments
T2 - bagged for third treatment
T3 - not bagged.

Branches will be covered with a garbage bag up to the tag just before spraying and uncovered afterwards.

5.3 Methods

5.3.1 Conelet Abortion Counts

Counts for conelet abortion will be carried out in early June after conelets have become pendant. The number of developing conelets and the number of aborted conelets will be recorded from the branch tip to the tag. At this time all aborted conelets will be collected, placed in labelled plastic bags and frozen. A sample may be examined at University of Victoria to evaluate the causes of abortion if damage by Fixed Copper spray is suspected.
5.32 Seed Set

In August, 3 cones from each sample branch will be collected, put in SEPARATE paper bags (1 cone per bag) for evaluation of seed set in treated and untreated cones. Bags will be labelled and taken to CLRS (with a list of samples) for extraction and counting of filled seed.

5.33 Phenology Records

Phenology records will be kept for female and male flowers on the sample branches twice a week from bud burst to the time the cones become pendant.

5.34 Ice-Nucleating Bacteria

Foliar samples will be taken from all sample branches, 4 days after bud burst. Samples consist of 2 branch tips 15 cm long (5 g fresh weight). Samples will be labelled with Tree #, Branch # and Treatment, placed in plastic bags and frozen immediately. Samples will be analyzed at University of Victoria.

As in Study 2 (Section 4.36) a sample of the groups of branches will be assessed for P. syringae population size depending on abortion counts to represent a range of abortion counts and a range of phenology.

5.4 Analysis

5.41 Conelet Abortion

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<td>Treatment</td>
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<td>Treatment x North/South</td>
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<tr>
<td>Trees x Treatment x North/South</td>
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<td>--</td>
</tr>
<tr>
<td>Total:</td>
<td>119</td>
<td></td>
</tr>
</tbody>
</table>

5.42 Bacterial Populations

The relationship between abortion levels and P. syringae populations will be plotted and a linear or curvilinear regression developed, if applicable, for early, intermediate and late phenology groups.
5.43 Seed Set

Filled seed per cone and % filled seed per cone (arcsine transformed) will be subjected to ANOVA to determine if the fixed Copper has any effect on seed set.

<table>
<thead>
<tr>
<th>SOURCE OF VARIATION</th>
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<td>Cones(Trees x Treatment x North/South)</td>
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<tr>
<td>Total:</td>
<td>239</td>
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6.0 REPORTS

Interim reports will be produced in December of each year and a final report January, 1992.

7.0 RESPONSIBILITIES

7.1 Study 1

Equipment & Instructions
Data Collection - temperature
QCL bud counts
Samples for Bacterial estimates
Bacterial Counts

Data Analysis & Report

7.2 Study 2 & 3

Orchard Spraying
Sample Collection
Phenology Records
Data Analysis & Report
Bacterial Counts

Seed Extraction

J. Parkinson
Orchard Techs
Orchard Techs
U of Victoria (contract)
J. Parkinson

J. Parkinson
Orchard Techs
Orchard Techs
J. Parkinson
U of Victoria (contract)
Research Br.
Mesachie Lake
8.0 SCHEDULE OF ACTIVITIES

8.1 STUDY 1 GEOGRAPHICAL SURVEY

1989

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<thead>
<tr>
<th>Activity/Task</th>
<th>MANDAYS</th>
<th>JP</th>
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<td>Apr. - obtain, calibrate &amp; set up hydrothermographs + CR10 (8 orchards)</td>
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<td>Apr. - samples for bacteria counts (40)</td>
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<td>Sept. - analysis</td>
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<td>Dec. - Report</td>
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1990, 1991

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<tr>
<td>Dec. - Report</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td>4.0</td>
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</tr>
</tbody>
</table>

8.2 STUDY 2 OPERATIONAL FIXED COPPER APPLICATION


<table>
<thead>
<tr>
<th>Activity/Task</th>
<th>MANDAYS</th>
<th>JP</th>
<th>ORCH. TECH.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr. - select sample trees (30) + branches (240)</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Spraying (including bagging 120 branches)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Foliage samples for bacterial counts (240)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Apr./May - phenology records (6-8x)</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>June - abortion counts (240 branches)</td>
<td>5.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Aug. - cone collection for seed est. (from 240 branches)</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Sept. - seed extraction (Mesachie Lake) 10 M.D. (480 cones)</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Oct. - data analysis</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Dec. - report</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>6.0</td>
<td>14.0</td>
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STUDY 3 TIMING OF FIXED COPPER APPLICATION


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<th>ORCH. TECH.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr. - select sample trees (15) + branches (120)</td>
<td>1.0</td>
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<tr>
<td>Spraying (3 times)</td>
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<tr>
<td>Foliage samples for bacterial counts (120)</td>
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</tr>
<tr>
<td>Apr./May - phenology records (6-8x)</td>
<td>4.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>June - abortion counts (120)</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Aug. - cone collection for seed est. (240)</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Sept. - seed extraction (Mesachie Lake) 6 M.D.</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Oct. - data analysis</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Dec. - report</td>
<td>1.0</td>
<td>1.0</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td>6.0</td>
<td>12.0</td>
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</tbody>
</table>
9.0 TIME AND COST SUMMARY

9.1 Time

<table>
<thead>
<tr>
<th>Study</th>
<th>1989</th>
<th>1990</th>
<th>1991</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1 (8 Orchards)</td>
<td>38</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Study 2</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Study 3</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>TOTAL</td>
<td>94</td>
<td>84</td>
<td>84</td>
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</table>

9.2 Materials

<table>
<thead>
<tr>
<th>Item</th>
<th>1989</th>
<th>1990</th>
<th>1991</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic bags, fasteners, wadding</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Branch labels</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Miscellaneous materials</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Fixed Copper</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Bacterial Counts</td>
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<td>6240</td>
<td>6240</td>
</tr>
<tr>
<td>TOTAL</td>
<td>$6840</td>
<td>$6940</td>
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</tr>
</tbody>
</table>

Details of materials cost is in Appendix I.
ACKNOWLEDGEMENT

My thanks to M. Crown for his suggestions and support in the development of this working plan, to A. Colangell for information regarding assessment of bacterial populations and to J. Boyd for typing this report. I appreciate the helpful reviews of the working plan by C. Bartram, C. Hewson, S. Ross and D. Summers and assistance from W. Bergerud with the statistical analyses.

10.0 REFERENCES


APPENDICES
APPENDIX I

COST/CALCULATIONS

Materials

Fixed fixed copper @ 1 L per tree
   Solution 400g/100 L = 4 g/tree
   Sechelt - 200 trees = 800 g Fixed Cu
   Snowdon - 600 trees = 2400 g Fixed Cu
   (Buckerfield's)

3200 kg - 4 kg Fixed Fixed Copper $100

Bacteria Counts

Study 1: 8 orchards
   2 sampling times
   2 samples/time
   32 sample counts @ $60 = $1920

Study 2: 4 samples @ $60 = $240 (monitoring effect of Fixed Copper spray)
   30 samples @ $60 = $1800
   $2040

Study 3: 8 samples @ $60 = $480 (monitoring effect of Fixed Copper Spray)
   30 samples @ $60 = $1800
   $2280
   $6240