THESIS

INVESTIGATIONS INTO THE NATURE OF
THE POTATO PSYLLID TOXIN

Submitted by

Rella L. Abernathy

Department of Entomology

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR
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Committee on Graduate Work

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ABSTRACT OF THESIS

INVESTIGATIONS INTO THE NATURE OF

THE POTATO PSYLLID TOXIN

The potato or tomato psyllid, *Paratrioza cockerelli* (Sulc) (Homoptera: Psyllidae) is a pest of many solanaceous crops in the western U.S. and causes a disease in potatoes and tomatoes known as "psyllid yellows". The disease is caused by a phytotoxin produced by the potato psyllid that is systemic and causes many morphological changes in the host plant which can lead to crop loss.

The effects of potato psyllid feeding on the yield of several varieties of potato, tomato and pepper were investigated. Tissue cultured potatoes were studied under greenhouse conditions by infesting the plants with potato psyllids. Dramatic yield losses occurred in four cultivars of potato studied. Varietal responses showed that early maturing cultivars had less damage, as measured by loss of tuber yield, than later cultivars. Another effect caused by potato psyllid feeding was premature sprouting of newly harvested tubers. In this case, early maturing cultivars had a greater proportion of tubers sprouting than later maturing cultivars.

Tomatoes and peppers were studied under field conditions and infested by naturally occurring psyllid populations. Of eight varieties of tomatoes evaluated, all sustained a loss in yield when infested with potato psyllids. A range of damage and different levels of infestation occurred within the varieties. Peppers, on the other hand did not show typical symptoms observed in tomatoes and potatoes. Of four varieties studied, only one showed a slight decrease in yield from potato psyllid infestation. Two other pepper varieties showed a gain in yield from potato psyllid feeding, though only one variety had a statistically significant increase in yield.

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A membrane feeding system was developed to collect potato psyllid nymph saliva. Several attempts were made to develop a bioassay for potato psyllid toxin, but no consistent method was found.

The secretions collected with the membrane feeding system and potato psyllid excrement were topically applied to potato minitubers to induce premature sprouting. Neither of these treatments were found to be statistically effective to break dormancy of minitubers.

Rella L. Abernathy
Entomology Department
Colorado State University
Fort Collins, CO 80523
Fall 1991

ACKNOWLEDGEMENTS

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INTRODUCTION

The potato or tomato psyllid, *Paratrioza cockerelli* (Sulc) (Homoptera: Psyllidae) is a pest of solanaceous crops in the western United States. The potato psyllid is thought to possess a phytotoxic compound in its saliva that is responsible for a disease of the potato and tomato called "psyllid yellows". The toxin is systemic and causes morphological changes throughout the host plant. Before the advent of modern insecticides, psyllid yellows was one of the most destructive potato diseases, resulting in enormous economic losses (Pletsch, 1947).

Early descriptions of "psyllid yellows". Richards *et al.* (1927) were the first to make a connection between an "unknown" psyllid and a disease causing a great deal of damage, up to complete crop loss, in potatoes in Utah. Later, Richards (1928) named this disease "psyllid yellows" and identified the psyllid as *Paratrioza cockerelli* (Sulc). He reported that the feeding of nymphs caused the disease and that he could induce early symptoms within nine days of feeding.

Several different theories emerged as to what caused the disease. Shapovalov (1929) speculated that the disease was transmitted through tubers, but only in some localities. Binkley (1929) performed experiments in which he was able to get disease transmission in healthy plants from nymphs that had previously fed on diseased plants, but did not get symptoms when eggs were allowed to hatch and the nymphs permitted to feed on healthy plants. This led Binkley to the conclusion that the disease was a virus that was not transmitted through the egg. Blood *et al.* (1933) demonstrated that more than 30 potato psyllids had to feed continuously to induce symptoms and that these plants recovered if the potato psyllids were removed. They hypothesized that the disease was caused by an insect toxin. Work by Schaal (1938) supported the toxic insect hypothesis. He could not induce symptoms in tubers from infested plants, though he did note that diseased tubers commonly exhibited early

sprouting. He also demonstrated that the number of feeding nymphs correlated with expression of the disease and that removing feeding potato psyllids often led to recovery in the plant. He had some success inoculating healthy plants with crude potato psyllid extract and observing some foliar symptoms. The most recent in-depth studies on the disease effects (Carter, 1950; Daniels, 1954) agreed with the theory that a phytotoxin produced by the potato psyllid was the factor.

Schaal (1938) also reported that environmental conditions could effect the severity of symptoms. In trying to control temperature by shading field plots, he found that direct sunlight was needed for expression of symptoms. Higher altitudes produced later infestation, but this could be due to lower temperature. Potatoes grown in alkaline soil, or which had fungal diseases or injuries showed more severe symptom expression with fewer potato psyllids present. Plants under stress were more susceptible.

Life history. Knowlton and Janes (1931) worked out the biology of the potato psyllid and Rowe and Knowlton the morphology (1935).

Egg. The eggs are deposited singly on the upper and lower surfaces of the leaves along the margins. The eggs are oblong-ovate, shiny yellow and borne on a stalk about 2/3 the length of the egg. The egg changes to more of an orange color as it develops. The average incubation period is five to six days.

Immature. There are five instars, with each instar possessing essentially the same morphological features. In later instars, the wing pads become more prominent but, overall, the nymph primarily becomes larger. Nymphs range in color from light tan to green to brown. The average time for nymphs to complete development to the adult stage depends on the time of year, but ranges from 12-19 days.

Adult. The teneral adult is a light amber color which changes to a dark, almost black color two to five days after emergence. Adults have pronounced white bands on the cuticle with an overall appearance of a tiny (1/12-1/10 inch long) cicada (Wallis, 1955). They have large, clear wings, which they hold roof-like over their backs. Adults are exceptional fliers and

jumpers as are all members of Psyllidae and are commonly called jumping plant lice. A sex ratio of 1:1 occurs. Females lay around 330 eggs over their lifetime and can live up to three times as long as males.

Host plants. Potato psyllids most often occur on members of Solanaceae but have been reported on Pinaceae, Salicaceae, Polygonaceae, Chenopodiaceae, Cruciferae, Leguminoseae, Lycophyllaceae and Convolvulaceae (Pletsch, 1947). Potato psyllids are generally found on solanaceous plants and, from oviposition studies, preferred host plants have been assessed (Wallis, 1955). The most preferred host by ovipositing females was Chinese lantern *Physalis francheti* (Mast.), an annual ornamental that is not widespread. The most preferred wild host plant was horsenettle *Solanum carolinense* L. Potato psyllids preferentially oviposited, in ascending order, on cultivated groundcherry *Physalis ixocarpa* (Brot.), wild groundcherry *Physalis lanceolata* (Michx.), buffalo-bur *Solanum rostratum* (Dunal), tomato *Lycopersicon esculentum* (Mill.), matrimony-vine *Lycium halmifolium* (Mill.), black nightshade *Solanum nigrum* L., wild tomato *Solanum triflorum* (Nutt.), bell pepper *Capsicum frutescens*, var. *grossum* (L.) Bailey, ornamental pepper *Capsicum frutescens* L., potato *Solanum tuberosum* L., tobacco *Nicotianan tobacum* L. and eggplant *Solanum melongena* L. Based on this study, wild host plants may well be very important in building epidemic populations which then move over to cultivated hosts. From our field observations, we noted that peppers were a particularly favored host in the early season, and as the season progressed, potato psyllid populations increased on tomatoes and potatoes.

Distribution. Specimens of potato psyllids have been collected from many vicinities in Colorado, New Mexico, Arizona and California. Specimens have been collected from specific areas of Utah, Texas, Oklahoma, Nebraska and South Dakota. Records of potato psyllid presence have been made from Nevada, Idaho, Montana, Wyoming, Alberta and Minnesota (Tuthill, 1943). These records are based on museum specimens. Pletch (1947) pooled the distribution records in the literature and mapped areas with the level of damage suffered from potato psyllids. Utah, Colorado, Wyoming and Nebraska displayed severe or

frequent damage. Montana and New Mexico had moderate or occasional damage. Light or rare damage occurred in California, Arizona, Texas, Idaho, North Dakota, South Dakota, Alberta and Saskatchewan. Isolated collections from wild or cultivated hosts have been recorded in Nevada, Kansas and Oklahoma. Desert breeding areas are thought to be in Texas, southern New Mexico, Arizona, California and northern Mexico.

Overwintering and migration. Potato psyllids appear to migrate annually to northerly locations from the desert breeding grounds listed in the previous section. According to Romney (1939), breeding in these areas occurs form January to May, and peak populations are reached in April or early May. By June, no adults are seen in the desert overwintering area and are not seen again until the fall, though the source of these insects is not known. It is likely that Colorado infestations generally originate from overwintering populations in Arizona. It is speculated that potato psyllid populations east of the Continental Divide (also including Colorado) arise from overwintering Texas psyllids. For epidemic populations to occur, favorable weather conditions have to occur during the migration in May and June (Wallis, 1954). Above average rainfall and below average temperatures in the areas north of the winter breeding grounds most likely retard migration. Above normal summer temperatures can hamper population growth once potato psyllids arrive in the north.

Host plant disease symptoms. Daniels (1954) thoroughly described the symptomology of psyllid yellows. He broke the symptoms down into primary and secondary categories. In potatoes, primary foliar symptoms include:

- Retarded growth
- Erectness of new foliage
- Mild chlorosis in new foliage with basal cupping of leaves
- A progression of red coloration in new leaves.

Secondary foliar symptoms for potatoes are:

- Upward rolling of leaves throughout the plant
- Shortened and thickened terminal internodes resulting in rosetting

- Enlarged nodes, aerial tubers or axillary branches

- Chlorosis increasing in intensity

- Growth at a standstill for weeks up to month.

The symptoms that occur in the underground parts of the potato plant demonstrate the systemic aspect of the disease.

The below-ground symptoms are:

- Setting of excessive number of tiny misshaped tubers (up to hundreds)

- Production of chain tubers

- Early breaking of dormancy of tubers.

The foliar symptoms in tomatoes are similar to those in potatoes. Secondary symptoms involve disruption of fruit set, which also results in lower yields. The symptoms are described by List (1939) and Daniels (1954).

The primary foliar symptoms in tomatoes are:

- Retarded growth

- Erectness of new growth

- Chlorosis

- Purpling of the leaves.

The secondary foliar symptoms are:

- Malformation and distortion of foliage

- Stimulated flower bloom

- Production of numerous and small, poor-quality fruit

- Widespread chlorosis

- Growth at a standstill for weeks to months.

Feeding habits and physiology of the disease. The disease symptoms of psyllid yellows are caused by a chlorotic toxemia that is produced solely by the insect, and that the toxic substance is injected into the plant during feeding (Carter, 1950). The feeding mechanisms of potato psyllids lend understanding to the mechanism of the disease.

Potato psyllids do most of their feeding on the undersurface of leaves (Eyer and Crawford, 1933) though, especially at high infestation levels, they may been seen feeding on the stem and petioles. The mouthparts of the nymph are long and bristlelike and are inserted into the border parenchyma where carbohydrates from photosynthesis are temporarily stored (Eyer, 1937). Feeding tracks, or setal sheaths, are found in the plant tissue when both adults and immatures feed. These tracks were found to follow an intercellular direct path through the cortical parenchyma cells (Carter, 1950). The depth and number of feeding tracks were found to be about the same for adults and nymphs. Teneral adults left very few feeding tracks.

In physiological studies, Eyer (1937) found that breakdown of proteins might be occurring from potato psyllid feeding. Diseased plants also had lower nitrogen levels than healthy plants. The injury to damaged border parenchyma cells appears to be spread across to the phloem where necrosis is seen. This may inhibit local translocation. As a result of this injury, chloroplasts appear smaller, lighter in color, and distorted. In heavily damaged tissue, the chloroplasts are completely disrupted. When plants were assayed for chloroplast pigments, diseased plants had lower levels. Eyer also demonstrated that the carbohydrate metabolism is effected by potato psyllid injury. Sucrose levels are abnormally high and starch levels low in damaged plants. Carter (1950) noted that the potato psyllid toxin may be a competitive inhibitor of auxin within the plant and this could account for the tuber symptoms. Indoleacetic acid (IAA) may decrease with the initiation of tuberization (Stallknecht, 1985), though bioassays are not conclusive. Kinetin has been shown to increase tuberization. It is possible that potato psyllid toxin may interact with auxins.

Very little is known about the toxin, its character or action. Because of the ability of the toxin to break dormancy in plants, if the toxin is a novel compound, it could be of value to the potato industry as a possible sprout initiator in dormant tubers. Dormancy breaking substances are used in countries where two crops are grown in the same season (Wurr, 1978) and are also of use in potato breeding programs.

OBJECTIVES OF STUDY

This study was concerned with gaining new knowledge about the potato psyllid toxin and clarifying past research. The aims of this study were to:

1. Quantify the effect of potato psyllid feeding on tissue cultured potatoes under controlled greenhouse conditions and to examine varietal responses of potatoes.

2. Quantify the effect of potato psyllid feeding on tomatoes under field conditions, and to determine if variety had an effect on host plant susceptibility and if potato psyllids preferred certain varieties of tomatoes over others.

3. Determine if peppers show disease symptoms under field conditions of potato psyllid infestation and if varietal responses occur in peppers.

4. Develop a bioassay for potato psyllid toxin so that the toxin might later be isolated and identified.

5. Determine if topical applications of potato psyllid toxin could break dormancy in minitubers.

CHAPTER ONE

VARIETAL RESPONSES OF TISSUE CULTURED POTATOES TO POTATO PSYLLID INJURY

Some secondary symptoms of psyllid yellows in potato are reduction in tuber yield due to the production of excessive small tubers, the presence of chain tubers, and premature sprouting of tubers (Richards and Blood, 1933). This chapter will investigate the yield reductions due to heavy potato psyllid infestation, as well as changes in dormancy of tubers.

Varietal responses of potatoes from potato psyllid injury have been reported in the literature. Schaal (1938) observed in the Greeley, Colorado area, that symptoms occurred in all varieties of potato grown there, but that earlier varieties showed the most obvious foliar damage and the most pronounced abnormality in tuber set. Arslan *et al..* (1985) noted varietal differences between three cultivars ("Kennebec", "Denali" and "Norgold Russet") when potato psyllids invaded a potato field being used for unrelated studies. Tissue cultured potato plantlets of different cultivars were used in this study to evaluate varietal differences of potatoes from potato psyllid injury. Cultivars were chosen based on times of maturity. Clones within cultivars and greenhouse conditions were used to reduce variation from genetic and environmental effects.

MATERIALS AND METHODS

Source and maintenance of insect colony. Immature and adult potato psyllids were field collected from the Horticulture Research Center of Colorado State University northeast of Fort Collins, Colorado, and from the Plant Environmental Research Center on the campus of Colorado State University from various vegetable hosts. Greenhouse colonies were reared on tomato and potato plants. During the summer, insects were frequently brought in from the

field to keep genetic variability within the colony. The host plants of the colony consistently showed severe symptoms of potato psyllid yellows.

1988 trial. In the summer of 1988, tissue cultured potatoes of three potato cultivars were used: "Norland", an early season cultivar; "Sangre", a mid to late season cultivar; and "Russet Burbank", a late season cultivar. Single node cuttings were made from a mother plant and propagated *in vitro* in Murashige and Skoog propagation medium (Espinoza *et al.* 1986). After the cuttings had rooted, they were planted in cell packs in a commercial seedling potting mix, fertilized and kept in the greenhouse. Artificial fluorescent lighting was used to maintain a 14:10 photoperiod. After the plantlets were established, about two weeks later, they were transplanted in 10-inch pots. Approximately six weeks later, plantlets were infested with potato psyllid nymphs and adults by removing infested leaves from colony plants and placing these infested leaves among plantlets. Control plantlets were sprayed with esfenvalerate (Asana XL) prior to infesting of experimental plants and at two-week intervals during the course of the experiment. This treatment was effective in keeping control plantlets free of potato psyllid feeding.

Approximately five weeks after potato psyllid infestation, the minitubers were harvested using a sieve with a 0.5 cm mesh. Those tubers that fell through the mesh were discarded. All others were kept and weighed. Minitubers also were checked for any sprouting activity. At the time of harvest, many potato psyllid infested tubers were seen to have small transparent leaves arising from the eyes. This was not observed in control tubers. One month later, minitubers were evaluated for any growth occurring from the eyes, including these small leaves.

1989 trial. The experiment was repeated in the spring of 1989 following the same method as given above. At the time of the experiment, Norland plantlets were not available. "Centennial" plantlets were used in place of Norland. Centennial is a mid- season cultivar.

Statistical analysis. Means and standard errors were calculated for the total yield per plant, the average tuber size per plant and the number of tubers per plant for each cultivar.

Each trial was analyzed separately. One-way analysis of variance (Weiss and Hassett, 1982; Spiegel, 1988) was used to compare potato psyllid infested and uninfested treatments for all of the above categories to determine if the treatments were significantly different.

RESULTS AND DISCUSSION

1988 trial. All infested plantlets showed foliar evidence of psyllid yellows. This included erectness of new foliage growth, severe cupping of leaves, dwarfing and chlorosis. Control plants appeared green and vigorous.

At harvest, marked tuber size differences occurred with all three cultivars. Potato psyllid infested tubers were extremely small compared to those from control plants. The tubers from infested plants had rough surfaces, making it difficult to remove soil particles, and had a softer consistency. Many of the infested plants, particularly Sangre, produced chain tubers.

Potato psyllid injury caused significant ($p \leq .0001$) reduction in yield, with an overall reduction for all cultivars combined of 71.1%. The yield per plant was significantly reduced in all three cultivars of infested plants (FIG.1.1). Norland potato psyllid infested plants produced a decrease in yield of 50.9%, as compared to uninfested controls. Sangre yield was decreased by 68.2% and Russet Burbank by 94.2%. Norland, the earliest maturing cultivar, showed the least reduction in overall yield and Russet Burbank, the latest maturing, showed the largest reduction. Sangre showed intermediate damage. These findings do not agree with early observations by Schaal (1938), who reported that early cultivars showed the most severe symptoms.

The average tuber size from potato psyllid infested plants showed a significant decrease ($p \leq .0001$) in all three cultivars (FIG. 1.2). The overall reduction in tuber size for all three cultivars was 82.5%. Norland was reduced by 73.8%, Sangre by 81.5% and Russet Burbank by 92.3%. Russet Burbank, the latest season cultivar, showed the most injury, as it did in yield reduction. Norland and Sangre showed about the same amount of reduction. Because yield

has the components of tuber size and tuber number, differences in tuber number will explain why Norland and Sangre didn't show differences in tuber size but did show differences due to yield.
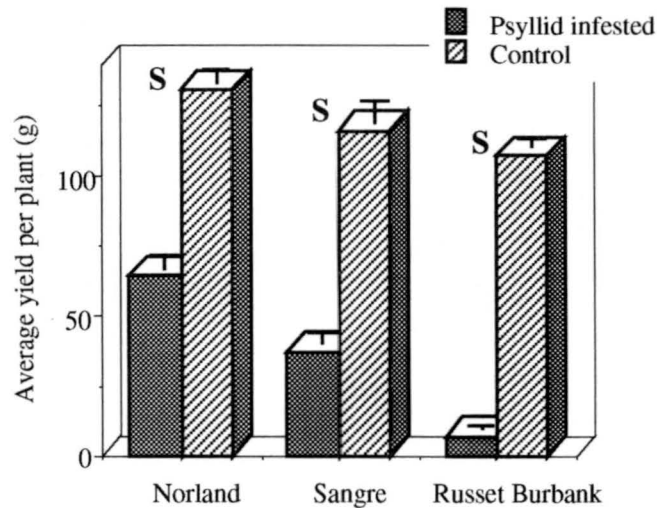

FIG. 1.1 . Comparative total yields from potato psyllid infested and control (uninfested) plants of three different cultivars for 1988 trials. Error bars indicate standard error. S denotes significance at $p \leq .0001$ (ANOVA; Statview™, 1985) between psyllid infested and control plants.
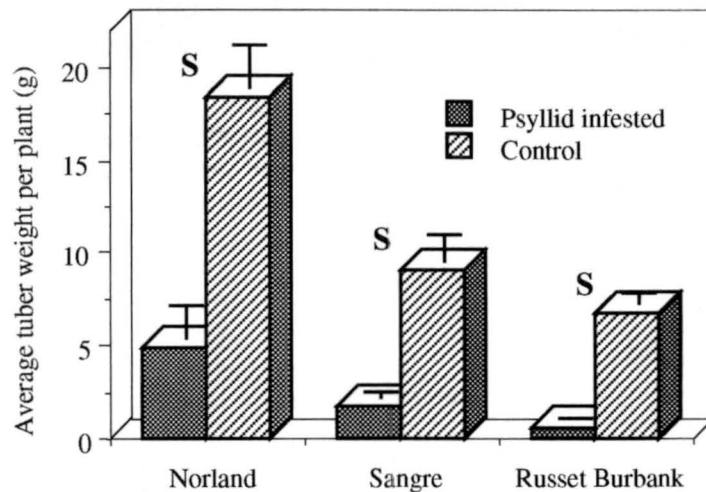

FIG. 1.2. Average tuber weights from potato psyllid infested and control plants for 1988 trials. Error bars indicate standard error. S denotes significance of $p \leq .0001$ (ANOVA; Statview™, 1985) between potato psyllid infested and control plants.

Although yield and tuber size decreased greatly in infested plants, the tubers set was higher in potato psyllid infested plants than in controls in two of the three cultivars (FIG. 1.3). Norland had an increase of 88.9% in tuber set of infested plants and Sangre increased by 85%. Excessive tuber set in infested plants is typical of potato psyllid injury (Daniels, 1954). Russet Burbank showed more tuber set in control plants, 24.9%, than in potato psyllid infested plants. This lower tuber set could be related to the later maturity of this cultivar.
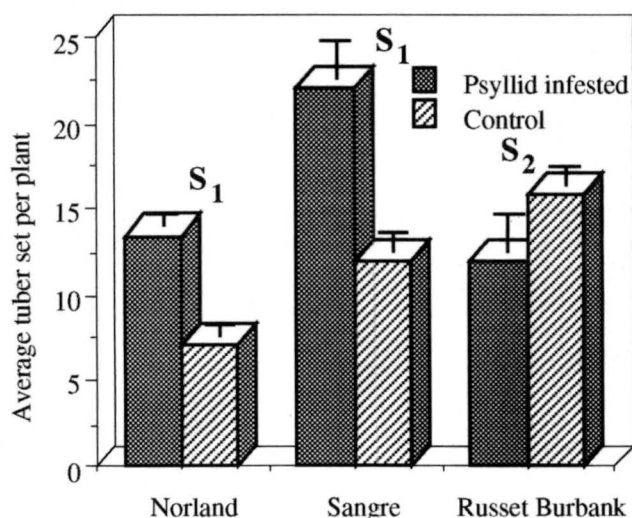

FIG. 1.3. The average number of tubers per plant of three potato cultivars for 1988 trials. Error bars indicate standard error. S_1 denotes significance of $.0001 < p \leq .005$ and S_2 denotes significance of $.05 < p \leq .10$ (ANOVA; Statview™, 1985) between psyllid infested and control plants.

At the time of harvest, it was observed that in the tubers of infested plants, several were showing premature sprouting. In those that were not sprouting, small pale leaves growing in the eyes were seen in many tubers. This was not observed in control plant tubers.

One month after harvest, tubers were evaluated for signs of premature breaking of dormancy, including sprouts, as well as small leaves. Results indicated that potato psyllid feeding greatly stimulated sprouting activity in all cultivars (FIG. 1.4). Almost all of the

tubers of Norland showed premature sprouting. Sangre and Russet Burbank also had large percentages of sprouting. This correlates with field observations, where heavily infested potatoes often showed sprouting at the time of harvest.
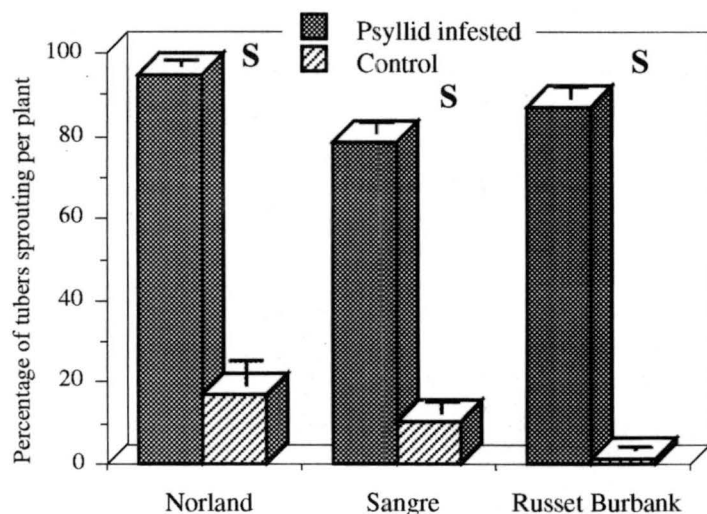

FIG. 1.4. Sprouting of potato tubers from potato psyllid infested and control plants one month after harvest for 1988 trials. S denotes significance at $p \leq .0001$ (ANOVA; Statview™, 1985) between psyllid infested and control plants.

1989 trial. In the second trial, fluctuating environmental conditions occurred, marked by periodic temperatures in excess of 32°C. Yields in both potato psyllid infested and control plants were greatly reduced compared to the 1988 trial and many plants failed to produce tubers (FIG. 1.5). Although a large proportion of control plants did not produce tubers, the additional factor of psyllid feeding almost entirely eliminated tuber production. Of the potato psyllid infested treatments, Russet Burbank had the highest number of plants not yielding (95%); Sangre (75%); Centennial (85%). These results indicate that potato psyllid injury in conjunction with severe environmental stress may have particular importance during tuber set.
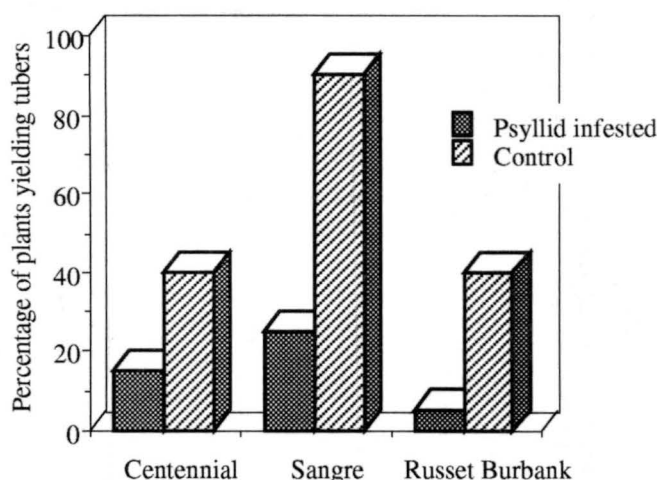
FIG. 1.5.   Percentage of potato plants that set tubers at the termination of the
1989 trial.

From those plants that did produce tubers, average yield, average tuber size, and tuber set

per plant were compared between potato psyllid infested and control plants. Russet Burbank,

however, was so severely affected by the combination of environmental conditions and

potato psyllid feeding that few plants produced tubers resulting in the sample size being

inadequate to determine statistical significance between psyllid infested and control plants.

Each of the cultivars showed a decrease in yield for potato psyllid infested plants (FIG.

1.6). Sangre had a decrease of 99.2% from psyllid feeding, Centennial 93.5% and though not

significant due to small sample size of psyllid infested plants, Russet Burbank had a decrease

of 76.4%. The overall decrease in yield for all 3 cultivars was 89.9%. In the first trial, the

yield loss was 71.1%. A comparison of both trials shows how environmental conditions may
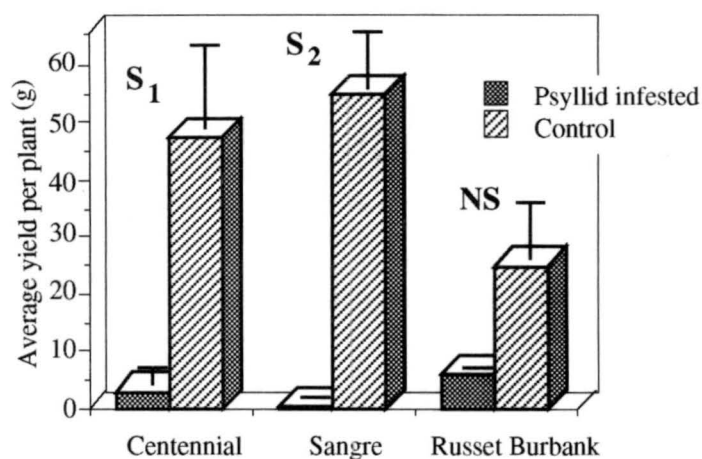
have affected yield loss (FIG. 1.7).

FIG. 1.6.   The average yield per plant from 1989 trial. Error bars indicat standard errors. S_1 denotes significance of .05 < p ≤ .10, S_2 denotes significance of .005 < p ≤ .01 and NS denotes no significance (ANOVA; Statview™, 1985) between psyllid infested and control plants.
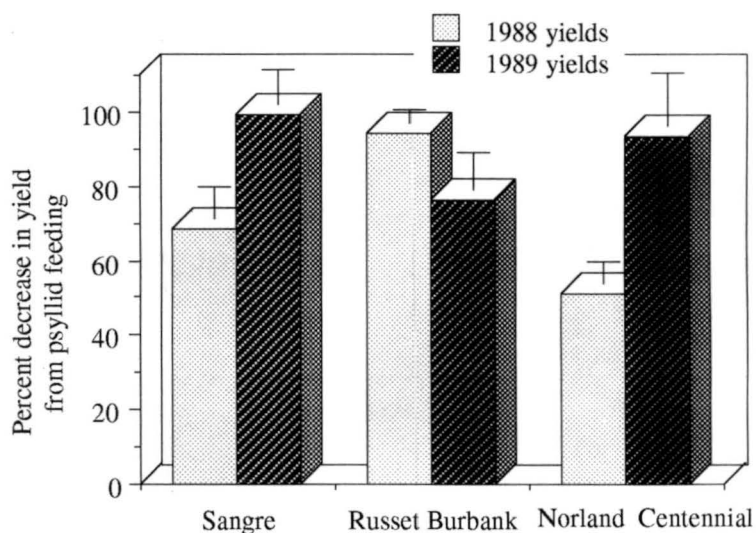

FIG. 1.7.   A comparison of the percent decrease in the yields of Sangre and Russet Burbank from 1988 and 1989 trials. Norland was only evaluated in the 1988 trial and Centennial in the 1989 trial. Error bars indicate standard error . Larger values indicate greater losses from psyllid injury.

Average tuber size was less in potato psyllid injured plants that in control plants (FIG. 1.8). The tuber size for Sangre infested decreased by 97.2%, Russet Burbank 72.8% and Centennial by 91.5%. A comparison of the decrease in average tuber size from both trials show that Sangre experienced greater psyllid injury in the 1988 trials, though Russet Burbank did not based on limited data (FIG. 1.9).
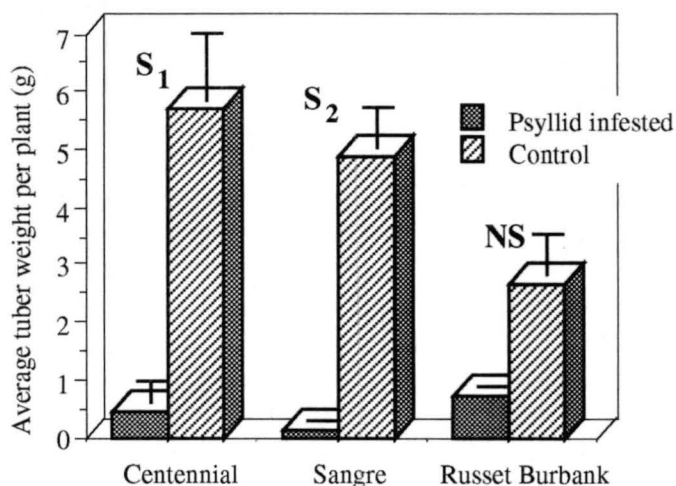

FIG. 1.8.   Average tuber weight per plant for 1989 trial. Error bars indicate standard error. S_1 indicates significance of $.1 < p \leq .25$; S_2 indicates significance of $.025 < p \leq .05$; NS indicates no signifcance (ANOVA; Statview™, 1985) between potato psyllid infested and control plants
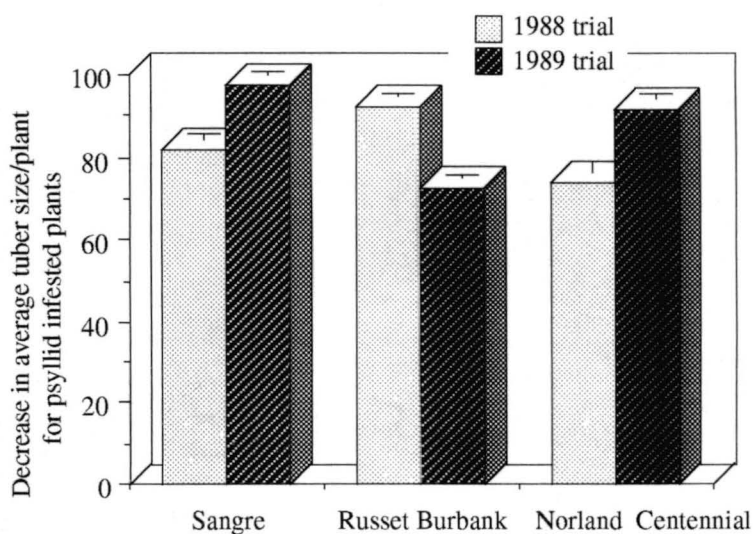

FIG. 1.9.   A comparison of the average tuber size per plant from 1988 and 1989 trials. Error bars indicate standard error. Larger values represent greater damage from psyllid injury.

Tuber set decreased in infested plants in the 1989 trial for both Centennial and Sangre (FIG. 1.10). An increase in tuber number is a typical symptom of psyllid injury (Daniels, 1954). This symptom may not be expressed when potato plants are highly stressed while infested with psyllids. A comparison of both trials (FIG. 1.11) indicate that tuber set may not always be excessive when heavy psyllid damage occurs, especially under extreme conditions due to other factors.
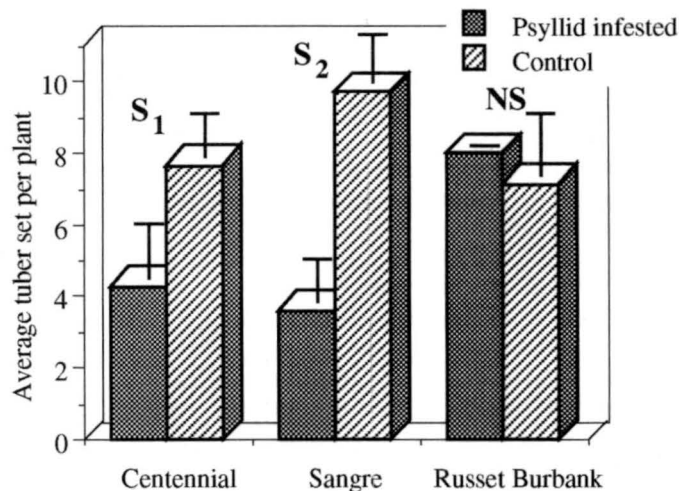

FIG. 1.10. Number of tubers per plant for 1989 trial. Error bars indicate standard error. S_1 denotes significance at $.1 < p \leq .25$, S_2 denotes significance at $.025 < p \leq .05$, and NS denotes no significance (ANOVA; Statview™, 1985) between potato psyllid infested and control plants.
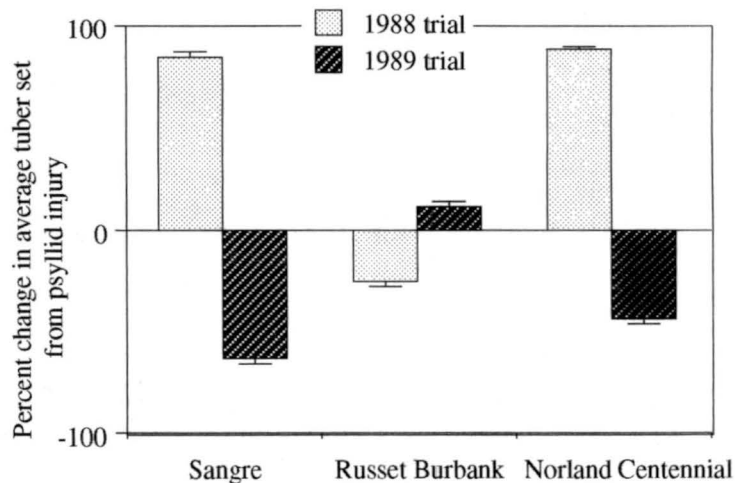

FIG. 1.11. A comparison of the percent increase or decrease in tuber set for 1988 and 1989 trials. Negative values signify a reduction in tuber number per plant and positive numbers an increase in tuber number when comparing psyllid infested plants with control plants. Error bars indicate standard error.

When the average tuber weights from both trials of all cultivars were plotted together, with control weights against infested weights, a high correlation was seen (FIG. 1.12). From these data, the reduction in tuber size from potato psyllid injury was modeled with a high degree of significance. Using this model, if the tuber size is known for uninfested cultivars, the reduction in tuber size due to potato psyllid injury is predictable.
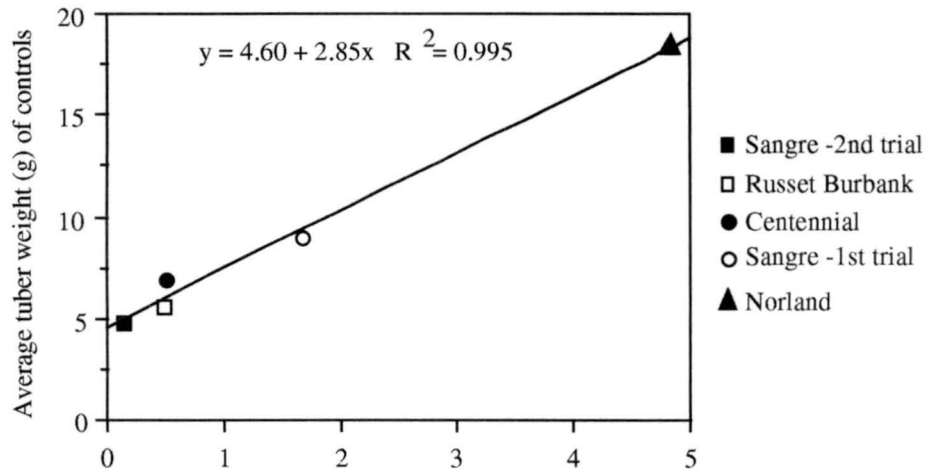

FIG. 1.12. Average tuber sizes of controls vs. potato psyllid infested plants. Data from 1988 and 1989 trials are included.

Tissue cultured plantlets are valuable in studying the effects of a toxin, such as the potato psyllid toxin, because they are propagated to be free of diseases, particularly viral diseases which produce foliar symptoms that can be mistaken for "psyllid yellows". The use of a greenhouse also helps to eliminate variability that could be an important factor in the field. Therefore, it is likely that the symptoms seen in the 1988 trial were a result of potato psyllid feeding. Even though there was much variability in the 1989 trials, control plants indicate that psyllid feeding impacted the yield and average tuber size.

Another important aspect of this study is varietal differences between the cultivars. The 1988 trial showed that the earlier the cultivar matured, the less effect potato psyllid feeding had on yield. This may indicate that potatoes have a sensitive developmental window and

as it gets later in the growing season when potato psyllid populations increase that earlier maturing cultivars may "escape" this vulnerable period when toxin levels become greatest. Norland, the earliest cultivar, showed a 51% decrease in yield. Though this is a significant loss, it is not as large a decrease as the other later season cultivars. Russet Burbank, being a late season cultivar, had an almost complete yield loss (94%), supporting this trend.

Tuber initiation generally occurs at lower temperatures (Cutter, 1978). The exceptionally high temperatures experienced during 1989 trial may be responsible for the low rate of tuberization. A difference between potato psyllid infested and uninfested plants is seen but, because little yield occurred in potato psyllid infested plants, varietal comparisons are likely not to be valid.

The presence of potato psyllid toxin caused premature sprouting in all three cultivars from the 1988 trial. The earlier a cultivar matured, the greater degree of premature sprouting occurred. Just as in the correlation with early vs. late cultivars and level of yield decrease, it is possible that potato psyllid levels are high enough to cause damage at a point where tubers are more developed in early varieties and at a particularly sensitive stage of development. When later cultivars reach this same stage of tuber development, potato psyllid populations may already be declining.

CHAPTER TWO

FIELD EVALUATION OF POTATO PSYLLID INJURY
TO TOMATO VARIETIES

Potato psyllids have been reported pests of tomatoes in the Fort Collins, Colorado, area for more than 50 years (List, 1939). Potato psyllid infestations have been reported to cause severe decreases in the yield of tomatoes (Wallis, 1946). Small, poor quality fruit often are produced and early infestation may result in such severe stunting that the plant may set little or no fruit (List, 1939).

In this study, the effect of natural field populations of potato psyllids was examined on several varieties of tomatoes. Decreases in yield due to potato psyllid injury as well as varietal responses were determined.

MATERIALS AND METHODS

In the spring of 1987, eight varieties of tomatoes, "Ace", "Columbian", "Early Girl", "Fantastic", "Jubilee", "Long Keeper", "Roma" and "Super Sioux" were grown to the seedling stage in the greenhouse. The seedlings were transplanted to the Horticulture Research Center of Colorado State University northeast of Fort Collins, Colorado. Each variety was planted in a split plot with a total of ten plants per plot. Half the plants were treated with esfenvalerate (Asana XL) and the other half of the plot was not treated. The esfenvalerate was effective in controlling potato psyllid populations. Six replicates of each variety were planted. During the growing season, potato psyllid populations were monitored by counting the three center plants of each 1/2 plot (six plants per whole plot). Fruit was checked weekly for ripening and when the fruit reached marketable size, weekly harvests were made. Each plant was harvested separately and each fruit was then weighed individually.

At the end of the season, the total yield, number of fruit and average fruit size were calculated for each plant. Data then were combined for each variety. Means and standard error were calculated for yield, fruit size and fruit set. One-way ANOVA (Statview™, 1985) was used to determine if potato psyllid infested and uninfested treatments were significantly different (Weiss and Hassett, 1982; Spiegel, 1988). The variation within each variety also was analyzed by this method. The counts of potato psyllids were compiled for each plant over the course of the season and combined for each variety.

RESULTS AND DISCUSSION

Injury from potato psyllids was seen in all varieties. The degree of significance between potato psyllid infested and control treatments varied for fruit size and fruit set due the great amount of variation within each variety. Seven varieties showed a high degree of significance (p=0.05) between potato psyllid infested and uninfested plants for yield (FIG. 2.1). All varieties show substantial reduction, except for Long Keeper, which was least affected with a percent decrease of 29.7%. The combined reduction in yield for all varieties was 41.5%. Of those varieties showing a significant yield reduction, a wide range of susceptibility was seen. The smallest percent decrease was seen in Fantastic, with a reduction of 30.4%, followed by Columbian and Super Sioux, with reductions of 35.3%, Early Girl with 37.6%, Roma with 43.3%, Jubilee with 59.3%, and the largest reduction in Ace with 60.9%.

Reduction in fruit size also resulted from potato psyllid injury (FIG. 2.2). The varieties showing significant differences between infested and uninfested were Ace, Columbian, Early Girl, Fantastic, Jubilee, Roma and Super Sioux. Long Keeper was the only variety not showing significance in average fruit size, with a reduction of 7.9%. The reductions of those varieties significantly reduced in fruit size from potato psyllid injury were Columbian with 13.4%, Roma with 17.1%, Super Sioux with 17.8%, Early Girl with 21.3%, Jubilee with 28.8%, Fantastic with 30.7%, and Ace with 32.0%. The varietal response to yield was somewhat different than average fruit size. For example, Fantastic showed the least damage when yield was used as a criterion for damage, but was one of the most severely affected in fruit size reduction. Yield has two components, fruit size and fruit number. Due to this, differences between total yield and average fruit size can occur.
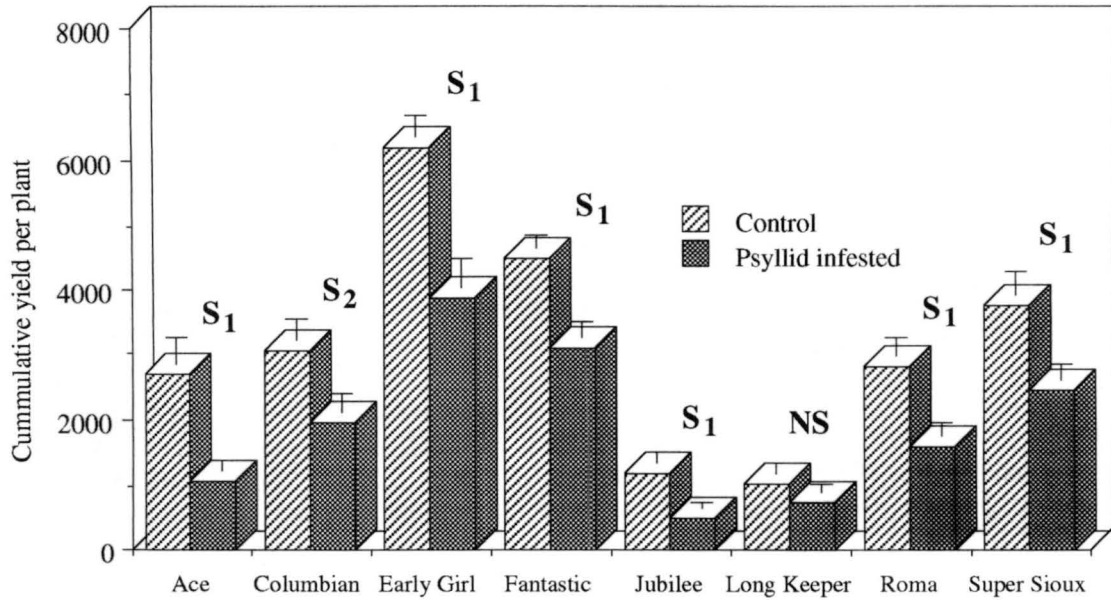
FIG. 2.1   The comparative yields of potato psyllid infested and uninfested
tomato plants, Fort Collins, Colorado, 1987. Error bars indicate
standard error. Letters denote significance (ANOVA; Staview™,
1985) between psyllid infested and control plants as follows: S_1,
$.0001 < p \le .005$; S_2, $.01 < p \le .025$; NS, no significance.

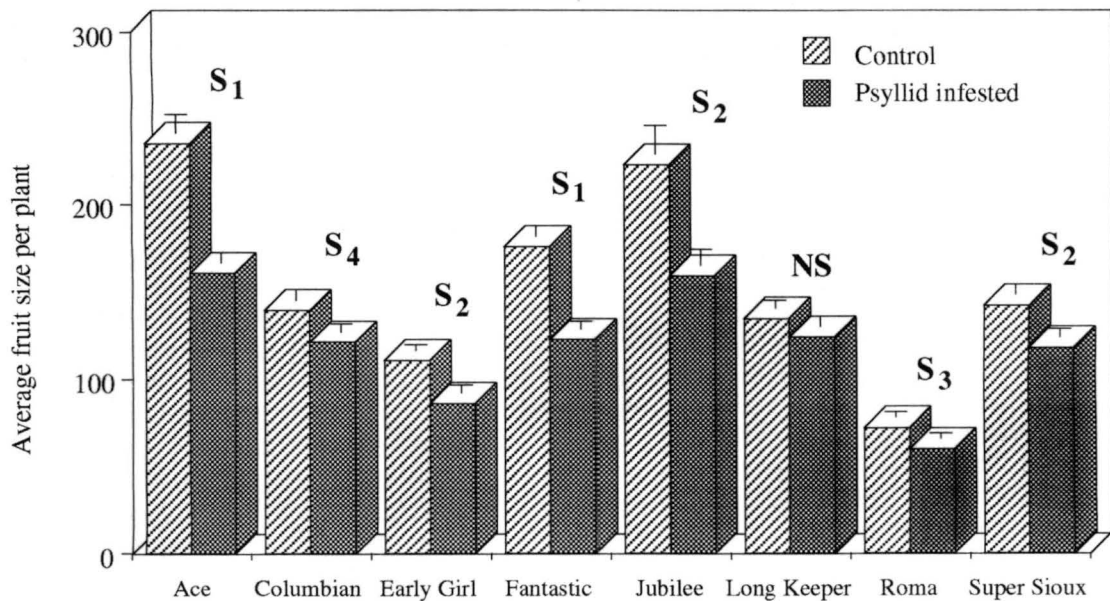

FIG. 2.2.   Average fruit size of tomatoes from potato psyllid infested and
uninfested plants, Fort Collins, Colorado, 1987. Error bars indicate
standard error. Letters denote significance (ANOVA; Staview™,
1985) between psyllid infested and control plants as follows: S_1,
$p \le .0001$; S_2, $.0001 < p \le .005$; S_3, $.005 < p \le .01$; S_4, $.01 < p \le .025$;
NS, no significance.

Fruit set was decreased in all varieties except Fantastic, which showed an increase in potato psyllid infested plants of 2.8%, which is not significant (FIG. 2.3). Only three varieties showed a significant difference between potato psyllid infested and uninfested plants. These were Ace, with 46.6% reduction in fruit set, Early Girl with 22.4% and Roma with 31.5%. Other varieties showed large reductions in number of fruit, but were not significant, due to considerable variation within the plants of each variety. These were Columbian, with 22.3% decrease, Jubilee with 41.0%, Long Keeper with 23.6%, and Super Sioux with 19.8%. Overall average loss in fruit set from potato psyllid infestation for all varieties was 25.6%.
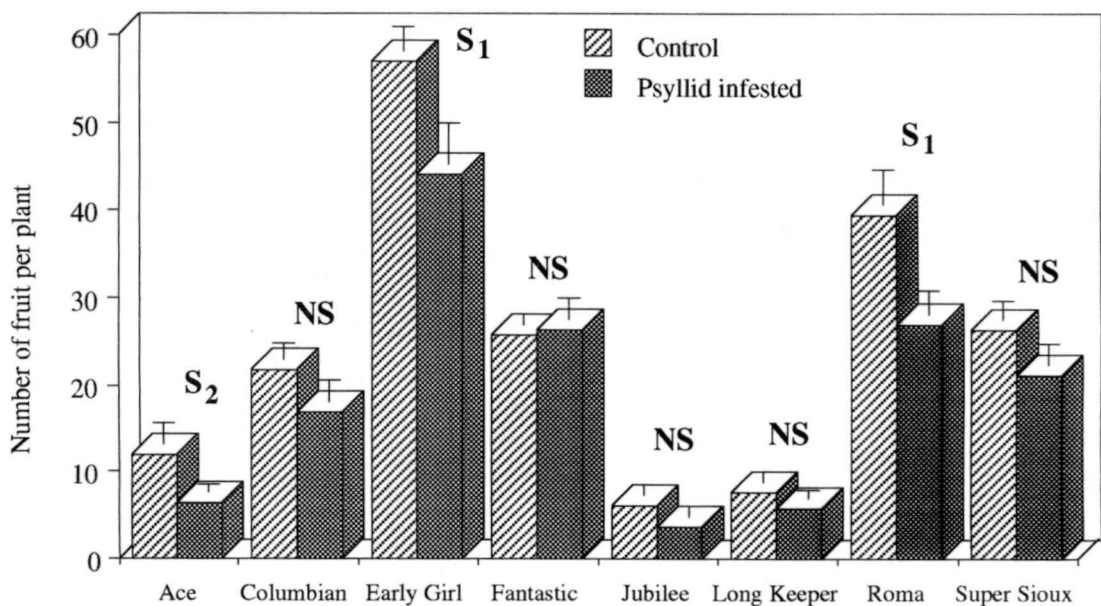

FIG. 2.3. The number of fruit per plant for infested and control tomatoes, Fort Collins, Colorado, 1987. Error bars indicate standard error. Letters denote significance (ANOVA; Staview™, 1985) between psyllid infested and control plants as follows: S_1, $.01 < p \le .025$; S_2, $.025 < p \le .05$; NS, no significance.

The yields of the varieties were plotted against the level of infestation or the number of potato psyllids per plant. The varieties fell into groups which then were denoted according to degree of infestation and of damage (FIG. 2.4). From the areas where the quadrant lines fit best between groups, reductions in yields less than 50% were considered to be low and

greater than 50% to be high levels of damage. Potato psyllid infestations of less than 45 per plant were assigned to be low infestation levels and those greater than 45 per plant as high infestations. Most varieties fell into the category (**III**) of low infestation and low damage; Columbian, Early Girl, Fantastic, Long Keeper and Super Sioux. Ace was category **I**, high infestation and high damage. Jubilee was in category **II**, low infestation and high damage. Roma was highly infested but had a low level of damage (**IV**). Most growers would probably not consider 50% damage to be an acceptable yield loss. When the data is analyzed this way, it shows that varieties have a range of susceptibility and resistance to potato psyllid infestation. More data is needed from more varieties to determine how much damage is economic. From this, growers would be able to determine the intensity of potato psyllid management that each variety required and establish scouting programs and economic thresholds.
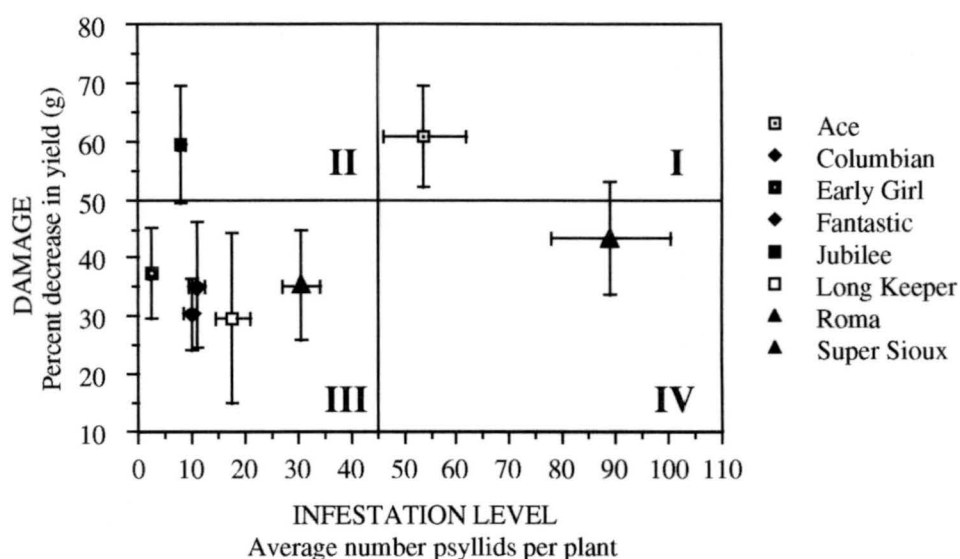

FIG. 2.4. The decrease in yield of tomato varieties per plant plotted against the number of potato psyllids per plant. Zones represent susceptibility to damage and to infestation. **I**: High infestation and high damage. **II**: Low infestation and high damage. **III**: Low infestation and low damage. **V**: High infestation and low damage. Error bars idicate standard error.

The data from yields, average fruit size and fruit per plant were analyzed by plotting

values from infested plants against values from controls to find if the same kind of correlation

occurred in field tomatoes as did in tissue cultured potatoes from the previous chapter (FIG.

1.9). The tomato data showed high correlations, with R^2 values of 0.949 for yield, 0.911 for

average fruit size and 0.951 for number of fruit per plant (FIG. 2.5, 2.6, and 2.7). This is the

same pattern seen in greenhouse grown potatoes. Although decreases in yield, fruit size and

set varied widely between varieties, presence of potato psyllids causes a change that can be

described for each variety by the same linear equation for each variable (yield, fruit size or

fruit set). These plots can be used to model what degree of damage can be expected if a potato

psyllid infestation occurs over the ranges in this study, the highest being an average of 90 per

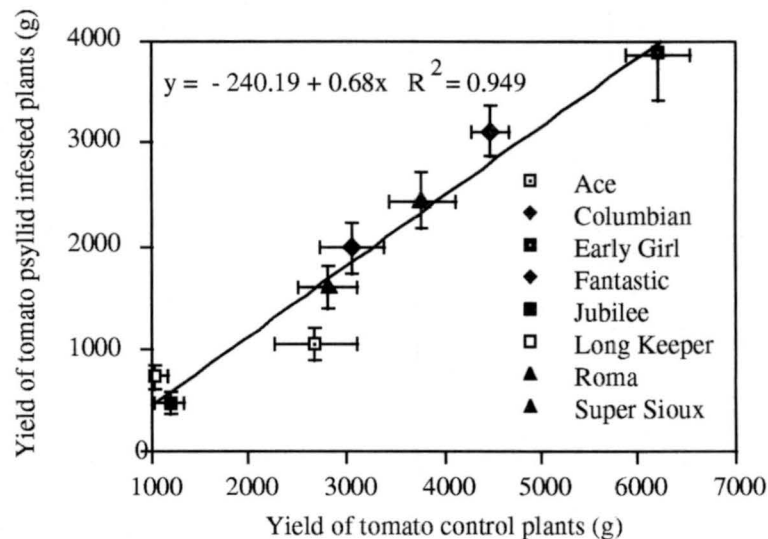plant in the case of Roma.

FIG. 2.5.   Plot showing the regression between the yields of potato psyllid infested and uninfested tomato plants for eight different varieties from Fort Collins, Colorado, 1987. Error bars indicate standard errors.
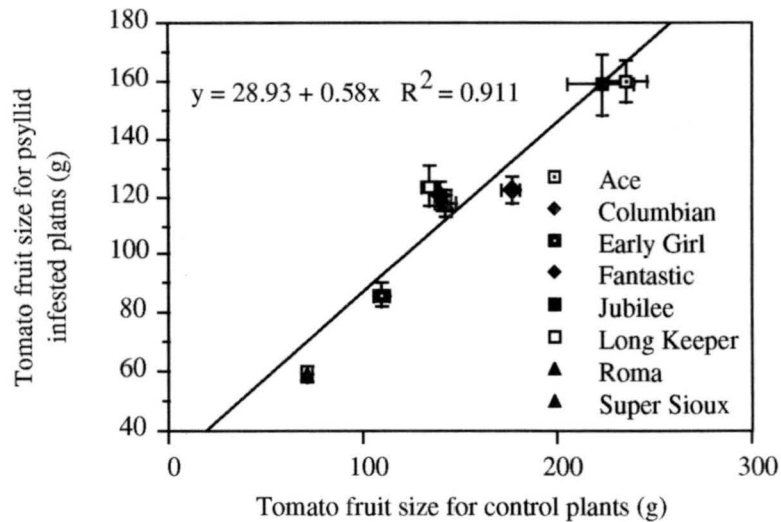
FIG. 2.6. The the regression between potato psyllid infested and control tomato fruit size for eight varieties from Fort Collins, Colorado, 1987. Error bars indicate standard errror.
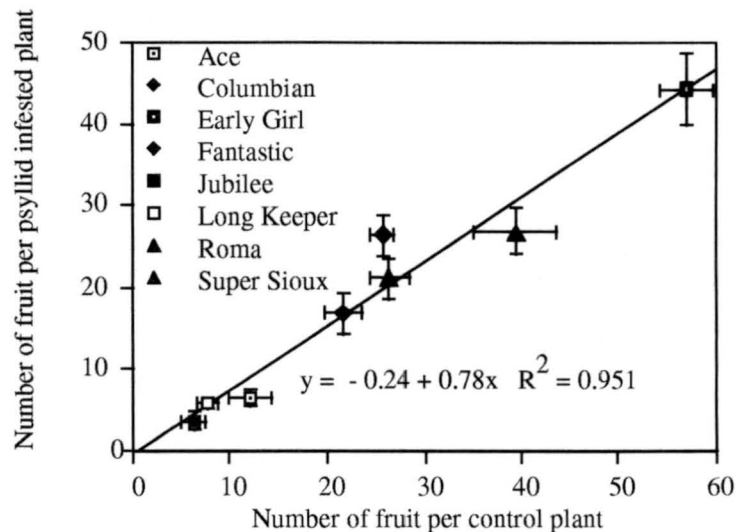

FIG. 2.7. The regression between potato psyllid infested and uninfested fruit number per plant for eight varieties of tomato from Fort Collins, Colorado, 1987. Error bars indicate standard error.

Linear regression analysis was conducted with uninfested plants as the independent variable and infested plants as the dependent variable. If the slope for the plot is less than one, this indicates a decrease in yield for infested versus uninfested. A slope greater than one indicates an increase. A y-intercept less than zero indicates a decrease in yield and a y-intercept greater than zero indicates an increase. The two effects are different and may stem from different physical causes. The same is true for the other variables of average fruit size and fruit number per plant. The regression plot for yield (FIG. 2.5) shows a negative y-intercept. This implies that potato psyllid injury causes an initial deficit of 240 grams when interpolated back to 0 for control plants. Since the slope is less than one, this indicates that the effect worsens as the yield gets greater in the uninfested plants. For fruit size and fruit set; the slopes of the regression plots (FIG. 2.6 and 2.7) are less than one, indicating significant damage in infested compared to uninfested plants. These plots show the overall effect of potato psyllid damage on the eight varieties of tomato that we tested. More varieties need to be evaluated to see if this trend holds true for all tomato varieties.

CHAPTER THREE

FIELD EVALUATION OF POTATO PSYLLID INJURY
TO PEPPER VARIETIES

Peppers are known to be a host for potato psyllids (Wallis 1951), but damage to peppers from potato psyllid infestation has not been reported. Daniels (1954) planted 64 varieties of pepper in 1934 and all but seven varieties were infested. He found no evidence of damage in any peppers. Records of varying levels of infestation were not given except to note those not infested. No record of control or uninfested plants is mentioned and only visual inspection appears to be used to measure if damage occurred. Because so little is known about peppers as potato psyllid host plants and no known study has ever thoroughly addressed whether damage occurs, the following study was conducted to quantify the effect of potato psyllid infestation on peppers as well as varietal responses.

MATERIALS AND METHODS

In the spring of 1987, four varieties of peppers, "Anaheim", "California Wonder", "Gypsy" and "Hungarian Yellow Wax" were grown to the seedling stage in the greenhouse. The seedlings were transplanted to the Horticulture Research Center of Colorado State University northeast of Fort Collins, Colorado. Each variety was planted in a split plot with a total of ten plants per plot. Half the plants were treated with esfenvalerate (Asana XL) and the other half of the plot was not treated. Six replicates of each variety were planted. During the growing season, potato psyllid populations were monitored by counting the three center plants of each 1/2 plot (six plants counted per whole plot). The fruit was checked weekly for ripening and when the fruit reached marketable size, it was harvested. The harvest from each

plant was kept separate and each fruit was weighed individually. At the end of the season, the total yield, number of fruit and average fruit size were calculated for each plant. Data were then combined for each variety. Means and standard error were calculated for yield, fruit size and fruit set. One-way analysis of variance (Staview™, 1985) was used to determine if potato psyllid infested and uninfested treatments were significantly different (Weiss and Hassett, 1982; Spiegel, 1988). The variation within each variety also was analyzed by this method. The number of psyllids counted on each plant were averaged for the season and combined for each cultivar.

RESULTS AND DISCUSSION

Whereas all the potato varieties tested showed significant injury from potato psyllids (Chapter 1), as well as most of the varieties of tomato (Chapter 2), the only variety of pepper showing significant difference between psyllid infested and control plants was Anaheim and, of the three factors yield, fruit size and fruit set, Anaheim showed significant differences only for yield (F=5.6; d.f.=35).

Of the four varieties of pepper, California Wonder showed no difference in yield between infested and uninfested plants, Gypsy showed a 14.6% decrease in yield and Anaheim and Hungarian Yellow Wax showed increases in yield in infested plants (FIG. 3.1). Anaheim had an increase of 49.1% and Hungarian Yellow had 17.3% increase in yield in potato psyllid infested plants. There is no prior record in the literature that could be found for an increase in yield due to potato psyllid feeding for any crop.

The average fruit weights of all four varieties showed small changes within each variety which were not significant (FIG. 3.2). Percent changes were small. Anaheim showed an increase in potato psyllid infested plants of 6.2%. The other three varieties showed slight decreases in potato psyllid infested plants. California Wonder had a 3.6% decrease, Hungarian Yellow Wax had a 2.4% decrease and Gypsy had a 7.2% decrease.

FIG.3.1. Pepper yields for potato psyllid infested and uninfested plants, Fort Collins, Colorado, 1987. Error bars indicate standard error. S denotes significance (ANOVA; Staview, 1985) at .01 < p ≤ .025 and NS denotes no significance.

FIG. 3.2. Comparison of the average fruit sizes of potato psyllid infested and potato psyllid control pepper plants, Fort Collins, Colorado, 1987. Error bars indicate standard error. NS denotes no significance (ANOVA; Statview, 1985) between psyllid infested and control plants.

The number of fruit per plant increased in the potato psyllid infested plants of Anaheim, California Wonder and Hungarian Yellow Wax. Gypsy was the only variety showing a decrease (FIG. 3.3). Anaheim had an increase in fruit number in potato psyllid infested plants of 34.3%, California Wonder had 8.1% and Hungarian Yellow Wax had 18.7%. Gypsy had a decrease of 12.9%. None were significant.
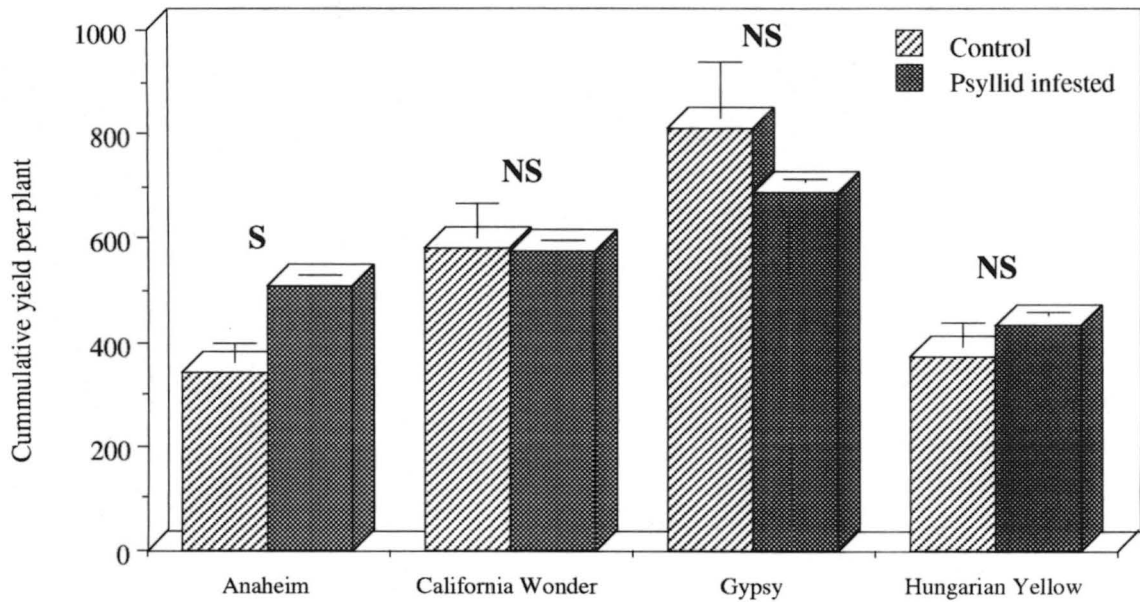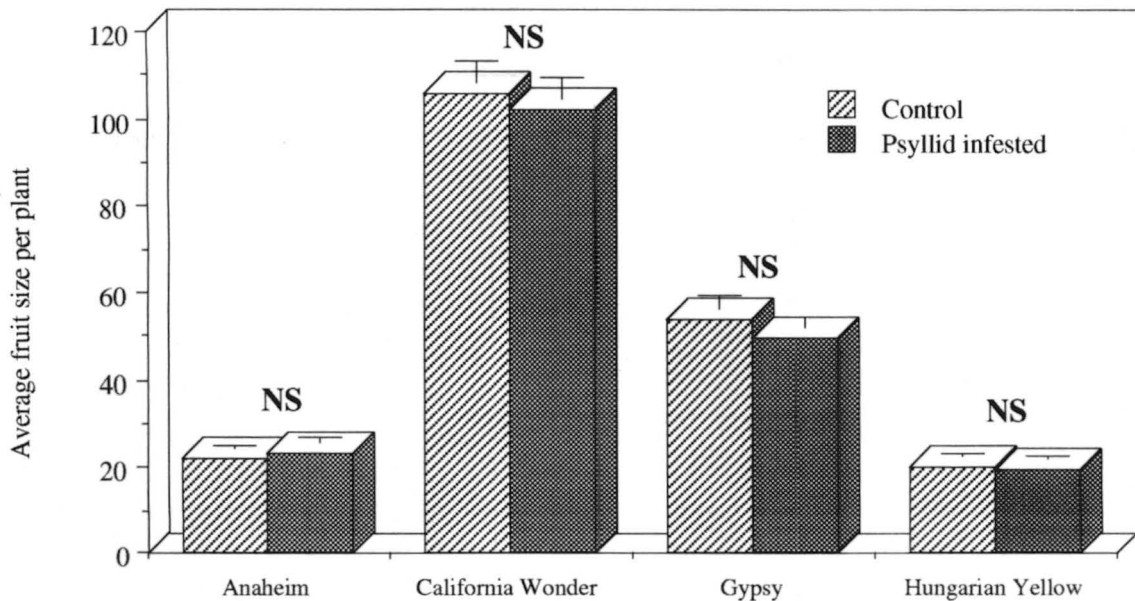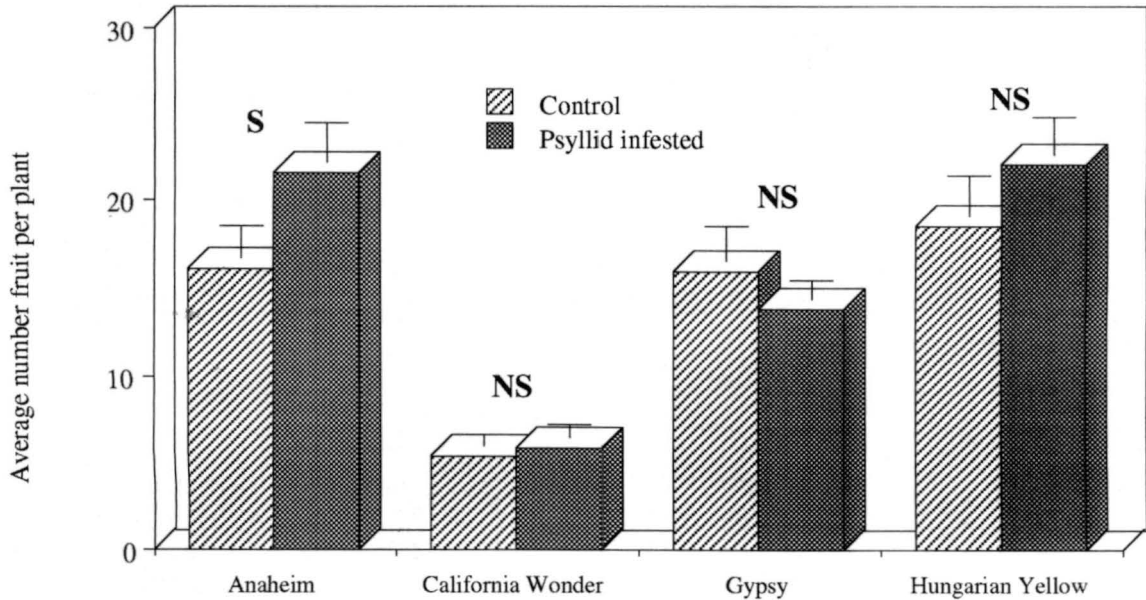

FIG. 3.3.   Number of fruit per plant for potato psyllid infested and uninfested pepper plants, Fort Collins, Colorado, 1987. Error bars indicate standard error.   NS denotes no significance (ANOVA; Staview, 1985) between psyllid infested and control plants.

The yields of the peppers were plotted against the level of infestation (the number of potato psyllids per plant). A curious trend is seen (FIG. 3.4); as the infestation increases the yield increases. In this case, instead of potato psyllid feeding causing injury, in three of the varieties, it benefited production. Tomatoes fell into categories depending on damage or susceptibility and number of potato psyllids that a variety could support (FIG. 2.4). But, all varieties of tomatoes showed reduction in yield at all infestation levels.
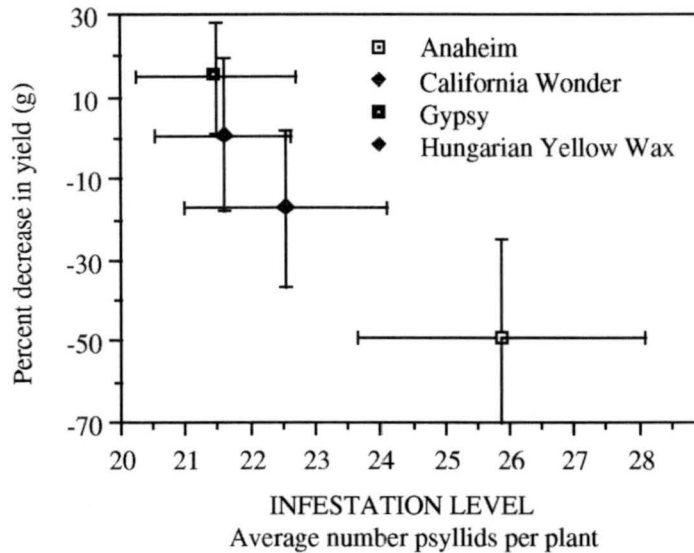
FIG. 3.4. The percent decrease in average yield per plant of pepper varieties
plotted against the number of potato psyllids per plant, Fort
Collins, Colorado, 1987. Error bars indicate standard error.

Regressions were made of infested against control for yield, average fruit weight and

number of fruit per plant (FIG. 3.5, 3.6 and 3.7). Linear regression analysis was used to

correlate infested, the dependent variable, to uninfested plants, the independent variable. If

the slope for the plot is less than one, this indicates a decrease in yield for infested versus

uninfested. A slope greater than one indicates an increase. A y-intercept less than zero

indicates a decrease in yield and a y-intercept greater than zero indicates an increase. The two

effects are different and may stem from different physical causes. The R^2 value of 0.885

implies that there is a linear relationship between infested and control yields for peppers (FIG.

3.5). The positive y-intercept indicates that there is an initial increase in yield in peppers from

potato psyllid feeding. This means that for low yield in uninfested plants, infested plants will

show a yield approximately 300 grams higher. The slope being less than one, as yield

increases in control plants the initial effect where infested plants have higher yields than

uninfested plants is progressively negated, so that by the time a large yield is produced in

uninfested plants, infested plants have a lower yield. Tomatoes showed (FIG 2.5) the same

slope effect in that infested yield drops as uninfested yield increases (this slope is also less than one). The major difference in response to injury is that instead of beginning with a high positive y-intercept, the y-intercept is negative in tomatoes. This implies that infested plants start in a deficit, thereby showing a worsening effect as yields increase. Peppers begin with potato psyllid infested plants having a yield advantage, but losing this advantage as yield increases until the initial effect is lost.

$$y = 306.00 + 0.47x \quad R^2 = 0.885$$

Legend:
- Anaheim
- California Wonder
- Gypsy
- Hungarian Yellow Wax

x-axis: Total pepper yield of control plants (g)
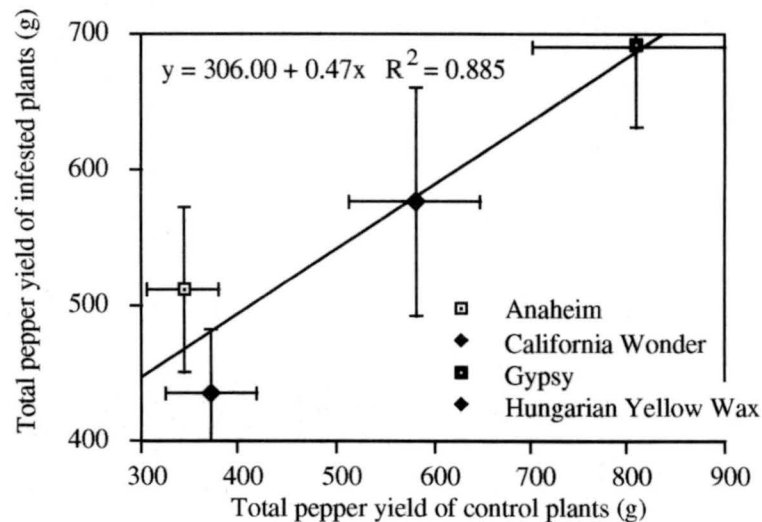y-axis: Total pepper yield of infested plants (g)

FIG.3.5.   Regression for infested vs. control yields for four varieties of pepper. Error bars indicate standard error.

The regression plots for average fruit size and number of fruit per plant are similar to the plot for yield as far as showing potato psyllid affect on pepper size and number (FIG. 3.5 and 3.6). The plot for pepper size shows a very high correlation coefficient of 0.998, but if the data is considered for average fruit size, it can be seen that in all varieties, there is no statistical difference between control and infested plants (FIG. 3.2).

The plot for fruit number per plant, showing the regression between potato psyllid infested and control plants, also is not useful for modeling potato psyllid effect for the same reasons as average pepper size (FIG. 2.12). This can be seen by referring to comparative yield data where no significant difference between potato psyllid infested and uninfested is shown (FIG. 2.9).
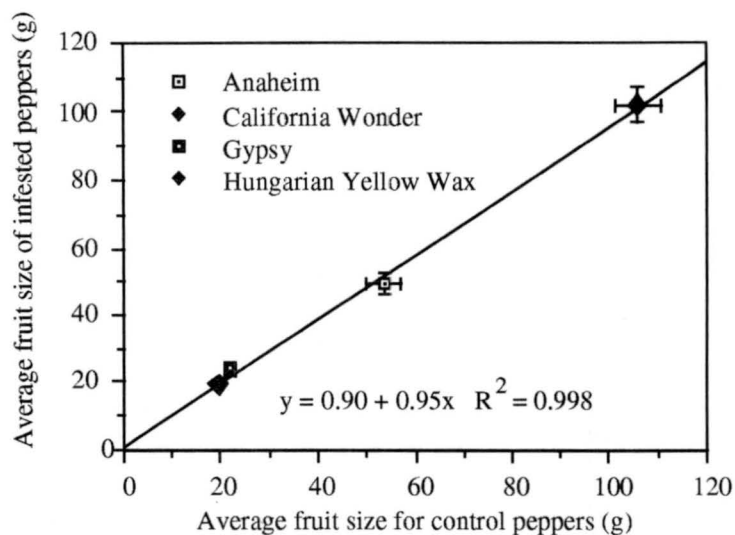
FIG.3.6.   Regression between infested and control peppers for average fruit weight per plant. Error bars indicate standard error.
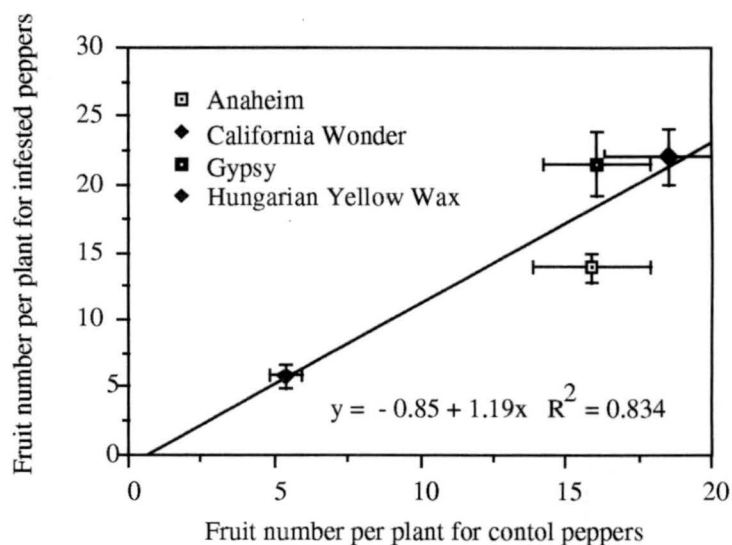
FIG.3.7.   Regression for fruit number per plant between potato psyllid infested and uninfested pepper varieties. Error bars indicate standard error.

The novel effects of potato psyllid injury on peppers show that the effects of the potato psyllid toxin may be more complex than what is indicated from tomato and potato damage. Physiological studies need to be carried out to find if peppers are affected at this level in a different way than what has been found in tomatoes and potatoes and may give a better understanding as to the action of the potato psyllid toxin.

CHAPTER FOUR

MEMBRANE FEEDING SYSTEM AND BIOASSAY DEVELOPMENT

A reliable bioassay is essential for the isolation of a biologically active compound and must first be established before any subsequent chemical analysis can occur. An ideal bioassay consistently shows a precise response and is relatively quick and easy to employ. Several different bioassays were assembled in an attempt to demonstrate a clear effect from either a crude extract of potato psyllid nymph bodies or by collecting the saliva of the potato psyllids. It is necessary to develop a bioassay for the effect of the potato psyllid toxin if it is to be isolated and later identified.

MATERIALS AND METHODS

Tissue cultured potato plantlet bioassay. Four cultivars ("Centennial", "Kennebec", "Russet Burbank" and "Sangre") of tissue cultured potato plantlets were used in the original bioassay development. Potato plantlets, provided by Dr. Kenneth Knutson of the Horticulture Department of Colorado State University, were propagated *in vitro* by making single node cuttings from a mother plant and then transferring the cutting to a container of Murashige and Skoog propagation medium as described by Espinoza *et al.* (1986). Within three to four weeks, the cuttings rooted. At this point, the plantlets were inoculated with live second instar potato psyllid nymphs at a rate of five per plantlet and the psyllids were left to feed for six days in a growth chamber at 27°C and with a 14:10 photoperiod. Plantlets were observed every few hours the first day and three times each subsequent day for any sign of "psyllid yellows" symptoms.

Excised tomato cotyledon and potato psyllid whole body extract bioassay. Tomato seeds (cultivar "Roma") were germinated in the dark at 28°C. After eight days, the cotyledons were excised from the seedlings with a sharp razor blade and ten cotyledons were placed in 9-cm sterile plastic Petri plates lined with filter paper and moistened with 20 mM KCl solution, much like a bioassay used to measure responses of cucumber cotyledons to cytokinins (Crane and Ross 1986). Tomatoes have been demonstrated to show greater degree of symptoms from potato psyllid feeding when whole intact plants were used to determine phytotoxic effects than did potatoes (Daniels, 1954). Therefore, tomato cotyledons were used with the expectation that they would be sensitive to potato psyllid treatments. After being weighed, cotyledons were placed abaxial side down in the Petri plate, after which the petri plates were sealed with Parafilm and placed in a growth chamber at 27°C with equal photoperiods.

After 24 hours, the cotyledons had expanded and become green and were treated in the following ways:

1) Five mid-instar potato psyllid nymphs (third and fourth instars) were placed on each cotyledon and allowed to feed.

2) An extract of macerated potato psyllid nymphs was applied with the blunt end of a wooden toothpick to the adaxial sides of the tomato cotyledons. The cotyledons were covered with the extract along their entire length and the application repeated twice. To make the extract, 70 potato psyllids were placed in 30 microliters of a 50:50 mixture of distilled water and dimethyl sulfoxide (DMSO) and macerated using a small mortar and pestle until a fine slurry was obtained.

3) Two controls were prepared in the same manner as treatment 2, with either distilled water or a 50:50 mixture of distilled water and DMSO being used in place of the potato psyllid extract.

DMSO was mixed with the potato psyllid extract to aid in the uptake of potential toxicogenic compounds from the potato psyllids. DMSO has been used successfully to increase plant absorption of external substances, e.g. luciferin in tobacco plants (Ow *et al.* 1986).

After each plate of cotyledons was treated, it was sealed with strips of *Parafilm*- "M"® and returned to the growth chamber. The tomato cotyledons were observed daily for any changes in color and any other symptom that may have been induced by any of the treatments. After six days, the cotyledons were weighed again.

The bioassay was repeated with the cultivar "Super Sioux". The extract of potato psyllid nymphs was conducted the same way as previously described and another control was added using green peach aphids [*Myzus persicae* (Sulzer)] of about the same size as the potato psyllids used in the psyllid extract. Because green peach aphids are not known to have any toxicogenic effects on tomato plants, this control was used to try to eliminate the effect of the insect body components in the potato psyllid extract, so that any effects observed would be due to the toxic properties of the potato psyllid nymphs. The treatment using live potato psyllids was not repeated because the nymphs either crawled off the cotyledons and would not feed or died. After treatment, weights of cotyledons were taken at two days and again at five days.

The bioassay was repeated with potato psyllid and green peach aphid extracts on the cotyledons of the Roma cultivar.

Development of a membrane feeding system. Mittler and Dadd (1964) described a technique feeding aphids an artificial diet by placing a diet solution in a sachet of stretched *Parafilm* "M"®. This membrane feeding system was adapted for potato psyllids using a 1.5 mL plastic Eppendorf® disposable centrifuge tube for the purpose of collecting potato psyllid salivary secretions. The tube itself was used as a cage to contain the potato psyllid nymphs and the attached cap was filled with the diet solution with a piece of tightly stretched *Parafilm* "M"® placed over the cap (FIG. 3.1A). The tube was punctured with a needle to make small

air holes around the circumference of the tube about halfway up its length. This provided a means of air exchange and a way to prevent moisture build up inside the tube.

During each trial, approximately 12 potato psyllid nymphs were placed inside the tube and the lid carefully closed. In original trials, the psyllids had difficulty locating and feeding on the diet, so the parafilm was smeared with the sap of fresh tomato leaves or rubbed with a leaf to leave cuticular waxes on the parafilm. The latter technique was found to work successfully in inducing potato psyllid feeding, and this method was adopted in all subsequent trials.

In studies with planthoppers and leafhoppers, Mitsuhashi (1974) found that these insects survived for short times when fed sugar solutions via membrane feeding systems, particularly sucrose solution with a concentration of 5% (w:w). Based on these findings, 5% sucrose solution was offered to potato psyllids. The potato psyllids fed vigorously on 5% sucrose solution and were able to survive up to ten days, the longest that membrane feeding systems were tested. Because the membrane feeding system was developed to collect salivary secretions, and not to rear potato psyllids, this solution was found to be satisfactory and was used in all subsequent trials.
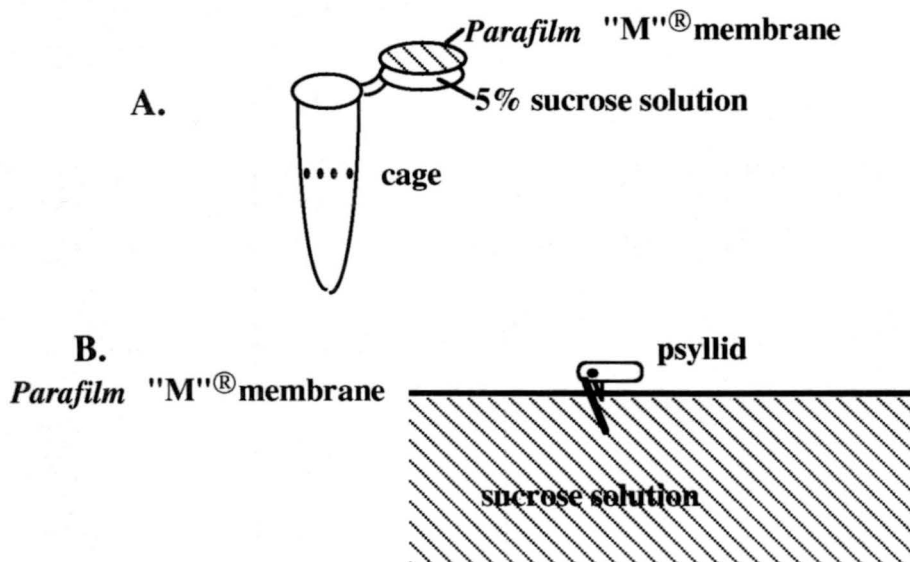

FIG.3.1 A.   An Eppendorf® centrifuge tube was used as a cage with the cap acting as a membrane feeding system where diet was contained by stretching parafilm tightly over it .

FIG. 3.1 B.   Diagram showing how the potato psyllid feeds by piercing the membranesurface with its stylets.

After the potato psyllid nymphs had been placed in the membrane feeding cages, the tubes were placed in the growth chamber at 27°C with 12:12 photoperiod.

Membrane feeding solution and the tomato cotyledon bioassay. After the potato psyllid nymphs had been allowed to feed for two days on the membrane feeding system, the diet solution was collected with a hypodermic syringe. The tomato cotyledon bioassay was rerun. In addition to the prior treatments, a new treatment using the fluid from the membrane feeding system was mixed with DMSO (50:50) and another control using 5% sucrose was mixed with DMSO (50:50).

The next run of the bioassay used all treatments previously described with the addition of an identical membrane feeding system with green peach aphids, to use their salivary secretions as a control.

RESULTS AND DISCUSSION

Tissue cultured potato plantlet bioassay. Tissue cultured potato plantlets used in the original bioassay were readily fed upon by potato psyllids. However, no obvious symptoms occurred for the first five days. On the sixth day, very slight erectness of new growth was observed in some cultivars, but was not easily or consistently measurable from controls so was not useful as a bioassay. Also, at that point fungal growth became heavy, probably due to potato psyllids introducing contaminants. The delay in symptom development along with fungal contamination caused this method to be discontinued.

Excised tomato cotyledon and potato psyllid whole body extract bioassay. Initial trials with the tomato cotyledon bioassay showed some marked effects from the whole potato psyllid body extract in Roma cotyledons. Initial trials showed the potato psyllid extract treatment to show a much greater weight gain than control treatments (FIG. 3.2). The difference was visually great enough in the potato psyllid extract treatment to be easily distinguishable. The cotyledons of the potato psyllid treatment appeared larger than the other treatments and looked thicker and more vigorous. Psyllid extract treated cotyledons weighed 35.8% more than controls.
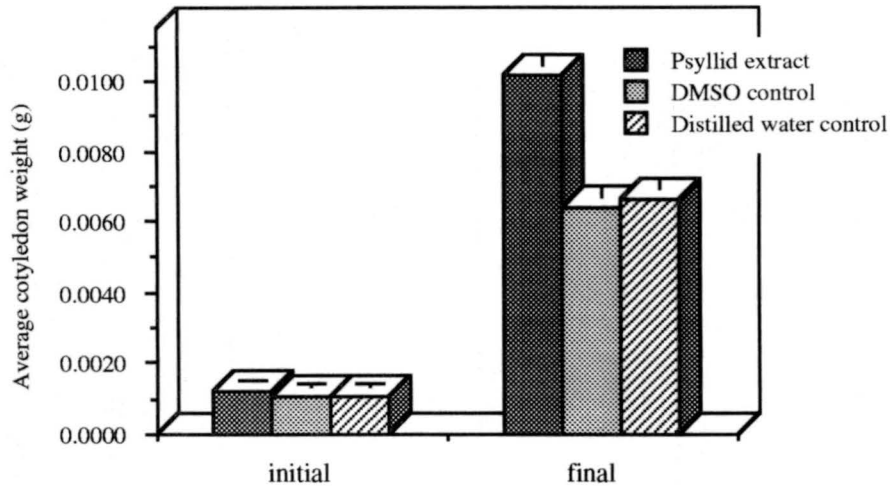
FIG. 3.2. The initial and final (6 days after treatment) weights of Roma
tomato cotyledons for a bioassay using whole body potato psyllid
extract applied in 50:50 water and DMSO. A control was made of
the same water and DMSO mixture as well as a water control.

The bioassay was repeated using Super Sioux tomato cotyledons, due to a temporary

unavailability of Roma seed. A green peach aphid extract was prepared identically to the

potato psyllid extract. Super Sioux cotyledons were not larger or did not weigh more than

control cotyledons (FIG. 3.3) as in the earlier bioassay with Roma. This may be because a

different tomato variety was used in place of Roma or because the method of preparing the

extract was slightly modified. In earlier bioassays with Roma, extract was immediately

placed on cotyledons as soon as it was made. In the Super Sioux bioassay, the extract was

made 1 1/2 to 2 hours before treatment and it is possible that some enzymes in the solution

may have inactivated the toxin. Because the results from the first trial looked promising, the

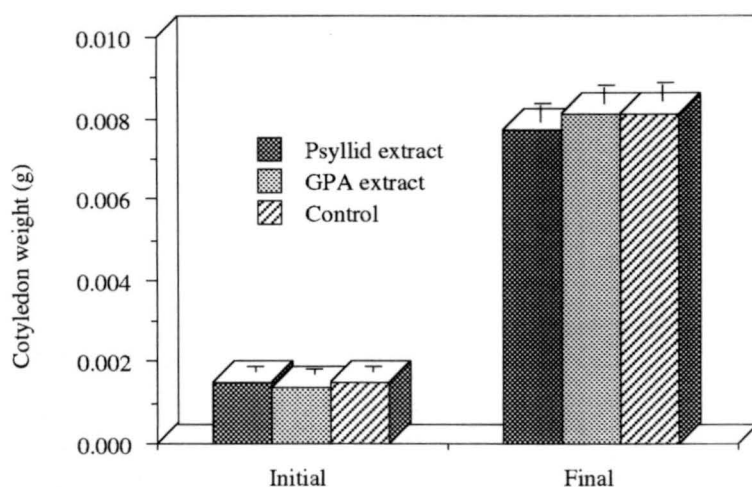bioassay was repeated with Roma cotyledons when the seed became available.

FIG. 3.3.   Tomato cotyledon bioassay using potato psyllid whole body
extract, green peach aphid extract and controls on Super Sioux
variety tomato. Error bars indicate standard error.

The bioassay was again performed using Roma seed as before including the green peach

aphid (GPA) extract prepared identically to the potato psyllid extract. The cotyledons treated

with the GPA extract had weight changes more like those of cotyledons of the controls, and

the potato psyllid extract treatment again showed a higher weight gain than the other

treatments (FIG. 3.4). After two days, the potato psyllid extract treatment cotyledons were

14.6% larger than the GPA extract treatment or controls. All treatments lost weight between

days two and five. However, potato psyllid extract treated cotyledons weighed 21.7% more

than GPA and 26.7% more than controls, though the difference was not as great as the first

successful trial (potato psyllid extract treatments weighting 35.8% more than controls).

Several trials of the bioassay were again performed using Roma seed and whole body

extract, but none showed any differences between any treatments whether potato psyllid,

GPA, DSMO:water controls or water controls. Due to the inconsistencies of this bioassay,

but also keeping earlier successes in mind, the bioassay was adjusted incorporating the same

treatments into the next phase of the bioassay using the membrane feeding solution in place
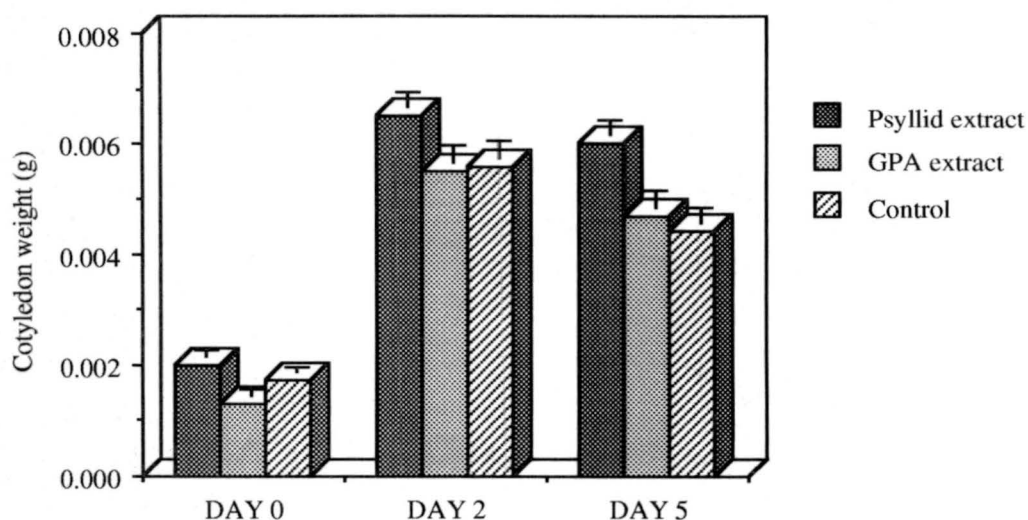
of crude body extracts.

FIG. 3.4. Tomato cotyledon weights two and five days after being treated with potato psyllid whole body extract and green peach aphid (GPA) extract prepared in 50:50 solutions of DMSO:water. The control consisted of 50:50 DMSO:water solution only. Error bars indicate from standard error.

Membrane feeding solution and the tomato cotyledon bioassay. The next treatment added was the sucrose diet obtained from membrane feeding systems after allowing potato psyllids and aphids to feed for two days. Observations were made on cotyledons about 24 hours following treatment. All treatments were greening and enlarging as previously observed with the exception of all cotyledons treated with the potato psyllid membrane feeding solution. These cotyledons were much smaller than other treatments, were laying flat and limp, and were severely yellowing. At two days, other treatments were weighed, all showing no differences in weight change between treatments, while the potato psyllid membrane feeding solution cotyledons had yellowed so severely that they disintegrated when lifted with forceps to be weighed, making any quantification of this treatment impossible.

Because of the immense difference between the potato psyllid treatment and the control treatments, it appeared that a fast and unambiguous bioassay had been developed. In later trials the same procedures were repeated several times, but the results were ambiguous and the validity of the approach is in question.

Inconsistent phytotoxicity using potato psyllids was common in the literature. Schaal (1938) was the only example of any worker achieving success with bioassays. He made macerated solutions of fourth instar nymphs in water, filtered the solution though cheesecloth and made a series of dilutions. He then injected the solution into the stems of healthy potato plants. He reported that typical symptoms only occurred with concentrated solutions (50 nymphs/10mL water). Foliar symptoms were used as an indicator of potato psyllid toxicity. Schaal said that the plants had to be under direct light for three to five days before symptoms would occur, but did not state how many days elapsed before he saw symptoms. The age of plants or the size the sample was not mentioned. Carter (1954) tried to repeat the bioassay, but was unsuccessful. He used the dilutions that Schaal used and, in addition, made more concentrated solutions. He used the injection technique, as well as fitting vials containing the solution over stumps of freshly cut petioles, another technique recommended by Schaal (1938). All of Carter's results were inconclusive.

In our study, although a bioassay was not developed, it was established that membrane feeding systems designed for other Homopterans could be successfully adapted for potato psyllids. If the salivary secretions of potato psyllids are responsible for disease symptoms in host plants, this knowledge may be useful in future investigations for the identification of the phytotoxin. This study also illustrated the difficulties that can be encountered during the development of a bioassay.

CHAPTER FIVE

TOPICAL TREATMENT OF MINITUBERS TO PROMOTE SPROUTING

Potatoes have a rest period, where even under favorable environmental conditions, the buds are unable to sprout. This period occurs in the last few weeks before harvest and after harvest (Hemberg, 1985). Many chemicals are used to break dormancy in potatoes. Due to the early sprouting of potatoes due to potato psyllid injury, the salivary secretions of potato psyllids were applied to minitubers to detect if a topical treatment could induce sprouting.

MATERIALS AND METHODS

Minitubers of the "Norkotah" potato cultivar were obtained from a commercial laboratory where they had been tested and shown to be free of viral diseases. The minitubers were approximately one cm in diameter. Many of the minitubers were beginning to sprout. Those not yet sprouting were chosen and treated with the following solutions:

1) Membrane feeding solution of 5% (w:w) sucrose collected after two days of potato psyllid feeding as described in chapter four.

2) Potato psyllid membrane feeding solution as above with 25% DMSO.

3) A 5% (w:w) sucrose solution as a control.

4) A 5% sucrose solution with 25% DMSO.

5) Potato psyllid excrement dissolved in distilled water (.2779 g/3 mL).

6) Distilled water.

The sucrose treatment was used as a check because the potato psyllid saliva was collected in sucrose. DMSO was added as a carrier in case the toxin was not able to penetrate by itself.

Excrement was used because it was thought that potato psyllids might ingest the toxin and it may accumulate in the excrement. The minitubers were dipped into a solution and continuously rotated to thoroughly cover the surface, for about two minutes each. After being dipped, the minitubers were allowed to dry on small screens and then placed in the dark in an incubator at 22°C and at high humidity. Sprouts were measured and recorded from each minituber ten days following treatment. Measurements were repeated 20 days and one month after treatment.

The experiment was repeated using "Ute Russet" minitubers. Due to a short supply of minitubers, only the first four solutions from the first trial were used for treatment. Many of these minitubers were sprouting and had measurable sprout lengths. Enough were available that were not sprouting to complete four treatments of membrane feeding solution, membrane feeding solution with 25% DMSO, 5% sucrose solution and 5% sucrose solution with 25% DMSO.

RESULTS AND DISCUSSION

The average sprout length, sum of sprout lengths, number of sprouts and length of apical sprout in Norkotah minitubers showed no statistical difference between all six treatments. The variation was extreme within each treatment. Therefore, box-and-whisker diagrams were constructed (Statview™, 1985) to show how the treatments compared to each other and the wide variation within each treatment. To plot the box-and-whisker diagrams, the median was calculated for the data from each treatment. From the lower half, the median was again calculated and also for the upper half. This divides the data into quarters (FIG. 5.1). This shows the spread of the data and how much variation is present (Weiss and Hassett, 1982). The box-and-whisker diagrams were constructed to show the average sprout lengths for all three dates when Norkotah minitubers were evaluated (FIG. 5.2, 5.3 and 5.4).
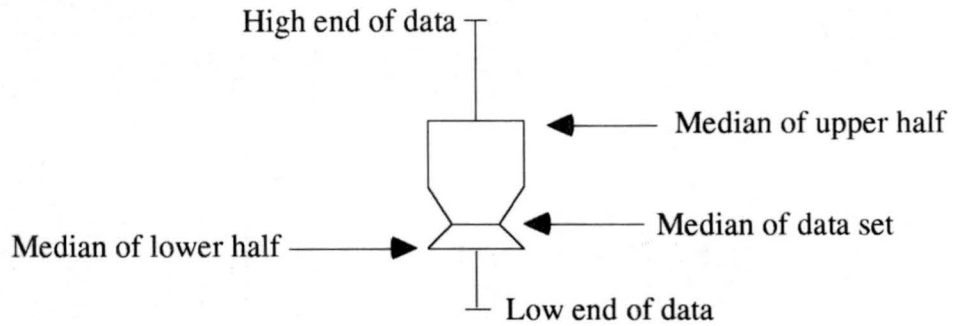
FIG. 5.1. Diagram of how box-and-whisker plots are constructed. The box
consists of the medians and the whisker lines of the high and low
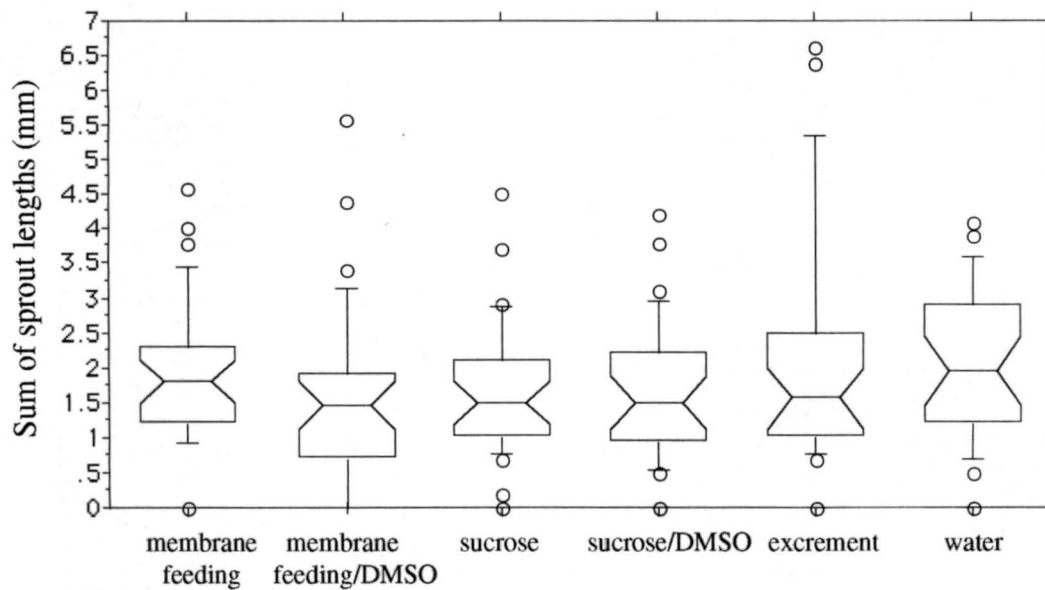ends of the data.

FIG. 5.2. Box-and whisker diagram for Norkotah minitubers topically
treated with various solutions showing the sum of sprout length.
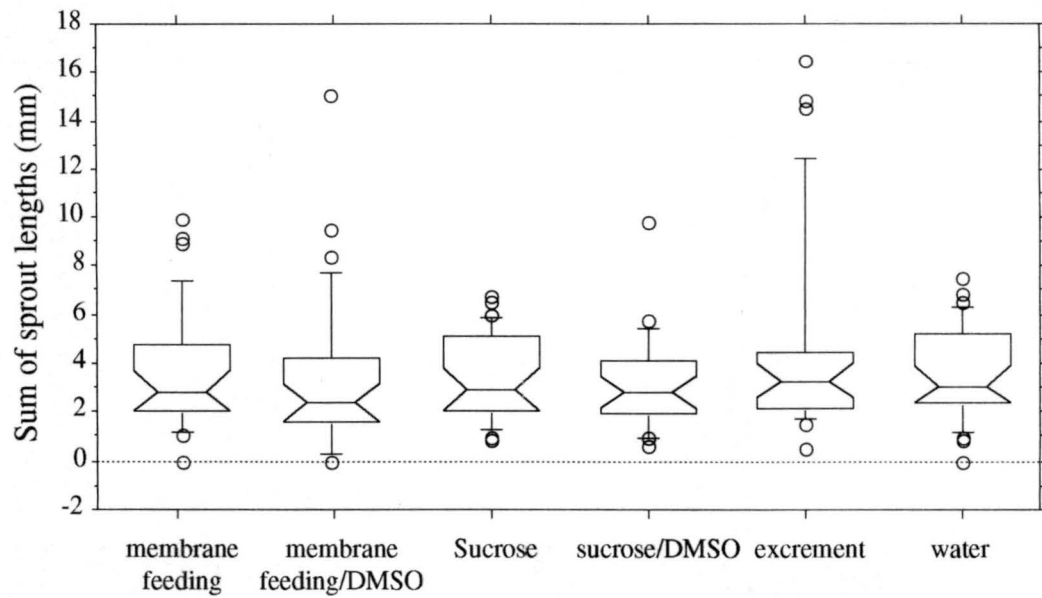Minitubers were evaluated 10 days after treatment.

FIG. 5.3.   Box-and-whisker diagram showing the sum of sprout length for
Norkotah minitubers treated with various solutions. Minitubers
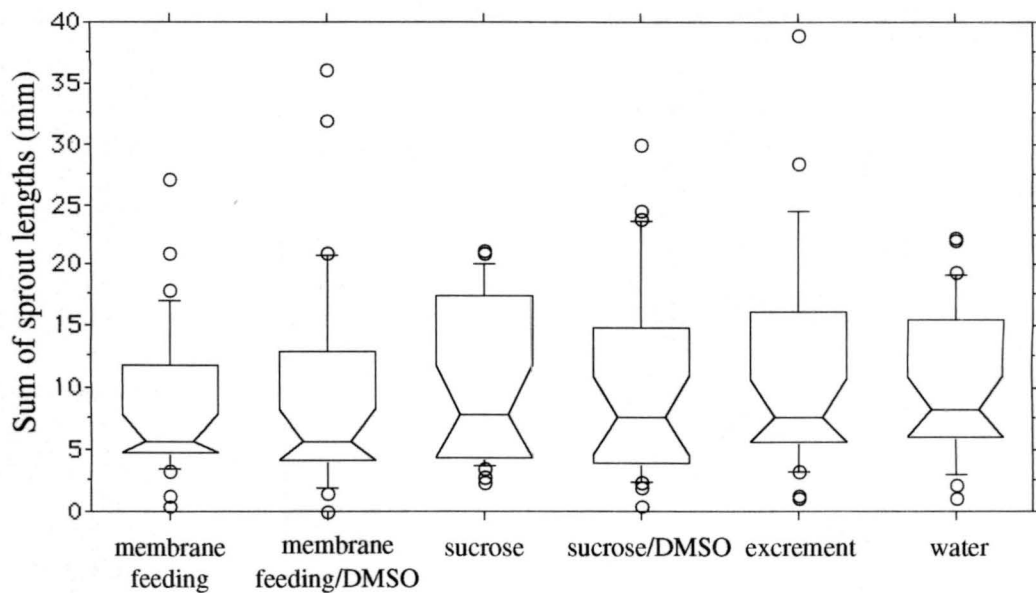were evaluated 20 days after treatment.

FIG. 5.4.   Box-and-whisker diagram for the sum of sprout length for Norkotah
minitubers treated with various solutions one month after treatment.

Variation was at its greatest within the treatments at the time of the last evaluation, one month following treatment (FIG. 5.4). With increasing age, there was more diversity in the number and size of sprouts. No trend is obvious. These methods of treatments do not show any value for inducing sprouting. Membrane feeding collection of salivary secretions may not be an effective means of collecting toxin. The toxin also may not be able to penetrate the skin of the potato. The excrement may not contain any toxin, or other products may have rendered it ineffective if it were present.

Ute Russet minitubers were extremely sensitive to DMSO, and the minitubers treated with solutions containing DMSO (sucrose and membrane feeding) showed no sprouting. Of the remaining two treatments of membrane feeding solution and sucrose, though a slight trend is seen, no statistical difference exists between treatments. Variation is very great within the treatments of Ute Russet minitubers (FIG. 5.5).
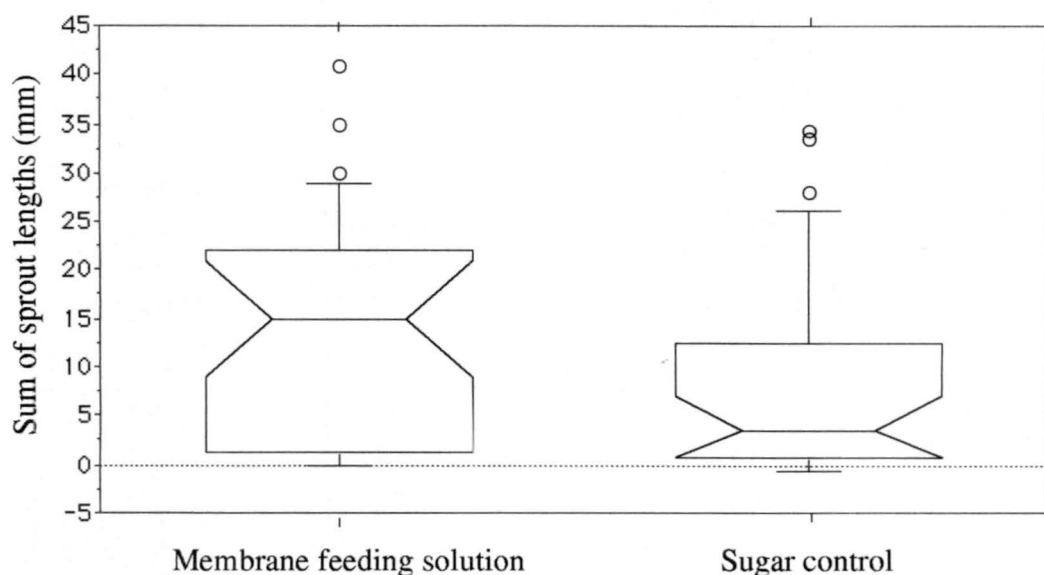

FIG. 5.5. Box-and-whisker diagram showing sum of sprout lengths for Ute Russet minitubers treated with potato psyllid membrane feeding solution in 5% sucrose. Minitubers were evaluated 2 weeks after treatment

This method for treating minitubers may be ineffectual for many reasons. The minitubers used for this study were already breaking dormancy, and this may have masked possible effects from any of the treatments. It this experiment were repeated, freshly harvested minitubers would be the best candidates for treatment. The toxin may not have been present in the treatments, may have been enzymatically deactivated or may not have been able to enter the minitubers. Another factor to consider is that the toxin may affect sprout growth by influencing the metabolism of the whole plant and may not directly affect the tuber.

SUMMARY

1. Tissue cultured potatoes reared in the greenhouse gave uniform environmental and genetic conditions to measure effects from potato psyllid injury. It was found that feeding by potato psyllids caused dramatic damage in all four cultivars tested. Varietal responses showed that early maturing cultivars had less damage than later cultivars. Russet Burbank, the latest cultivar tested, had the greatest amount of damage. Breakage of dormancy showed a different varietal response than yield loss. Cultivars that were early showed greater breakage of dormancy and later cultivars did not have as large a proportion of sprouting.

2. Eight varieties of tomatoes were evaluated in the field for potato psyllid damage. All sustained a loss in yield when infested with potato psyllids. A range of damage and level of infestation occurred. Five varieties had low levels of infestation, with low amounts of damage relative to the other varieties. Ace sustained high infestation and high damage. Jubilee had low infestation but high damage, and Roma was highly infested with low damage.

3. Pepper varieties did not respond to potato psyllid infestation in the same way potatoes and tomatoes did. California Wonder showed no difference between uninfested and infested plants Gypsy showed a slight and insignificant decrease, but two varieties showed an increase in yield from potato psyllid feeding. Of these two, Anaheim was statistically significant, while Hungarian Yellow Wax showed no statistically significant yield loss. The differences between the yields for tomatoes and peppers may be due to some physiological differences between the two. These differences may give clues as to the action of the potato psyllid toxin.

4. A membrane feeding system was developed to collect the salivary secretions of potato psyllid nymphs. Several attempts were made to develop a bioassay for the potato psyllid toxin, but no consistent technique was found.

5. Potato minitubers were topically treated with potato psyllid salivary secretions collected in membrane feeding systems and with potato psyllid excrement to attempt to promote sprouting. The treatments with potato psyllid products did not perform any differently than controls. The toxin may not have been present in the treatments, may have been degraded, or may not have been taken up by the minituber. Another possibility is that the toxin does not act directly on the minituber, but acts through the system of the whole plant.

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