\$1-0<sup>3</sup>

Project Number 81-O3 DECEIVE JAN 2 6 1982 ALMOND BOARD

1981 Final Report

## EPIDEMIOLOGY AND CONTROL OF FROST INJURY TO ALMOND INCITED BY LEAF SURFACE ICE NUCLEATION ACTIVE BACTERIA

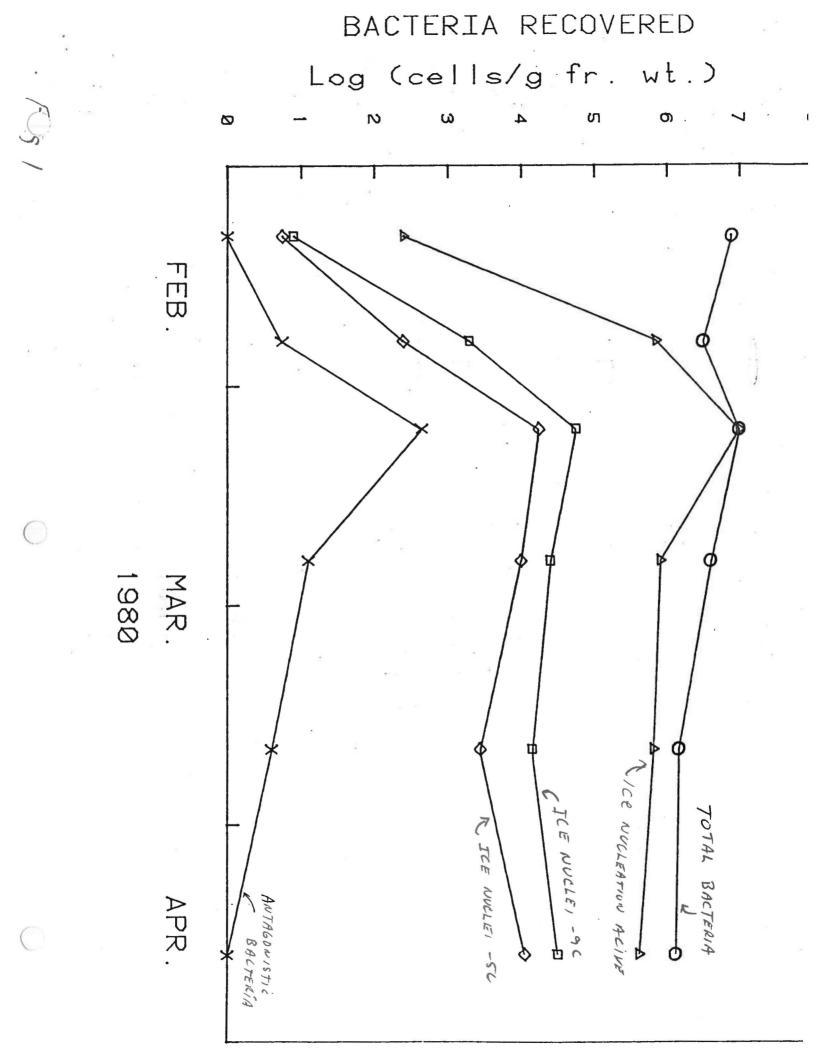
Steven E. Lindow Department of Plant Pathology University of California, Berkeley

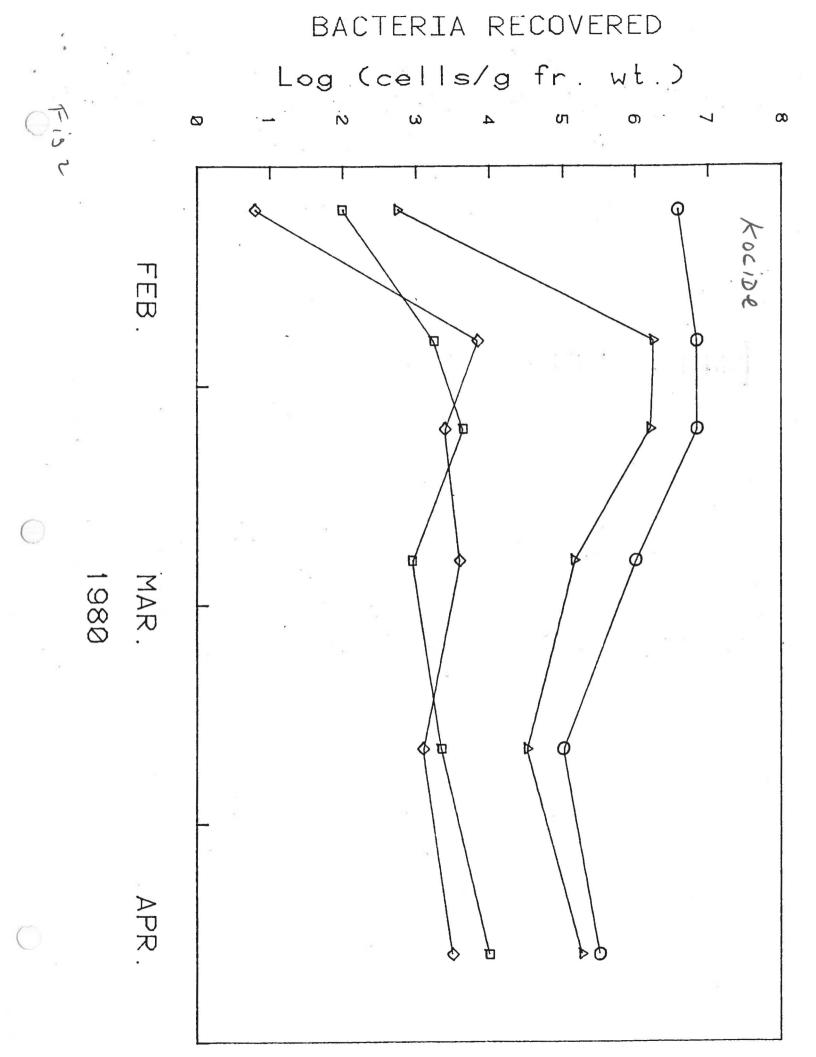
Almond leaves and flowers are colonized with extremely high numbers of ice nucleation active bacteria during 1980 and 1981. Over 10 million ice nucleation active bacteria per gram of leaf tissue were observed on trees during periods of maximum frost hazard on untreated trees. The overwhelming majority of ice nucleation active bacteria were strains of the bacterium Pseudomonas syringae which also cause blast of almond. Small overwintering populations of P. syringae appeared to be the major source of inoculum of ice nucleation active bacteria on almond. Grasses and weeds on the orchard floor also appeared to be potential sources of early season inoculum. Populations of ice nucleation active bacteria were at their lowest level at approximately the period of first bud swelling. Populations of ice nucleation active bacteria on almond leaves and flowers increased rapidly for approximately 4 weeks following bud swelling and reached maximum populations of about 10<sup>7</sup> cells/gram fresh weight of tissue on untreated tissue in the Turlock area (Figure I) and 106 cells/gram fresh weight in the Fresno Although populations of ice nucleation active bacteria declined in area, late spring, maximum populations were observed during the period of maximum frost hazard. Field plots were established in 1981 near Turlock and Chico. Two bactericides, eleven non-ice nucleation active bacterial antagonists, and seven bacterial ice nucleation inhibitors were evaluated for control of frost injury to almond. No significant natural frosts occurred during 1981 but the effectiveness of these treatments was evaluated by freezing detached plant parts under controlled laboratory conditions. Bactericides reduced populations of ice nucleation active bacteria on treated trees from 10- to 100-fold compared to untreated trees. (Figures 2-4). A mixture of streptomycin and terramycin was slightly more effective than Kocide 101 in reducing populations of ice nucleation active bacteria when both were applied at 10 day intervals starting at bud break. Additional reductions in ice nucleation active bacterial populations were observed on trees which had received a dormant application of Kocide 101 in addition to periodic applications of Kocide 101 (Figure 7). The numbers of ice nuclei active at -5C or higher were also highly reduced on plants treated with bactericides compared to untreated plants (Figures 1-4). The frost sensitivity of treated plant material at -4C was reduced proportionally to the reduction in populations of ice nucleation active bacteria. Dormant applications of fixed copper fungicides such as Kocide 101 appear promising as frost control procedures if followed by applications of bactericide at 10 day intervals following bud break.

Six of eleven non-ice nucleation active bacteria evaluated, readily colonized almond flower and leaf surfaces from a single foliar application made at approximately 30% bloom (Figure  $\leq$  ). More than 10% of the total bacteria on antagonistic bacteria treated trees were the strains applied. The populations of ice nucleation active bacteria and number of ice nuclei active at -5C or higher were reduced significantly on trees treated with antagonistic bacteria. Mutants of an ice nucleation active strain of <u>P</u>. syringae from almond leaf surfaces were induced and selected that were deficient in ice nucleation activity. These mutants had no ice nucleation activity at temperatures above -5C. However, these mutants multiplied as rapidly as wild type strains <u>in vitro</u> and colonized both greenhouse grown corn as well as almond tissue as well as wild type strains. Because <u>P</u>. syringae appears to be ideally suited for growth and survival on almond foliage the colonization of almond by ice nucleation deficient mutants of <u>P</u>. syringae apears to utilize sites and/or nutrients that might otherwise be used by ice nucleation strains of this same species. Non-ice nucleation deficient mutants of <u>P</u>. syringae readily colonized almond foliage following a single application at about 10% bloom and comprised over 50% of the total bacteria on plants at many samplings. The frost sensitivity of almond was significantly reduced in laboratory freezing assays following treatment with these non-ice nucleation active bacteria compared with untreated trees. Three non-ice nucleation active mutants of <u>P</u>. syringae appeared very promising during 1981 and will be further evaluated during 1982. Because of their dynamic colonization of rapidly expanding almond foliage, antagonistic bacteria have considerable promise as frost control agents that would not require frequent reapplication such as bactericides.

Bacterial ice nucleation inhibitors significantly reduced the ice nuclei associated with almond tissue and thus the frost sensitivity of treated trees. Frost sensitivity of treated trees was reduced for several days following application. Although certain ice nucleation inhibitors such as dilute solutions of phosphoric acid reduced the populations of ice nucleation active bacteria, the primary effect of nucleation inhibitors was to reduce the ice nucleation activity of ice nucleation active bacteria already on trees. The reduction of frost sensitivity of treated trees was correlated with the reduction of the number of ice nuclei present in the trees at the time of freezing.

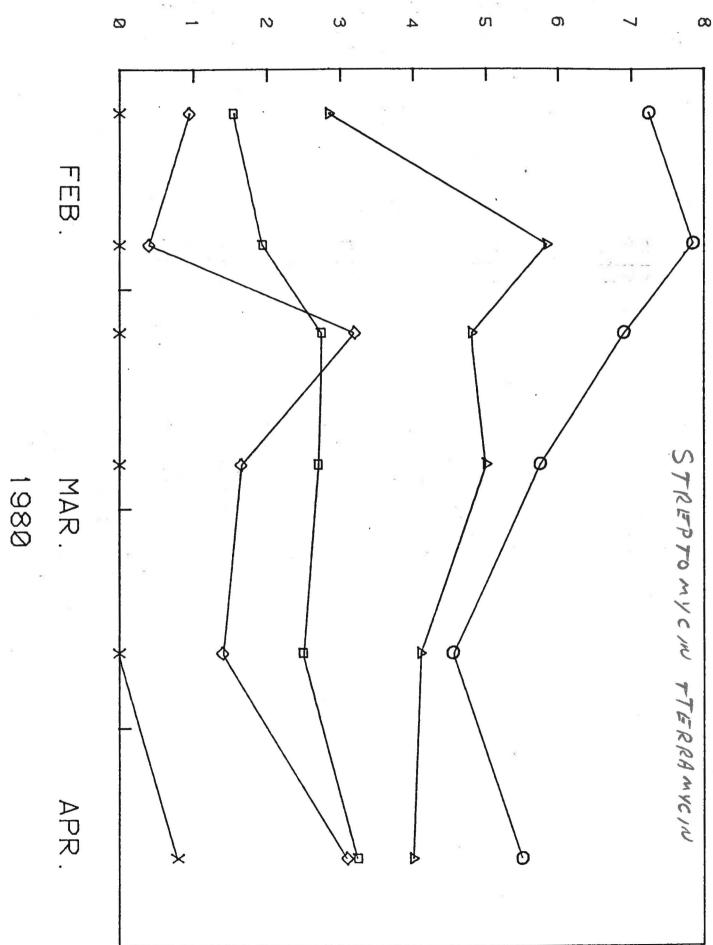
These results suggest that frost injury to almond may be controlled by use of either bactericides, some of which are already registered for use on almond, or bacterial ice nucleation inhibitors which, although not registered for use on almond, may be granted exempt status and thus made available for use in the near future. Antagonistic bacteria also offer considerable promise as biological control agents of frost injury.





BACTERIA RECOVERED

Log (cells/g fr. wt.)



QT-15 3

.

 $\bigcirc$ 

