OVERWINTERING BIOLOGY OF THE TARNISHED PLANT BUG, *LYGUS LINEOLARIS* (Palisot de Beauvois) (HEMIPTERA: MIRIDAE), IN NOVA SCOTIA, AND THE POTENTIAL USE OF ICE NUCLEATING ACTIVE BACTERIA FOR INSECT PEST CONTROL

by

CORY SILAS SHEFFIELD

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ABSTRACT

Many agricultural crops are susceptible to pests at the early stages of fruit development, which usually occur early in the growing season. In Nova Scotia, the tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois), an important pest of apple and strawberry, overwinters as an adult, and its emergence time in the spring corresponds with the vulnerable stages of fruit development for both crops.

One of the objectives of this study was to verify the number of generations of TPB in the Annapolis Valley of Nova Scotia, Canada, and determine which generations attack apple and strawberry. Although there are two adult summer generations (a possible third on some hosts), only the overwintered adults attack apple, and these adults and the first generation nymphs attack strawberry. Fruit quality and overall yield for both crops can be significantly reduced by TPB feeding.

The second area of research focused on the overwintering of the TPB in Nova Scotia, focusing particularly on the potential of an ice-nucleating bacterium for its control. The TPB is freeze intolerant, and "summer" adults supercool

(freeze) around -12°C, which is well below ambient conditions for this time of year. As seasonal day length decreases, TPB's go through many physiological changes, including suppressing their supercooling capacity to well below -20°C. They overwinter in a variety of areas, the most sheltered sites offering the greatest protection from winter conditions. Combined with their enhanced supercooling capacity, the TPB is able to survive the winter in temperate zones, and emerge the following spring to cause significant economic loss to many crops.

The ice-nucleating bacterium, *Pseudomonas syringae*, significantly decreased the supercooling capacity of "cold-hardy" TPB's. By increasing the susceptibility of TPB adults to milder sub-zero temperatures, winter mortality may be increased so that spring numbers are not high enough to reach damaging levels for crops. The potential of ice-nucleating bacteria for use in biological control programs, and preparation methods are discussed.

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INTRODUCTION

Very few phytophagous pest species have as wide a range of feeding and oviposition sites as the [1]tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois)(Hemiptera: Miridae). This species has been reported using at least 385 species from 55 plant families (Young, 1986), including up to 130 economically important agricultural crops such as apple, *Malus domestica* (Hammer, 1939; Parker and Hauschild, 1975; Hauschild and Parker, 1976; Boivin and Stewart, 1983a and 1983b), strawberry, *Fragaria* X *ananassa* (Handley, 1991; Handley and Pollard, 1993; Bostanian, 1994), brambles, *Rubus* spp. (Spangler *et al.*, 1993), celery, *Apium graveolens* var. *dulce* (Boivin *et al.*, 1991) alfalfa, *Medicago sativa* (Craig, 1983; Walstrom, 1983; Jensen *et al.*, 1991; Schwartz and Foottit, 1992), trefoil, *Lotus corniculatus* (Wipfli *et al.*, 1990 and 1992, Peterson *et al.*, 1992), canola, *Brassica napus* (Butts and Lamb, 1990a and 1990b; Leferink and Gerber, 1997) cotton, *Gossypium hirsutum* (Laster and Meredith, 1974; Scales and Hacskaylo, 1974; Bailey, 1982; Fleischer *et al.*, 1985 and

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[1] The common name "tarnished plant bug" is used for several "*Lygus*" bugs worldwide (see Schwartz and Foottit, 1992). The species in this work, *Lygus lineolaris*, is indigenous to the western hemisphere.

1988) as well as many weed species (Painter, 1929; Reid *et al.*, 1976; Snodgrass *et al.*, 1984). Its status as a pest is well documented (Kelton, 1975; Bostanian, 1994), and for some crops it is considered one of the main causes of damage and economic loss.

Life History

The TPB is ubiquitous, and its distribution ranges throughout North America (Schwartz and Foottit, 1992). The number of generations observed per year varies with geographic and climatic regions. In northern Alberta and Saskatchewan, the TPB is univoltine (Craig, 1983; Butts and Lamb, 1991; Schwartz and Foottit, 1992), while in the northern United States (Peterson *et al.*, 1992), southern Canada (Guppy, 1958; Gerber and Wise, 1995) and the eastern provinces (Brittain and Saunders, 1917), two generations are common, with a possible third in some areas with late season hosts such as alfalfa, ragweed (*Ambrosia* spp.), golden rod (*Solidago* spp.), and asters (*Aster* spp.) (Ridgway and Gyrisco, 1960; Stewart and Khoury, 1976; Bostanian, 1994). The TPB is active year round in the southern United States, with four or more generations per year (Strong *et al.*, 1970; Day, 1987).

Generation times vary throughout the season; in Quebec, the first summer generation requires 26-47 days, the second 20-25 days, and the third 25-30 days, to go from egg hatch to adult respectively (Bostanian, 1994).

The life cycle of the TPB includes three stages; the egg, five nymphal instars, and the adult. The elongate eggs (0.85 mm - 1.06 mm) are laid within the soft stem and petiole tissues of herbaceous plants, hatching time being faster at higher temperatures; temperatures above 35°C are detrimental (Ridgway and Gyrisco, 1960; Slaymaker and Tugwell, 1982). In Quebec, oviposition (for the overwintered females) takes place from the first week of May to the third week of June (approximately 50 days), when temperatures are above 16°C. Bostanian (1994) reports that female TPB's can lay 0-3 eggs per day, which require up to 18 days of incubation.

The first three nymphal instars range in length from 0.85 mm to 2.2 mm, are pale yellow-green and look somewhat like aphids. The forth and fifth instars are 2.1 mm and 4.2 mm respectively, and are distinguishable by the well developed wing pads and dark green colour (Bostanian, 1994).

The adults vary in colour from light brown to dark green or black, and have characteristic markings on the wings and

body (Schwartz and Foottit, 1992). Adults range in size from 4.9 mm to 6.0 mm, females being slightly larger than males (Bostanian, 1994).

Nature of Feeding Injury and Plant Damage

The TPB is a "direct pest" in that it feeds directly on the developing fruit (or fruit forming structures) of the plants. Like all "true bugs" (Order Hemiptera), the TPB is equipped with piercing-sucking mouthparts. For *Lygus* bugs (Family Miridae, or "plant bugs"), succulent green and reproductive plant tissues, especially developing and fully ripened fruit, are the primary feeding and oviposition sites (Crosby and Leonard, 1914; Tingey and Pillemer, 1977; Pedigo, 1989). The feeding mechanism involves piercing and probing the plant tissue with stylet-like mouthparts to obtain fluids. According to Strong (1970) and Tingey and Pillemer (1977), *Lygus* feeding can cause several types of damage in plants. These include complete abscission of the fruiting body, the production of shrivelled seeds or seeds without embryos, fruit deformity ("catfacing" and "apical seediness"), tissue necrosis around the feeding site, and reduction of vegetative growth. The damage associated with feeding is due to physical injury

by the mouthparts at the site of tissue penetration (see Handley, 1991; Handley and Pollard, 1993). The saliva, which contains digestive enzymes and other compounds, can also contribute to tissue damage (Handley, 1991 and references cited therein; Madhusudhan *et al.*, 1994) and plant [2]disease (Nault, 1997). Damage as such has a potential to result in significant economic loss, particularly in crops where the fruit or seeds are harvested.

Some pest species cause economic damage at only one stage in their development. For example, many Lepidoptera are considered agricultural pests as larvae (caterpillars), which are the primary feeding and growing stages for these insects. Therefore, it is only these stages which are of concern in crop production. The TPB is a threat during both the adult and nymphal stages, and both are found and monitored at the same time on many crops. In fact, it is the nymphs which are considered the most serious problem in strawberry production (Bostanian, 1994).

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The tarnished plant bug and many other sap feeders may be important vectors in the transmission of many plant pathogens.

Tarnished Plant Bug Overwintering Biology

The TPB, unlike most Miridae (Cobben, 1968), overwinters as a diapausing adult in and beneath leaf litter or other dead vegetation (Cleveland, 1982), under rocks or bark, in between blades of grass; usually in or near areas of their autumn host (Khattat and Stewart, 1980). Craig (1983) suggests that in Saskatchewan, all of the second summer generation adults, and a portion of the first summer generation adults in reproductive diapause (0-20% of population), overwinter.

Overwinter mortality for the TPB is very high and even under favourable conditions winter survivorship is usually 40-60% (Beirne, 1972), but 80-90% mortality is not uncommon in some sites (Painter, 1929). Fye (1982) found that moisture levels within *Lygus* overwintering sites may be a critical factor affecting winter mortality, especially during very dry winters. He also suggests that overwintering sites consisting of large overlapping leaves provide the most suitable type of cover.

High overwinter mortality is characteristic of insects which are typically described as "freeze intolerant" (Block, 1991 and 1995). Freeze intolerance means that these insects

must [3]prevent the formation and growth of ice crystals within their bodies. There are several methods by which this is accomplished, including the production of antifreeze or cryoprotectant compounds within the body, ridding the body of or masking the activity of potential ice nucleating compounds, and overwintering in sites which do not reach intolerable temperatures (Danks, 1978, Leather *et al.*, 1993). Little work has been done on the TPB in terms of its cold-hardiness, overwinter biology and physiology, and factors which determine and contribute to overwinter survival and mortality have not been studied in any detail.

In early spring, TPB's emerge from their overwintering sites and migrate to areas which support plants exhibiting early season growth. Such sites, which consist primarily of weed species, are very important in terms of supporting TPB populations as they develop. Fye (1980) reports that weeds can support and maintain TPB populations during times when cultivated crops are not favourable or available to them. Migration of gravid females (Stewart and Gaylor, 1991) from these areas into crops is one of the main causes of economic

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[3] Cold related damage at temperatures above the supercooling or freezing point can occur, and will be discussed later.

loss. In Nova Scotia, this time corresponds with the pre-bloom and bloom stages of apple, as well as the bloom and fruit development stages of strawberry and other crops. Despite the relatively high winter mortality, a sufficient number of adults survive to cause damage; this is especially true for strawberry. Its bloom and fruiting period overlap the egg-laying and developmental period for first summer generation TPBs. Although the number of emerged overwintered adults is low, the fecundity of the TPB is reported to approach 300 viable eggs per female (Gerber, 1995). Rapid growth of a population, if not suppressed, can lead to extensive crop damage and loss of revenue.

Need for Control

Most present day pest management practices involve the use of timed insecticide applications combined with the encouragement of natural biological control. Integrated pest management (IPM), a program for pest control which attempts to use all available means to effectively and safely manage pest populations (Crozier, 1993a), works using concepts such as economic injury levels (EIL), which can be defined as the lowest number of insects (pests) per unit area required to

cause economic damage to a crop, and economic threshold levels (ETL's; also called the action threshold or AT), which is the number of insects present per unit area at which management action should be taken to prevent economic loss (Pedigo, 1989; Crozier, 1993a). EIL's and ETL's vary from species to species and crop to crop, depending on such factors as type of injury (direct versus indirect crop damage), and plant (or cultivar) resistance to different species.

EIL's and ETL's have been established for the TPB on both apple (Boivin *et al.*, 1982; Bostanian and Coulombe, 1986) for Quebec, and strawberry in Quebec (Mailloux and Bostanian, 1988 and 1989; Bostanian, 1994) Ontario (Cermak and Walker, 1992) and Nova Scotia (Crozier, 1993b). Careful monitoring of pest densities by various means (depending on the species) allows growers to determine need for insecticide sprays. This has proven to be a more environmentally sound practice, and more economical (Crozier, 1993a).

Damage to crops has several contributing factors. Minor components include soil type, temperature and precipitation levels throughout the growing season, fertilizer levels, the cultivar or variety, and the abundance of natural pest control agents (predators and parasites). The main components of

damage, like that caused by the TPB, are the result of **1)** the abundance of the pest, and **2)** the stages of fruit development which are susceptible to the pests feeding (Bale, 1991). For apple (Prokopy and Hubbell, 1981; Boivin and Stewart, 1982 and 1983; Michaud *et al.*, 1989) and June bearing strawberry production, overwintered TPB adults and their progeny are the only stages of the active season generations which are considered to cause damage. These crops, like many others (Bale, 1991), are most susceptible to this type of damage early in the growing season. The pest population levels at these times are mainly determined by the initial colony size, i.e., the overwintered adults, which in turn is related to the magnitude of the overwintering generation which survived (Bale, 1991).

The role that successful overwintering plays in determining the population dynamics of a species the following season has received little attention (Bale, 1991), although monitoring the overwintering stages prior to or during the winter has proved very effective for predicting early season numbers for several species (Bale, 1991; Leather *et al.*, 1993, and references therein; Smith and Borden, 1990).

A method which could increase winter mortality for the

TPB (and other pests) would contribute to keeping spring population levels below ETL's for crops and their respective pests, thereby decreasing the number of insecticide treatments needed for control. To resolve such a method, extensive study must be conducted on the biology of the species, focusing particularly on the species overwintering strategies and susceptibilities. It is not only important to consider the species' cold-hardiness in terms of supercooling points (SCPs), antifreeze and cryoprotectant synthesis, and triggers for the synthesis of such compounds. For this type of control to be effective, one must also look at the insects overwinter ecology, and consider in detail the characteristics of the actual overwintering sites. Temperatures (and other environmental factors) within these sites, which can be significantly different than those of ambient (Danks, 1978; Leather *et al.*, 1993), are the actual conditions the insects are exposed to throughout the winter. These should be considered for studies looking at overwintering, and also in models which predict spring emergence based on temperature and degree-day accumulations. Other causes of naturally occuring winter mortality should also be investigated.

The objectives of this study are:

a) to determine the number of generations of TPB occuring in the Annapolis Valley area of Nova Scotia,

b) to determine which stages of apple development are most susceptible to TPB feeding,

c) to confirm the relationship between TPB feeding density, and strawberry fruit quality,

d) resolving token stimuli which initiate overwinter preparation,

e) to determine the extent of TPB cold hardening capacity throughout the year through the use of supercooling point determination, and

f) to investigate the potential use of an ice-nucleating-active bacterium for biological control of the TPB in Nova Scotia. A part of this objective will involve developing preparation methods for the bacterium, and determining how to achieve the simplest and most effective control potential.

MATERIALS AND METHODS

Seasonal Population Dynamics of the TPB

1) *Seasonal abundance in alfalfa*

The dynamics of a population of TPB's was followed throughout the growing seasons of [4]1994 through 1996 in an un-mowed alfalfa field (150 m x 50 m) at the Sheffield Farm, Atlantic Food and Horticulture Research Centre (Agriculture and Agri-Food Canada) sub-station, Kings County, Nova Scotia. Alfalfa was chosen because it exhibits vegetative growth from early spring until mid-October, which makes it attractive to the TPB throughout most of the growing season (Gerber and Wise, 1995). The sampling method used was a wide, 180° pendulum sweep with a net (diameter of 38 cm) through the vegetation (Hutchins, 1994) while walking a "U-shaped" pattern throughout the field (after Wipfli *et al.*, 1992). Sampling commenced in April and continued into November; samples being taken approximately once a week, and under similar environmental conditions (sunny and dry). The number of adults and nymphs caught in each of the 20 sweeps per sample

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[4] The data from 1994 were collected in the first half of the season from an alfalfa field at Agriculture and Agri-Food Canada Research Station in Kentville. Because of an unforseen mowing incident, the site at the Sheffield Farm was used for the remainder of this study.

date was recorded (Figures 5a - 5c).

2) *Migration of adult TPB*

To account for the migratory nature of the TPB, which may cause fluctuations in the population dynamics in one area, other sites around the alfalfa field were examined before and during their bloom periods. The alfalfa field used was bordered on the east and west by pear, *Pyrus communis*, orchards, and to the south by meadows containing primarily golden rod, *Solidago* spp. (in bloom in late summer) and other weeds. A plot of canola was grown in late summer of 1995 within the alfalfa field, and potatoes, *Solanum tuberosum*, were planted in 1996 north of the alfalfa field, and were in flower from July until August.

3) *Predator population dynamics*

During the alfalfa growing season of 1996, the number of important TPB predators caught in the TPB population sweeps was also recorded. These predators included minute pirate bugs, *Orius* spp. (Hemiptera: Anthocoridae), damsel bugs, *Nabis* spp. (Hemiptera: Nabidae), and the two-spotted stink bug, *Perillus bioculatus* (Hemiptera: Pentatomidae). The first two are considered the most prominent TPB nymph predators within

alfalfa fields in Nova Scotia. There are many other generalist predators in alfalfa which were not included, such as lady bird beetles (Coleoptera: Coccinellidae), green lacewings, *Chrysoperla* and *Chrysopa* spp. (Neuroptera: Chrysopidae) and many species of spiders (Araneida). Wasp parasitoids of the TPB were also not included in the counts.

Susceptible Stages of Apple Development

In 1994, "Cortland" and "McIntosh" apple varieties within the Latin Square orchard block at the Atlantic Food and Horticulture Research Centre, Kentville, Nova Scotia, were used to determine which stages of apple fruit development were most susceptible to TPB feeding. Forty nylon sleeve cages (30 cm x 60 cm) were placed over individual branches (Figure 1) containing ten blossom clusters at the "green-tip" stage of development. The cages were left on the branches until harvest, with the exception of one week (May 30 - June 6), during which time they were removed to allow for natural pollination. During this time, the orchard was monitored for TPB activity using a one minute observation period of randomly selected branches throughout the orchard.

One of two "treatments" were tested for impact on fruit

quality. Eight adult TPB were put in each of four treatment cages for both apple varieties and confined for a one week interval starting at the "pink bud" stage of fruit development. Two blossom clusters were removed from both the treatment and control cages after the one week period, and each blossom and calyx was inspected for damage (after fruit set, individual apples were removed after the feeding episode and inspected). The remaining blossom clusters and/or developing apples were left until harvest. This protocol was repeated four times, with a three week period between each feeding episode.

At harvest, individual apple weight was measured, and visual damage was recorded. The proportion of damaged apples in the treatment cages at each of the feeding episodes were compared.

Strawberry Damage at Different TPB Densities

"Kent" variety strawberry plants were planted in mid-July, 1996 as dormant crowns in 12.5 cm standard pots, and were grown under ambient temperature and photoperiod conditions within a greenhouse at the Atlantic Food and Horticulture Research Centre. The secondary bloom was used for these

experiments. At anthesis, the flowers were hand pollinated using a camel-hair brush, with several visits per flower throughout the bloom period to ensure full pollination. A number of blossoms were left untouched, and used as non-pollinated controls.

The TPB's used in this experiment were caught in the field and deprived of food for 24 hours. At the time of achene separation, TPB's were released into plastic "deli-type" containers which were fitted over the developing fruit (Figure 2). The containers were supported with plastic sticks braced in the soil, and had holes punched in the top for ventilation. The bottom was cut out to fit the blossom into the container, and the opening was sealed with Parafilm™ "M" laboratory film (American National Can) to keep bugs in, and prevent the blossom stems from being damaged by the rough edge of the container. TPB's were placed in the containers at the following randomly allocated feeding density treatments: 0 (pollinated control), 2, 4, and 6 adults per berry; and were left to feed for 48 hours (after Handley, 1991). Following this period, both the TPB's and the containers were removed and the berries were left to develop.

Fully ripened fruit were examined for damage and

photographed. Mean berry weight and standard error (S.E.) was determined for each treatment. A ranking system was developed to categorize the observed damage. A grade of "0" was given to perfectly shaped berries; "1" described berries with slight apical seediness or buttoning; "2", berries with apical seediness, and slight deformity or irregularities in shape; "3", berries with apical seediness and deformity; and "4" and "5", berries which were very deformed as a result of TPB feeding (the grades "4" and "5" were distinguished based on the severity of the deformation).

Overwinter Physiology

To determine token stimuli for "cold-hardening", first summer generation adult TPB's ("summer" adults) were caught in the field from mid- to late-July (1995 and 1996), during their reproductive season (Gerber and Wise, 1995), and placed in one of four environmental cabinets; two at 20°C and two at 10°C; each treatment having a cabinet with a light:dark photoperiod regime of 18h:6h (long day length) and 12h:12h (short day length) respectively. The insects were reared on etiolated potato tubers (after Slaymaker and Tugwell, 1982) and green and/or yellow beans, which were placed in a bed of

moist sand within a 18.4 L bucket which had holes cut into the top and sides (for air circulation) that were covered with nylon screen. The acclimation period under these conditions was approximately four weeks. During this time, potato tubers and beans were replaced as needed, and any eggs laid (or nymphs present) during, or at the end of, the acclimation period were noted.

The supercooling point (SCP) was used as a measure of cold-hardiness. After the acclimation period, live insects were attached, with petroleum jelly, to a thermocouple within a Nalgene™ 2.0 mL cryovial, and submerged in an FTS-Systems ultra-low temperature bath (Stone Ridge, NY) interfaced with a MacIIci computer using virtual instrumentation (VI) Lab View 3 (National Instruments, Austin, TX) software and hardware (Nubuss port and A/D boards). From an initial temperature of 15°C, the temperature was dropped at a constant rate of 1°C /min (after Salt, 1966; Panneton *et al.*, 1995), and SCP's of the TPB were measured to the nearest 0.1°C. The SCP was the lowest temperature recorded prior to the release of latent heat energy (exotherm) (after Lee, 1991).

The experiment was repeated again in mid-September to early October, when the second summer generation of adult

insects ("autumn" adults) were suspected of being in reproductive diapause (Gerber and Wise, 1995).

Using the SCP of the individual bugs as a measure of "cold-hardiness", the proportion of "summer" and "autumn" adult TPB's which had enhanced supercooling capacity after acclimatization were compared. A SCP value of -14°C was used as a cut-off; TPB's with SCP's above this temperature did not have enhanced supercooling capacity, and were considered "not cold-hardy"; those with enhanced supercooling capacity were considered "cold-hardy".

Seasonal Supercooling Profile

To determine seasonal changes in "cold hardiness" for the TPB, adults were caught throughout the active season, and supercooling points were determined using the previously mentioned technique. These data were plotted with daily minimum temperatures from 1995 (obtained from Agriculture and Agri-Food Canada weather station) to determine TPB susceptibility to cold-temperatures throughout the year.

Preparation of *Pseudomonas syringae* Spray

Slant cultures of *Pseudomonas syringae* van Hall, culture SRSFB-45 (DEK-1), a known ice-nucleating active (INA+)

bacterium, grown on nutrient agar containing 2.5% glycerol, were obtained from [5]Dr. Claude Richard and Dr. Luc Couture (Agriculture and Agri-Food Canada, Sainte Foy, Quebec). Upon arrival, the cultures were held in a refrigerator at 5°C. Plates and more slants were prepared from these cultures, using nutrient agar containing 2.5% glycerol. All cultures were grown at 20°C until sufficient growth was observed, and were then stored in a cold room. This was repeated every month to keep cultures fresh.

Two methods of bacterial spray preparation were compared. For the first spray preparation, *P. syringae* was grown in nutrient broth. A "loopful" of bacteria was removed from a culture plate and placed in an erlenmyer flask containing 70 mL of nutrient broth with 2.5% glycerol, and set on a shaker in an environmental cabinet at 20°C. Absorbance measurements were taken hourly, and a growth curve was created for this bacterium (Figure 3). Cultures were then grown using this preparation method, and during the time of linear growth (as determined by absorbance measurements from the growth curve), the flasks were removed and the contents centrifuged at 5000-

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[5] Dr. Claude Richard provided original cultures (1995) used for growth curve determination and preliminary data. Dr. Luc Couture provided addition cultures (1997).

6000 rpm. The pellets of bacteria were then re-suspended in E-Pure water, and absorbance measurements were taken for this and diluted solutions of the stock. The Acridine Orange Direct Count method (Peele and Colwell, 1981) was used to enumerate cells from the *P. syringae* solutions of known optical density. A curve of cell number/volume verses absorbance (600 nm) was plotted, and an equation for predicting cell number from absorbance measurements was calculated (Figure 4a).

The second method of spray preparation was done using *P. syringae* cultured on nutrient agar containing 2.5% glycerol for 3 days at 20°C. Cells were removed from the plates by rubbing a sterile cotton swab through their growth, and then suspended in distilled E-pure water by swirling the swab in the water, until the solution became cloudy. Absorbance measurements ([6]550 nm) were made for this solution and for the dilution series made from the stock. The Acridine Orange Direct Count method was used as before to develop a graph and equation to predict cell number/volume from absorbance (Figure 4b).

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[6] Absorbance at 550 nm was used after a discussion with Dr. Marcia Lee, Department of Microbiology, Miami University, Oxford, OH.

Using agar grown bacterial solutions of known concentrations, supercooling points were determined by placing 12.5 μL of solution into 1.5 mL microcentrifuge tubes (equipped with thermocouples) weighted on one end to keep the liquid in contact with the thermocouple. Mean supercooling points (±S.E.) were determined for all concentrations

The Effect of *P. syringae* Spray on SCP

To determine the potential of *P. syringae* to reduce TPB cold-hardiness (through reduction of supercooling capacity), adult TPB's were collected in mid-October, 1996, when they were suspected of being in reproductive diapause, and were reared on etiolated potato tubers and /or yellow beans at 10°C with no lights for at least four weeks to induce or maintain diapause.

TPB's were removed from the containers and placed in petri dishes, and then misted with sterile E-pure water (control) or *P. syringae* (10^9 bacteria /mL water, prepared in nutrient broth as mentioned before). Supercooling points were determined using the above methods and instrumentation after intervals 1, 3, and 7 days.

RESULTS

The Number of TPB Generations in Nova Scotia

In Nova Scotia, the TPB has three separate (though slightly overlapping) adult generations per year; the first being the overwintered adults from the previous year, the second having a peak in abundance occurring from mid- to late July, and the third in late August-September (Figure 5a to 5c).

Adults were first observed on alfalfa in late April, but were observed and caught on other plants (*Epigea repens* within an experimental blueberry plot) as early as April 1st. These overwintered adults were primarily brown, and their body markings were dull, almost yellow in colour and not easily distinguished.

The number of overwintered adults observed in the field at their peak of abundance was low (<1 per sweep) for 1994 and 1995 in comparison to the first and second summer generations. Alfalfa at this time was just beginning the vegetative stages of growth. High numbers of TPB's were found concentrated on the heads of dandelion (*Taraxacum*) flowers (sometimes five per flower) within the field, and in the adjacent orchards.

Adults of the first and second summer generation were easily distinguished from the overwintered adults. They were dark green to almost black in colour, and the body markings (which were white) were very distinguishable. As the season progressed, adults of a generation appeared to change colour, becoming lighter and browner.

The number of emerged overwintered adults caught at the peak of abundance in the spring of 1996 was significantly less than those observed prior to winter in 1995 (Figure 5b and 5c). This trend was also observed for the winter of 1994 and spring of 1995 (Figure 5a and 5b).

Population data collected in 1996 showed that TPB numbers within the alfalfa field never reached the mid-season levels observed in 1995, and remained low (<1 per sweep) throughout the year. The quality of the alfalfa plants in 1996 was very poor; many plants covering large areas within the field showed vegetative growth only, and most were unhealthy. These areas had significantly fewer adults (Two sample T-Test, T=4.09, DF=2 $p<0.01$) and [7]nymphs than areas with healthy alfalfa (Figure 6).

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[7] No analysis could be done on the nymphs, as none were found in the field in poor quality areas. This is therefore assumed to be significant.

Two overlapping generations of nymphs were also observed within the alfalfa field. Nymphs were sampled in alfalfa by early June, but were observed in strawberry fields as early as late May. During mid-summer, nymphs at all instars were observed concurrently within the field.

Predator numbers within the alfalfa field were usually very low compared to the TPB. Of the three species studied, the damsel bugs appeared to be most common (Figure 5c).

Alternative Hosts

Many other plant species served as hosts for TPB populations. TPB's were not detected in notable numbers prior to the bloom period of these plants, but were found in high numbers during bloom.

The floor of the pear orchards surrounding the alfalfa field had high numbers of TPB adults early in the spring, primarily on dandelion. Very few adults were observed on the pear blossoms.

The sites containing *Solidago* spp., *Aster* spp., *Brassica* and other mustards were important hosts for late season adult TPB's, as they were found in very high numbers. These sites yielded high numbers of adults for collection purposes (i.e.,

supercooling point determinations), but very few nymphs. The canola plot (1995) also had many adults within it throughout its short bloom period.

A third summer generation may occur on ragweed, as early instar nymphs were observed as late as September in areas with many plants.

Susceptible Stages of Apple Development

During fruit development, TPB feeding damage was observed at significant levels from the pink tip stage of development to the time the apples were approximately one centimetre in diameter for "McIntosh" (Log-likelihood Ratio, G= 9.82 and 6.08 for stages 1 and 2 respectively, DF=1, $p<0.025$) and "Cortland" (Log-likelihood Ratio, G= 7.54 and 5.15 for stages 1 and 2 respectively, DF=1, $p<0.025$) apples (Table 1a and 1b). Damage at the end of the 1 week feeding intervals appeared as puncture marks on the calyx or the developing fruit (depending on the stage), often with exuded plant fluids surrounding the damaged area (Figures 7, 8, and 9).

At harvest, significant amounts of punctures were found only in stages 4 and 5 for "McIntosh" apples (Log-likelihood Ratio, G= 4.03 and 4.50 respectively, DF=1, $p<0.05$) (Table 2a

and 2b). Puncture marks were the only signs of damage observed at harvest, although in some cases, the number of punctures per apple varied (as high as 10), as did the surface area over which the damage covered.

Weights of treatment and control apples did not differ significantly for "McIntosh" (Non-parametric multiple comparison, $Q<3.317$, $DF=11$, $p>0.05$) or "Cortland" (Non-parametric multiple comparison, $Q<3.317$, $DF=11$, $p>0.05$) varieties (Figure 11a and 11b respectively). Uncaged "McIntosh" control weights did not differ significantly from caged controls, but did differ significantly from the stage 2, 3, and 4 caged treatments (Non-parametric multiple comparison, $Q>3.317$, $DF=11$, $p<0.05$). Uncaged "Cortland" apples weighed significantly more than all caged apples, with the exceptions of the stage 2 and 5 treatment cages (Non-parametric multiple comparison, $Q\geq3.567$, $DF=11$, $p<0.05$).

Mature fruit number (as compared to the initial blossom count) was very low, and many fruit were aborted immaturely. Fruit at various stages of development and sizes were often found at the bottom of cages, and in some cases, no mature fruit were harvested.

TPB Impact on Strawberry

TPB feeding at all three of the densities tested significantly reduced the weight of berries from that of the pollinated control, but no difference was detected between the three feeding densities and the non-pollinated control (Tukey, $F=17.54$, $DF=100$, $p<0.001$; square root transformation of data) (Figure 12).

Fruit quality was also affected with the introduction of TPB (Figure 13). As with weights, fruit receiving 2, 4, and 6 TPB showed no significant difference in grade among treatments, but all were found to differ significantly in quality from the pollinated control (Nonparametric multiple comparison with adjustments for tied ranks and unequal sample size, $Q=4.41$ for 2 TPB, $Q=4.974$ for 4 TPB, and $Q=6.483$ for 6 TPB respectively, $DF=4$, $p<0.001$). Berries with various grades are shown in Figures 14a - 14e.

Grading of the non-pollinated controls was not done as these berries did not show the symptoms of reduced fruit quality in the order used in the grading scheme. For example, although some of these fruit were deformed, apical seediness was not the first symptom of injury.

Achenes size was also examined between the pollinated

control (Figure 15), the TPB damaged berries (Figure 16a), and the non-pollinated control (Figure 16b). The achenes on poorly pollinated berries were not of uniform size, while those on well pollinated and TPB damaged berries were of uniform size, even in areas of apical seediness.

TPB Overwintering Strategies

"Summer" TPB adults held at high temperature and long day length had SCP's of $\bar{x}$= -12.3 ± 0.9°C (n= 40, range -23.2°C to -5.8°C), which were significantly higher than those acclimated at high temperature with short day length; $\bar{x}$= -18.2 ± 0.7°C (n=49, range -24.3°C to -7.0°C). "Summer" TPB's reared at 10°C with long day length had SCP's similar to adults acclimated at the same temperature with short day length; $\bar{x}$= -16.5 ± 0.9°C (n=50, range -24.7°C to -5.8°C), $\bar{x}$= -17.9 ± 1.0°C (n=40, range -27.1°C to -6.3°C) respectively, and did not differ significantly from those acclimated at high temperature and short day length (Figure 17) (Tukey, F=15.19, DF=352, p <0.001).

Adults caught in the autumn and acclimated at high temperature with short day length and low temperature with long day length, had SCP's $\bar{x}$= -20.3 ± 0.7°C (n=46, range -

24.1°C to -7.0°C), $\bar{x}$= -20.4 ± 0.9°C (n=41, range -26.5°C to -6.0°C) respectively, which were not significantly different from those acclimated at low temperature with short day length, $\bar{x}$= -22.5 ± 0.5°C (n=45, range -27.1°C to -10.6°C). "Autumn" adults acclimated at high temperature with long daylength, $\bar{x}$=-19.0 ± 0.5°C (n=42, range -22.9°C to -11.4°C), did differ significantly from those acclimated at low temperature and short day length. All "autumn" adult SCP's were significantly lower than summer adults acclimated at high temperature with long day length, and those acclimated at low temperature with short day length were significantly lower than all "summer" adults.

These same trends were observed for the proportion of "summer" and "autumn" TPB's from the acclimation cabinets which had enhanced supercooling capacity (Figure 18a). Less than half of the "summer" adults acclimated at high temperature and long day length were cold-hardy, which was significantly less than "summer" adults from the other three cabinets, of which over 60% were cold-hardy (Chi-square analysis, χ^2=15.41, DF=1, $p<0.001$). Almost all of the "autumn" adults had enhanced supercooling capacity (more than 80% of population in each acclimation cabinet), and were not

significantly different from one another (Figure 18b).

With the "summer" adults, nymphs were observed in the buckets reared at high temperature, regardless of photoperiod, indicating that these adults laid eggs. No nymphs were found in buckets containing "summer" adults at low temperature, or in any containing "autumn" adults.

Seasonal Influence on SCP

SCP's for adults during all three generations are reported (Figure 19). Emerged overwintered adults and 1st summer adults showed no difference in SCP's, while late summer adults had significantly lower SCP's.

SCP values throughout the year were significantly lower than ambient temperatures except during a few incidences in the winter.

Agar as Growth Media

The SCP's of various concentrations of bacterial sprays, prepared on nutrient agar containing 2.5% glycerol, are given in Figure 20. Concentrations between 70,000 and 5.3 billion cells per mL ("D" to "O" on Figure 20) had SCP's significantly higher than water (Tukey, 67.96, DF=89, $p<0.001$). The largest increase in SCP was at concentrations above 300,000 cells per

mL ("H" to "O" on Figure 20).

***Pseudomonas syringae* Effect on SCP**

Supercooling points of adult TPB's sprayed with *P. syringae* grown in nutrient broth (Figure 21) were significantly higher than those sprayed with water, on all of the days tested (Non-parametric two-factor Analysis of Variance, $H=25.11$, $DF=1$, $p<0.001$), and the spray was equally effective on all days tested ($H=0.0483$, $DF=2$, $p>0.05$).

DISCUSSION

TPB Life History in Nova Scotia

The tarnished plant bug is a true opportunist. Throughout the growing season, it switches its food resources as host plants become unsuitable. Many studies report the highly motile adults of this species (Snodgrass *et al.*, 1984; Gerber and Wise, 1995) migrating into areas as the plants in these locations enter their reproductive stages (Stewart and Khoury, 1976; Khattat and Stewart, 1980; Cleveland, 1982; Snodgrass *et al.*, 1984; Womack and Schuster, 1987; Fleisher and Gaylor, 1987 and 1988; Bostanian, 1994). Their migratory nature was observed in this study as well. In 1996, very few TPB's were found in the unhealthy section of the alfalfa field, while the more recently planted "healthy" section had significantly more adults and nymphs. The old area had very poor vegetative growth, and no reproductive growth was visible on these plants. It is likely that adults left this section of the field and populated the higher quality area, or, because TPB numbers were low in the field in 1996, migrated to other areas.

TPB's leave overwintering sites in the spring soon after

snow melt (Khattat and Stewart, 1980), at temperatures as low as 8°C (Bostanian, 1994), and seek areas with weed and wild plant species, especially those with early season succulent growth. The saps in young foliage, reproductive meristems, and floral and fruit structures are ideal as food (Soroka, 1997), as these contain many of the nutrients required by phytophagous species (Friend, 1953). Females begin to develop eggs shortly after leaving the overwintering sites, and by May, 90% of the females are ready to lay eggs (Painter, 1929). The presence of nymphs within the alfalfa field by mid-May supports these findings. These wild sites serve as important early season host for population development (Fye, 1980; Khattat and Stewart, 1980; Womack and Schuster, 1987; Fleisher and Gaylor, 1987). The TPB will migrate into other, more favourable areas, such as crops, as the population develops, and these areas become available.

In this study, overwintered adults were observed as individuals on isolated plants almost two months prior to being found in the alfalfa field. Craig (1983) suggested that *Lygus* adults generally do not overwinter within alfalfa fields; instead, they leave these areas and seek sites which provide more succulent hosts and better shelter from winter

temperatures. It is unclear whether the TPB overwintered in the alfalfa field in this study, as snow and ice cover, frozen plants and frozen ground made it very difficult to obtain samples during winter and early spring. The alfalfa was left standing in the field throughout the year, so the dead plants probably would have provided suitable cover for overwintering. The population data suggested that overwintered adults are found within the alfalfa in the spring, but it was not determined whether these adults overwintered within this site, or were migrants from early spring hosts. As well, data collected in the fall indicated that numbers within the field declined, which would indicate early "hibernation", a metabolically costly event (Danks, 1978), or migration into more suitable areas. The latter or a combination of the two seems reasonable to assume, as adults were still observed on other species of plant (see below) after the decline in number was observed in alfalfa.

Like alfalfa, strawberry is also colonized by overwintered adults which migrate in from weedy areas. Gravid females (Stewart and Gaylor, 1991) migrate into the fields and lay eggs within the soft stem tissues. From bloom on, both overwintered adults and nymphs are present in the field. The

high fecundity of these females makes the population in these areas grow incredibly fast. It is these first generation nymphs, whose abundance is well synchronized with the bloom phenology of strawberry, which cause a majority of the damage to this crop (Bostanian, 1994).

Very few plant species are able to support TPB populations consistently throughout the year. Many hosts are ephemeral, so a succession of host plants is needed to complete the yearly cycle (Fleischer and Gaylor, 1988). Many of these short term hosts are probably used by the motile adults only, although some egg laying may occur. In this study, areas containing *Solidago* (primarily *S. canadensis*) had high numbers of adults during bloom, but very few prior to flower bud development. Very few nymphs were observed, indicating that these sites are used primarily by the adults. Reid *et al.* (1976) report that *S. canadensis* may serve as a host for up to forty-six species of adult Miridae in southeastern Ontario; the TPB (one of the most common) was found primarily during late season (July-August). They did find TPB nymphs on *S. canadensis* in this study, but suggest that it serves more importantly for migratory adults as a late

season host.

Ambrosia spp., another late season host, supported both adult and nymph populations. Early instar nymphs were found in high numbers on this species, indicating that oviposition had occurred. Therefore, this plant appears important, in that it provides food and suitable oviposition material late in the season, possibly supporting a third summer generation in Nova Scotia.

Plant species with longer bloom periods, or which have developing fruit or herbaceous tissues which are soft enough to be used for oviposition and support nymph populations, are more important for maintaining TPB populations throughout the year. The alfalfa field in the study was ideal for monitoring seasonal phenology; it was not sprayed or mowed, and populations remained undisturbed. This type of habitat allows accurate seasonal population dynamics to be studied, as the populations do not have to be followed as they move from host to host. At least two summer adult and nymph generations were observed in this plot, with a slight overlap between generations. In all three years studied, the TPB used alfalfa as a host throughout the year.

The opportunistic nature of the TPB is also demonstrated by reported incidences of predation (Culliney *et al.*, 1986; Cleveland, 1987). It has been observed feeding on soft bodied insects such as aphids, and nymphs of several bug species, including their own (pers. observation). This situation is not common, and probably occurs when there are pressures on the population, such as shortage of food.

These characteristics make this species difficult to control. Although one can spray insecticides to control a population on a crop, new adults can migrate into these areas and quickly establish new populations (Fleischer *et al.*, 1988). The overwintered adults are very important in terms of establishing populations early in the year, and targeting these seems reasonable to keep numbers below critical levels.

Apple Fruit Damage

Results in this study indicate that the TPB feeds on apple during the early stages of fruit development, from the pre-bloom stages until the fruit is approximately 1-2 cm in diameter. These findings support those of other studies (Hammer, 1939; Mailloux *et al.*, 1979; Prokopy and Hubbell, 1981; Boivin and Stewart, 1982 and 1983b). Feeding during the

early stages of fruit development is favoured because the blossom buds, blossoms, and small developing fruit are relatively soft and easily penetrated by the mouthparts.

Feeding can have significant effects on the formation and quality of the fruit. If buds and blossoms are attacked, or damage is particularly severe, abortion will occur. In this experiment, fruit set and mature fruit number were low (as compared with original blossom counts). The majority of this was probably due to natural thinning which occurs in apple. A full crop will have 5% of the blossoms set fruit and reach maturity (Roland, 1978), and often, growers spray trees with "thinning chemicals" to achieve this. There was a significant amount of damage observed in the treatment cages at the early stages of development, so a portion of the dropped blossoms and fruit may have been the result of TPB feeding.

Fruit abortion is most common when damage is inflicted at the earliest stages of development. At later stages (i.e., well after fruit set), TPB damage is likely to result in misshapen or dimpled fruit, with circular depressions and small scabs around the stalk or calyx area (Hammer, 1939). The intensity of this damage is the result of several contributing factors such as the number of punctures and the

stage of development; feeding damage is less severe if done at later stages. Also, apple varieties differ in susceptibility to the feeding and/or saliva constituents secreted by the TPB. The apple varieties in this study, "McIntosh" and "Cortland", are considered mildly susceptible to "stinging bug" injury. This could explain why in some cases, TPB feeding evidence was observed as little dimples and scabs on the fruit surface. More severe damage is observed in other varieties, such as "Red Delicious", "Spy", and "Spartan" (Bent, pers. comm.). TPB feeding on these varieties can result in very misshapen fruit, which are unsuitable for fresh sale. An example of such fruit is shown in Figure 10.

The TPB is one of several economically important phytophagous mirid pests of apple in North America (Boivin and Stewart, 1982). Although it is considered to be the most important in northeastern North America (Parker and Hauschild, 1975; Hauschild and Parker, 1976), the other mirids; the green apple bug, *Lygocoris communis* (Knight), the red apple bug, *Lygidea mendax* Reuter, the mullein bug, *Campylomma verbasci* (Meyer), and the apple brown bug, *Atractotomus mali* (Meyer), cause significant damage in other areas. The TPB differs from

the other mirid pests of apple in that it overwinters as an adult, the others as eggs. The overwintered adults fly into orchards and visit developing apple buds, blossoms, and foliage (Prokopy *et al.*, 1979), early in the developmental stages of apple. These adults will lay eggs on weeds within the orchard. Most of these adults do not live beyond these early stages of fruit development (Prokopy *et al.*, 1979). Therefore, it is only the overwintered adult TPB's which cause damage to apple. The nymphs and summer adults are not believed to feed on these later stages of fruit development, and do not contribute significantly to apple damage (Boivin and Stewart, 1982).

Caging TPB adults on the branches throughout the season forced them to feed on structures within the cage; mainly leaves, stems, and developing apples. At harvest, no significant signs of feeding were observed on the apples for any of the feeding intervals, with the exception of the last two stages for "McIntosh". The damage observed was small puncture marks on the apple surface. These findings are contradictory to those of others. Small sample size may have contributed to these results, as towards the end of the

season, some of the cages had very few apples, even the control cages.

The cages themselves appeared to affect apple quality. All caged "Cortland" apples (with two exceptions) weighed less than uncaged apples, which were selected randomly from the orchard. Caged "McIntosh" apples receiving TPB's weighed less than the caged controls, which did not differ significantly from the uncaged controls. These results seem reasonable if TPB's caused significant deformity to the fruit, which is not the case in this experiment. The reverse was observed for "Cortland" apples. Therefore, these results are inconclusive, due to the effects of this type of cage, which limited light, moisture, and probably elevated temperature. Differences in weight are therefore not attributed to TPB feeding.

Other "apple stinging bugs" are common on apple in Nova Scotia. They overwinter as eggs on the apple trees, and emerge just prior to or during bloom. These species, particularly the apple brown bug, are considered to be very important pests of apple in Nova Scotia (MacPhee, 1976; Bent, pers. comm.), possibly more so than the TPB. In other areas of North America, attributing apple damage to the TPB is warranted, as the apple brown bug is not common or present.

In Nova Scotia, the true status of the TPB as an apple pest is unknown. It is found here in high enough numbers to significantly contribute to damage, but no literature exists on its importance in Nova Scotia. Its presence is monitored in orchards not as a species, but as part of the "stinging bug" complex (Bent, pers. comm.). More research is needed to identify the actual "stinging bugs" causing the damage in Nova Scotia, and their relative importance.

Strawberry Fruit Damage

The TPB has long been recognized as a major pest of strawberry (Painter, 1929), and IPM programs have been implemented for many years (Maillioux and Bostanian, 1988a, 1988b and 1989; Bostanian, 1994). In order to obtain an understanding of how and why damage occurs, knowledge of the processes of pollination and fertilization, and their influence on fruit formation is needed.

Most strawberry cultivars are self-fertile (Free, 1993) and pollination occurs by three means; 1) by gravity (which is believed to be the most important), 2) by wind, and 3) by insect pollinators. Although bees and other pollinators may not be necessary for successful pollination (Strand, 1994),

cross pollination by insects is favoured (Free, 1993), and some studies have reported lower fruit yield in the absence of insects (Hughes, 1961 and 1962). Other studies (Jaycox, 1970) have shown that 11-15 bee visits are necessary to obtain full pollination.

Inadequate pollination results in deformed fruit (Figure 16b), which is unmarketable for fresh sale. Without adequate pollination, fertilization does not occur, the ovules within the achenes do not develop, and no growth hormones are produced. The role of growth hormones in strawberry fruit development has been investigated extensively (Gustafson, 1939; Nitsch, 1950; Archibold and Dennis, 1985; Southwick and Poovaiah, 1987). These compounds, particularly the auxins, are produced after the ovules are fertilized, and cause growth of the receptacle tissue around the achenes (Nitsch, 1950). By removing the achenes of well-pollinated fruit, symptoms of poor pollination result (Nitsch, 1950), and applying auxins to receptacle tissue with the achenes removed resulted in normal fruit development (Nitsch, 1950; Archibold and Dennis, 1985; Southwick and Poovaiah, 1987).

Poorly pollinated fruit may be mistaken for TPB damage. Results in this study suggest that fruit quality can be very

similar between poorly pollinated and TPB damaged fruit. In both cases, the result is fruit of reduced quality and weight. Nitsch (1950) reported that the weight of the fleshy part of the fruit (the receptacle) is roughly proportional to the number of fertilized ovules. This also would apply to the proportion of undamaged achenes. Close examination of the fruit reveals differences (Figures 16a and 16b). Poorly pollinated fruit do not exhibit apical seediness as pollination (or lack of) can occur at any location on the fruit. TPB feeding is focussed around the site of penetration, resulting in apical seediness. Increased feeding results in further fruit damage and deformity.

Another distinguishing characteristic between poorly pollinated and TPB damaged fruit is the difference in achene size. Achenes of poorly pollinated berries show a range of sizes, while berries showing apical seediness have achenes of similar size around the area of damage, and damaged achenes are usually discoloured (Handley and Pollard, 1993).

TPB feeding during early fruit development is focused on the developing achenes (Handley and Pollard, 1993). Damage to these has the same effect as lack of fertilization or removal of achenes in terms of hormone production. Most TPB damage in

developing fruit can be attributed to the absence of plant growth regulators.

The control berries were of significantly higher weight and quality than berries receiving TPB. No significant differences in weight and quality were observed between any of the berries receiving 2, 4, and 6 TPB feeding treatments, or with weight in the non-pollinated controls in this study, indicating that any TPB feeding can lead to reduced fruit weight.

In this study, TPB's were confined to the developing berries at the achene separation stage of development, in order to assess the impact of feeding densities on fruit quality. Handley (1991) reports that TPB feeding at stages prior to this usually results in blossom death, and that the duration of exposure to TPB feeding is also important. Therefore, high numbers of nymphs within strawberry fields have the potential to cause significant losses in both overall yield and quality. This susceptibility to damage at low feeding densities is demonstrated in the ETL used in strawberry IPM programs; 0.26 nymphs per blossom cluster, as suggested by Mailloux and Bostanian (1988). Bostanian (1994) suggests that 0.15 nymphs per blossom cluster is more

practical, as the higher value requires immediate action. In Nova Scotia, a ETL of 0.50 nymphs per blossom cluster is suggested (Crozier, 1993b). Careful monitoring of this crop is essential to obtain high quality, marketable fruit.

Cold-hardiness

An insect's ability to survive the winter in temperate zones, i.e., its cold-hardiness, is the result of many interacting factors (Danks, 1996). Of these, biochemical and physiological aspects of overwintering have received much attention, while equally important ecological aspects of cold-hardiness (winter conditions within the micro-habitat or hibernacula, insect behavioural response to winter conditions), and other adaptations to cold environments have been overlooked or seldom studied (Danks, 1978; Bale, 1987, Leather *et al.*, 1993). Species are seldom studied from all viewpoints (Danks, 1978 and 1996).

Choice of overwintering sites is of utmost importance for ensuring survival, especially for freeze intolerant species. Ideal locations not only provide adequate buffering from ambient winter temperatures, but are also protected from water-logging and flooding during the spring (Danks, 1991),

hidden from potential predators (Danks, 1991; Leather *et al.*, 1993), and allow commencement of activity at suitable times the following spring (Danks, 1978). The conditions within the hibernacula are very important, as these are the actual conditions the insects are exposed to throughout the winter months. Humidity levels, temperature, and other factors interact within these sites, and are all important aspects of the insects cold-hardiness (Danks, 1978 and 1991; Leather *et al.*, 1993). A critical balance of these factors exists for insect winter survival.

Early reports (Painter, 1929) have described some overwintering sites of the tarnished plant bug; these and others have also been confirmed (Cleveland, 1982). Survival can differ greatly between these sites, being highest in those offering the most protection from ambient conditions. Sites consisting of large, overlapping leaves have been found to yield higher rates of survival for the TPB (Fye, 1982). One of the primary overwinter sites of the TPB, orchard floors (Fye, 1982), usually consist of large leaves, forming a continuous layer over the ground. Other protected sites include under mullein, *Verbascum* spp., other surface wood

trash (Cleveland, 1982), and strawberry mulch (Schaefers, 1980). Conditions in unprotected overwintering sites, such as on bark or between blades of grass probably do not offer the same degree of protection as the previously mentioned sites, and therefore more closely follow ambient conditions. Overwinter survival in these areas is significantly lower than in protected areas (Painter, 1929).

Humidity levels within the overwintering sites of *Lygus* bugs have previously been investigated (Fye, 1982), and relative humidity levels of 10% or less were concluded to be detrimental to survival, especially during dry winters. On the other hand, high humidity and contact moisture can be very harmful at sub-freezing temperatures within the overwintering sites (Danks, 1991).

Snow cover enhances survival for many species. Snow has very good insulating properties (Danks, 1991), and temperatures at ground level under a blanket of snow (where many arthropods, including the TPB, overwinter) are not only significantly warmer than exposed ground surfaces (which more or less follows the trends of ambient temperatures), but offer a more stable, less fluctuating range of temperatures. Temperatures within these sites are often at, or slightly

below, 0°C (Danks, 1991), which is above the critical levels of supercooling for most cold-hardy freeze intolerant species. Stable temperatures within the sites are very important. Highly fluctuating temperatures have been reported to be very harmful for overwintering insects, even at temperatures well above the SCP (Danks, 1991). A blanket of snow and a thick layer of leaves offers a great deal of protection to TPB's overwintering in these sites. Despite the variety of overwintering sites and differing degrees of protection, winter mortality for the TPB can often be quite high (Painter, 1929). Block (1995) suggests that high winter mortality in freeze intolerant species is common. Spring numbers of overwintered TPB within the alfalfa field, as compared to the previous fall, were significantly lower. This supports the observations of high mortality, but also could indicate that alfalfa may not be used as an overwintering site, as mentioned earlier.

This study investigated the environmental triggers for the induction of one aspect of cold-hardiness, the supercooling capacity, as well as the effect of reversing these cues on insects already in the cold-hardened state. Reproducing "summer" adult TPB's have SCP's between -12°C

and -13°C, which for the season, is well below any recorded temperatures for this time of year (see Figure 19). This work suggests that day length is the main token stimulus for enhancing supercooling capacity for this species, a strategy not uncommon in many insects (Baust, 1982; Leather *et al.*, 1993). "Summer" adults placed in cabinets at high temperature and long day length, maintaining summer conditions, had SCP's significantly higher than those acclimated at the other conditions, and a high proportion of these individuals did not have enhanced supercooling capacity. Bostanian (1994) states that the first four nymphal instars are the photosensitive stages, and that photoperiods of 12.5 h or less will induce diapause in the adult (photoperiods of 13.5 h prevent diapause and promote reproductive adults). He also suggests that continuous light prevents diapause in young adults and terminates it in diapausing adults. Diapause, as referred to here, is in the reproductive sense (i.e. atrophy of ovaries and testes). Diapause, as a whole, is an endocrine-mediated dormancy, characterized by reduced metabolic rate (and food intake) and behavioural changes, but does not necessarily imply "cold-hardiness" (Denlinger, 1991). The relationship between cold-hardiness and diapause is not completely

understood, and cannot be generalized (Denlinger, 1991). Aspects of cold-hardiness, such as changes in supercooling capacity, may be a component of the "diapause program", as are changes in the reproductive status of a species. Therefore, the role of photoperiod in inducing "reproductive diapause" in the TPB may be similar for enhancing supercooling capacity. Photoperiod is very predictable with seasonal change and may act as a reliable cue for several aspects of insect biology (Leather *et al.*, 1993; Danks, 1994). Often, abiotic parameters, such as photoperiod and temperature, work together as cues for these processes, as can biotic cues such as food shortages and crowding (Leather *et al.*, 1993).

Egg laying occurred when "summer" adults were placed in warm temperatures, regardless of photoperiod. This provided evidence that as a population, these adults were not in reproductive diapause at the time of collection. Because the decreased photoperiod within the cabinet at high temperature and short day length acted as a cue for enhancing supercooling point, and photoperiod was short enough to cause reproductive diapause (Bostanian, 1994), it is likely that egg laying took place within a few days of the TPB's being placed in these

cabinets, before the insects had time to acclimatize to these new conditions. However, Bostanian (1994) also reports that fifth instar nymphs and adults are not photosensitive, and therefore, should not have been in reproductive diapause if placed in these cabinets as adults. Reproductive status was not assessed by dissection, so if the above is true, then enhancement of supercooling capacity and reproductive diapause would have to occur independently, as photoperiod did influence supercooling capacity.

Also evident from these results is the protective mechanism of insects with enhanced supercooling capacity. "Autumn" insects, which were assumed to be in reproductive diapause, and "cold-hardened", had similar SCP's regardless of acclimatization conditions, and almost all had enhanced supercooling. This indicates that TPB's in this "cold-hardened" and reproductive state are not easily reversed by late season changes in environmental conditions. Such mechanisms protect insects from harsh conditions that "cold-hardiness" and diapause would normally protect them from (Leather *et al.* 1993).

The TPB supercooling profile follows the annual ambient temperature profile closely. For the majority of the year,

the supercooling capacity of the TPB is well below seasonal ambient weather conditions. This is not to say they are not vulnerable to cold temperatures. Many species suffer deleterious effects of cold temperature in the absence of ice at temperatures well above their SCP, called chill injury (Denlinger *et al.*, 1991). Chill injury or "cold shock" is dependent on the rate of cooling, and different species vary in their susceptibility. The importance of overwintering sites which are sheltered with less fluctuating temperatures is stressed again.

The Potential for Biological Control

Pseudomonas syringae and other biological ice-nucleators have received much attention in the last decade as a possible biological control agent (Strong-Gunderson *et al.*, 1990 and 1992; Steigerwald *et al.*, 1995; Fields *et al.*, 1995; Landry and Phillips, 1996; Lee *et al.*, 1991, 1993, 1994 and 1996). These agents have significantly reduced the supercooling capacity of several freeze-intolerant insect species, sometimes elevating the SCP of the insect by as much as 11°C (Fields *et al.*, 1995). Insects which have come in contact with INA bacteria, whether by surface contact (Strong-

Gunderson *et al.*, 1992; Steigerwald *et al.*, 1995; Lee *et al.*, 1996) or by ingestion (Strong-Gunderson *et al.*, 1990), become susceptible to subzero temperatures as high as -4°C, much higher then normal. Therefore, control of pest could be gained by exploiting the insects intolerance of ice-formation, and by making them more susceptible at higher temperatures.

These compounds are effective in causing death in insects because they help initiate the formation of ice. The spontaneous growth of ice or ice crystals requires a nucleus; water molecules aggregate around the nucleus until a critical size is reached (Lee, 1991; Steigerwald *et al.*, 1995). For most biological systems, ice nucleation is believed to be a result of a heterogenous mechanism (i.e., the nucleus is a substance other then water) (Lee, 1991). The nucleus for ice formation in this case is a protein produced by the bacteria.

For this type of control to be effective, more knowledge than just the SCP's and cold-hardiness of the target insect must be considered. Although the SCP of a freeze-intolerant species is ultimately the temperature at which it will die by freezing, many insects are able to reduce the likelihood of freezing by choosing hibernation sites which are sheltered

from these temperatures.

In this study, the SCP's of TPB's sprayed with a solution containing INA+ bacteria were significantly higher than those sprayed with water. However, the elevation in SCP observed, approximately 5°C, was not as large as expected. Drs. R.E. Lee, Jr. and M. Lee (pers. comm.) suggested that the method used to prepare the bacteria (in nutrient broth) was inferior to growing it on agar, as the bacteria do not express ice-nucleation as strongly. Although INA+ bacteria solutions prepared from agar were not tested on the TPB, solutions of equal concentration to those prepared in nutrient broth had SCP's between -3°C and -4°C. In fact, all solutions containing 3×10^5 cells / mL or higher were found to significantly increase the SCP of water. At smaller volumes, fewer cells are present, so sprays of 1×10^9 cells / mL, as suggested by Dr. R.E. Lee, Jr. (pers. comm.) are suitable for observing elevated SCP's in insects, and should be used in future studies of this type.

Other Considerations for Control

The TPB has a number of important natural enemies (Clancy and Pierce, 1966; Soroka, 1997). The generalist predators

include damsel bugs (Nabidae), big-eyed bugs (Geocoridae), assassin bugs (Reduviidae), minute pirate bugs (Anthocoridae), spiders (Oxyopidae, Tetragnathidae, and Thomisidae), ladybird beetles (Coccinellidae), green lacewings (Chrysopidae), sphecid wasps (Sphecidae), and syrphid flies (Syrphidae).

Many of these natural enemies have recently received attention in terms of being managed for biological control. One study concluded that the big-eyed bugs appeared to be the most effective predators for *Lygus* bugs, but it was suggested that these are very hard to rear (Idaho Alfalfa Seed Commission Newsletter, 1995). Soroka (1995 and 1997) looked at the potential of releasing gravid female lacewings into crops subject to TPB damage. Although lacewings (particularly the larvae) are voracious predators, high enough numbers were not obtained. Many of these females did not stay in the release area, and no differences in *Lygus* bug numbers were observed between treatment and control plots.

Several parasitoids are also associated with *Lygus* bugs in North America, and attack it at different developmental stages. Egg parasitoids include the genera *Anaphes*, *Erythemelus*, and *Polynema* (Mymaridae) (Sohati *et al.*, 1989)

and *Telenomus* (Scelionidae) (Al-Ghamdi *et al.*, 1995). There are two important native braconid parasitoids, which attack the nymphs (Soroka, pers. comm.). *Peristenus pallipes* (Curtis) develop in the first summer generation of nymphs, *P. pseudopallipes* (Loan) in later generations (Lim and Stewart, 1976). Both of these species emerge as larvae from fifth instar nymphs or adults.

Rates of parasitism are higher in wild, un-managed sites (Lim and Stewart, 1976; Snodgrass and Fayad, 1991), some report rates as high as 62% (Scales, 1973) and 85% (Soroka, 1997). These levels are probably high enough to reduce local populations (Snodgrass and Fayad, 1991). Population levels of parasitism are significantly less in managed areas (Soroka, 1997), primarily due to insecticide use, and fall and spring farming practices, which kills the overwintering pupae within the soil (Scales, 1973). Another explanation states that many of *Lygus* host plants are not native, and parasitoid numbers are usually only high in weeds (Soroka, 1997) Levels in these areas are insufficient to manage TPB populations.

Imported parasitoid species have shown potential as control agents (Day *et al.*, 1990; Day, 1996). In Canada, this

research began in 1977, after parasitoids collected in Europe were released here (Soroka, 1997). This work began earlier in the United States (Hedlund and Graham, 1987), and establishment has been successful in some areas (Day *et al.*, 1990; Day, 1996). Progress is being made in Quebec and Ontario, but no evidence of establishment in the prairies has been reported (Soroka, 1997). Reviews of the history of these programs in North America are found in Hedlund and Graham (1987) and Soroka (1997).

The importance of early season hosts in supporting TPB populations has been previously mentioned. Some of these species have been found to support higher TPB population densities than others (Bostanian, 1994). Using highly attractive plants, trap crops could be established early in the season to deter TPB adults from important crops. Bostanian (1994) suggests that yellow rocket, *Barbarea vulgaris* L., may be a suitable trap crop for strawberry. As populations establish, these sites could be burned. Burning has also shown potential for *Lygus* control in other areas (Schaber and Entz, 1994). Populations were reported to decrease if alfalfa was burned after 50 mm of growth occurred,

by which time many eggs had been laid, and nymphs had developed.

Conclusions

Control of insect pests using cold temperature is not a new idea, and several studies have been conducted on the potential of its use for control of structural insect pests (Rust *et al.*, 1997, and references sited therein), and pests of stored products (Yokoyama and Miller, 1989; Moffit and Burditt, 1989a and b; Hoy and Whiting, 1997; Mitcham *et al.*, 1997). Cold treatment has been used for many years to kill many of the insects found in museum specimens. Under the right circumstances, exploiting the "freeze-intolerance" or "chill injury" susceptibility of insect pests seems very promising as a control procedure, as it is both effective, and environmentally safe.

Many insect pest species enhance their supercooling capacity as a part of their "cold-hardiness" strategies. In these situations, heterogeneous ice-nucleating compounds or microorganisms, like *P. syringae*, cause significant reductions in the supercooling capacity, making treated insects susceptible to milder sub-zero temperatures. If applied

properly, such substances would allow effective control of the pests.

The benefits of using *P. syringae* for pest control are numerous. This and similar organisms are found naturally in the environment, and have been isolated from many sites, including freeze-tolerant frogs (Lee *et al.*, 1995) and insects (Tsumuki *et al.*,1995), plants (Cody *et al.*, 1987; Lindow, 1988; Marshall, 1988; Legrad and Hunter, 1990; Montesinos and Vilardell, 1991; Whitesides and Spotts, 1991), and rainwater (Constantinidou *et al.*, 1990). If these field isolates of INA+ microorganisms are used, problems associated with the release of genetically engineered organisms would be prevented. These treatments would essentially be biodegradable, with no chemical residues.

There are several precautions to adhere to when considering using INA+ microorganisms. The microorganisms were originally isolated from plants, during investigations into the causes of frost injury (Lindow *et al.*, 1982; Lindow, 1983). Therefore, the impact on freeze susceptible plants must be considered. As well, many beneficial arthropods share overwintering sites with the pests, and therefore would be

susceptible to heterogeneous ice-nucleation as well. Non-target arthropods are always of concern in pest management programs, and always should be considered with new types of control. However, if used under controlled conditions, and in overwintering sites which are very specific, many of these problems can be avoided. For instance, Lee *et al.*(1994) suggests the use of trap crops for the Colorado potato beetle; sites where this species would aggregate and could be controlled, without detrimental effects on beneficial insects and plants. The use of these microorganisms have shown promise in stored product sites as well (Lee *et al.*, 1992; Fields, 1993; Fields *et al.*, 1995); very specific areas, with little potential for damage to non-target species.

Another point to consider is the site of overwintering. This strategy could be ineffective if the species of concern overwintered in areas which were above the SCP of treated insects.

One promising aspect of this type of control is that it is compatible with other types of control, including insecticide spraying, release of predators, parasites, and pathogens, and other possible methods. Further work on pest

species, particularly their overwintering habits and sites, must be looked at in greater detail, and more research is needed on the bacteria. It can be used effectively, not for immediate control, as seen with traditional insecticides, but as a way of reducing pest numbers the following season. For many crops, including apple and strawberry, many of the pest are most damaging at the early stages of crop development (Bale, 1991). Targeting these species with an environmentally sound method of control, before they become a significant problem to the crop, is an environmental and economical alternative to traditional control.

LITERATURE CITED

Al-Ghamdi, K.M., R.S. Stewart, and G. Boivin. 1995. Synchrony Between Populations of the Tarnished Plant Bug, *Lygus lineolaris* (Palisot de Beauvois)(Hemiptera: Miridae), and Its Egg Parasitoids in Southwestern Quebec. Can. Ent. 127: 457-472.

Archbold, D.D., and F.G. Dennis, Jr. 1985. Strawberry Receptacle Growth and Endogenous IAA Content as Affected by Growth Regulator Application and Achene Removal. J. Amer. Soc. Hort. Sci. 110(6):816-820.

Bailey, J.C. 1982. Influence of Plant Bug and Leafhopper Populations on Glabrous and Nectariless Cottons. Environ. Entomol. 11: 1011-1013.

Bale, J.S. 1987. Insect Cold Hardiness: Freezing and Supercooling - An Ecophysiological Perspective. J. Insect Physiol. 12: 899-908.

Bale, J.S. 1991. Implications of Cold Hardiness for Pest Management. *In*: Insects at Low Temperature. R.E. Lee Jr., and D.L. Denlinger (Eds.). Chapman and Hall. 513pp.

Baust, J.G. 1982. Environmental Triggers to Cold Hardening. Comp. Biochem. Physiol. 73A(4): 563-570.

Beirne, B.P. 1972. Pest Insects of Annual Crop Plants in Canada. IV. Hemiptera-Homoptera. V. Orthoptera. VI. Other Groups. Mem. Ent Soc. Can. 85., 73pp.

Block, W. 1991. To Freeze or Not to Freeze? Invertebrate Survival of Sub-zero Temperatures. Functional Ecol. 5: 284-290.

Block, W. 1995. Insects and Freezing. Science Progress. 78(4): 349-372.

Boivin, G., R.K. Stewart, and I. Rivard. 1982. Sticky Traps for Monitoring Phytophagous Mirids (Hemiptera: Miridae) in an Apple Orchard in Southwestern Quebec. Environ. Entomol. 11: 1067-1070.

Boivin, G., and R.K. Stewart. 1982. Identification and Evaluation of Damage to McIntosh Apples by Phytophagous Mirids (Hemiptera: Miridae) in Southwestern Quebec. Can. Ent. 114: 1037-1045.

Boivin, G., and R.K. Stewart. 1983a. Sampling Technique and Seasonal Development of Phytophagous Mirids (Hemiptera: Miridae) on Apple in Southwestern Quebec. Ann. Entomol. Soc. Am. 76: 359-364.

Boivin, G., and R.K. Stewart. 1983b. Seasonal Development and Interplant Movements of Phytophagous Mirids (Hemiptera: Miridae) on Alternate Host Plants in and Around an Apple Orchard. Ann. Entomol. Soc. Am. 76: 776-780.

Boivin, G., J.-P.R. Le Blanc, and J.A. Adams. 1991. Spatial Dispersion and Sequential Sampling Plan for the Tarnished Plant Bug (Hemiptera: Miridae) on Celery. J. Econ. Entomol. 84(1): 158-164.

Bostanian, N.J., and L.J. Coulombe. 1986. An Integrated Pest Management Program for Apple Orchards in Southwestern Quebec. Can. Ent. 118: 1131-1142.

Bostanian, N.J. 1994. The Tarnished Plant Bug and Strawberry Production. Tech. Bull. 1994-1 E. Agriculture and Agri-Food Canada. 28pp.

Brittain, W.H., and L.G. Saunders. 1917. Notes on the Biology of *Lygus pratensis* Linn., in Nova Scotia. Proc. ent. Soc. N.S. 3: 85-91.

Butts, R.A., and R.J. Lamb. 1990a. Injury to Oilseed Rape caused by Mirid Bugs (*Lygus*) (Heteroptera: Miridae) and its Effect on Seed Production. Ann. Appl. Biol. 117: 253-266.

Butts, R.A., and R.J. Lamb. 1990b. Comparison of Oilseed *Brassica* Crops with High or Low Levels of Glucosinolates and alfalfa as Hosts for Three Species of *Lygus* (Hemiptera: Heteroptera: Miridae). J. Econ. Entomol. 83(6): 2258-2262.

Butts, R.A., and R.J. Lamb. 1991. Seasonal Abundance of Three *Lygus* Species (Heteroptera: Miridae) in Oilseed Rape and Alfalfa in Alberta. J. Econ. Entomol. 84(2): 450-456.

Cermak, P., and G.M. Walker. 1992. Tarnished Plant Bug: A Major Pest of Strawberry. Ontario Ministry of Agriculture and Food. No. 92-108.

Clancy, D.W., and H. D. Pierce. 1966. Natural Enemies of Some *Lygus* Bugs. J. Econ. Entomol. 59(4): 853-858.

Cleveland, T.C. 1982. Hibernation and Host Plant Seqeunce Studies of Tarnished Plant Bugs, *Lygus lineolaris*, in the Mississippi Delta. Environ. Entomol. 11: 1049-1052.

Cleveland, T.C. 1987. Predation by Tarnished Plant Bugs (Heteroptera: Miridae) of Heliothis (Lepidoptera: Noctuidae) Eggs and Larvae. Environ. Entomol. 16: 37-40.

Cobben, R.H. 1968. Evolutionary Trends in Heteroptera. Part I. Eggs, Architecture of the Shell, Gross Embryology and Eclosion. Centre for Agriculture Publishing and Documentation, Wageningen. 475 pp.

Cody, Y.S., D.C. Gross, E.L. Proebsting,Jr., and R.A. Spotts. 1987. Suppression of Ice Nucleation-Active *Pseudomonas syringae* by Antagonistic Bacteria in Fruit Tree Orchards and Evaluations of Frost Control. Phytopathology. 77: 1036-1044.

Constantinidou, H.A., S.S. Hirano, L.S. Baker, and C.D. Upper. 1990. Atmospheric Dispersal of Ice Nucleation-Active Bacteria: the Role of Rain. Phytopathology. 80: 934-937.

Craig, C.H. 1983. Seasonal Occurrence of *Lygus* spp. (Heteroptera: Miridae) on Alfalfa in Saskatchewan. Can.Ent. 115: 329-331.

Crosby, C.R., and M.D. Leonard. 1914. The Tarnished Plant-Bug. Cornell Agric. Exp. Stn. Bull. 346: 463-526.

Crozier, L. 1993a. Integrated Pest Management in Nova Scotia Agriculture. Nova Scotia Department of Agriculture and Marketing, Plant Industry Fact Sheet.

Crozier, L. 1993b. The Tarnished Plant Bug. Nova Scotia Department of Agriculture and Marketing, Strawberry Fact Sheet.

Culliney, T.W., D. Pimentel, O.S. Namuco, and B.A. Capwell. 1986. New Observations of Predation by Plant Bugs (Hemiptera: Miridae). Can. Ent. 118: 729-730.

Danks, H.V. 1978. Modes of Seasonal Adaptation in the Insects. Can. Ent. 110: 1167-1205.

Danks, H.V. 1991. Winter Habitats and Ecological Adaptations for Winter Survival. *In*: Insects at Low Temperature. R.E. Lee Jr., and D.L. Denlinger (Eds.). Chapman and Hall. 513pp.

Danks, H.V. 1994. Diversity and Integration of Life-Cycle Controls in Insects. pp. 5-40 *In*: Insect Life-Cycle Polymorphism. H.V. Danks (Ed.). Kluwer Academic Publishers, Netherlands.

Danks, H.V. 1996. The Wider Integration of Studies on Insect Cold-Hardiness. Eur. J. Entomol. 93: 383-403.

Day, W.H. 1987. Biological Control Efforts Against *Lygus* and *Adelphocoris* spp. Infesting Alfalfa in the United States, with Notes on other Associated Mirid Species. *In*: Economic Importance and Biological Control of *Lygus* and *Adelphocoris* in North America. Hedlund, R. and H.M. Graham (Eds.). U.S.D.A., Agric. Res. Publ. ARS-64. 95 pp.

Day, W.H. 1996. Evaluation of Biological Control of the Tarnished Plant Bug (Hemiptera: Miridae) in Alfalfa by the Introoduced Parasite *Peristenus digoneutis* (Hymenoptera: Braconidae). Environ. Entomol. 25(2): 512-518.

Day, W.H., R.C. Hedlund, L.B. Saunders, and D. Coutinot. 1990. Establishment of *Peristenus digoneutis* (Hymenoptera: Braconidae), a Parasite of the Tarnished Plant Bug (Hemiptera: Miridae) in the United States. Environ. Entomol. 19(5): 1528-1533.

Denlinger, D.L. 1991. Relationship Between Cold Hardiness and Diapause. *In*: Insects at Low Temperature. R.E. Lee Jr., and D.L. Denlinger (Eds.). Chapman and Hall. 513pp.

Denlinger, D.L., K.H. Joplin, C.P. Chen, and R.E. Lee, Jr. 1991. Cold Shock and Heat Shock. *In*: Insects at Low Temperature. R.E. Lee Jr., and D.L. Denlinger (Eds.). Chapman and Hall. 513pp.

Fields, P.S. 1993. Reduction of Cold Tolerance of Stored-Product Insects by Ice-Nucleating Active Bacteria. Environ. Entomol. 22: 470-476.

Fields, P., S. Pouleur, and C. Richard. 1995. The Effect of High Temperature Storage on the Capacity of an Ice-Nucleating-Active Bacterium and Fungus to Reduce Insect Cold-Tolerance. Can. Ent. 127: 33-40.

Fleischer, S.J., M.J. Gaylor, and J.V. Edelson. 1985. Estimating Absolute Density from Relatively Sampling of *Lygus lineolaris* (Heteroptera: Miridae) and Selected Predators in Early to Mid-season Cotton. Environ. Entomol. 14: 709-717.

Fleischer, S.J., M.J. Gaylor. 1987. Seasonal Abundance of *Lygus lineolaris* (Heteroptera: Miridae) and Selected Predators in Early Season Uncultivated Hosts: Implications for Managing Movement into Cotton. Environ. Entomol. 16: 379-389.

Fleischer, S.J., M.J. Gaylor. 1988. *Lygus lineolaris* (Heteroptera: Miridae) Population Dynamics: Nymphal Development, Life Tables, and Leslie Matrices on Selected Weeds and Cotton. Environ. Entomol. 17(2): 246-253.

Fleischer, S.J., M.J. Gaylor, and N.V. Hue. 1988. Dispersal of *Lygus lineolaris* (Heteroptera: Miridae) Adults Through Cotton Following Nursery Host Destruction. Environ. Entomol. 17(3): 533-541.

Free, J.B. 1993. Insect Pollination of Crops. Second Edition. Academic Press Inc. San Diego. 684 pp.

Friend, W.G. 1953. Nutritional Requirements of Phytophagous Insects. Ann. Rev. Entomol. 3: 57-74.

Fye, R.E. 1980. Weed Sources of *Lygus* Bugs in the Yakima Valley and Columbia Basin in Washington. J. Econ. Entomol. 73: 469-473.

Fye, R.E. 1982. Overwintering of *Lygus* Bugs in Central Washington: Effects of Pre-Overwintering Host Plants, Moisture, and Temperature. Environ. Entomol. 11: 204-206.

Gerber, G.H. 1995. Fecundity of *Lygus lineolaris* (Heteroptera: Miridae). Can. Entomol. 127: 263-264.

Gerber, G.H., and I.L. Wise. 1995. Seasonal Occurrence and Number of Generations of *Lygus lineolaris* and *L. borealis* (Heteroptera: Miridae) in Southern Manitoba. Can. Entomol. 127: 543-559.

Guppy, J.C. 1958. Insect Surveys of Clovers, Alfalfa, and Birdsfoot Trefoil in Eastern Ontario. Can. Entomol. 90: 523-531.

Gustafson, F.G. 1939. Auxin Distribution in Fruits and its Significance in Fruit Development. Amer. J. Bot. 33: 189-194.

Hammer, O.H. 1939. The Tarnished Plant Bug as an Apple Pest. J. Econ. Entomol. 32(2): 259-264.

Handley, D.T. 1991. Strawberry Fruit Development and the Effects of Feeding by the Tarnished Plant Bug (*Lygus lineolaris*). *In*: The Strawberry Into the 21st Century. Timber Press, Inc. 288 pp.

Handley, D.T., and J.E. Pollard. 1993. Microscopic Examination of Tarnished Plant Bug (Heteroptera: Miridae) Feeding Damage to Strawberry. J. Econ. Entomol. 86(2): 505-510.

Hauschild, K.I., and B.L. Parker. 1976. Seasonal Development of the Tarnished Plant Bug on Apple in Vermont. Environ. Entomol. 5(4): 675-679.

Hedlund, R.C., and H.M. Graham. 1987. Economic Importance and Biological Control of *Lygus* and *Adelphocoris* in North America. U.S.D.A., Agric. Res. Publ. ARS-64. 95 pp.

Hoy, L.E., and D.C. Whiting. 1997. Low-Temperature Storage as a Postharvest Treatment to Control *Pseudococcus affinis* (Homoptera: Pseudococcidae) on Royal Gala Apples. J. Econ. Entomol. 90(5): 1377-1381.

Hughes, H.M. 1961. Preliminary Studies on the Insect Pollination of Soft Fruits. Expl. Hort. 6: 44.

Hughes, H.M. 1962. Pollination Studies. Progress Report. M.A.A.F., N.A.A.S., Rep. Efford. Exp. Hort. Stn.

Hutchins, S.H. 1994. Techniques for Sampling Arthropods in Integrated Pest Management. *In*: Handbook of Sampling Methods for Arthropods in Agriculture. L.P. Pedigo and C.D. Buntin (Eds.). CRC Press, Inc. Boca Raton. 714 pp.

Idaho Alfalfa Seed Commission Newsletter. 1995. Biological Pest Control in Alfalfa Seed IPM Work Group Meets in Fresno. *In*: Forage Seed News. Saskatchewan Agriculture and Food. No. 9: 10.

Jaycox, E.R. 1970. Pollination of Strawberries. Am. Bee. J. 110: 176-177.

Jensen, B.M., J.L. Wedberg, and D.B. Hogg. 1991. Assessment of Damage Caused by Tarnished Plant Bug and Alfalfa Plant Bug (Hemiptera: Miridae) on Alfalfa Grown for Forage in Wisconsin. J. Econ. Entomol. 84(3): 1024-1027.

Kelton, L.A. 1975. The Lygus Bugs (genus *Lygus* Hahn) of North America (Heteroptera: Miridae). Mem. Ent. Soc. Can. 95: 1-101.

Khattat, A.R., ans R.K. Stewart. 1980. Population Fluctuations and Interplant Movement of *Lygus lineolaris*. Ann. Entomol. Soc. Am. 73: 282-287.

Landry, C.E., and S.A. Phillip, Jr. 1996. Potential of Ice-Nucleating Active Bacteria for Management of the Red Imported Fire Ant (Hymenoptera: Formicidae). Environ. Entomol. 25(4): 859-866.

Laster, M.L., and W.R. Meredith, Jr. 1974. Evaluating the Response of Cotton Cultivars to Tarnished Plant Bug Injury. J. Econ. Entomol. 67(5): 686-688.

Leather, S.R., K.F.A. Walters, and J.S. Bale. 1993. The Ecology of Insect Overwintering. University Press, Cambridge. 255 pp.

Lee, M.R., Lee, R.E., Jr., J.M. Strong-Gunderson, and S.R. Minges. 1995. Isolation of Ice-Nucleating Active Bacteria from the Freeze-Tolerant Frog, *Rana sylvatica*. Cryobiology. 32: 358-365.

Lee, R.E., Jr. 1991. Principles of Insect Low Temperature Tolerance. *In*: Insects at Low Temperature. R.E. Lee, Jr., and D.L. Denlinger (Eds.). Chapman and Hall. 513pp.

Lee, R.E., Jr., J.M. Strong-Gunderson, M.R. Lee, K.S. Grove, and T.J. Riga. 1991. Isolation of Ice Nucleating Bacteria from Insects. J. Exp. Zool. 257: 124-127.

Lee, R.E., Jr., J.M. Strong-Gunderson, M.R. Lee, and E.C. Davidson. 1992. Ice-Nucleating Active Bacteria Decrease the Cold-Hardiness of Stored Grain Insects. J. Econ. Entomol. 85(2): 371-374.

Lee, R.E., Jr., M.R. Lee, and J.M. Strong-Gunderson. 1993. Insect Cold-Hardiness and Ice Nucleating Active Microorganisms Including Their Potential Use for Biological Control. J. Insect Physiol. 39(1): 1-12.

Lee, R.E., Jr., J.P. Costanzo, P.E. Kaufman, M.R. Lee, and J.A. Wyman. 1994. Ice-Nucleating Active Bacteria Reduce the Cold-Hardiness of the Freeze-Intolerant Colorado Potato Beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 87(2): 377-381.

Lee, R.E., Jr., K.A. Steigerwald, J.A. Wyman, J.P. Costanzo, and M.R. Lee. 1996. Anatomical Site of Application of Ice-Nucleating Active Bacteria Affects Supercooling in the Colorado Potato Beetle (Coleoptera: Chrysomelidae). Environ. Entomol. 25(2): 465-469.

Leferink, J.H.M., and G.H. Gerber. 1997. Development of Adult and Nymphal Populations of *Lygus lineolaris* (Palisot de Beauvois), *L. elisus* Van Duzee, and *L. borealis* (Kelton) (Heteroptera: Miridae) in Relation to Seeding Date and Stage of Plant Development on Canola (Brassicaceae) in Southern Manitoba. Can. Ent. 129: 777-787.

Lim, K.P., and R.K. Stewart. 1976. Parasitism of the Tarnished Plant Bug, *Lygus lineolaris* (Hemiptera: Miridae), by *Peristenus pallipes* and *P. pseudopallipes* (Hymenoptera: Braconidae). Can. Ent. 108: 601-608.

Lindow, S.E., D.C. Arny, and C.D. Upper. 1982. Bacterial Ice Nucleation: A factor in Frost Injury to Plants. Plant Physiol. 70: 1084-1089.

Lindow, S.E. 1983. The Role of Bacterial Ice Nucleation in Frost Injury to Plants. Ann. Rev. Phytopathol. 21: 363-384.

Lindow, S.E. 1988. Lack of Correlation of In Vitro Antibiosis with Antagonism of Ice Nucleation Active Bacteria on Leaf Surfaces by Non-Ice Nucleation Active Bacteria. Phytopathology. 78: 444-450.

MacPhee, A.W. 1976. Predictions of Destructive Levels of the Apple-Stinging Bugs *Atractotomus mali* and *Campylomma verbasci* (Hemiptera: Miridae). Can. Ent. 108: 423-426.

Madhusudhan, V.V., G.S. Taylor, and P.W. Miles. 1994. The Detection of Salivary Enzymes of Phytophagous Hemiptera: A Compilation of Methods. Ann. Appl. Biol. 124: 405-412.

Mailloux, G., R.O. Paradis, and J.G. Pilon. 1979. Développement saisonnier de la punaise terne, *Lygus lineolaris* (P. De B.) (Hémiptères: Miridae), sur fraisiers, framboisiers et pommiers dans le sud-ouest de Québec. Ann. Soc. Ent. Québec. 24: 48-64.

Mailloux, G., and N.J. Bostanian. 1988. Economic Injury Level Model for Tarnished Plant Bug, *Lygus lineolaris* (Palisot de Beauvois)(Hemiptera: Miridae), in Strawberry Fields. Environ. Entomol. 17(3): 581-586.

Mailloux, G., and N.J. Bostanian. 1989. Presence-Absence Sequential Decision Plans for Management of *Lygus lineolaris* (Hemiptera: Miridae) on Strawberry. Environ. Entomol. 18(5): 829-834.

Marshall, D. 1988. A Relationship between Ice-Nucleation-Active Bacteria, Freeze Damage, and Genotype in Oats. Phytopathology. 78: 952-957.

Michaud, O.D., G. Boivin, and R.K. Stewart. 1989. Economic Threshold for Tarnished Plant Bug (Hemiptera: Miridae) in Apple Orchards. J. Econ. Entomol. 82(6): 1722-1728.

Mitcham, E.J., S. Zhou, and V. Bikoba. 1997. Controlled Atmospheres for Quarantine Control of Three Pests of Table Grape. J. Econ. Entomol. 90(5): 1360-1370.

Moffitt, H.R., and A.K. Burditt, Jr. 1989a. Effects of Low Temperature on Three Embryonic Stages of the Codling Moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 82(5): 1379-1381.

Moffitt, H.R., and A.K. Burditt, Jr. 1989b. Low-Temperature Storage as a Postharvest Treatment for Codling Moth (Lepidoptera: Tortricidae) Eggs on Apple. J. Econ. Entomol. 82(6): 1679-1681.

Montesinos, E., and P. Vilardell. 1991. Reltionships Among Population Levels of *Pseudomonas syringae*, Amount of Ice Nuclei, and Incidence of Blast of Dormant Flower Buds in Commercial Pear Orchards in Catalunya, Spain. Phytopathology. 81: 113-119.

Nault, L.R. 1997. Arthropod Transmission of Plant Viruses: A New Synthesis. Ann. Ent. Soc. Am. 90(5): 521-541.

Nitsch, J.P. 1950. Growth and Morphogenesis of the Strawberry as Related to Auxin. Amer. J. Bot. 37: 211-215.

Painter, R.H. 1929. The Biology of the Tarnished Plant Bug *Lygus pratensis* L. MSc. Thesis, McGill University. 171 pp.

Parker, B.L., and K.I. Hauschild. 1975. A Bibliography of the Tarnished Plant Bug, *Lygus lineolaris* (Hemiptera: Miridae) on Apple. Bull. Entomol. Soc. Am. 21: 119-121.

Pedigo, L.P. 1989. Entomology and Pest Management. Macmillan Publishing Company, New York. 646 pp.

Peele, E.R., and R.R. Colwell. 1981. Application of a Direct Microscopic Method for the Enumeration of Substrate-Responsive Marine Bacteria. Can. J. Microbiol. 27: 1071-1075.

Peterson, S.S., J.L. Wedberg, and D.B. Hogg. 1992. Plant Bug (Hemiptera: Miridae) Damage to Birdsfoot Trefoil Seed Production. J. Econ. Entomol. 85(1): 250-255.

Prokopy, R.J., R.G. Adams, and K.I. Hauschild. 1979. Visual Responses of Tarnished Plant Bug Adults on Apple. Environ. Entomol. 8: 202-205.

Prokopy, R.J., and G.L. Hubbell. 1981. Susceptibility of Apple to Injury by Tarnished Plant Bug Adults. Environ. Entomol. 10: 977-979.

Reid, D.G., C.C. Loan, and R. Harmsen. 1976. The Mirid (Hemiptera) Fauna of *Solidago canadensis* (Asteracea) in South-eastern Ontario. Can. Ent. 108: 561-567.

Ridgway, R.L., and G.G. Gyrisco. 1960. Effect of Temperature on the Rate of Development of *Lygus lineolaris* (Hemiptera: Miridae). Ann. Ent. Soc. Amer. 53: 691-694.

Roland, A.E. 1978. Production and Pollination: Anatomy of Pollination. Nova Scotia Fruit Growers' Association 115th Annual Report. 61-62.

Rust, M.K., E.O. Paine, D.A. Reierson. 1997. Evaluation of Freezing to Control Wood-Destroying Insects (Isoptera, Coleoptera). J. Econ. Entomol. 90(5): 1215: 1221.

Salt, R.W. 1966. Effect of Cooling Rate on the Freezing Temperatures of Supercooled Insects. Can. J. Zool. 44: 655-659.

Scales, A.L. 1973. Parasites of the Tarnished Plant Bug in the Mississippi Delta. Environ. Entomol. 2(2): 304-306.

Scales, A.L., and J. Hacskaylo. 1974. Interaction of Three Cotton Cultivars to Infestation of the Tarnished Plant Bug. J. Econ. Entomol. 67(5): 602-604.

Schaber, B.D., and T. Entz. 1994. Effect of Annual and Biennial Burning of Seed Alfalfa (Lucerne) Stubble on Populations of Lygus (*Lygus* spp.), and Alfalfa Plant Bug (*Adelphocoris lineolatus* (Goeze)) and Their Predators. Ann. Appl. Biol. 124: 1-9.

Schaefers, G.A. 1980. Yield Effects of Tarnished Plant Bug Feeding on June-Bearing Strawberry Varieties in New York State. J. Econ. Entomol. 73: 721-725.

Schwartz, M.D., and R.G. Foottit. 1992. Lygus Bugs on the Prairies: Biology, Systematics, and Distribution. Tech. Bull. 1992-4E.

Slaymaker, P.H., and N.P. Tugwell. 1982. Low-Labor Method for Rearing the Tarnished Plant Bug (Hemiptera: Miridae). J. Econ. Entomol. 75: 487-488.

Smith, R.F., and J.H. Borden. 1990. Relationship between Fall Catches of *Campylomma verbasci* (Heteroptera: Miridae) in Traps Baited with Females and Density of Nymphs in the Spring. J. Econ. Entomol. 83(4): 1506-1509.

Snodgrass, G.L., W.P. Scott, and J.W. Smith. 1984. Host Plants and Seasonal Distribution of the Tarnished Plant Bug (Hemiptera: Miridae) in the Delta of Arkansas, Louisiana, and Mississippi. Environ. Entomol. 13: 110-116.

Snodgrass, G.L., and Y.H. Fayad. 1991. Euphorine (Hymenoptera: Braconidae) Parasitism of the Tarnished Plant Bug (Heteroptera: Miridae) in Areas of Washington County, Mississippi Disturbed and Undisturbed by Agricultural Production. J. Entomol. Sci. 26(3): 350-356.

Sohati, P.H., R.K. Stewart, and G. Boivin. 1989. Egg Parasitoids of the Tarnished Plant Bug, *Lygus lineolaris* (P. De B.)(Hemiptera: Miridae), in Quebec. Can. Ent. 121: 1127-1128.

Soroka, J. 1995. The Use of Lacewings (Neuroptera: Chrysopidae) for the Biological Control of Lygus Bugs in Alfalfa Seed Fields. Saskatchewan Alfalfa Seed Producers' Association. Pest Management Research Report-Insects and Diseases. Report 001: 2-3.

Soroka, J. 1997. Proceedings of the 1996 *Lygus* Working Group Meeting. Agriculture and Agri-food Canada. 51 pp.

Southwick, S.M., and B.W. Poovaiah. 1987. Auxin Movement in Strawberry Fruit Corresponds to Its Growth-Promoting Activity. J. Amer. Soc. Hort. Sci. 112(1): 139-142.

Sprangler, S.M., A.M. Agnello, and M.D. Schwartz. 1993. Seasonal Densities of Tarnished Plant Bug (Heteroptera: Miridae) and Other Phytophagous Heteroptera in Brambles. J. Econ. Entomol. 86(1): 110-116.

Steigerwald, K.A., M.R. Lee, R.E. Lee, and J.C. Marshall. 1995. Effect of Biological Ice Nucleators on Insect Supercooling Capacity Varies with Anatomical Site of Application. J. Insect Physiol. 41(7): 603-608.

Stewart, R.K., and H. Khoury. 1976. The Biology of *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) in Quebec. Ann. Ent. Soc. Quebec. 21:52-63.

Stewart, S.D., and M.J. Gaylor. 1991. Age, Sex, and Reproductive Status of the Tarnished Plant Bug (Heteroptera: Miridae) Colonizing Mustard. Environ. Entomol. 20(5): 1387-1392.

Strand, L.L. 1994. Integrated Pest Management for Strawberries. University of California Publication 3351.

Strong, F.E. 1970. Physiology of Injury Caused by *Lygus hesperus*. J. Econ. Entomol. 63: 808-814.

Strong, F.E., J.A. Sheldahl, P.R. Hughes, and E.M.K. Hussein. 1970. Reproductive Biology of *Lygus hesperus* Knight. Hilgardia. 40: 105-147.

Strong-Gunderson, J.M., R.E. Lee, M.R. Lee, T.J. Riga. 1990. Ingestion of Ice-Nucleating Active Bacteria Increases the Supercooling Point of the Lady Beetle *Hippodamia convergens*. J. Insect Physiol. 36(3): 153-157.

Strong-Gunderson, J.M., R.E. Lee, and M.R. Lee. 1992. Topical Application of Ice-Nucleating-Active Bacteria Decreases Insect Cold Tolerance. App. Environ. Microbiol. 58(9): 2711-2716.

Tingey, W.M., and E.A. Pillemer. 1977. Lygus Bugs: Crop Resistence and Physiological Nature of Feeding Injury. Bull. Entomol. Soc. Am. 23: 277-287.

Tsumuki, H., H. Yanai, and T. Aoki. 1995. Identification of Ice-Nucleating Active Fungus Isolated from the Gut of the Rice Stem Borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) and a Search for Ice-Nucleating Active *Fusarium* Species. Ann. Phytopathol. Soc. Jpn. 61: 334-339.

Walstrom, R.J. 1983. Plant Bug (Heteroptera: Miridae) Damage to First-Crop Alfalfa in South Dakota. J. Econ. Entomol. 76: 1309-1311.

Whitesides, S.K., and R.A. Spotts. 1991. Frequency, Distribution, and Characteristics of Endophytic *Pseudomonas syringae* in Pear Trees. Phytopathology. 81: 453-457.

Wipfli, M.S., J.L. Wedberg, and D.B. Hogg. 1990. Damage Potentials of Three Plant Bug (Hemiptera: Heteroptera: Miridae) Species to Birdsfoot Trefoil Grown for Seed in Wisconsin. J. Econ. Entomol. 83(2): 580-584.

Wipfli, M.S., S.S. Peterson, D.B. Hogg, and J.L. Wedberg. 1992. Dispersion Patterns and Optimum Sample Size Analyses for Three Plant Bug (Heteroptera: Miridae) Species Associated with Birdsfoot Trefoil in Wisconsin. Environ. Entomol. 21(6): 1248-1252.

Womack, C.L., and M.F. Schuster. 1987. Host Plants of the Tarnished Plant Bug (Heteroptera: Miridae) in the Northern Blackland Prairies of Texas. Environ. Entomol. 16(6): 1266-1272.

Yokoyama, V.Y., and G.T. Miller. 1989. Response of Codling Moth and Oriental Fruit Moth (Lepidoptera: Tortricidae) Immatures to Low-Temperature Storage of Stone Fruits. J. Econ. Entomol. 82(4): 1152-1156.

Young, O.P. 1986. Host Plants of the Tarnished Plant Bug, *Lygus lineolaris* (Heteroptera: Miridae). Ann. Entomol. Soc. Am. 79: 747-762.

Zar, J.H. 1984. Biostatistical Analysis: Second Edition. Prentice-Hall, Inc. New Jersey. 718 pp.

TABLES

Table 1a. Proportion of "McIntosh" apples with tarnished plant bug injury, as recorded at the end of each 1 week feeding episode, for each stage of fruit development. Statistical summaries are presented for "treatment" verses "control", using the Log-likelihood Ratio for Contingency Tables (after Zar, 1984).

Table 1b. Proportion of "Cortland" apples with tarnished plant bug injury, as recorded at the end of each 1 week feeding episode, for each stage of fruit development. Statistical summaries are presented for "treatment" verses "control", using the Log-likelihood Ratio for Contingency Tables (after Zar, 1984).

Treatment Category	Blossoms or Fruit Inspected	Blossoms or Fruit Damaged	Log-likelihood Ratio ($G=\chi^2$ value)	Significant vs. Control	P-value
Pink Tip-Bloom	37	6	9.82	Yes	< 0.005
Calyx- 2 weeks after	24	4	6.08	Yes	< 0.025
1.5 cm- 2 cm	32	2	1.58	No	0.10 <
3 cm +	4	0	0	No	0.975 <

Treatment Category	Blossoms or Fruit Inspected	Blossoms or Fruit Damaged	Log-likelihood Ratio ($G=\chi^2$ value)	Significant vs. Control	P-value
Pink Tip-Bloom	39	5	7.54	Yes	< 0.01
Calyx- 2 weeks after	17	3	5.15	Yes	<0.025
1.5 cm- 2 cm	27	2	1.82	No	0.10 <
3 cm +	5	0	0	No	0.975 <

Table 2a. Proportion of "McIntosh" apples with tarnished plant bug injury, as recorded at harvest, for each stage of fruit development. Statistical summaries are presented for "treatment" verses "control", using the Log-likelihood Ratio for Contingency Tables(after Zar, 1984).

Table 2b. Proportion of "Cortland" apples with tarnished plant bug injury, as recorded at harvest, for each stage of fruit development. Statistical summaries are presented for "treatment" verses "control", using the Log-likelihood Ratio for Contingency Tables(after Zar, 1984).

TPB Introduction Period	Total Apples Examined	Total Apples with Damage	Log-likelihood Ratio (G= χ^2 value)	Significant	P-value
Pink Tip-Bloom	13	3	3.77	No	0.05 <
Calyx- 2 weeks after	7	2	3.02	No	0.05 <
1.5 cm-2 cm	7	1	0.52	No	0.25 <
3 cm- 4cm	12	3	4.03	Yes	< 0.05
5 cm +	14	2	4.50	Yes	< 0.05

TPB Introduction Period	Total Apples Examined	Total Apples with Damage	Log-likelihood Ratio (G= χ^2 value)	Significant	P-value
Pink Tip-Bloom	5	0	0	No	< 0.975
Calyx- 2 weeks after	3	0	0	No	< 0.975
1.5 cm-2 cm	6	0	1.70	No	0.10 <
3 cm- 4cm	13	1	0.43	No	0.50 <
5 cm +	4	0	0	No	< 0.975

FIGURES

Figure 1. Nylon sleeve cage (30 cm x 60 cm) used to enclose apple branches for both treatments (containing tarnished plant bugs) and controls (no tarnished plant bugs).

Figure 2. Plastic "deli-type" containers used to examine the relationship between tarnished plant bug feeding densities and strawberry fruit quality.

Figure 3. Growth curve for *Pseudomonas syringae*, showing absorbance (600 nm) verses time (min).

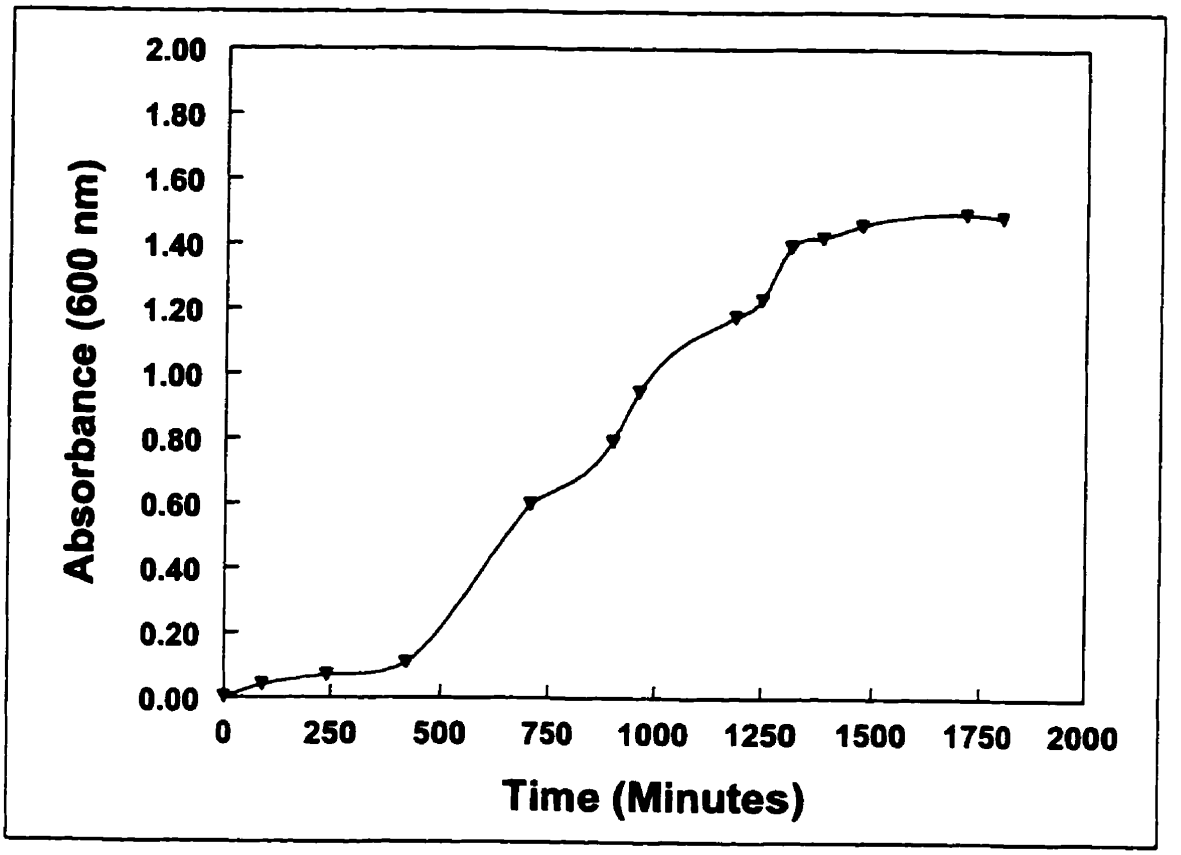
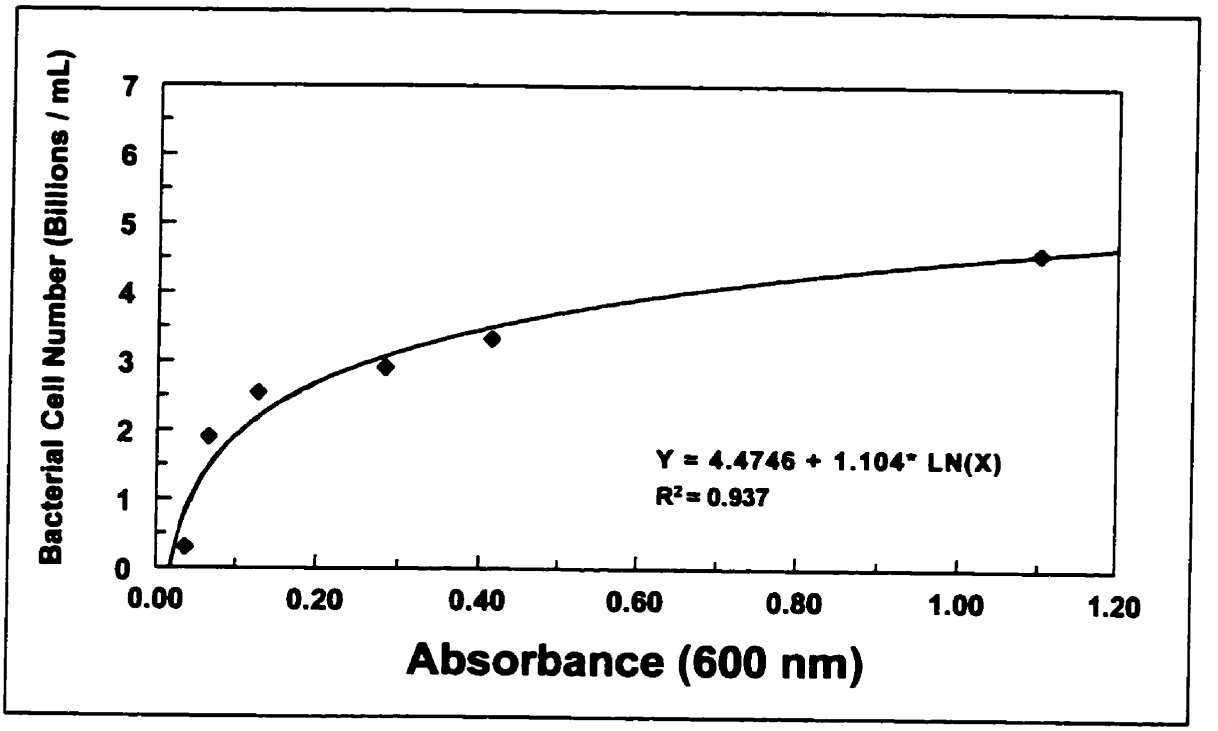

Absorbance (600 nm)
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0
250
500
750
1000
1250
1500
1750
2000
Time (Minutes)

Figure 4a. Bacterial cell number (billions per mL) verses absorbance (600 nm) for *Pseudomonas syringae* grown in nutrient broth containing 2.5% glycerol. Bacterial cell number was calculated using the Acridine Orange method (after Peele and Colwell, 1981).

Figure 4b. Bacterial cell number (billions per mL) verses absorbance (550 nm) for *Pseudomonas syringae* grown on nutrient agar containing 2.5% glycerol, and then suspended in water. Bacterial cell number was calculated using the Acridine Orange method (after Peele and Colwell, 1981).


Bacterial Cell Number (Billions / mL)
0
1
2
3
4
5
6
7
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Y = 4.4746 + 1.104* LN(X)
R² = 0.937
Absorbance (600 nm)

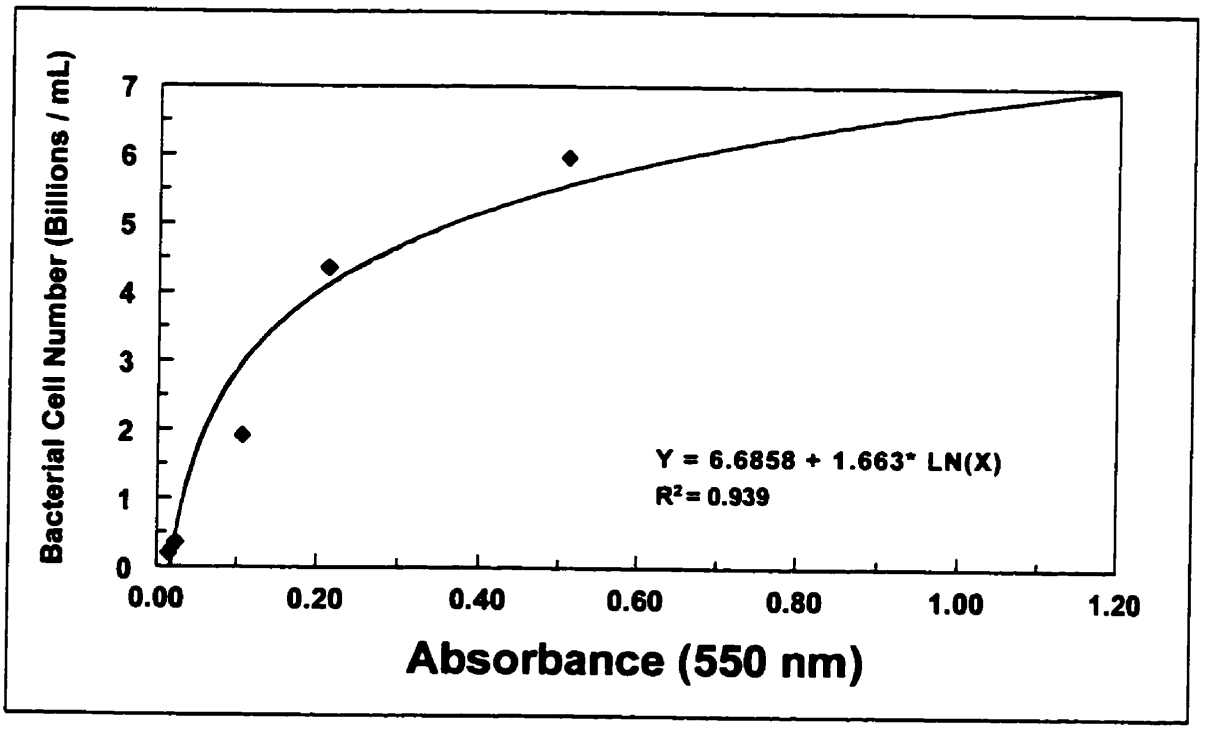
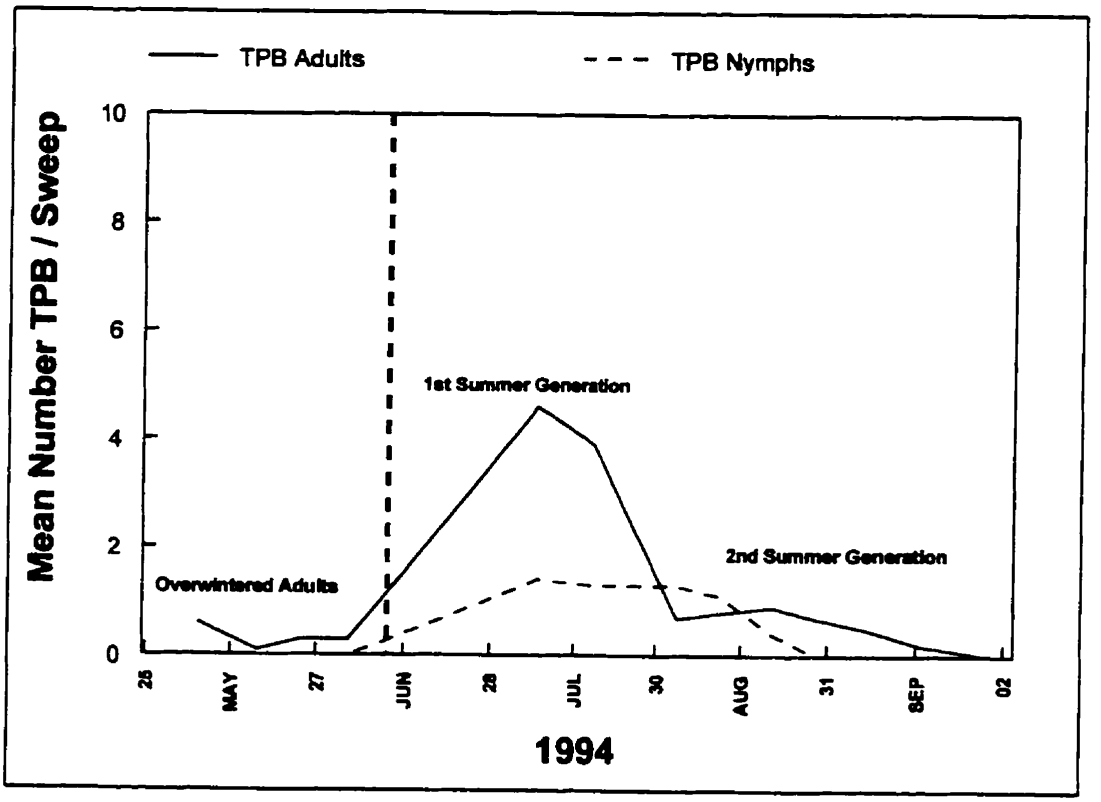

Bacterial Cell Number (Billions / mL)
0
1
2
3
4
5
6
7
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Y = 6.6858 + 1.663* LN(X)
R² = 0.939
Absorbance (550 nm)

Figure 5a. Seasonal population dynamics of tarnished plant bug adults and nymphs within an alfalfa field at Sheffield Farm, Sheffield Mills, Nova Scotia in 1994. The vertical dashed line represents the approximate time of switching from a field at the Atlantic Food and Horticulture Research Centre, Kentville, to the Sheffield field.

Figure 5b. Seasonal population dynamics of tarnished plant bug adults and nymphs within an alfalfa field at Sheffield Farm, Sheffield Mills, Nova Scotia in 1995.


TPB Adults
TPB Nymphs
Mean Number TPB / Sweep
10
8
6
4
2
0
1st Summer Generation
2nd Summer Generation
Overwintered Adults
25
MAY
27
JUN
28
JUL
30
AUG
31
SEP
02
1994

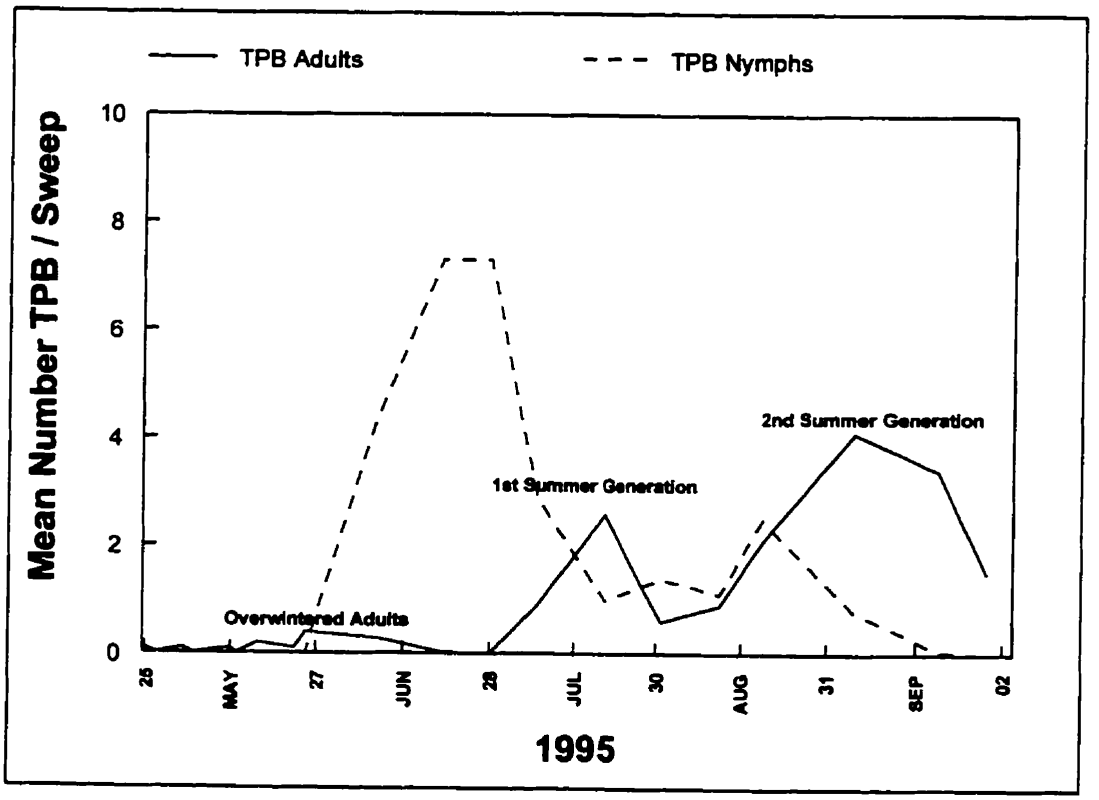

TPB Adults
TPB Nymphs
Mean Number TPB / Sweep
10
8
6
4
2
0
2nd Summer Generation
1st Summer Generation
Overwintered Adults
25
MAY
27
JUN
28
JUL
30
AUG
31
SEP
02
1995

Figure 5c. Seasonal population dynamics of tarnished plant bug adults and nymphs, and some predators, within an alfalfa field at Sheffield Farm, Sheffield Mills, Nova Scotia in 1996. Nym=Nymph, Damsel=Damsel Bugs, M.P. Bugs=Minute Pirate Bugs, T.S.S.B.=Two-spotted Stink Bug.

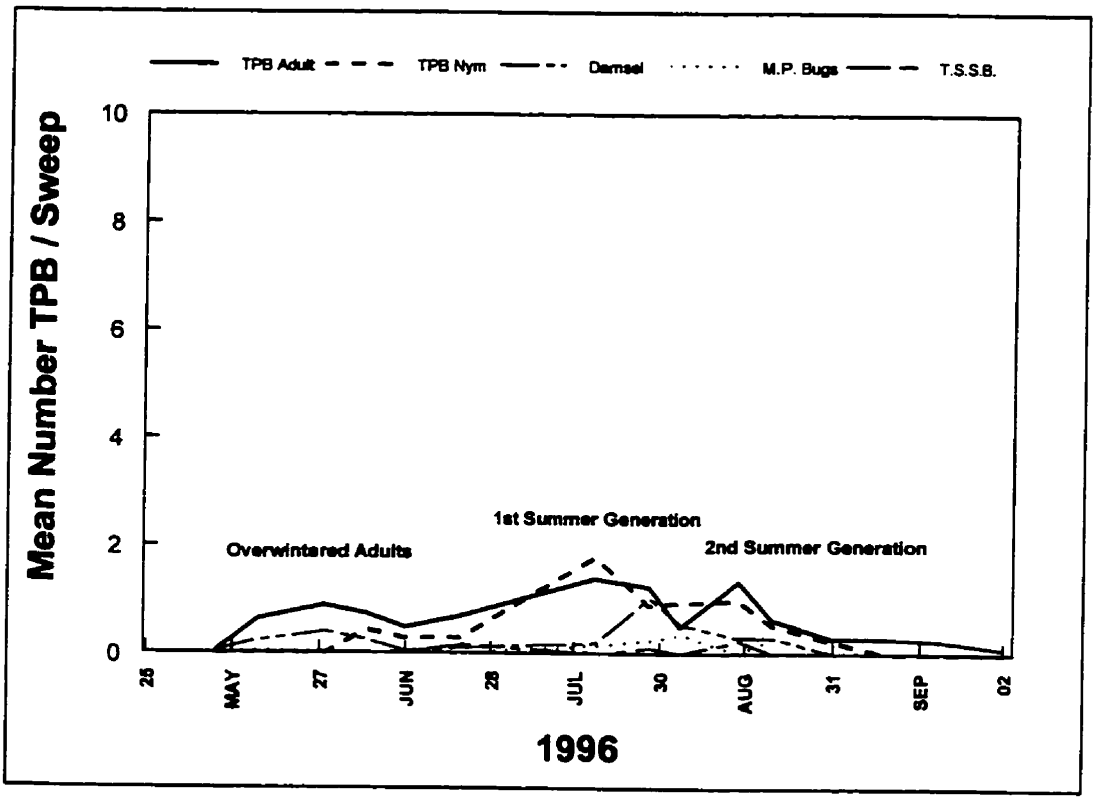

TPB Adult
TPB Nym
Damsel
M.P. Bugs
T.S.S.B.
Mean Number TPB / Sweep
10
8
6
4
2
0
Overwintered Adults
1st Summer Generation
2nd Summer Generation
25
MAY
27
JUN
28
JUL
30
AUG
31
SEP
02
1996

Figure 6. Mean (± S.E.) adult and nymph tarnished plant bug numbers within healthy and unhealthy areas of an alfalfa field at the Sheffield Farm, Sheffield Mills, Nova Scotia. Adult numbers were significantly less in the unhealthy area (Two sample T-Test, $T=4.09$, $DF=2$, $p<0.01$); no analysis could be done on nymphs.

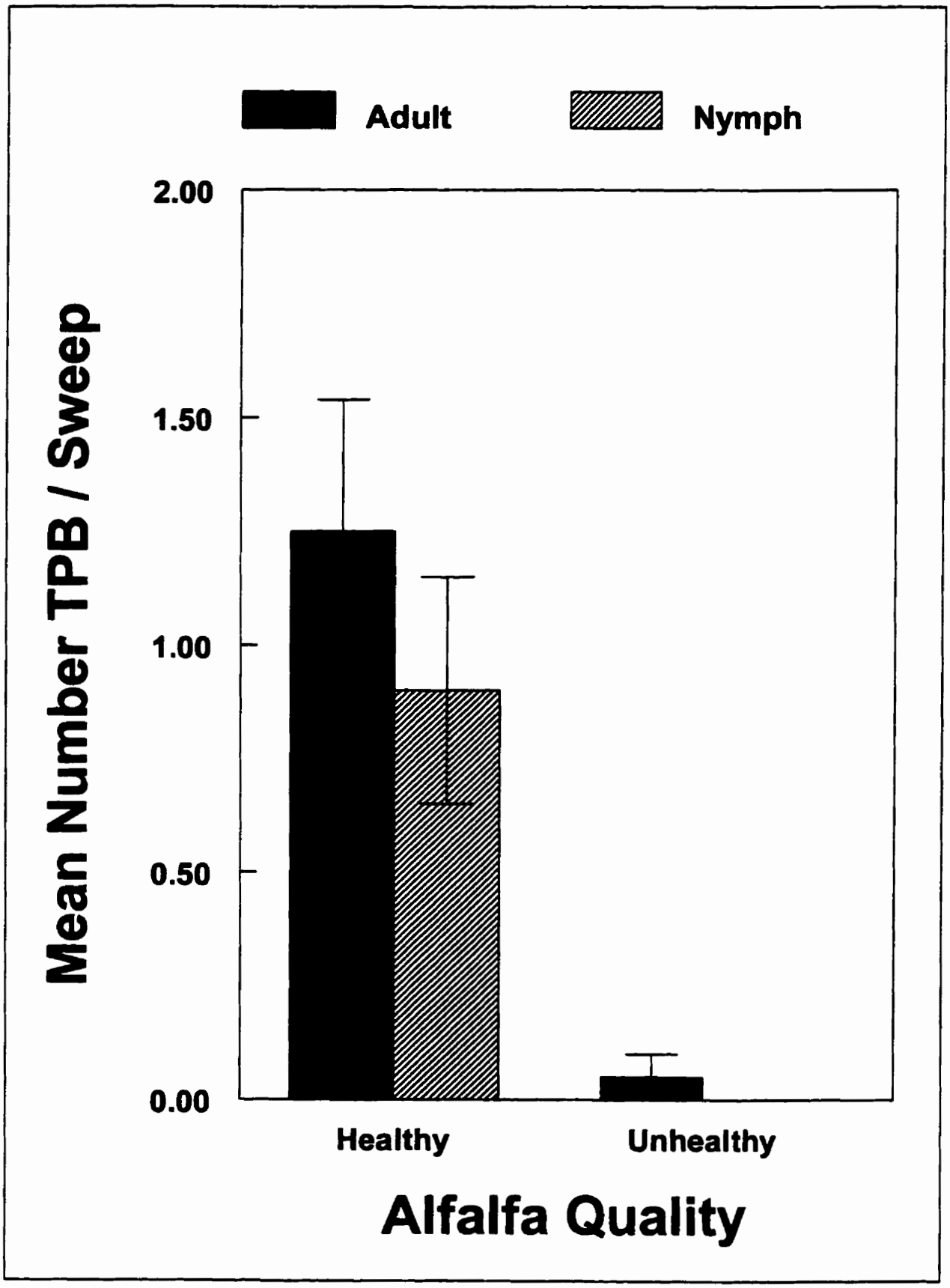

Adult
Nymph
2.00
1.50
1.00
0.50
0.00
Mean Number TPB / Sweep
Healthy
Unhealthy
Alfalfa Quality

Figure 7. Tarnished plant bug feeding injury at Stage 1 of fruit development (bloom-petal fall). Notice the dried sap at the edges of the penetration mark.

Figure 8. Tarnished plant bug feeding injury at Stage 2 of fruit development (calyx). The brown bruised area on the developing calyx indicates tissue penetration.

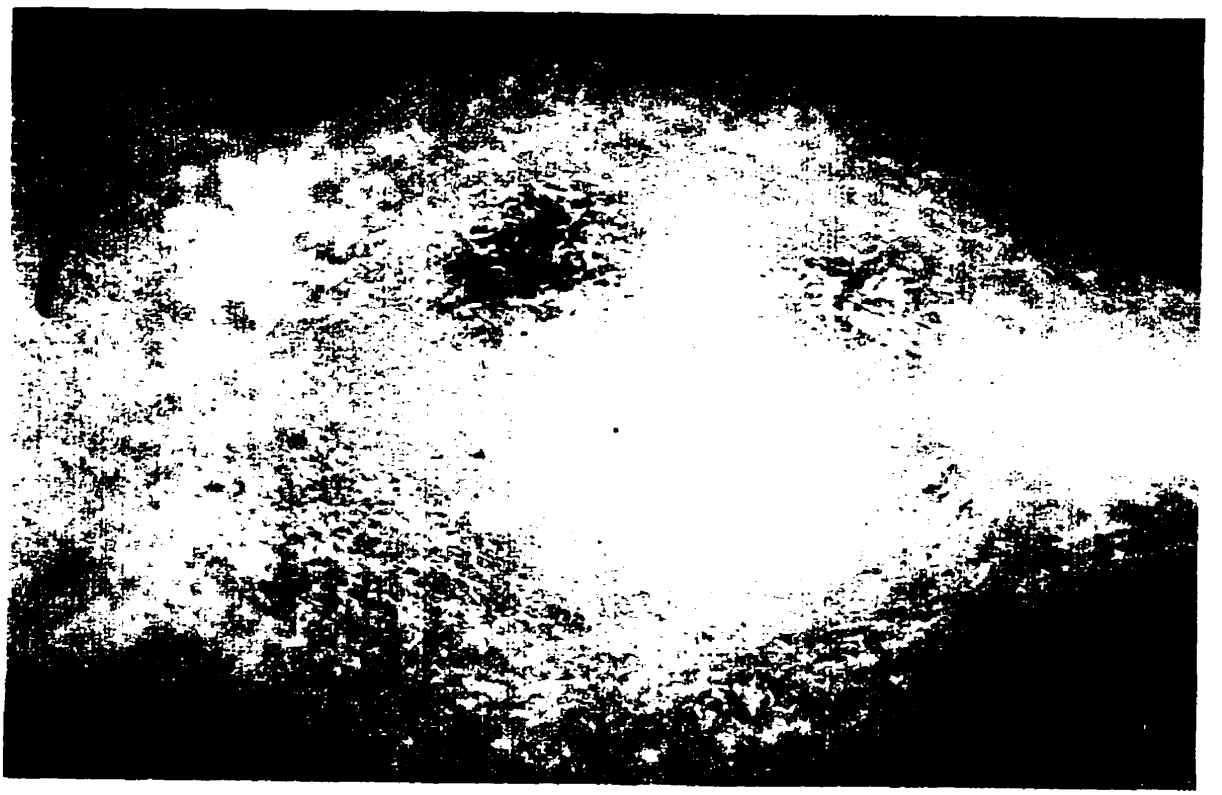


Figure 9. Tarnished plant bug feeding injury at Stage 3 of fruit development (1.5 cm -2 cm in diameter). Notice the dried sap at the edges of the penetration mark.

Figure 10. Typical "stinging bug" injury to apple, as seen at harvest.

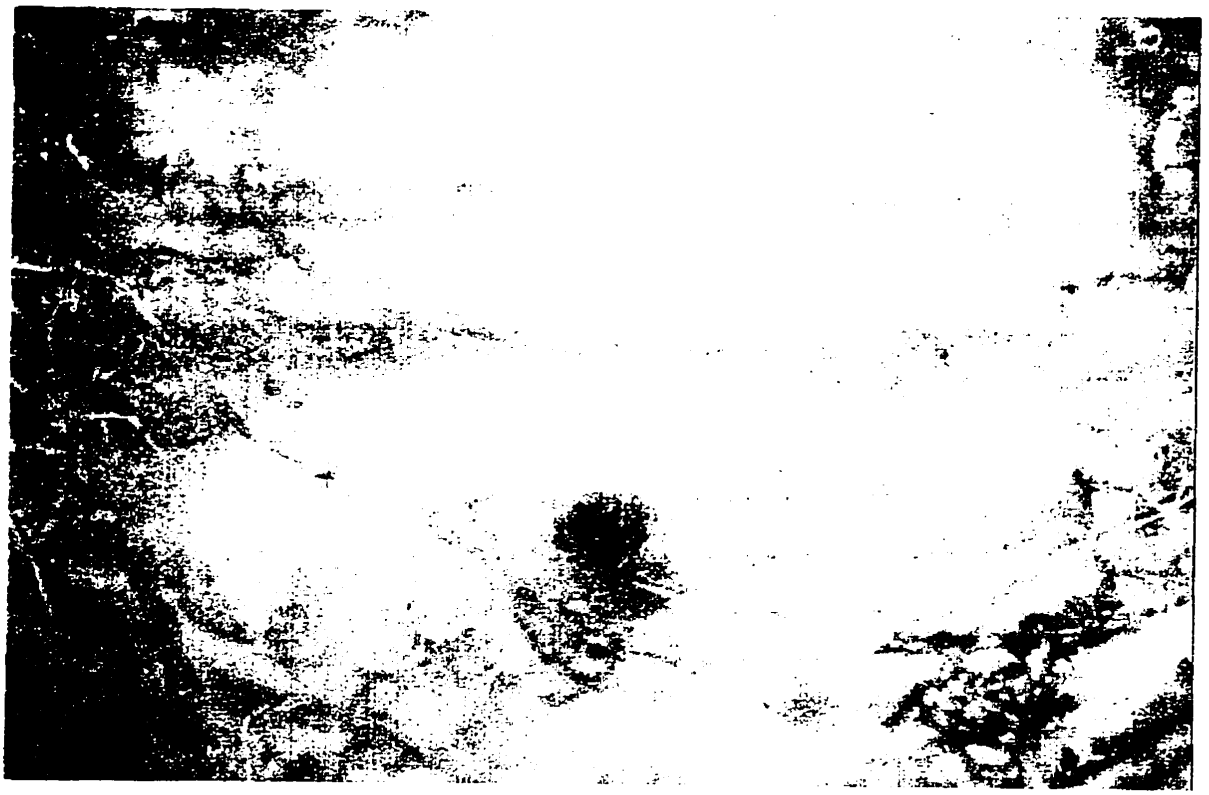
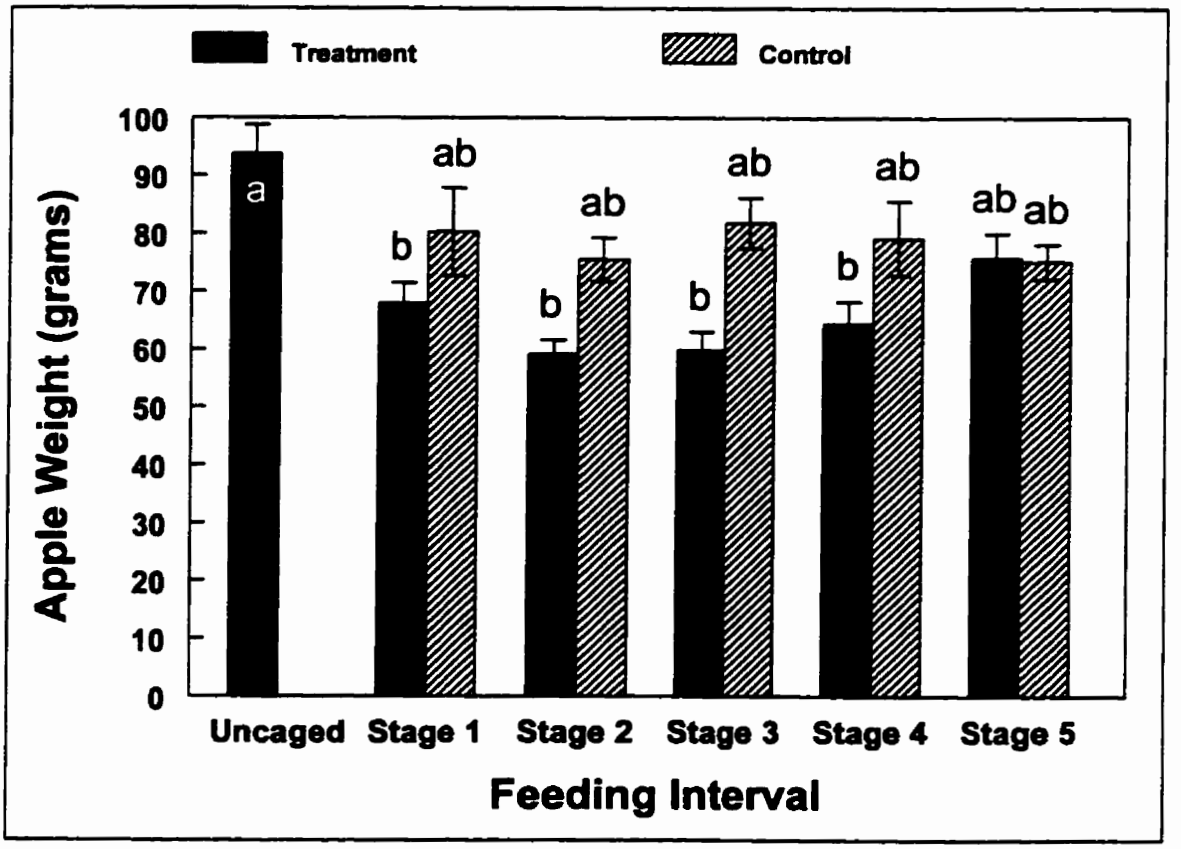


Figure 11a. Mean (± S.E.) weight (grams) of uncaged and caged control "McIntosh" apples, and treatment apples which received eight tarnished plant bugs for one week at different stages of fruit development. Bars with shared letters are not significantly different (Non-parametric multiple comparison, $Q_{0.05,11}=3.317$).

Figure 11b. Mean (± S.E.) weight (grams) of uncaged and caged control "Cortland" apples, and treatment apples which received eight tarnished plant bugs for one week at different stages of fruit development. Bars with shared letters are not significantly different (Non-parametric multiple comparison, $Q_{0.05,11}=3.317$).


Treatment
Control
Apple Weight (grams)
100
90
80
70
60
50
40
30
20
10
0
a
b
ab
b
ab
b
ab
b
ab
ab
ab
Uncaged
Stage 1
Stage 2
Stage 3
Stage 4
Stage 5
Feeding Interval

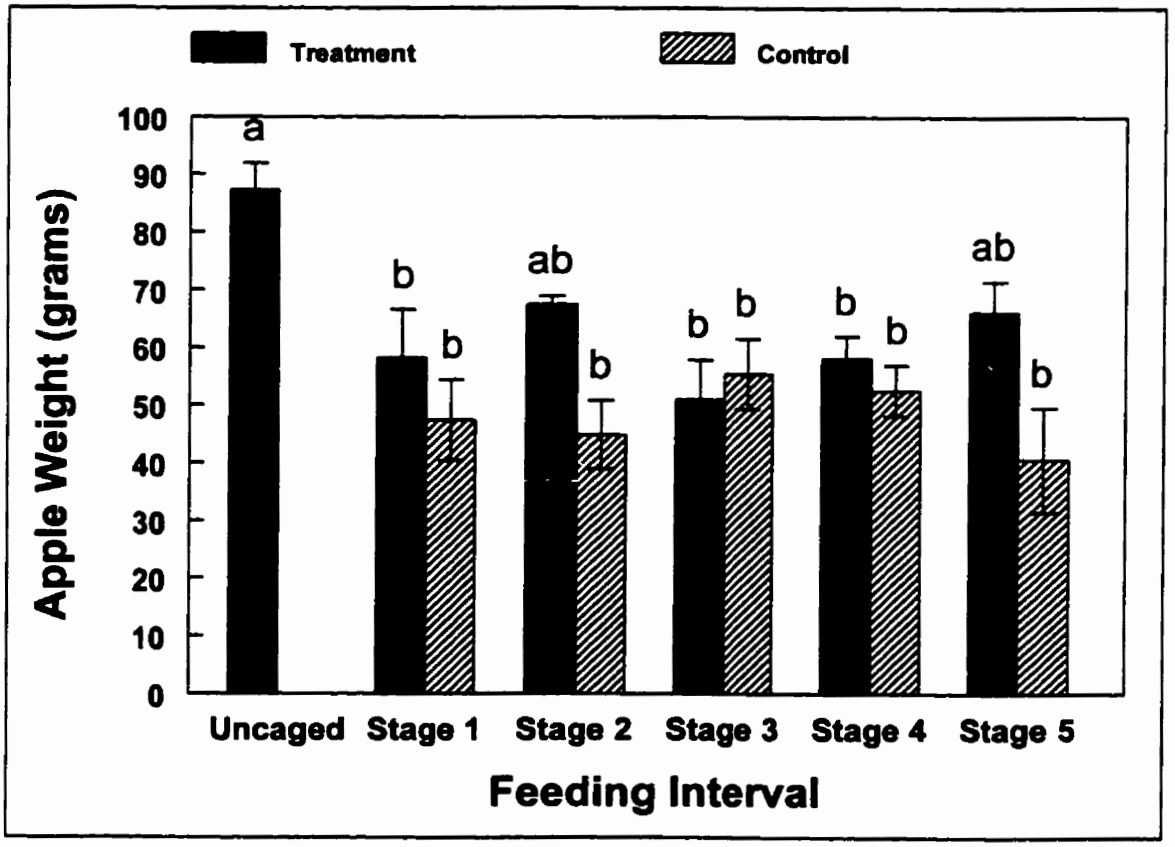
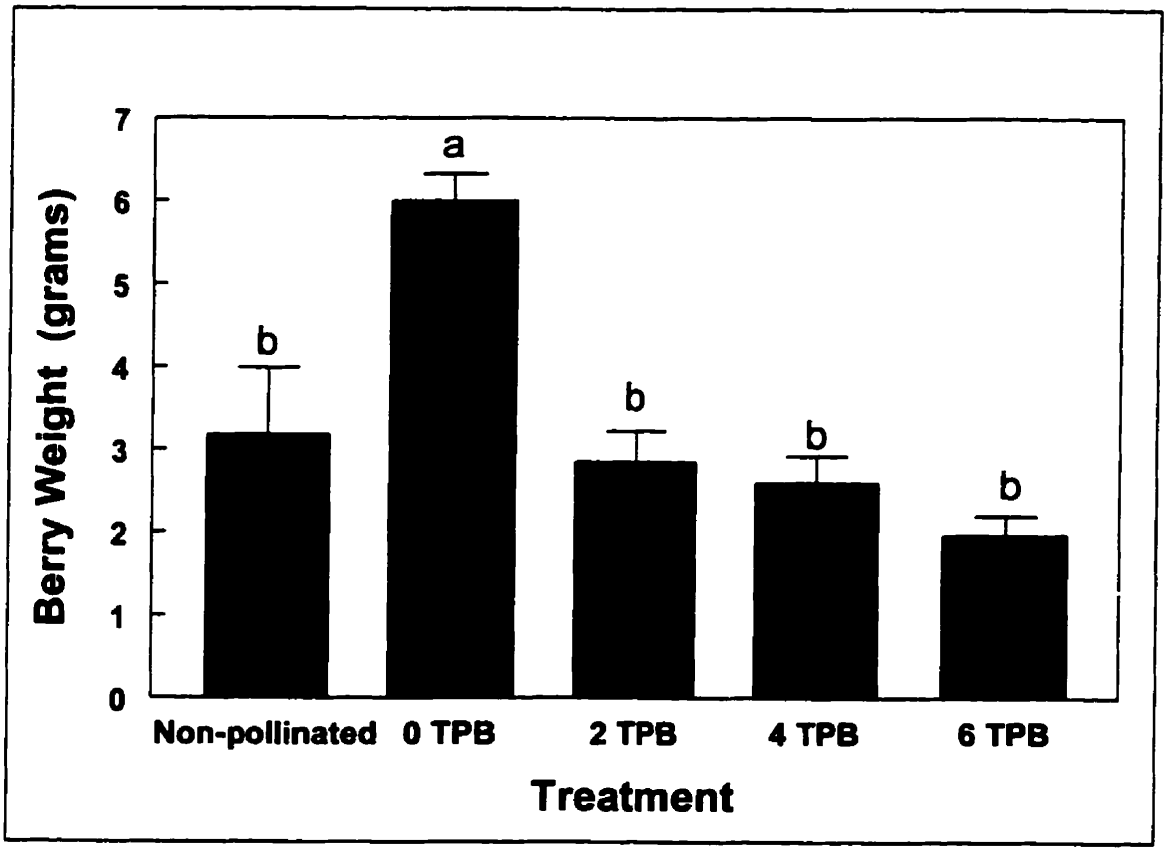

Treatment
Control
Apple Weight (grams)
100
90
80
70
60
50
40
30
20
10
0
a
b
b
ab
b
b
b
b
b
ab
b
Uncaged
Stage 1
Stage 2
Stage 3
Stage 4
Stage 5
Feeding Interval

Figure 12. Mean (± S.E.) strawberry weight (grams) for non-pollinated and pollinated controls, and three tarnished plant bug feeding densities. Bars sharing letters are not significantly different (Tukey, $F=17.54$, $DF=100$, $p<0.001$); square root transformation of data).

Figure 13. Frequency distribution of berry grade for four tarnished plant bug feeding densities. Grades of berries receiving Feeding densities of 2 ($Q=4.41$), 4 ($Q=4.97$), and 6 ($Q=6.48$) tarnished plant bugs were significantly poorer than the control, but not from one another (Non-parametric multiple comparison with adjustments for tied ranks and unequal sample size, $DF=4$, $p<0.001$).


Berry Weight (grams)
7
6
5
4
3
2
1
0
a
b
b
b
b
Non-pollinated
0 TPB
2 TPB
4 TPB
6 TPB
Treatment

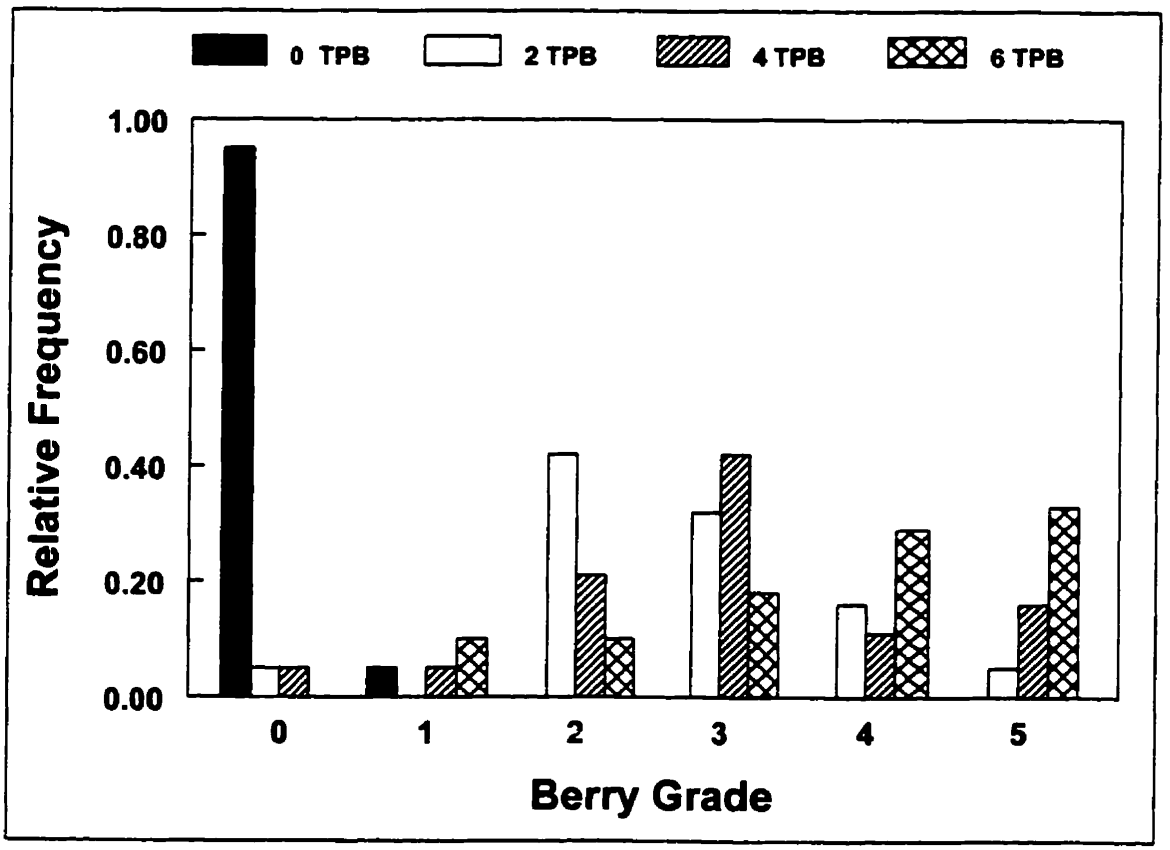

0 TPB
2 TPB
4 TPB
6 TPB
Relative Frequency
1.00
0.80
0.60
0.40
0.20
0.00
0
1
2
3
4
5
Berry Grade

Figure 14. A) A well pollinated, undamaged strawberry with an assigned grade of "0". Strawberries damaged by tarnished plant bug feeding; B) a grade of "1" showing apical seediness, C) a grade of "2", apical seediness and some deformity, D) a grade of "3", apical seediness and deformity, and E) a severely damaged berry, with a grade of "4".

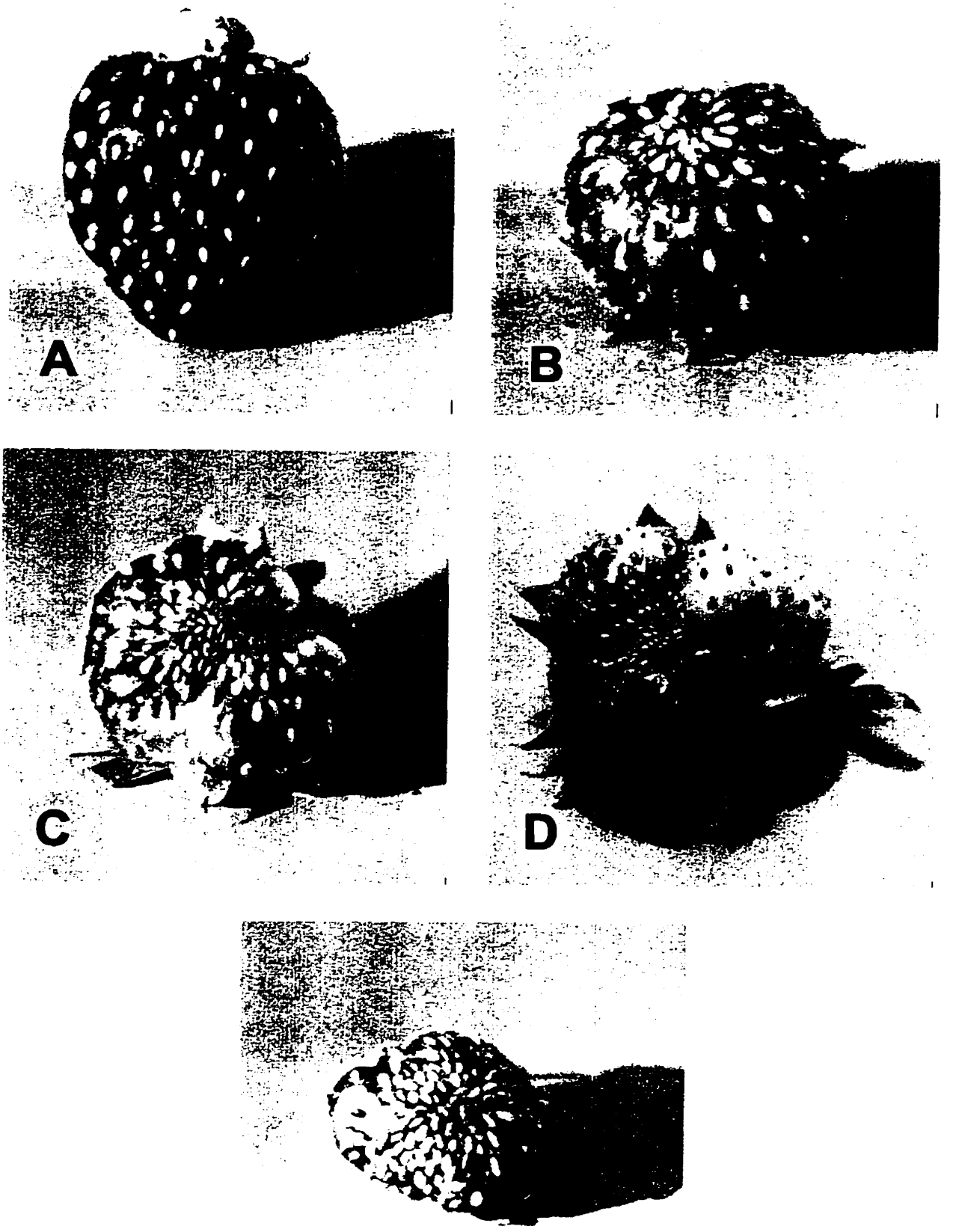

A
B
C
D
E

Figure 15. The apex of a well pollinated, undamaged strawberry.

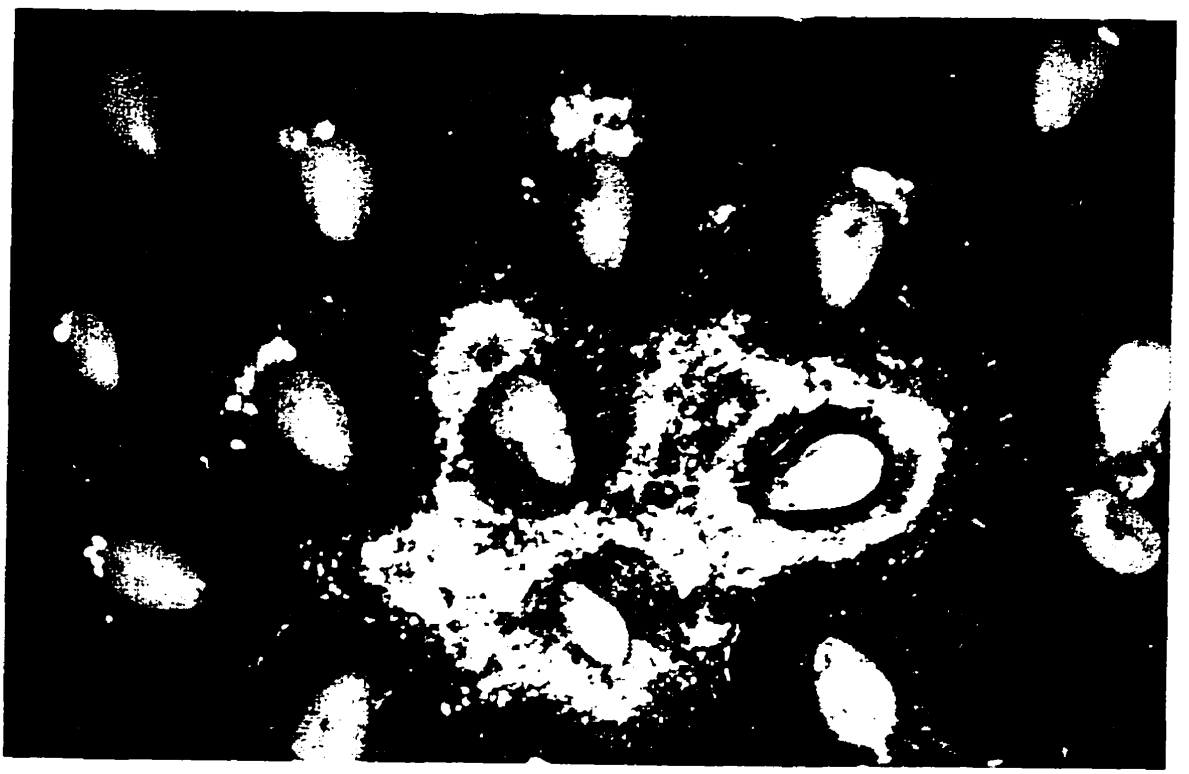


Figure 16a. A strawberry with apical seediness; damage caused by tarnished plant bug feeding. Notice that the achenes of damaged and undamaged areas are approximately the same size.

Figure 16b. A poorly pollinated strawberry. Notice the irregular shape of berry, and achenes of varying size.

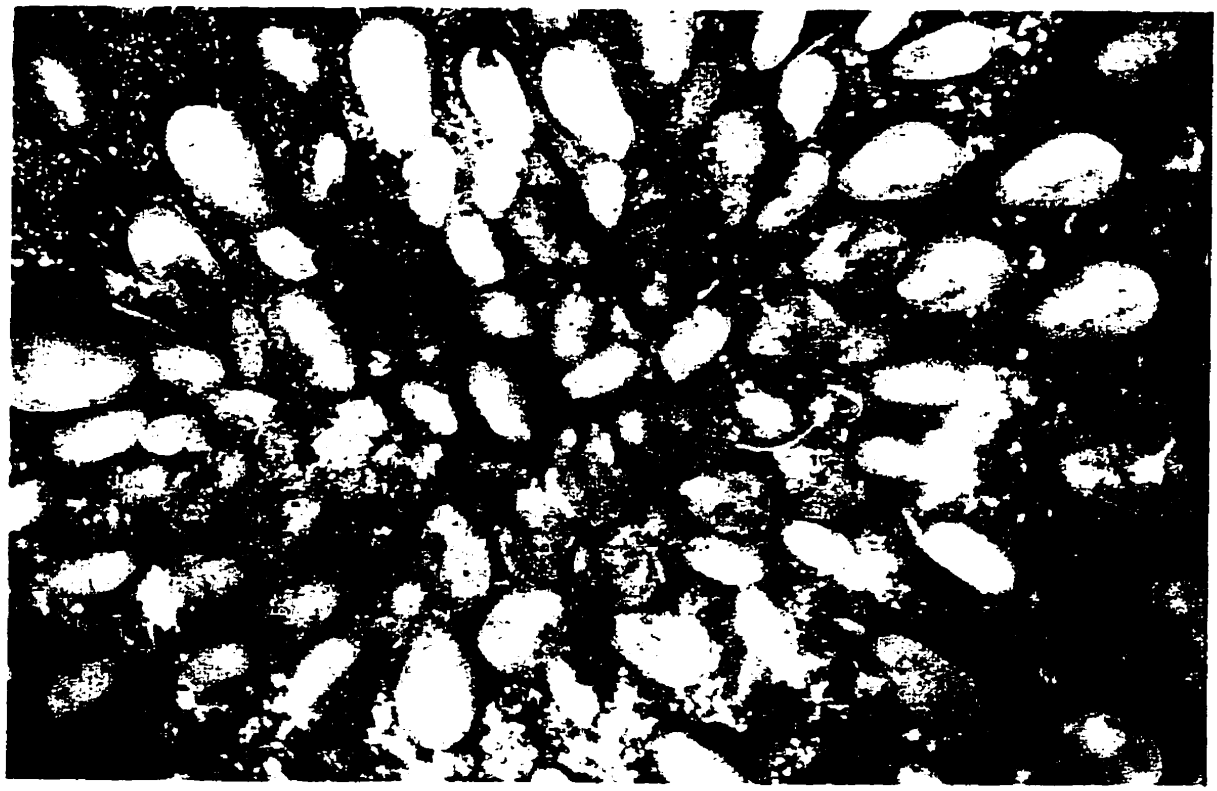


Figure 17. Mean (± S.E.) supercooling points (°C) for "summer" and "autumn" collected tarnished plant bugs acclimated for 4 weeks at the indicated conditions ("L" indicates photoperiod of 18:6 light: dark, "S", a photoperiod of 12:12 light: dark). Bars which share letters are not significantly different (Tukey, F= 15.19, p=0.000, df=352).

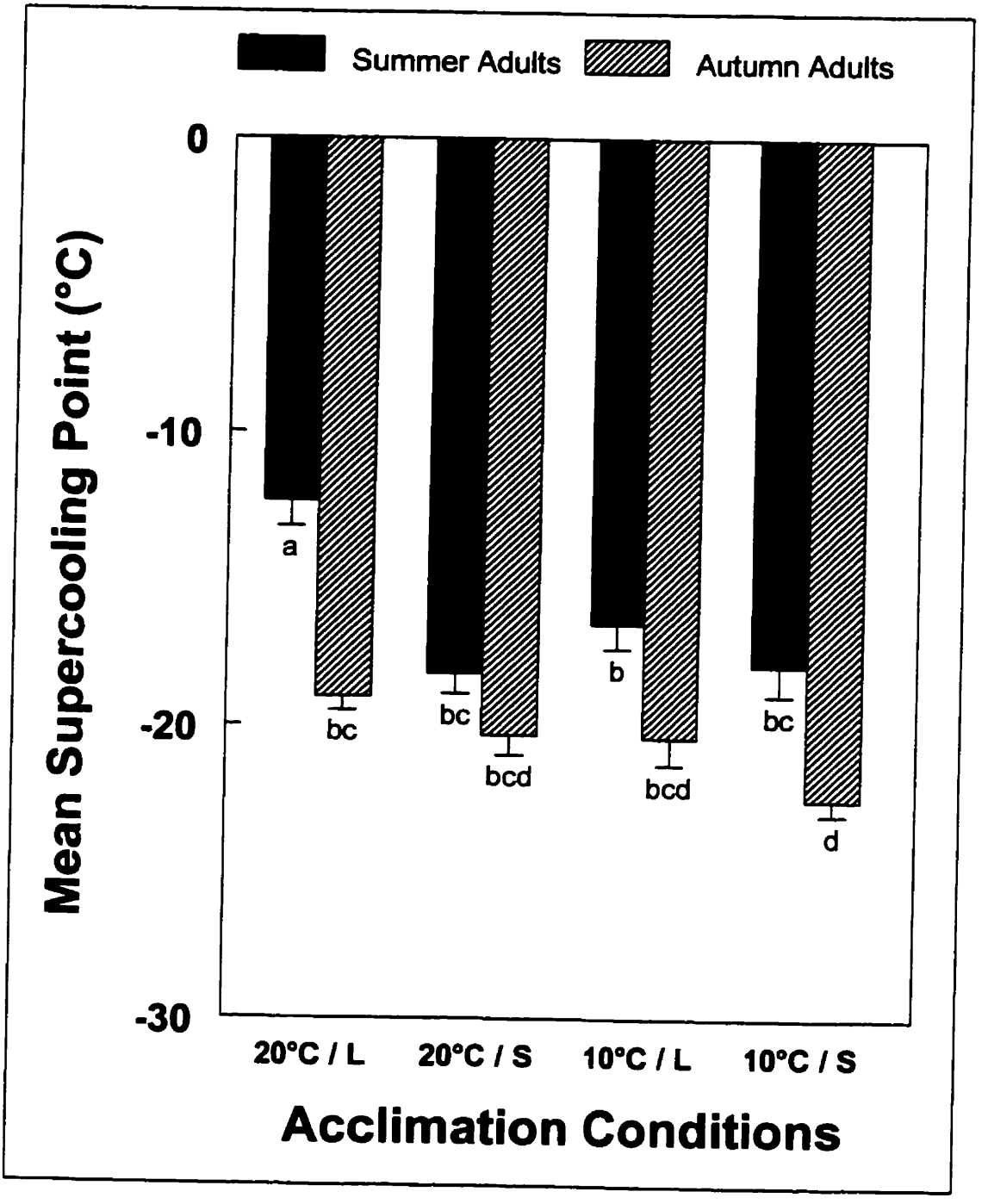
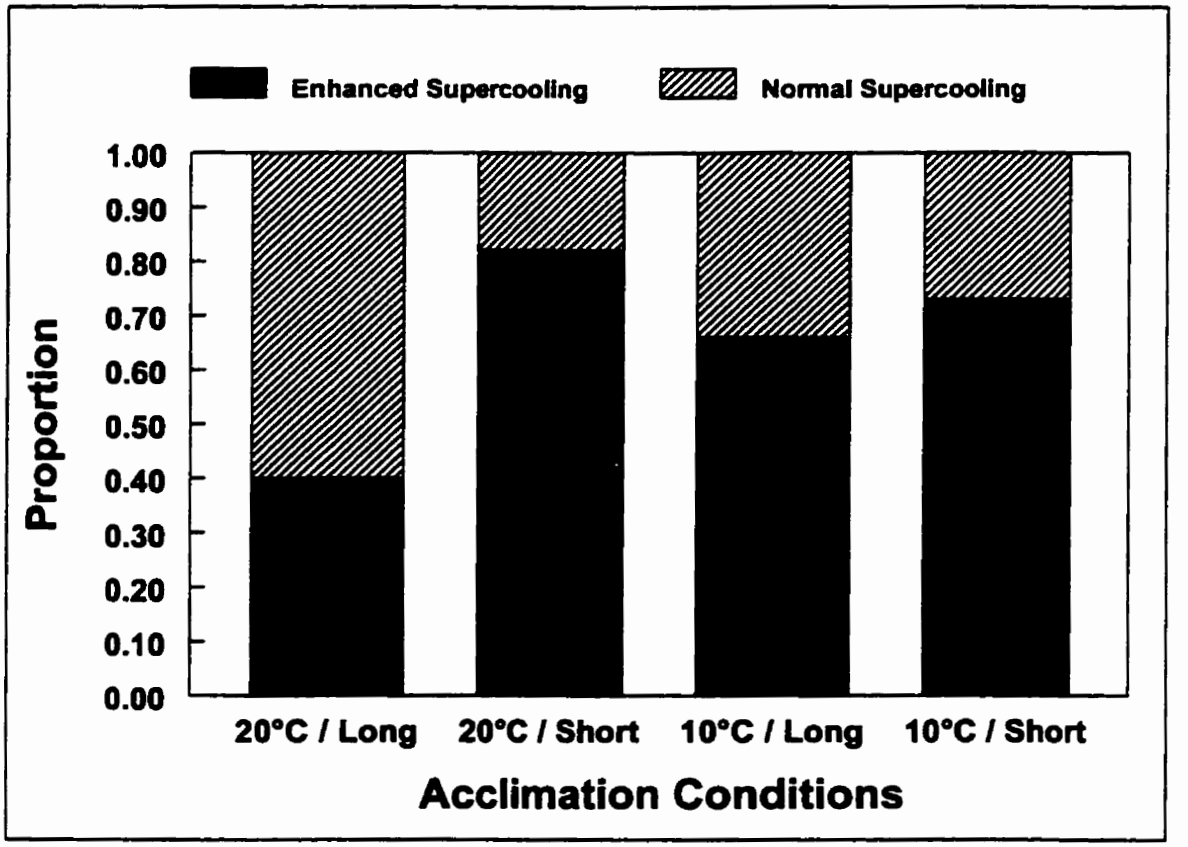

Summer Adults
Autumn Adults
0
-10
-20
-30
Mean Supercooling Point (°C)
a
bc
bc
bcd
b
bcd
bc
d
20°C / L
20°C / S
10°C / L
10°C / S
Acclimation Conditions

Figure 18a. The proportion of cold hardy "summer" adult tarnished plant bugs, as indicated by their individual SCP's ("Long" indicates photoperiod of 18:6 light: dark, "Short", a photoperiod of 12:12 light: dark). A critical value of -14°C was used; TPB's with SCP's above this were considered not cold-hardy, those with SCP's below this were cold-hardy. TPB's acclimated at 20°C / Long were significantly less "cold-hardy" than all others (Chi-square analysis, $x^2=15.41$, DF=1, $p<0.001$).

Figure 18b. The proportion of cold hardy "autumn" adult tarnished plant bugs, as indicated by their individual SCP's ("Long" indicates photoperiod of 18:6 light: dark, "Short", a photoperiod of 12:12 light: dark). A critical value of -14°C was used; TPB's with SCP's above this were considered not cold-hardy, those with SCP's below this were cold-hardy. No difference in "cold-hardiness" was found between any of the acclimation conditions.


Enhanced Supercooling
Normal Supercooling
Proportion
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
20°C / Long
20°C / Short
10°C / Long
10°C / Short
Acclimation Conditions

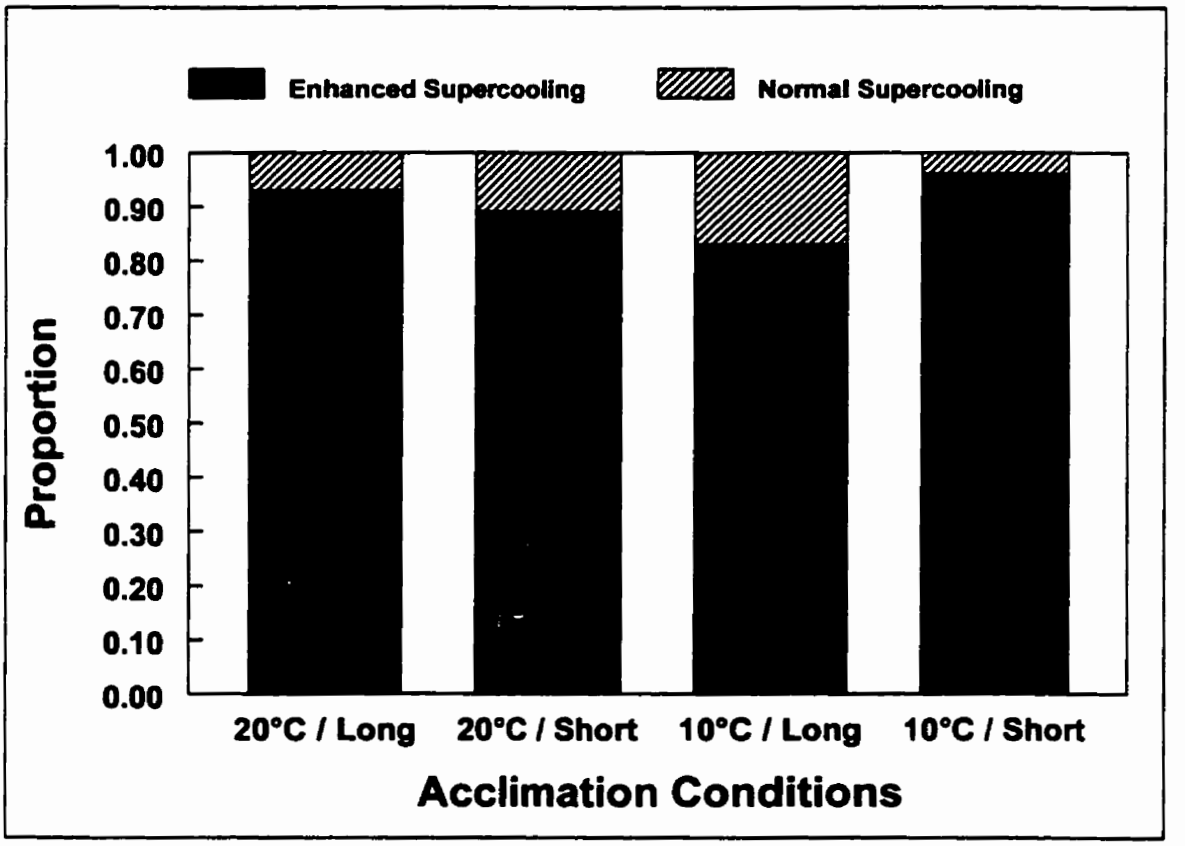

Enhanced Supercooling
Normal Supercooling
Proportion
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
20°C / Long
20°C / Short
10°C / Long
10°C / Short
Acclimation Conditions

Figure 19. Daily minimum ambient temperatures (°C) from Kentville, N.S. (Agriculture and Agri-Food Canada Weather station) for 1995, and approximate seasonal supercooling profile for the tarnished plant bug.

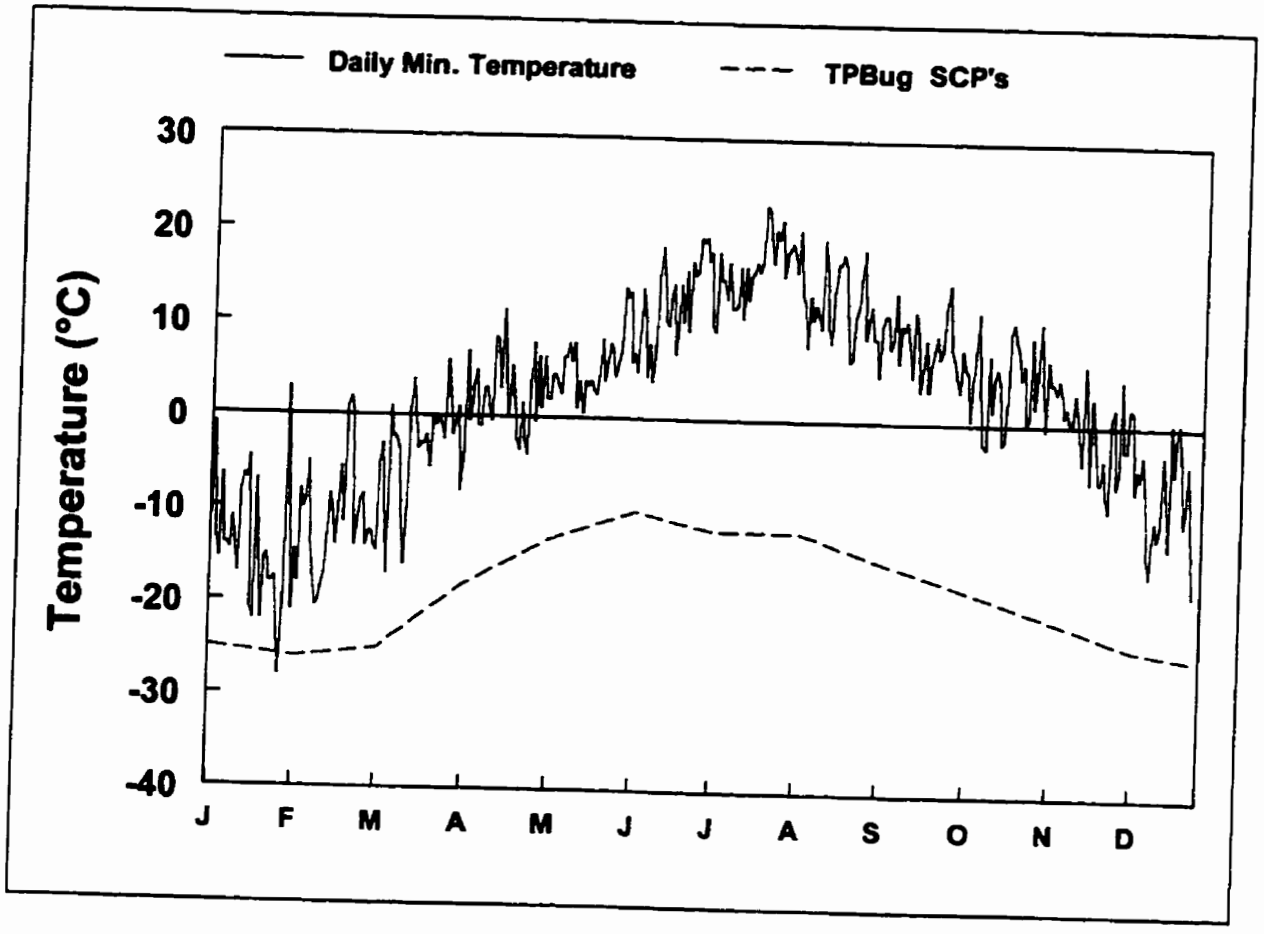

Daily Min. Temperature
TPBug SCP's
Temperature (°C)
30
20
10
0
-10
-20
-30
-40
J
F
M
A
M
J
J
A
S
O
N
D

Figure 20. The mean (± S.E.) supercooling points (°C) of *Pseudomonas syringae* solutions of various concentrations. **A**= water, **B**=300, **C**=30,000, **D**=75,000, **E**=150,000, **F**=260,000, **G**=330,000, **H**=3,300,000, **I**= 16,500,000, **J**=165,000,000, **K**= 330,000,000, **L**=660,000,000, **M**=1,300,000,000, **N**= 2,600,000,000, and **O**=5,300,000,000 cells per mL respectively. Bars which share letters are not significantly different (Tukey, $F=67.96$, $DF=89$, $p<0.001$).

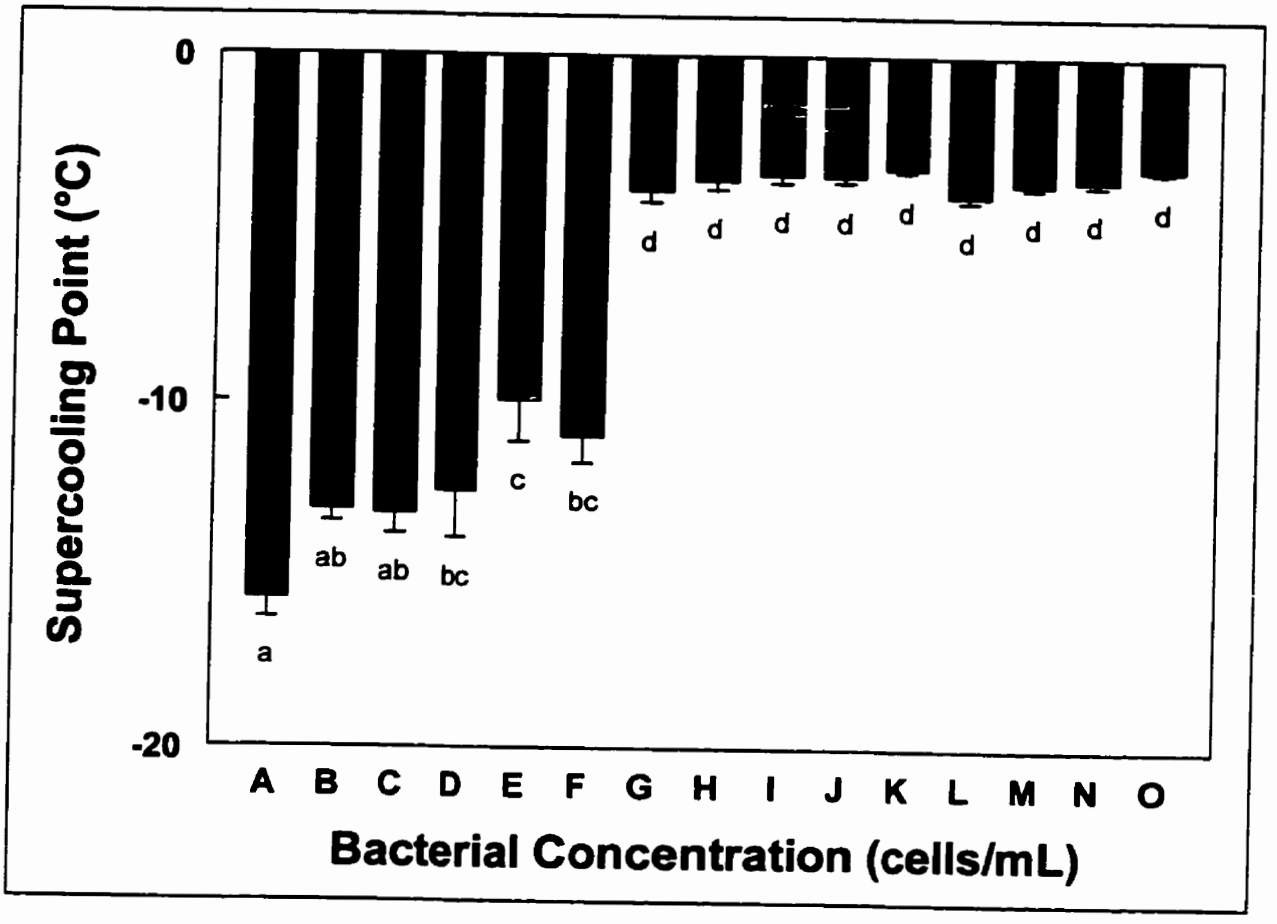

0
-10
-20
Supercooling Point (°C)
a
ab
ab
bc
c
bc
d
d
d
d
d
d
d
d
d
A B C D E F G H I J K L M N O
Bacterial Concentration (cells/mL)

Figure 21. Mean (± S.E.) supercooling points (°C) for TPB's sprayed with an INA spray (containing $1x10^9$ *Pseudomonas syringae* cells per mL) and water, at 1, 3, and 7 days after spraying. TPB's sprayed with the INA solution had SCP's which were significantly higher than those sprayed with water on all days tested (Non-parametric two-factor Analysis of Variance, $H=25.11$, $DF=1$, $p<0.001$), and the spray was equally effective on all days tested ($H=0.0483$, $DF=2$, $p>0.05$).

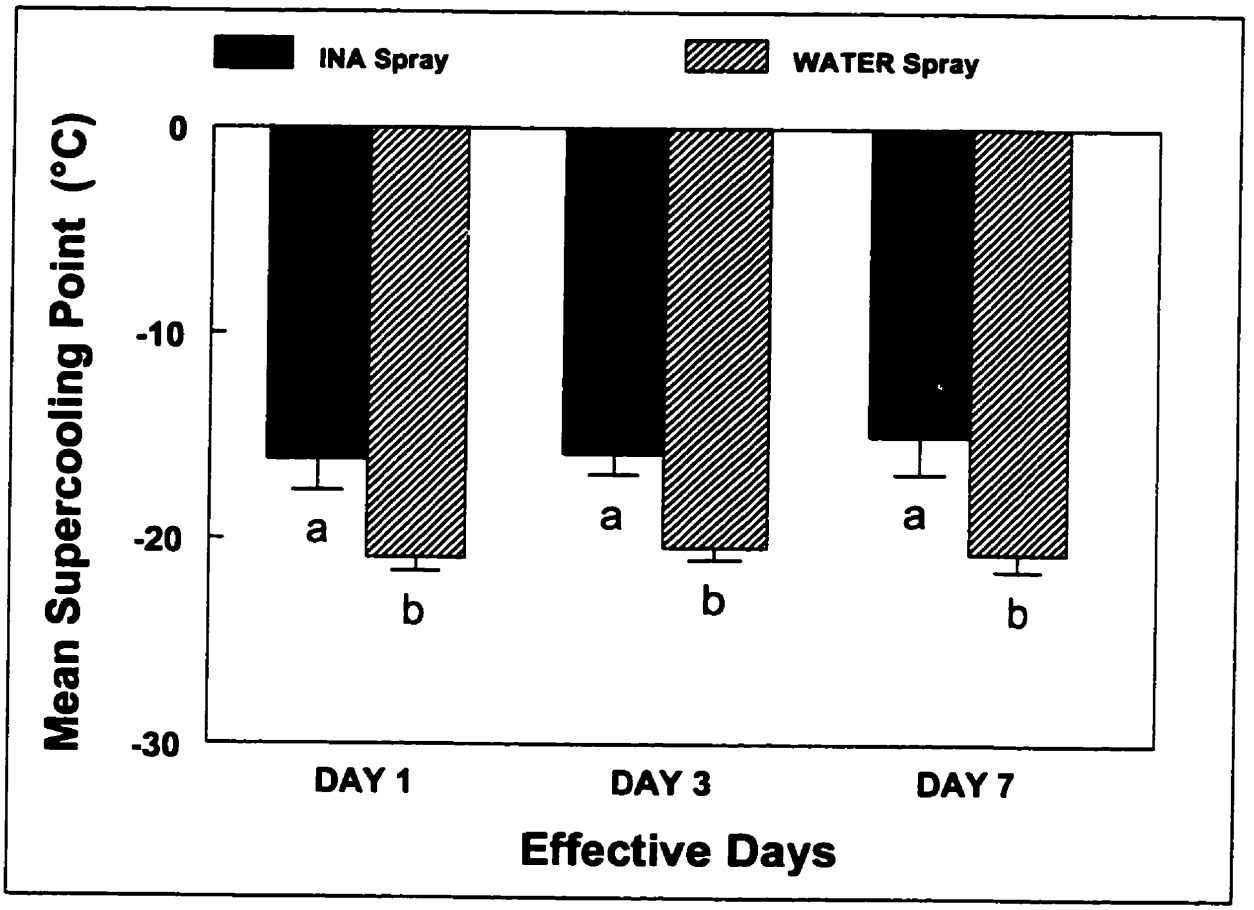
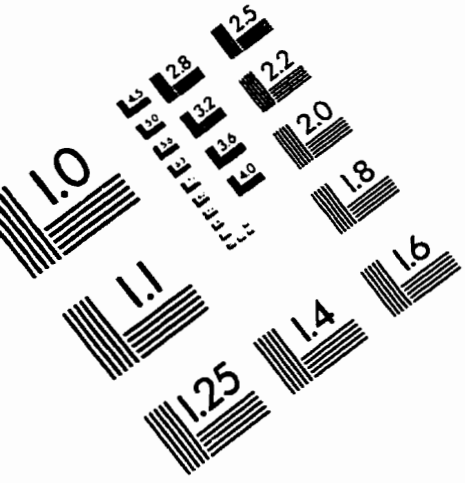
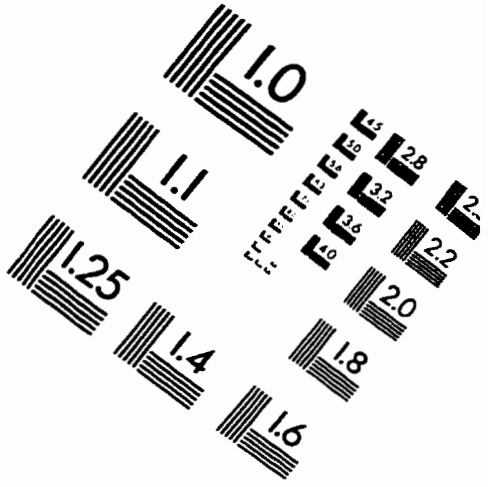
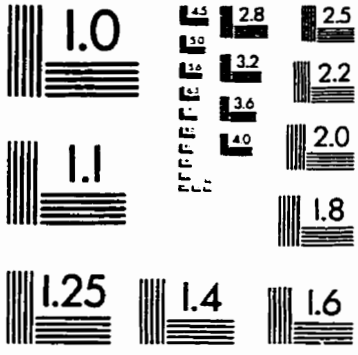
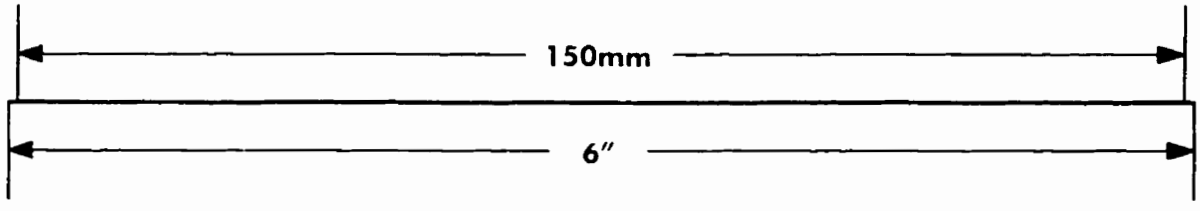

INA Spray
WATER Spray
Mean Supercooling Point (°C)
0
-10
-20
-30
a
b
a
b
a
b
DAY 1
DAY 3
DAY 7
Effective Days

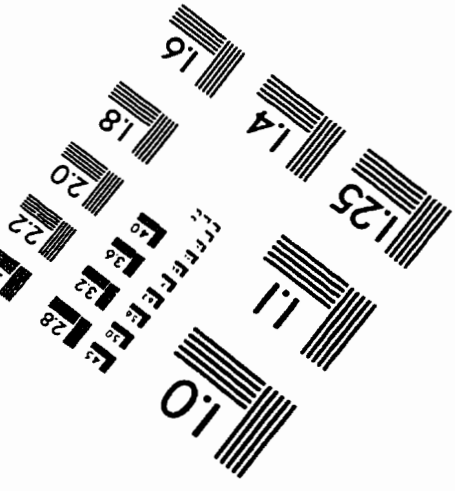
IMAGE EVALUATION TEST TARGET (QA-3)

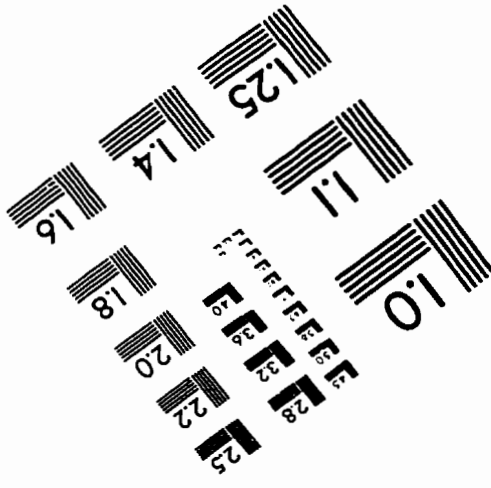












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989