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DEICING SALT INJURY TO Highbush Blueberry  
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**DEICING SALT INJURY TO Highbush Blueberry (*Vaccinium corymbosum*  
L.) in West Michigan**

**By**

**Steven F. Berkheimer**

**A THESIS**

**Submitted to  
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## ABSTRACT

### DEICING SALT INJURY TO Highbush Blueberry (*Vaccinium corymbosum* L.) IN WEST MICHIGAN.

By

Steven F. Berkheimer

Varying rates of flower bud mortality on blueberries growing adjacent to roads in West Michigan have been observed since the early to mid-1990s; the purpose of these studies was to investigate the influence of deicing salt (primarily sodium chloride, NaCl) to this injury. Injury was surveyed in the spring on 12 farms by recording flower bud mortality at numerous locations, and mapped graphically with GPS technology. The most severe injury occurred closest to heavily traveled roads. Potted plants sprayed with NaCl or receiving soil applications exhibited similar symptoms observed on roadside plants, with injury severity proportional to the NaCl concentration. Salts rinsed from twigs sampled prior to each successive spray were in the same range as salts rinsed from twigs sampled from field-grown plants in the winter months. The highest soil salt levels killed most above ground growth, suggesting that injury in the field results primarily from salt deposition on buds and twigs.

Twigs were also excised from salt treated and control branches and frozen incrementally to measure LT<sub>50</sub>. Salt exposure (e.g., chloride salts [NaCl, KCl, CaCl<sub>2</sub>, MgCl<sub>2</sub>] and sodium salts [NaCl, NaAC]) reduced the LT<sub>50</sub> of flower buds by as much as 11.0 °C relative to the control, even within 7 hours of treatment.

## **DEDICATION**

**This work is dedicated to the memory of Francis Tucker Berkheimer (1937 – 1984) and  
Michael Kiçkova (1913 – 1978). I miss you both very much.**

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## **REVIEW OF LITERATURE**

Many roads in northern areas are treated with deicing salts, primarily NaCl. Salting maintains safe travel surfaces during the winter, but this practice also may damage roadside vegetation in the northern U.S. (Davidson, 1970; Sucoff, 1975; Sucoff et al., 1975a; Bowers and Hesterberg, 1976; Simini and Leone, 1986; Herrick, 1988; Bryson and Barker, 2002;), Canada (Hofstra and Hall, 1971; Hofstra et al., 1979; Gibbs and Palmer, 1994;), the U.K. (Gibbs and Burdekin, 1986; Thompson et al., 1986; Gibbs and Palmer, 1994;), and European countries (Gibbs and Burdekin, 1983; Buschbom, 1980; Bogemans et al., 1989; Pedersen et al., 2000; Paludan-Muller et al., 2002). Deicing salt usage may also result in concrete spalling (Amerhein et al., 1992; Shanley, 1994;), vehicular corrosion (van de Voorde and van Dijck, 1973; Buschbom, 1980; Amerhein et al., 1992; Shanley, 1994; U.S. E.P.A., 1999; Robidoux and Delisle, 2001), increased Cl<sup>-</sup> levels in surface waters (Zelazny et al., 1970; van de Voorde and van Dijck, 1973; Peters and Turk, 1981; Wilcox, 1986; Demers and Sage, 1990; Amerhein et al., 1992; Shanley, 1994; Robidoux and Delisle, 2001) and damage to bridges (Amerhein et al., 1992; U.S. E.P.A., 1999; Robidoux and Delisle, 2001).

Deicing salts may injure plants by runoff and salt loading of the soil (Langille, 1976; Thompson and Rutter, 1986) or when salt-laden spray or dust is generated by traffic and deposited directly on plants by wind (Sucoff, 1975). High soil salt levels tend to occur in close proximity to roadways (Bowers and Hesterberg, 1976). The consequences of high soil salt levels are well understood since saline soils limit crop production in many arid areas of the world (Kaya and Higgs, 2002; Borsani et al., 2003). Agricultural production on high salt or saline soils requires the use of tolerant species and

genotypes and careful water management (Maas, 1986). Less well understood is how elevated soil salt levels affect the plant community structure of the roadside environment, as well as regeneration of this community (Bicknell and Smith, 1975).

This work is focused on the effect of wind-blown salt spray on blueberries in Michigan. Eaton et al. (1999, 2002) and Percival et al. (2002) described the effects of deicing salts on lowbush blueberry. Damage to fruit crops other than blueberry has been reported on apple (Hofstra and Lumis, 1974) and peach (Northover, 1987). Understanding the nature of deicing compounds and the conditions under which they function best can help to understand where and when they should be applied.

### Characteristics of Deicing Salts

In general, deicing salts work by depressing the freezing point and turn ice and snow into slush. Solid salts (i.e., NaCl, CaCl<sub>2</sub> pellets, et al.) 'bore' through ice and snow and produce a concentrated brine solution that spreads beneath the ice or packed snow and breaks the bond to the pavement. Loosened ice and snow is easily removed by plows or traffic.

Deicing agents must be water soluble to lower freezing points. Calcium chloride (CaCl<sub>2</sub>) is approximately 1.5 times more soluble than NaCl at 0 °C, has a lower freezing point and will thus melt more ice. Both NaCl and CaCl<sub>2</sub> are deliquescent (Roth and Wall, 1976). Calcium chloride generates more heat of solution (680.3 kJ·kg<sup>-1</sup>) than NaCl (102.8 kJ·kg<sup>-1</sup>). Due to their individual chemical properties, the efficacy of these two salts varies under different environmental conditions (Roth and Wall, 1976). Sodium chloride is only effective above 7 °C, whereas CaCl<sub>2</sub> works best below this temperature (Roth and Wall, 1976). Calcium chloride is more effective over a broad range of temperatures, it reacts

and achieves results more quickly than NaCl, but NaCl remains effective over longer periods of time (Table 1).

Rock salt is 94 to 97% NaCl, and contains about 39%  $\text{Na}^+$  and 61%  $\text{Cl}^-$  (Schraufnagel, 1967). Calcium chloride contains about 36% calcium ( $\text{Ca}^{++}$ ) and 64%  $\text{Cl}^-$ . Sodium chloride returns readily to a solid form, forming a residue of white dust that can become airborne with traffic movement and high winds. Calcium chloride tends to remain as brine that is resistant to movement by traffic and wind. This could mean that under certain circumstances  $\text{CaCl}_2$  is more likely to run off into drains and ditches rather than drift onto roadside vegetation.

Calcium magnesium acetate was first identified by the U.S. Highway Dept. as a possible deicing material in the late 1970s (MDOT, 1993). It is produced from dolomitic lime and acetic acid. CMA has low corrosive potential, and is frequently blended with NaCl at rates of 20% or more CMA to reduce corrosion.

Magnesium chloride attacks concrete more slowly than NaCl, and when used as labeled, it reportedly does not harm vegetation (MDOT, 1993). However, it contains 17 - 56% more  $\text{Cl}^-$  than other salt deicers, and 51% water. It leaves no residue and melts ice down to  $-15^\circ\text{C}$ . Magnesium chloride begins deicing rapidly after application like  $\text{CaCl}_2$ , but it may become diluted and lose efficacy (MDOT, 1993).

Urea and potassium chloride (KCl) have similar deicing properties. Both do not attack concrete by way of a chemical reaction. Urea works to  $-9^\circ\text{C}$ , and KCl works to  $-11^\circ\text{C}$ , thus, they must be used at slightly higher rates. Urea has been used on airport runways for deicing purposes. Sodium acetate is also known as 'solid runway deicer', and is exothermic (evolves heat while melting ice). Sodium acetate has low toxicity, is

non-persistent and biodegradable, and has less impact on the biochemical oxygen demand than urea. This deicer is active down to -18 °C and does not track indoor like other deicers.

### Toxicity of Deicing Compounds

Information on the relative toxicities of various deicing compounds to woody plants is limited. The majority of studies dealing with salt injury to plants have been conducted with NaCl, the most widely used deicer. However, the effects of NaCl and CaCl<sub>2</sub> on woody vegetation have been compared (Holmes, 1961; Walton, 1969; Bogemans et al., 1989). Calcium chloride appears to be more phytotoxic than NaCl when applied to the roots of Norway maple (*Acer platanoides*) during the dormant season (Walton, 1969). Injury was attributed to direct chloride toxicity as well as other factors that induced water stress in the trees. An earlier study (Holmes, 1961) also compared the effects of CaCl<sub>2</sub> and NaCl on six deciduous and evergreen tree species. Little phytotoxicity was observed when these salts were applied to the roots over six years. While a few trees exhibited damage symptoms, most appeared healthy, leading the author to conclude that winter deicing practices in Massachusetts as practiced at the time probably does little harm to trees. The author conceded that the applied salt solutions may have run off and away from the tree roots due to frozen soil in the winter months, thus sparing the tree of salt injury.

When equal doses of Cl<sup>-</sup> were applied to the roots of spruce trees, uptake of Cl<sup>-</sup> was inhibited when Ca<sup>++</sup> was present (Bogemans et al., 1989). Consistent with Dirr (1975), these authors found that shoot and needle Cl<sup>-</sup> concentrations correlated well with visual damage symptoms. Twig Cl<sup>-</sup> levels of 0.8%, and Na<sup>+</sup> levels of 0.5% typically

arrested growth in the 12 deciduous and coniferous species tested (Lumis et al., 1976). Although  $\text{Cl}^-$  is preferentially accumulated in plant cells over  $\text{Na}^+$ ,  $\text{Na}^+$  may also reach toxic concentrations inside plant cells (Dirr, 1976).

### Chemical Properties of Sodium Chloride

The salt content of seawater is about 3.3%, and may range as low as 1.9%, depending on location (Schraufnagel, 1967). Total annual salt production in the U.S. is about 43 million tons per year, and 75% of this is used to make other compounds, such as chlorine gas; hydrochloric acid (HCl); and sodium hydroxide (NaOH). For human consumption, the oceans could provide us with our needs, but rock salt or halite from underground deposits is the other principal salt source.

Sodium chloride has a molecular weight of 58.4 g, melting point of 804 °C, the density is 2.17  $\text{g mL}^{-1}$ , and a solubility of 36  $\text{g } 100\text{mL}^{-1} \text{ H}_2\text{O}$  (Moxley, 1973). The eutectic temperature (lowest possible freezing point of a saturated solution) is -21 °C.

Chlorine ( $\text{Cl}_2$ ) is a yellow-green, poisonous gas, but nearly always occurs as a chemical combination with other elements (Schraufnagel, 1967). The earth's crust averages about 0.15%  $\text{Cl}^-$ . Although humans can adapt to  $\text{Cl}^-$  concentrations in drinking water up to 2000  $\text{mg L}^{-1}$ , excessive  $\text{Cl}^-$  can be harmful to people with heart or kidney disorders (Schraufnagel, 1967). Under natural conditions in most humid parts of the United States, groundwater contains less than 10  $\text{mg L}^{-1} \text{ Cl}^-$  (Hutchinson, 1970).

Chloride can be an environmental pollutant if  $\text{Cl}^-$  levels are high in ponds or wells. Twenty percent of farms in one study had ponds with  $\text{Cl}^-$  levels greater than the Public Health Service standard for potable water sources of 250  $\text{mg L}^{-1}$  (Hutchinson, 1970; Peters and Turk, 1981). Chloride greater than 250  $\text{mg L}^{-1}$  may also corrode

stainless steel and other metals (Schraufnagel, 1967), compromising industrial structures, such as bridges, water treatment pipes, and equipment.

Sodium is a semi-white metal that reacts readily with other inorganic elements. The earth's crust is comprised of 2.6%  $\text{Na}^+$ , the sixth most abundant element. Sodium compounds are generally very water soluble, and most  $\text{Na}^+$  related problems arise from excesses as opposed to deficiencies. Clay and organic matter in soils are negatively charged, and adsorb cations (positively charged ions) readily; this includes  $\text{Na}^+$ , magnesium ( $\text{Mg}^{++}$ ),  $\text{Ca}^{++}$  and potassium ( $\text{K}^+$ ). Anions (negatively charged ions) are repelled by the negatively charged soil surfaces. This means that  $\text{Na}^+$  cations can accumulate in soils, whereas  $\text{Cl}^-$  tends to leach through the soil profile.

#### Salt Spray Injury to Plants

Salt spray from roads can directly damage adjacent vegetation (Westing, 1969; Davison, 1970; Sucoff, 1975; Bowers and Hesterburg, 1976; Buschbom, 1980; Simini and Leone, 1986; Eaton et al., 1999). Early work in Michigan concentrated on the effects of  $\text{CaCl}_2$ , which was applied to gravel roads to control dust as early as the 1920s (Strong, 1944). Even though many roads carried fewer than 500 cars per day, traffic and prevailing winds appeared to carry enough  $\text{CaCl}_2$ -laden dust onto nearby vegetation to cause damage. Strong (1944) investigated whether this injury was the result of direct contact with foliage or excessive soil levels. He observed that soil applications of  $\text{CaCl}_2$  injured trees, but species varied in their tolerance. Strong's findings indicated that it was not possible to reproduce the same damage symptoms under controlled conditions as those seen in the field. As well, the higher soil salt treatments frequently killed the plants outright, although the  $\text{Cl}^-$ -laden dust rarely did.

Davidson (1970) surveyed injury to four pine species: Austrian pine, *Pinus nigra*; eastern white pine, *Pinus strobus*; red pine, *Pinus resinosa*; Scots pine, *Pinus sylvestris* planted in 1966 along Michigan highways. About 40% of the trees were dead or severely injured a year after planting. White pine appeared most sensitive to salt injury, Scots and Red pine were intermediate, and Austrian pine was more tolerant than the other species. The most severely affected plants were nearest to the highway in the splash and drift zone. The author concluded that deicing salts caused the observed mortality, and that Austrian pine appeared to be the most suitable *Pinus* species for highway median plantings.

Some NaCl can leave treated roadways. As much as 10% of the deicing materials applied to roads in Marquette County, Michigan became airborne on any day (Bowers and Hesterberg, 1976). The researchers sampled soil at various distances from the road for Na<sup>+</sup> and Ca<sup>++</sup>. They also sampled white pine needles for the same cations at various distances from the road and at varying heights within the tree. Injury to the trees was related to Na<sup>+</sup> levels in the soil, sampling depth, distance from the roadway, and time of year (spring/fall). Sodium levels deposited on the needles increased up to 34m from the roadway, and were highest on needles closest to ground level.

Numerous site characteristics contribute to the extent to which airborne deicing salts damage roadside vegetation. The distance of the vegetation from the road, width of the roadside shoulder, percent slope, elevation, and aspect (Piatt and Krause, 1974), quantity of salt applied, application method, average daily traffic (ADT), speed limit of the road adjacent to susceptible, exposed vegetation, and prevailing wind speeds and direction all influence injury levels (Sucoff, 1975). This may also explain why some salt-

sensitive vegetation at very exposed sites sometimes persist in spite of their high salt exposure (personal observation). Salt spray is generated relative to the quantity of deicing material applied, and this will vary with winter severity (i.e., greater snow accumulations typically require more deicing salt applications, resulting in increased salt spray levels). Damage is usually consistent with patterns of deicing salt distribution; i.e., one can expect to find more damage to blueberry bushes on heavily traveled divided highways than in fields on the secondary highways, or on minor secondary roads. However, dramatic differences in damage to blueberry plants along minor roads can appear from one year to the next, again depending on winter severity.

As traffic volumes and speeds increase, more salt spray will be generated. A shift in the usual direction of the prevailing winds can effectively evacuate windborne salts away from susceptible vegetation.

Deicing salts are applied to many Michigan roads to maintain safe road surfaces during the winter. There are 193,121 linear kilometers of highway in Michigan (MDOT, 1993). Most roads are maintained by local or county jurisdictions in Michigan. The Michigan Department of Transportation (MDOT) maintains about 8% of roads (MDOT, 1993). During the winter of 1991-92, the last date for which accurate data were available, MDOT applied 401,178 metric tons of NaCl (MDOT, 1993), a rate of about 28 metric tons/equivalent for the entire winter, with an equivalent being defined as 1.6 kilometers of 7.3 meter-wide, two-lane road surface (MDOT, 1993). While it has not been possible to obtain the quantity of deicing salt applied in Ottawa County, data from Macomb County, Michigan, indicate that annual salt use may vary by more than 200 percent (Mykytiak, pers. comm.)



Small trees are typically damaged at greater distances from the road than large trees (Piatt and Krause, 1974). Roadside vegetation at higher elevation relative to the road is also less damaged (Piatt and Krause, 1974). Soils down slope of the roadway also typically contain higher  $\text{Cl}^-$  levels, but distance to the farthest affected vegetation also increases with increasing slope. It is believed that  $\text{Cl}^-$  dispersal is facilitated by the increased water movement on steeper slopes (Piatt and Krause, 1974).

Woodcock (1950) used test plates of varying shapes to simulate leaves and showed that long, narrow plates exposed in a salt laden air stream accumulated much more salt per unit area than broad plates. Edges appeared to collect more salts than centers, and very few of the smaller salt nuclei (droplets of concentrated salt water that weigh  $10^{-14}$  g or less) were deposited on broad surfaces. This suggests that narrow twigs and leaves (evergreen needles) may collect more salt than broad leaves.

Salt damage to roadside turf is less commonly reported than injury to woody plants. This is somewhat puzzling because the long and narrow foliage of many grasses would be expected to intercept large quantities of salt in the absence of snow cover. There is some conflict as to which form of vegetation is more salt sensitive, woody or herbaceous. Holmes (1961) reported grass species to be more sensitive than trees, observing that grass underneath some sugar maples sustained more damage from high soil salts than the trees themselves. Cordukes and Parups (1971) reported considerable  $\text{Cl}^-$  tolerance of all 12 grass cultivars tested, representing eight species. Others have concluded that woody plants sustain more damage than herbaceous species (Westing, 1969; Davison, 1975; Sucoff, 1975; MDOT, 1993).

## Physiological Basis of Salt Injury to Woody Plants

Woody plants absorbed less salt when exposed to salt spray in the middle of the winter when they are fully dormant than when they began growing in March and April (Rostra and Lumis, 1974; Lumis et al., 1976). Lumis et al. (1976) found the highest internal shoot  $\text{Na}^+$  and  $\text{Cl}^-$  levels in the dormant buds of Norway maple (*Acer platanoides* L.), beech (*Fagus grandifolia* Ehrh.) and lilac (*Syringa vulgaris* L.) Button (1964) reported increasing levels of  $\text{Na}^+$  and  $\text{Cl}^-$  in tissues and sap of sugar maple trees with increasing exposure to roadside drainage. Symptoms of decline were most severe on trees exposed to roadside drainage. A carry-over of accumulated chloride from one season to the next was thought to contribute to rapid accumulation of critical quantities of salt in the foliage. However, some trees with very high tissue  $\text{Cl}^-$  concentrations exhibited no symptoms of injury. Simini and Leone (1982) found that most actively growing plants absorbed more salt when exposed to shorter photoperiods, higher relative humidities, and lower temperatures. However, salt spray injury to dormant Norway maple (*Acer platanoides* 'Emerald Queen') buds was not influenced by air temperature and humidity (Barrick and Davidson, 1980). They also concluded that injury in dormant tissue was more closely related to penetration of phytotoxic ions than to osmotic stress.

High exogenous salt concentrations may cause an imbalance of cellular ions resulting in ion toxicity and osmotic stress (Chang-Le et al., 2003). This type of salt stress can also impair electron transport within the various subcellular compartments leading to the generation of reactive oxygen species, such as hydroxyl radicals, and the superoxide radical (Chang-Le et al., 2003). Whether this occurs when dormant plants are exposed to salt spray is not known. Caya and Higgs (2002) found that applying small

amounts of calcium nitrate to saline soils reduces the effects of salts on chlorophyll concentrations, plant growth, and fruit yield, and increased membrane permeability of cucumber (*Cucumis sativus*).

Zobel and Nighswander (1990) hypothesized that salt spray enters dormant Austrian and red pine needles through the stomata, and that the resulting necrosis was caused by accumulation of phenolic compounds produced as a defense mechanism. This may lead to the degradation of the cytoplasm, the disappearance of organelles, the release of vacuolar contents, damage to membranes and death of mesophyll cells (Zobel and Nighswander, 1990). Barrick et al. (1979) concluded that differences in sensitivity of Austrian, scots, and white pine to salt spray injury reflected different protoplasmic sensitivity rather than penetration patterns of  $\text{Na}^+$  and  $\text{Cl}^-$  ions. Sucoff (1975) believed salt entered dormant deciduous twigs via bundle traces. It is unknown if lenticels could also provide an entry court for salts into dormant twig tissues.

Twigs and stems of woody plants exposed to wind-blown salt spray often exhibited dieback (Davidson, 1970; Lumis et al., 1973; Dirr, 1975; Sucoff, 1975; MDOT, 1993). Cold injury caused similar symptoms. Some investigators speculate that salt spray causes more injury to vegetation than run-off and soil accumulation of deicing salts (Hofstra et al., 1979), while others argue that woody plants are more sensitive to soil salinity than salt spray (Thompson and Rutter, 1986a). Thompson and Rutter (1986) observed that some plants of all species studied died as a result of soil salinity, while salt spray killed only a small proportion of shoots which were replaced by the development of latent buds (witches-broom development). The sensitivity of plant species to salt spray is not necessarily related to their tolerance to excess soil salt levels (Thompson and Rutter,

1986).

How salt spray injures aerial plant parts is not clear. Injury may result from toxic  $\text{Cl}^-$  or  $\text{Na}^+$  in the tissues (Dirr, 1975). Internal shoot concentrations of sodium and chloride were correlated with injury to apple (*Malus sylvestris*) twigs (Hofstra and Lewis, 1975), suggesting toxicity. However, internal  $\text{Na}^+$  and/or  $\text{Cl}^-$  levels are not consistently correlated with the relative sensitivity of the species. Paludan-Miller et al. (2002) studied the effects of NaCl applied to the soil or above ground plant parts of seedlings of four deciduous tree species (maple, *Acer pseudoplatanus*; beech, *Fagus sylvatica*; horse chestnut, *Aesculus hippocastanum*; and basswood, *Tilia cordata*). While the four species differed in their responses, the amount of injury could not be predicted based on foliar concentration of  $\text{Na}^+$  and  $\text{Cl}^-$ .

Injury may also be related to water loss associated with salt exposure. However, Northover (1987) found no evidence of desiccation to peach shoots killed by salt spray. Westing (1969) observed that abundant rainfall in the northern states helps plants avoid salt injury by flushing salts out of the soil profile and away from plant roots, and points out that the high soil salt levels in the western U.S. results partly from low rainfall. Button (1964) provided evidence that saline soils can have a gradual, cumulative negative effect on roadside sugar maples, even at low concentrations. Trees in advanced states of decline had low twig and leaf  $\text{Cl}^-$  levels. The author accounted for this paradox by theorizing that translocation, transpiration and metabolism had already declined to the extent that during the season before sampling, the trees had taken up little water and little chloride. If this trend were operating for at least several years prior to Button's survey, then the cycles of leaf abscission coupled with negligible  $\text{Cl}^-$  uptake might have removed

enough  $\text{Cl}^-$  from the twig and leaf samples the author collected to account for the low  $\text{Cl}^-$  levels. Lumis et al. (1976) theorized that salt accumulation in several species resulted from accumulation from previous years' salt applications. The annual increases in internal salt concentration helps explain the gradual decline in vigor observed by several workers (Button, 1964; Pedersen et al., 2000). This could mean that  $\text{Na}^+$  and  $\text{Cl}^-$  are not being completely removed from the plants by leaf abscission in the fall.

### Woody Plant Cold Hardiness

Since salt exposure reduces the cold hardiness of various woody plants (Sucoff, 1975), and causes similar symptoms as lethal winter temperatures, salt spray injury may be analogous to winter injury.

Low temperature stress limits crop production in many areas of the world (Chen, 1994; Howell, 2000), and includes *chilling stress* and *freeze stress* (Chen, 1994). Chilling injury can occur at temperatures of 10 °C to 15 °C. Freezing low-temperature stress occurs when temperatures fall below 0 °C; and is associated with ice formation within plant tissues (Chen, 1994). Plants have evolved the ability to acclimate to cold temperatures as a response to seasonal changes in climatic patterns, thereby allowing them to complete their life cycles in spite of these challenges (Wisniewski and Bassett, 2003). Symptoms of freezing injury include frost cracks and sun scalding of tree trunks, blackheart in shoots of trees and shrubs, winter burn (evergreen foliage), soil heaving and crown kill damage of herbaceous plants, autumnal and spring frost injury of succulent annuals, and mid-winter kill of dormant flower buds (Weiser, 1970).

Weiser (1970) described the sequences of freezing death in hardened bark cells based upon analysis of results from freezing curve, calorimetric, and microscopic studies.

supercooling→extracellular freezing in non-living xylem cells, and between cells→rapid growth of ice throughout stem, resulting in→an exotherm (release of a substantial amount of heat of fusion from free-water) resulting in a warming of the tissue temperature from the supercooling point ( $-2^{\circ}\text{C}$  to  $-8^{\circ}\text{C}$ ) to a crest at the freezing point of the available water in the stem tissues ( $-0.3^{\circ}\text{C}$  to  $-1^{\circ}\text{C}$ )→additional cooling after the free water is frozen→movement of protoplasmic water out of cells to extracellular ice nuclei, as a response to the extracellular aqueous vapor pressure deficit→a second pronounced exotherm, followed by decreasing tissue temperature and a continuous slow migration of cellular water out to extracellular ice nuclei→protoplasmic shrinkage, plasmolysis, and concentration of solutes in the cell→a continued slow movement of cellular free water out to extracellular ice as the temperature decreases→a calorimetric decrease which indicates that movement of water out of the cell (freezing) is halting→a third exotherm→protoplasmic granulation→necrosis.

Cold hardiness is a complex trait controlled by many genes (Chen, 1994; Wisniewski and Bassett, 2003), and changes throughout the dormancy period (Wisniewski and Bassett, 2003). Detailed reviews of cold hardiness at the molecular level are available (Chen, 1994; Wisniewski and Bassett, 2003).

How salt compromises cold hardiness is not understood, but could involve cell membrane changes that result in more succulent and less hardy tissues (Sucoff and Hong, 1976a), or an increase in tissue sensitivity to NaCl associated with low temperature stress (Sucoff and Hong, 1976a). Even small losses in cold hardiness can cause significant crop losses, because each cold stress event can have cumulative effects in flower bud loss, or damage to woody tissues (Howell, 1988).

Freezing injury may begin when ice crystals form within the intercellular spaces of plant tissue (Chen, 1994). This will occur when the plant tissue is exposed to temperatures below the freezing point of the tissue. Live plant cells are surrounded by a hydrophobic plasma membrane, preventing the growth of ice crystals into the cytoplasm. Thus, the ice is absent within the cell, but may continue to spread throughout the intercellular space. This is known as *extracellular freezing*. If the temperature drops below a critical lethal point, ice crystals form rapidly inside the cytoplasm (*intracellular freezing*), rupturing membranes and killing cells. When tissues thaw, cytoplasm leaks from cells, and tissues lose of turgor. Necrotic tissues may appear ‘water-soaked’ due to electrolyte leakage (Howell and Weiser, 1970). Sakai (1973) observed a lack of intracellular freezing in the twigs of extremely cold hardy taxa, such as poplar (*Populus gelricha*) willow (*Salix sachalinensis*) and white birch (*Betula pubescens*).

Freezing rate affects the amount of injury to plant tissues (Weiser, 1970; Sakai, 1973; Vorsa, 1992). Rapid temperature decreases result in more injury. Temperature decreases seldom exceed 1.0 to 2.0 °C/hour (Flinn and Ashworth, 1994a), although decreases as much as 20.9 °C in one hour were recorded which resulted in devastating blueberry flower mortality in numerous fields in New Jersey (Vorsa, 1992).

Differential thermal analysis (DTA) is a useful tool in estimating the cold hardiness of woody plant tissues (Wisniewski and Bassett, 2003). Investigators performing DTA will often ‘seed’ or induce ice crystal formation in the tissue by wrapping tissue samples in moistened cheesecloth, or misting the plant tissue with water before DTA begins (Warmund, 1993; Warmund and English 1995; Warmund and English, 1998). The process of ice formation is known as ‘ice nucleation’. The non-

lethal *high-temperature exotherm* represents extracellular ice formation (Warmund et al., 1991). This is followed by one or more *low temperature exotherms*, which are associated with intracellular freezing of supercooled water in the flower primordia. Thermal analysis (TA), as well as DTA, is usually employed to measure cold hardiness of tissues that avoid freezing by supercooling, such as peach (*Prunus persica*) and cherry (*Prunus cerasus*) flower buds.

### Supercooling

Supercooling is a freeze-avoidance mechanism (Sakai, 1979; Wisniewski and Bassett, 2003), whereby tissues or organs are able to retain water in a liquid phase at low temperatures by remaining free from internal and heterogenous ice nuclei, and isolated from the nucleating effect of extracellular ice. Supercooling occurs in flower buds, xylem ray parenchyma cells [XRPCs], and some other tissues, and occurs at higher temperatures than deep supercooling. The detection of low temperature exotherms (LTEs) can accurately estimate the level of cold hardiness in many, but not all, species that deep supercool (Lindstrom et al., 1995).

### Deep Supercooling

Deep supercooling is a freeze-avoidance mechanism that occurs in certain stem tissues such as axial and XRPCs, as well as flower buds, (Quamme, 1991), and differs from supercooling by occurring at lower temperatures (below -20 °C ). This is characteristic of many woody plants, but especially deciduous hardwoods (Quamme, 1991). Intracellular freezing of the deep supercooled water in these tissues will sustain permanent injury as temperature decreases below a nucleation point (Quamme, 1991). Andrews and Proebsting (1986) found no effect of photoperiod on the development of the



deep supercooling mechanism in peach and sweet cherry.

Deep supercooling remains a rather elusive phenomenon, in that apparently the properties that allow deep supercooling to occur seem to rely on the structural organization of the tissue/organ (Wisniewski and Bassett, 2003). Quamme (1976) posited that the spontaneous nucleation temperature of supercooled xylem water correlated with the northern limit of apple (*Malus pumila*) and pear (*Pyrus communis*) production in North America. Four exotherms occur as apple twigs are cooled, and the fourth was associated with the freezing of xylem and pith tissues, and coincided with the killing temperature of xylem ray and pith parenchyma cells (Quamme et al., 1972). Apple bud and bark tissues were hardier than xylem in mid-winter, and that pith and xylem were hardier than bark and buds in early autumn and late spring (Quamme et al., 1972).

In order to rapidly determine cold tolerance of woody plants in the field, a method for determining 'LT<sub>50</sub>', or the temperature at which 50% of flower buds will be killed, has been developed (Bittenbender and Howell, 1974; Proebsting and Sakai, 1979). Bittenbender and Howell's adaptation of the Spearman-Kärber formula makes this computation easy, even for uneven temperature decrements. DTA is often useful for determining LT<sub>50</sub>, but can require many (200 or more) flower buds (Proebsting and Sakai, 1979). Field mortality levels are often accurately bracketed by determinations of LT<sub>50</sub>, but not always (Bittenbender and Howell, 1974; Proebsting and Sakai, 1979; Flinn and Ashworth, 1994a; Wolf and Cook, 1994).

Hardiness has been associated with maximum tolerance to desiccation (Vertucci and Stushnoff, 1992). Hardiness is also hypothesized as being dependent upon the

moisture-retaining power of the cell (Vertucci and Stushnoff, 1992), depending on the tissues. Bittenbender and Howell (1975) found that dehydration of blueberry flower buds increased hardiness, and increased moisture reduced hardiness. At 25 °C, hardiness was apparently unaffected by moisture. This implies that a small amount of NaCl could remove enough free cellular water so as to decrease the incidence of intracellular freezing, which could increase cold hardiness. It is just as likely that exposure to increasing NaCl concentrations could remove enough cellular water to desiccate and kill cells. This would be more devastating to flower buds than woody bark tissues, as the flower buds are the critical sensitive tissues (Bittenbender and Howell, 1976; Hancock et al., 1987).

Other factors affect the cold hardiness of woody plants. Flore et al. (1983) found that shading during the growing season reduced cold hardiness in sour cherry and peach, possibly by reducing photosynthesis and carbohydrate accumulation. Howell (1988) reported the same finding in grape (*Vitis* spp.), and concluded that vine hardiness can be viewed as a carbohydrate sink, albeit a weak one. Proebsting and Mills (1973) reported increased survival of flower buds after exposure to ethephon [(2-chloroethyl)phosphonic acid]. Delayed bloom, as well as anthesis, caused by ethephon treatment was attributed to increased yields. Proebsting and Mills (1974) found that ‘Bing’ cherry trees (*P. avium*) treated with gibberellic acid (GA<sub>3</sub>) had reduced hardiness, depending on the time of application because of the dormancy-breaking and growth stimulating properties. Raese (1996) found that summer sprays of CaCl<sub>2</sub> or calcium nitrate [Ca(NO<sub>3</sub>)<sub>2</sub>] increased cold hardiness of ‘Anjou’ pear trees (*Pyrus communis*). This study was conducted over many years in Washington State, where severe winter injury occurs about every 8 years.

Raese (1996) suggested that calcium is an important component of plant cells, and thus may be important in maintaining cold hardiness of certain plant tissues.

### Blueberry Cold Hardiness

Commercial production of blueberries in Michigan is generally limited to southern Michigan because winters are too severe in more northern climes. Even in the southern production area, annual winter injury ranges from slight to severe, so even a slight reduction in the cold tolerance of flower buds can result in substantial yield losses. Blueberry bushes may be more susceptible to salt spray injury than some other woody species because the flower buds are borne on the exposed terminal portions of the previous year's growth. Each flower bud can give rise to from four to 18 fruits (Hancock, pers. comm.), depending on the cultivar.

The ability to acclimate rapidly and early in the fall is an important trait for highbush blueberry cultivars grown in the North Central states (Fear and Lawson, 1987). Highbush blueberry undergoes a rest period, identified as a three-stage process (onset, deep rest, and release from dormancy), while simultaneously developing cold hardiness (Gough, 1994). The rest phase is a period of dormancy, and the plant will not respond to external stimuli until certain internal conditions have been met; i.e., the *chilling requirement* being the main factor. External conditions such as low temperatures and decreasing photoperiod are thought to trigger the onset of rest (Gough, 1994). The plant begins to shutdown because of the influence these external factors have on respiration, plant growth regulators, and metabolism (Gough, 1994). Roots are reported to maintain growth into mid-winter and enter a quiescent state rather than dormancy, in response to low soil temperatures and the lack of available water (Gough, 1994). The chilling

requirement is the critical amount of time that blueberry must be exposed to temperatures below 7 °C in order to resume growth (Gough, 1994). The chilling requirement is cultivar-dependent, and there is disagreement regarding the actual temperature below which the chill requirement is best satisfied (Gough, 1994; Eck, 1988). Northern highbush cultivars typically require a greater number of chilling hours, from 800 to 1100 chilling hours (Eck, 1988), whereas southern, low-chill, highbush cultivars adapted to central Florida require less than 400 hours (Gough, 1988). A 'chill hour' is defined as the amount of time a dormant plant is exposed to the minimum low temperature that satisfies the chill requirement. Vegetative buds may have a higher chilling requirement than floral buds (Eck, 1988; Gough, 1994), since they often open later than floral buds in the spring.

New growth cannot commence in the spring until the chilling requirement has been met. The chill requirement is often satisfied before external conditions are conducive to growth in the northern blueberry production areas. However, plants cannot resume growth until external conditions will allow flower and vegetative buds to emerge from the bud scales. Spring growth and fruiting will be less vigorous on those plants that have only marginally met their chill requirements, and may be even more vigorous on those that have received more than their necessary chill requirements (Eck, 1988; Gough, 1994).

Bittenbender and Howell (1976) and Johnston (1939), reported highbush blueberry is most susceptible to freezing injury in the spring. The reason for this is late-spring frosts are common in Michigan and, at this stage in their development, flower buds have usually lost hardiness to the extent that the bud scales are being pushed open by the emerging flowers. Blueberry sites are also typically low areas where cold air

accumulates. Olson and Eaton (2001) also observed spring freeze damage to lowbush blueberry flower buds that occurred in Nova Scotia, Canada. Bittenbender and Howell (1976) reported that dehardening occurred in highbush blueberry between mid-January and early March in Michigan, and that the ovaries are the tissues most sensitive to cold temperatures. Proebsting (1970) reported that mid-winter flower bud hardiness declined as much as 0.9 °C/hour at temperatures above -2.0 °C. Doughty and Hemmerick (1974) reported that the same was true for highbush blueberry, and that certain nutrient sprays (i.e., ZnPK, ZnPCa and ZnPCaMn) may reduce freeze injury to flower buds, although differences were noted among tested cultivars. These authors also reported that bushes treated with ZnPN and ZnPKCa showed serious injury to flower buds and wood.

The position of flower buds on blueberry twigs also affects their hardiness. Terminal buds are less hardy than more basal buds in secondary, tertiary and quarternary positions (Bittenbender and Howell, 1976; Hancock et al., 1987; Clark et al., 1991). Hancock et al. (1987) observed a positive inverse linear relationship between percent brown ovaries to bud position since the apical buds typically begin developing earlier in the spring than more basal buds (Hancock et al., 1987). More fully developed buds (terminal buds, in most cases) may contain more water that increases the likelihood of intracellular freezing (Bittenbender and Howell, 1976).

If terminal buds are less hardy than more basal buds, then a high number of flower buds per stem may increase a blueberry cultivar's productivity, regardless of its ability to tolerate freezing low-temperature stress (Bittenbender and Howell, 1976). It would seem that this should be a goal for blueberry breeders interested in producing more cold-tolerant cultivars (Hancock et al., 1987).

Clark et al. (1991) also observed that origin of germplasm, and the season of flower bud development affect the ability of blueberry flower buds to tolerate cold. Southern highbush blueberry cultivars were more cold tolerant than rabbiteye cultivars (in which flower buds may form during the fall growth flush, or during the spring growth flush) at similar stages of flower bud development (Clark et al. 1991).

In highbush blueberry, the low-temperature exotherm (LTE) is associated with xylem injury, rather than bark injury (Quamme et al., 1972a). Xylem, phloem and cambium injury in highbush blueberry is not as important for survival as bark injury, and bark injury occurred at a higher temperature than xylem, and was not associated with an exotherm (Quamme et al., 1972a). Flinn and Ashworth (1994) observed that highbush blueberry lacks detectable low-temperature exotherms under conditions of slow-cooling ( $-2.0^{\circ}\text{C/hr}$ ). These same authors also found that DTA was incapable of detecting LTEs in 'Berkeley' flower buds attached to 5 cm stem segments, and cooled at a rate of  $2.0^{\circ}\text{C/hour}$  (Flinn and Ashworth, 1994). This is significant because the relationship between ice distribution and vascular development has been studied only in those species that exhibit the low-temperature exotherm (Flinn and Ashworth, 1994). Flinn and Ashworth (1994a) studied the rate of vascular development in highbush blueberry, and noted that both acclimated and deacclimated floral buds possessed long, narrow extracellular splits in bracts, bud and floret scales; nonacclimated buds were deficient in this feature. They observed that mature, continuous vessels had differentiated further toward the apex of corollas into December. Fuchsin stain was used to detect this anatomical development, and vascular development was suspended from December through the end of February.

The highbush blueberry cultivar 'Berkley' is not separated from xylem at the

onset of dormancy, and thus cannot supercool (Howell, pers. comm.) As a result, it is less hardy under Michigan conditions than some other cultivars.

By mid-April, deacclimated florets were four times wider and longer than acclimated florets, because of water uptake (bud swelling). A densely stained vascular system was observed to spread throughout the florets, and all portions of the florets were apparently connected to the parent plant via mature xylem (Flinn and Ashworth, 1994a). Sakai (1979) also found that flower buds of flowering dogwood (*Cornus florida*) had higher water content in the spring (66% water, fresh weight) than did winter buds (33% water, fresh weight). The latter author also reported no detectable LTEs in these spring buds.

Bittenbender and Howell (1975a) developed useful models to describe the level of cold hardiness obtained in highbush blueberry. It was found that increasing photoperiod correlated better with dehardening. Such models can be of value in selecting a planting site, or evaluating seedling cold hardiness.

Quamme (1978) observed masses of ice formation in bud scales and flower bud axis of peach flower buds, but not the primordia. He proposed the existence of two barriers that operated below  $-10.0^{\circ}\text{C}$  that prevented external nucleation of supercooled tissue, and that ultimately an explosive growth of ice formation killed flower primordia, presumed to be intracellular ice formation (Quamme, 1978). He also proposed the mechanism in supercooling involves migration of water from the bud axis to the scales, resulting in a dry region (or sink) which may function as a barrier against ice growth from the peduncle, while water in the primordia does not migrate during cooling of the flower buds. Graham and Mullin (1976) had previously suggested that the water content of

Rhododendron flower buds is related to their exotherm temperature, and that flower bud scales may function as an ice sink for reduction of water in the florets

Ishikawa and Sakai (1981) studied the mechanisms involved in supercooling in Rhododendron flower buds, drawing on the earlier work by Graham and Mullin (1976) and Quamme (1978). The former authors reported the exotherm temperature in excised Rhododendron florets was inversely proportional to the water content. They also found a decrease in water content of florets and peduncle of *R. japonicum*, and an increase in that of the scales. These authors dissented with the use of the term 'supercooling' to describe the strategy employed by florets to survive cold stress, since this involves a migration of water and subsequent dehydration, as in extracellular freezing.

In the early/middle 1990s blueberry growers in west Michigan began observing severe loss of flower buds on their plants closest to the nearest salted roads, with subsequently reduced yields. Distance to the nearest road appeared to be closely related to flower bud mortality. This thesis was initiated to study this phenomenon in order to determine whether deicing salts were contributing to the loss of flower buds of blueberry plants in West Michigan.



Table 1. Comparison of various deicing chemicals. Note: For general comparison only; published values of lowest effective temperatures vary widely.

\*BOD = Biochemical oxygen demand.

| Deicing chemical   | Lowest effective temp (°C) <sup>Y</sup> | General characteristics <sup>Y</sup>  | Cost/metric ton (\$) <sup>Y</sup> |
|--------------------|---|---|-----------------------------------|
| Sodium chloride    | -8                                      | <ul style="list-style-type: none"> <li>-Harms vegetation</li> <li>-10 – 25% can leave the roadway after application</li> <li>-Can cause environmental pollution</li> <li>-Chemically attacks concrete</li> <li>-Degrades soil structure</li> <li>-Contains cyanide (sodium ferrocyanate - toxic to fish)</li> </ul> | 33                                |
| Calcium chloride   | -25                                     | <ul style="list-style-type: none"> <li>-No cyanide</li> <li>-Works faster</li> <li>-Less harmful to soil structure</li> <li>-Exothermic</li> <li>-More corrosive than MgCl<sub>2</sub></li> <li>-6x more expensive than NaCl</li> <li>-Harms vegetation</li> </ul>  | 220                               |
| Potassium chloride | -11                                     | <ul style="list-style-type: none"> <li>-Less harmful to vegetation</li> <li>-No cyanide</li> <li>-Does not attack concrete</li> <li>-Can be slow-acting</li> <li>-Needs to be used at slightly higher rates</li> </ul>  | 240                               |
| Magnesium chloride | -25                                     | <ul style="list-style-type: none"> <li>-Less harmful to vegetation</li> <li>-No cyanide</li> <li>-Does not attack concrete</li> <li>-Chemically attacks concrete</li> <li>-May become diluted and ineffective</li> <li>-Contains 17 – 56% more chlorides than other 'salt' deicers</li> </ul>                       | 240                               |

Continued on next page

Table 1. *Continued*

| <b>Deicing chemical</b>   | <b>Lowest effective temp (°C)<sup>Y</sup></b> | <b>General characteristics<sup>Y</sup></b>   | <b>Cost/metric ton (\$) <sup>Y</sup></b> |
|---------------------------|---|--|--|
| Sodium acetate            | Below -18                                     | <ul style="list-style-type: none"> <li>-Does not track</li> <li>-Exothermic</li> <li>-Biodegradable</li> <li>-No cyanide</li> <li>-Does not attack concrete</li> <li>-Slightly difficult to work with (can cake or dust easily)</li> </ul>   | 1610 – 1920 <sup>Z</sup>                 |
| Urea                      | -10   | <ul style="list-style-type: none"> <li>-Contains no chlorides (less corrosive)</li> <li>-No cyanide</li> <li>-Does not attack concrete</li> <li>-Can increase BOD</li> <li>-Needs to be used at slightly higher rates</li> </ul>   | 280                                      |
| Calcium magnesium acetate | Below -18                                     | <ul style="list-style-type: none"> <li>-Biodegradable</li> <li>-Adheres to pavement longer</li> <li>-Less toxic to aquatic life</li> <li>-No cyanide</li> <li>-No new application equipment necessary</li> <li>-Improves soil structure</li> <li>-Less harmful to vegetation</li> <li>-20x + more expensive than NaCl</li> <li>-Can be slower-acting under certain conditions</li> <li>-Can increase BOD</li> <li>-Blown easily by wind</li> <li>-Cakes easily</li> <li>-Not as effective below -5 °C</li> </ul> | 712 – 1889 <sup>Z</sup>                  |

<sup>Y</sup>Cryotech Deicing Technology (2004)<sup>Z</sup>MDOT (1993)

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## **CHAPTER II**

### **FLOWER BUD MORTALITY AND NaCl LEVELS IN BLUEBERRY FIELDS ADJACENT TO SALTED ROADS IN MICHIGAN**

## **ABSTRACT**

### **FLOWER BUD MORTALITY AND SALT LEVELS IN BLUEBERRY FIELDS ADJACENT TO MICHIGAN ROADS TREATED WITH DEICING SALT**

Blueberry fields adjacent to roads in southwest Michigan exhibit abnormally high levels of flower bud mortality and twig dieback, even following relatively 'mild' Michigan winters. This work was conducted to determine if this injury was caused by deicing salts (primarily NaCl) blown directly onto bushes. During these surveys, flower primordia injury was evident by mid-January, and increased throughout the winter. Flower bud mortality was recorded in the spring in several locations within 12 farms, and mapped graphically with Global Positioning System (GPS) technology. Survey farms were adjacent to salted highways and secondary roads. The numbers of living and dead flower buds were counted on two plants at varying distances to the salt source at bloom-time, in May. The greatest number of dead flower buds occurred closest to the most heavily traveled roads.

Flower bud mortality was dependent upon minimum daily temperatures and the orientation to and distance from the salted road(s). Dormant twig sampling for flower primordia mortality and salt deposition indicated that flower primordia mortality was generally greatest on twigs sampled close to salted roads. Lower amounts of salt were found on twigs from plants farther away from the salt source, or from farms west of major salted roads.

Salt deposition on twigs often increased through the winter months, but decreased in those months that experienced precipitation events, which apparently rinsed away salts.

## **INTRODUCTION**

Michigan is the largest producer of blueberries in the nation, averaging 27 million kilograms per year (1996 – 2000), with a mean value of 45 million dollars (MASS, MDA, 2001). There are about 620 blueberry farms and 6,800 hectares in Michigan (ERS, USDA, 2004). Most of the production occurs along Lake Michigan, from Berrien County north to Muskegon County.

In 1999, several Michigan blueberry growers in Ottawa County expressed concern about low yields and twig dieback on plants close to roads treated with deicing salts, primarily NaCl. Preliminary observations in 2000 indicated that high levels of salt were deposited on twigs during the winter and that flower primordia mortality was greatest close to the road, suggesting that injury may result from exposure to deicing salt applied to the roads.

This was not the first report of deicing salt injury to woody vegetation in Michigan. Davidson (1970) reported that *Pinus* spp. vary in their sensitivity to deicing salt. Barrick et al. (1979) hypothesized that differences in the sensitivity of *Pinus* spp. to deicing salt spray may relate to morphological differences in needles.

Deicing salts may injure plants by runoff and salt loading of the soil (Langille, 1976; Thompson et al., 1986) or when salt-laden spray or dust is generated by traffic and deposited directly on plants by wind (Sucoff, 1975). Salt spray is generated relative to the quantity of deicing material applied, and this will vary with winter severity (i.e., greater snow accumulations typically require more deicing salt applications, resulting in increased salt spray levels). Deicing salt spray injury to woody vegetation has been reported in the northern United States (Davidson, 1970; Sucoff, 1975; Bowers and

Hesterberg, 1976; Simini and Leone, 1986; Herrick, 1988; Bryson and Barker, 2002), Canada (Hofstra and Hall, 1971; Hofstra et al., 1979; Northover, 1987), European countries (Buschbom, 1980; Gibbs and Burdekin, 1983; Bogemans et al., 1989; Pedersen et al., 2000; Paludan-Muller et al., 2002), and the United Kingdom (Thompson et al., 1986; Gibbs and Palmer, 1994).

Twigs and stems of woody plants exposed to wind-blown salt spray often exhibited dieback (Davidson, 1970; Lumis et al., 1973; Dirr, 1975; Sucoff, 1975; MDOT, 1993; Eaton, 1999; Eaton et al., 2004). Some investigators speculate that salt spray causes more injury to vegetation than run-off and soil accumulation of deicing salts (Hofstra et al., 1979), while others argue that woody plants are more sensitive to soil salinity than salt spray (Thompson and Rutter, 1986). Thompson and Rutter (1986) observed that some plants of all species studied died as a result of soil salinity, while salt spray killed only a small proportion of shoots which were replaced by the development of latent buds (witches-broom development). High soil salt levels can cause severe dieback of tree crowns and branches, whereas deicing salt spray seldom affects woody plants to this degree. The sensitivity of plant species to salt spray is not necessarily related to their tolerance to excess soil salt levels (Thompson and Rutter, 1986). How salt spray injures aerial plant parts is not clear. Injury may result from toxic  $\text{Cl}^-$  or  $\text{Na}^+$  in the tissues, or from desiccation of cells (by osmosis) (Dirr, 1975). Internal shoot concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  were correlated with injury to apple (*Malus sylvestris*) twigs (Hofstra and Lumis, 1974), suggesting toxicity. Tissue sampling may provide evidence for  $\text{Na}^+$  and/or  $\text{Cl}^-$  toxicity; however, internal  $\text{Na}^+$  and/or  $\text{Cl}^-$  levels are not consistently correlated with the relative sensitivity of the species. Unfortunately, there are conflicting reports regarding



the correlation between observed phytotoxicity, and toxic ion levels in plant tissues (Button, 1964; Sucoff, 1975; Pedersen et al., 2000).

Eaton et al. (1999) investigated deicing salt injury to lowbush blueberry (*V. angustifolium* Ait.) next to highways in Nova Scotia, Canada. They found that flower numbers and yield increased with distance from the road, and were inversely related to the concentration of salt on the shoots. They also found that plants protected by plastic shelters had more live flowers and higher yields than exposed plants at the same distances from the road (Eaton et al., 1999).

Considerable amounts of deicing salts may leave treated roadways as drift (Sucoff, 1975; MDOT, 1993), and travel away from the roadside (Moxley, 1973; Sucoff, 1975; MDOT, 1993). Pre-treating the deicing salts before application with liquid calcium chloride or some other brine decreases the amount of salts leaving the roadway (MDOT, 1993). If as much as 10% of deicing salt leaves roadways after application (Bowers and Hesterberg, 1976), and 100 to 141 kg NaCl are applied per kilometer of divided highway in Ottawa County (personal communication, Ottawa County Road Commission, December, 2001), this means that anywhere from 10 to 14 kilograms of NaCl are leaving the roadway soon after every application.

Vegetation adds to roadway aesthetics, reduces glare, and prevents erosion (Westing, 1969; Sucoff, 1975). Westing (1969) concluded that spring and summer precipitation helped preserve the roadside vegetation by leaching salts through the soil profile. Others have speculated that deicing salts may accumulate in the soil due to insufficient precipitation (Davison, 1971; Pedersen et al., 2000), thus accumulating within plant tissues (Button, 1964).

The purpose of this study was to assess whether deicing salts contribute to flower bud mortality to highbush blueberry growing adjacent to roads in western Michigan.

## **MATERIALS AND METHODS**

Twelve blueberry fields in Ottawa and Muskegon Counties, Michigan, (Figure 1, Table 3), were surveyed in May of 2002, 2003, and 2004 for flower bud damage. Farms were selected based on their proximity to roads that received deicing salts during the winter. Sampling locations were chosen at varying distances and orientations relative to the road. At each sampling location, five twigs were selected from each of two adjacent bushes (ten twigs were sampled at farms 'I' and 'J' in 2002), and the number of dead and live flower buds were recorded. Twigs were selected from the side of the plants nearest the road. Assessments were during bloom, so that dead buds could be distinguished from live buds. The GPS coordinates were determined for each sampling location with a TSC1 Datalogger (Trimble Navigation Limited, Sunnyvale, CA). Maps of each farm were constructed by mapping the GPS data points on aerial photographic images on Ottawa County using ArcView software version 3.2 (ESRI, Redlands, CA). The relationship between bud mortality and distance to the road was determined by regression analyses (TableCurve™ 2D, V. 2.00, San Rafael, Calif.) using pooled data (by year) from farms adjacent to (including orientation) a specific road type; e.g., west of a divided highway (farms 'FG', 'H'), east of a divided highway ('A', 'I', 'J', 'L'), north of a 2-lane highway ('B'), south of a two-lane highway ('C'), east of a secondary road ('K'), and north of a secondary road ('D', 'E').

To determine how salt deposition on twigs and injury to buds varied during the winter, twigs were sampled at farms 'C' and 'I' in 2001, 2002 and 2003, and from farms 'B' and 'K' in 2003. At farm 'B', twigs were sampled from nine locations, three each at

25, 50 - 55, and 80 - 90 m from the road. At farm 'C', twigs were sampled from four locations 35 - 50 m from the road, and from four locations, 85 - 105 m from the road. Twigs from farm 'I' were sampled from eight locations, varying from 34 to 116 m from the road. Twigs from farm 'K' were sampled from 9 locations 14, 27, and 57 m from the road. Five samples at each location consisted of 15 cm long twigs from a single plant. Twigs were selected from the side of the plant closest to the road.

Twigs were placed in plastic bags and on ice, transported to East Lansing, Mich., and shortened to 10 cm. The five twig samples were placed in 50 mL centrifuge tubes containing 20 mL of double deionized water, capped, and inverted ten times to rinse off surface salts. The rinsate was poured into separate tubes, and the electrical conductivity was measured with an EC Meter (Orion Research, Boston, MA). EC reading was multiplied by a conversion factor of 0.584 to determine salt concentration in  $\mu\text{g cm}^{-1}$  (Soil and Plant Analysis Council, 1992). Twigs were then wrapped in moist paper towels, placed in another plastic bag, and held at 18 °C for 5 d, and dissected to determine the number of dead and live flower primordia. Mean flower bud mortality was computed by dividing the number of dead buds from all twigs sampled from each plant location by the total number of flower buds and multiplying this number times 100%.

Regression analyses were performed by road type because previous work has shown that type of road can dramatically influence the degree of salt spray injury (Sucoff, 1975). Data for all farms east of a divided highway, those west of the divided highway, those north of a secondary road, and those east of a secondary road were combined. (Farms 'F' and 'G' were combined, thus comprising one farm, 'FG'.) The regression models were selected that predicted mortality between 0 and 100% over the range of

distances measured at each farm, and highest  $R^2$  values, and those presented here were considered the best of those generated. The regression models were generated by TableCurve™ (San Rafael, CA), and the PROC NLIN command of SAS version 8.0 (Cary, NC) was used to estimated probability values for the chosen models.

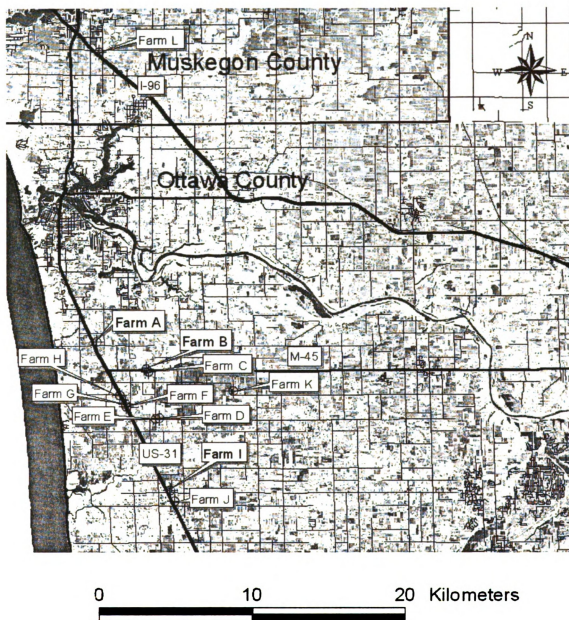


Figure 1. Map of Ottawa and Muskegon County farms surveyed for flower bud mortality, May 2002 – 2004.

**Table 3: Location, number of observations, and surface area of Michigan Blueberry farms surveyed for flower bud mortality in May.**

| Farm | Orientation<br>relative to<br>adjacent<br>roadway | <u>Observations / farm</u> |      |      | Area<br>surveyed<br>(ha) |
|------|---|----------------------------|------|------|--------------------------|
|      |   | 2002                       | 2003 | 2004 |                          |
| A    | East of US 31                                     | 105                        | 40   | 105  | 5.7                      |
| B    | North of M-45                                     | 94                         | 60   | 94   | 5.0                      |
| C    | South of M-45                                     | 133                        | 29   | 133  | 5.5                      |
| D    | East of 144 <sup>th</sup>                         | 22                         | 23   | 22   | 2.9                      |
| E    | West of 144 <sup>th</sup>                         | 19                         | 19   | 19   | 0.4                      |
| FG   | West of US 31                                     | 13                         | 22   | 13   | 0.5                      |
| H    | West of US 31                                     | 31                         | 16   | 31   | 0.9                      |
| I    | East of US 31                                     | 57                         | 41   | 57   | 3.8                      |
| J    | East of US 31                                     | 21                         | 21   | 21   | 0.5                      |
| K    | East of 120 <sup>th</sup>                         | 38                         | 28   | 38   | 2.3                      |
| L    | East of I-96                                      | 68                         | 22   | 68   | 6.2                      |

## **RESULTS**

**Flower bud mortality in May:** Flower bud mortality varied by year (Table 4). Overall mortality levels were low in 2002 (mean of 9%), high in 2003 (41%) and intermediate in 2004 (24%). Generally, those farms with higher mean mortality had a higher range of mortality across the farms (Table 4). Farms with the highest average mortality over all three years were farm 'J' (45%), farm 'L' (39%), and farm 'K' (35%) whereas farms with lower levels were farm 'D' (5%), and farms 'B' and 'C' each averaged 15%.

**Table 4. Results of May farm surveys, 2002 – 2004, showing mean and range of flower bud mortality**

| Farm | Flower bud mortality (%) |        |      |          |      |          |
|------|--------------------------|--------|------|----------|------|----------|
|      | 2002                     |        | 2003 |          | 2004 |          |
|      | Mean                     | Range  | Mean | Range    | Mean | Range    |
| A    | 3                        | 0 - 63 | 45   | 0 - 100  | 44   | 0 - 100  |
| B    | 2                        | 0 - 40 | 22   | 0 - 99   | 20   | 0 - 100  |
| C    | 1                        | 0 - 14 | 23   | 0 - 93   | 22   | 0 - 100  |
| D    | 0                        | 0 - 1  | 11   | 0 - 91   | 4    | 0 - 50   |
| E    | 9                        | 0 - 38 | 20   | 0 - 51   | 15   | 0 - 57   |
| FG   | 0                        | 0      | 35   | 2 - 100  | 25   | 0 - 67   |
| H    | 3                        | 0 - 6  | 29   | 0 - 92   | 16   | 0 - 100  |
| I    | 22                       | 0 - 93 | 48   | 0 - 100  | 19   | 0 - 100  |
| J    | 20                       | 8 - 50 | 83   | 49 - 100 | 31   | 49 - 94  |
| K    | 4                        | 0 - 29 | 64   | 8 - 100  | 36   | 8 - 100  |
| L    | 11                       | 0 - 66 | 72   | 22 - 100 | 33   | 22 - 100 |

Flower bud mortality levels are presented graphically for farm 'H', which was west of a divided highway (Figures 5, 6, and 7), farm 'I', which was east of the same divided highway (Figures 8, 9, and 10), and farm 'K', which was east of a secondary road (Figures 11, 12, and 13). Although there was a great deal of mortality from year to year,



generally the greatest amount of injury was closest to the road(s). There was some indication on farms 'H' and 'I' that mortality was also influenced by proximity to a secondary road.

**Regression Analyses.** The relationship between proximity to the road and flower bud mortality were also examined by regression analysis, where data from farms adjacent to a similar road type were combined, and regression models were chosen that best described the relationships (Table 5). There were significant relationships between proximity to the road and bud mortality in 16 of 18 cases. All of the 16 significant models described decreasing mortality with increasing distance to the road. The farms east of a divided highway (farms 'A', 'I', 'J', and 'L'), south of a two-lane highway (farm 'C'), and east of a secondary (farm 'K'), had the strongest relationship between bud mortality and proximity to the road.

The regression models and data for farms east of a divided highway, west of the divided highway, and east of a secondary road are presented to show the relationships more clearly (Figures 2, 3, and 4).

Table 5. Relationship between flower bud mortality (y) and distance from road (in meters) (x), on blueberry farms, by road type, 2002 – 2004.

| Road type and orientation                                     | Function                        | R <sup>2</sup> | P-val. <sup>z</sup> |
|---|---------------------------------|----------------|---------------------|
| <b>East of divided highway (farms 'A', 'I', 'J', and 'L')</b> |                                 |                |                     |
| 2002  | $y = 1.84 + 3091.9/x^{1.5}$     | 0.21           | <0.0001             |
| 2003  | $y = 263 - 51.5 \ln x$          | 0.63           | <0.0001             |
| 2004  | $y = 144 - 26.7 \ln x$          | 0.16           | <0.0001             |
| <b>West of divided highway (farms 'FG', and 'H')</b>          |                                 |                |                     |
| 2002  | $y = 11 - 2.54 \ln x$           | 0.18           | 0.0038              |
| 2003  | $y = 51.2 - 0.0009x^{2.5}$      | 0.14           | 0.0486              |
| 2004  | $y = 24.4 - 415.0/x^{1.5}$      | 0.07           | 0.0748              |
| <b>North of 2 lane highway (farm 'B')</b>                     |                                 |                |                     |
| 2002  | $y = 2.18 - 0.015x$             | 0.05           | 0.0284              |
| 2003  | $y = -0.93 + 583614.5x^{-2.88}$ | 0.88           | <0.0001             |
| 2004  | $y = 10.9 + 0.140x$             | 0.04           | 0.0408              |
| <b>South of 2 lane highway (farm 'C')</b>                     |                                 |                |                     |
| 2002  | $y = -1.13 + 7192/x^2$          | 0.48           | <0.0001             |
| 2003  | $y = -16 + 1979/x$              | 0.74           | <0.0001             |
| 2004  | $y = 10.0 + 29139/x^2$          | 0.39           | <0.0001             |
| <b>North of secondary road (farms 'D' and 'E')</b>            |                                 |                |                     |
| 2002  | $y = 5.31 - 0.11x^{0.5} \ln x$  | 0.04           | 0.22                |
| 2003  | $y = -49.3 + 215/\ln x$         | 0.34           | 0.0001              |
| 2004  | $y = 45.2 - 10.7 \ln x$         | 0.25           | 0.0007              |
| <b>East of secondary road (farm 'K')</b>                      |                                 |                |                     |
| 2002  | $y = 0.209 + 1039280x^{-4.57}$  | 0.47           | <0.0001             |
| 2003  | $y = 104 - 0.03x^2$             | 0.81           | <0.0001             |
| 2004  | $y = -2.97 + 17738.9/x^2$       | 0.77           | <0.0001             |

<sup>z</sup>Probability values estimated using PROC NLIN command in SAS.

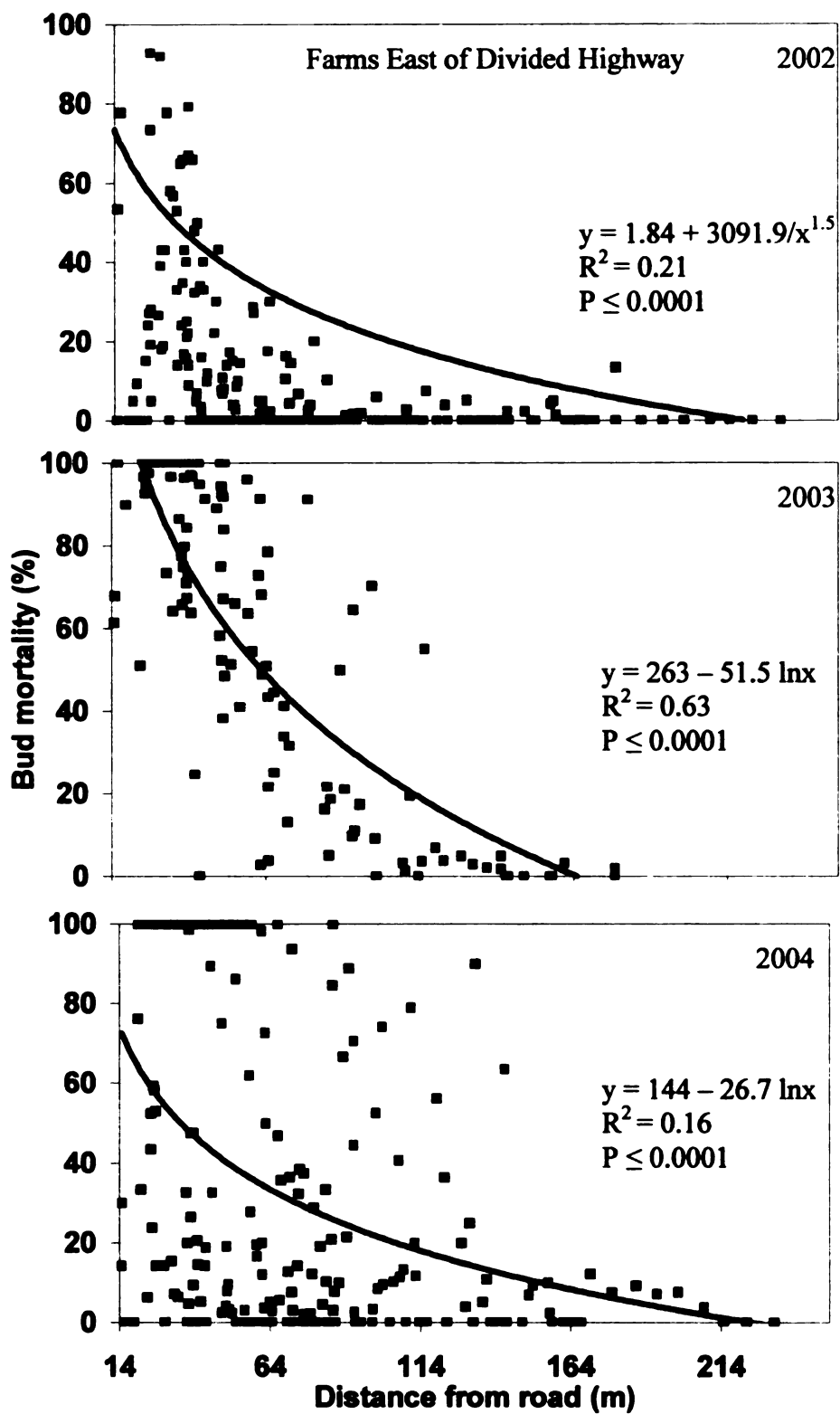


Figure 2. Relationship between flower bud mortality (y) and distance from road (x) at farms 'A', 'I', 'J', and 'L', in 2002, 2003, and 2004.

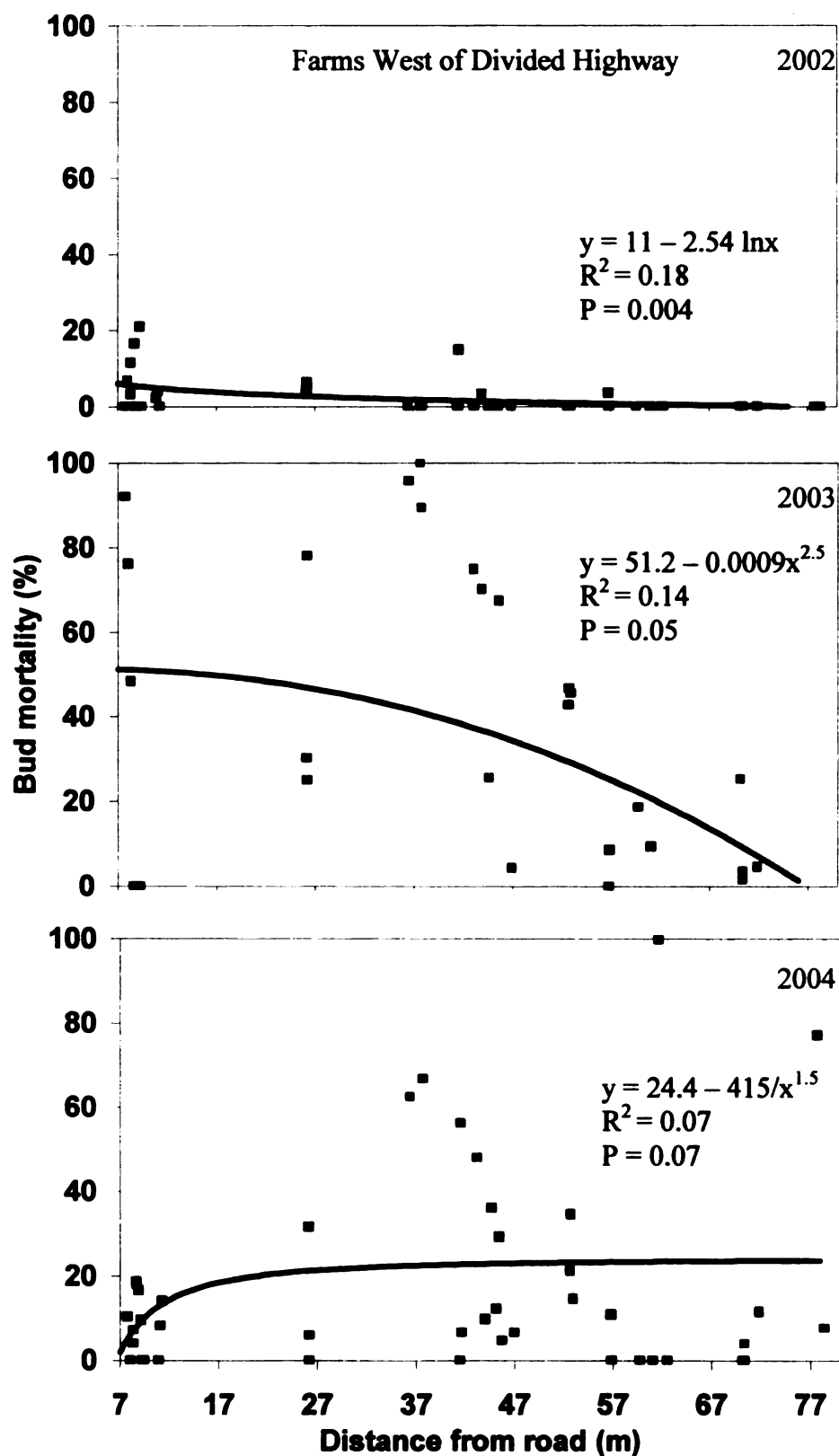


Figure 3. Relationship between flower bud mortality (y) and distance from road (x) at farms 'FG', and 'H', in 2002, 2003, and 2004.



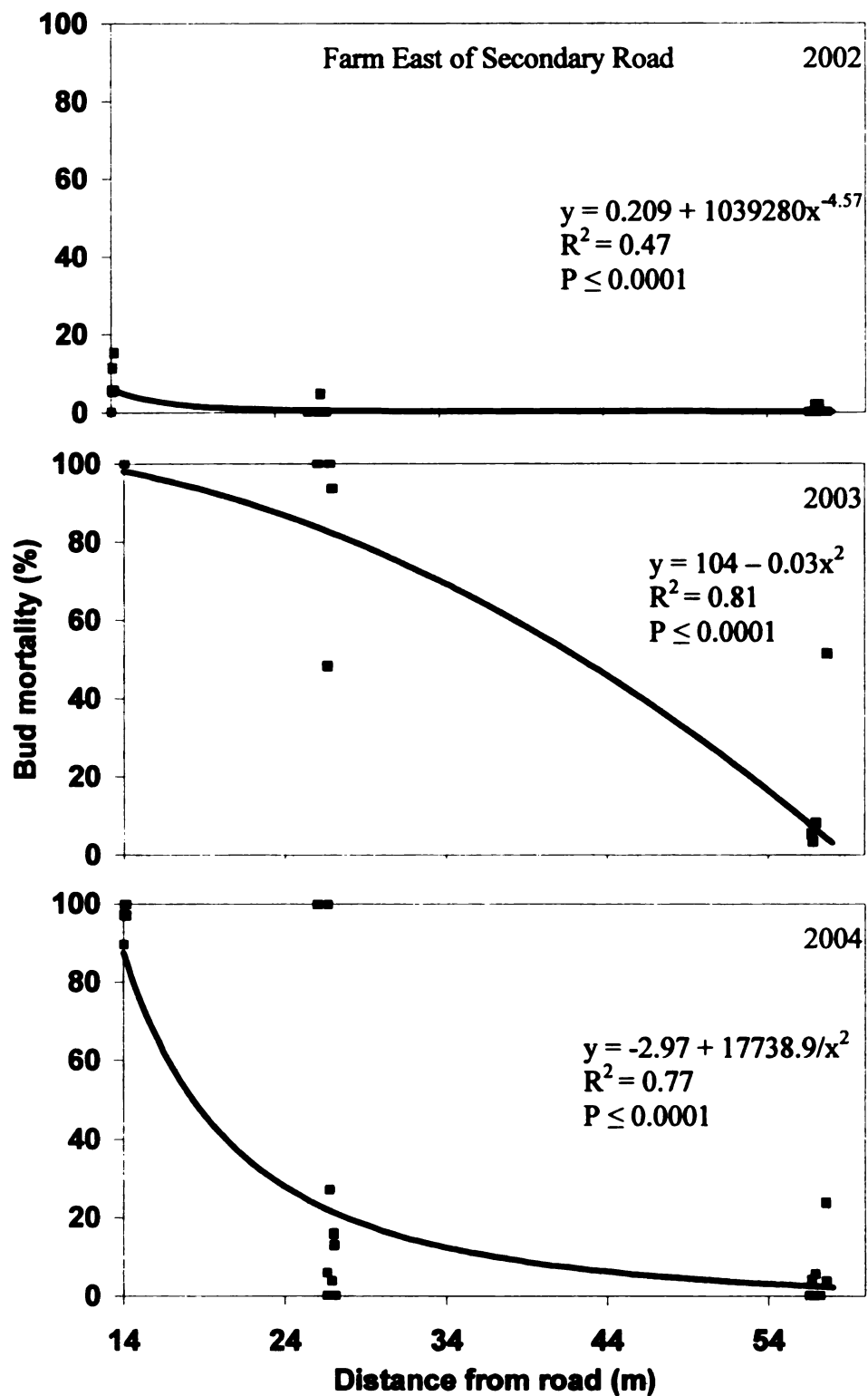


Figure 4. Relationship between flower bud mortality (y) and distance from road (x) at farm 'K', in 2002, 2003, and 2004.

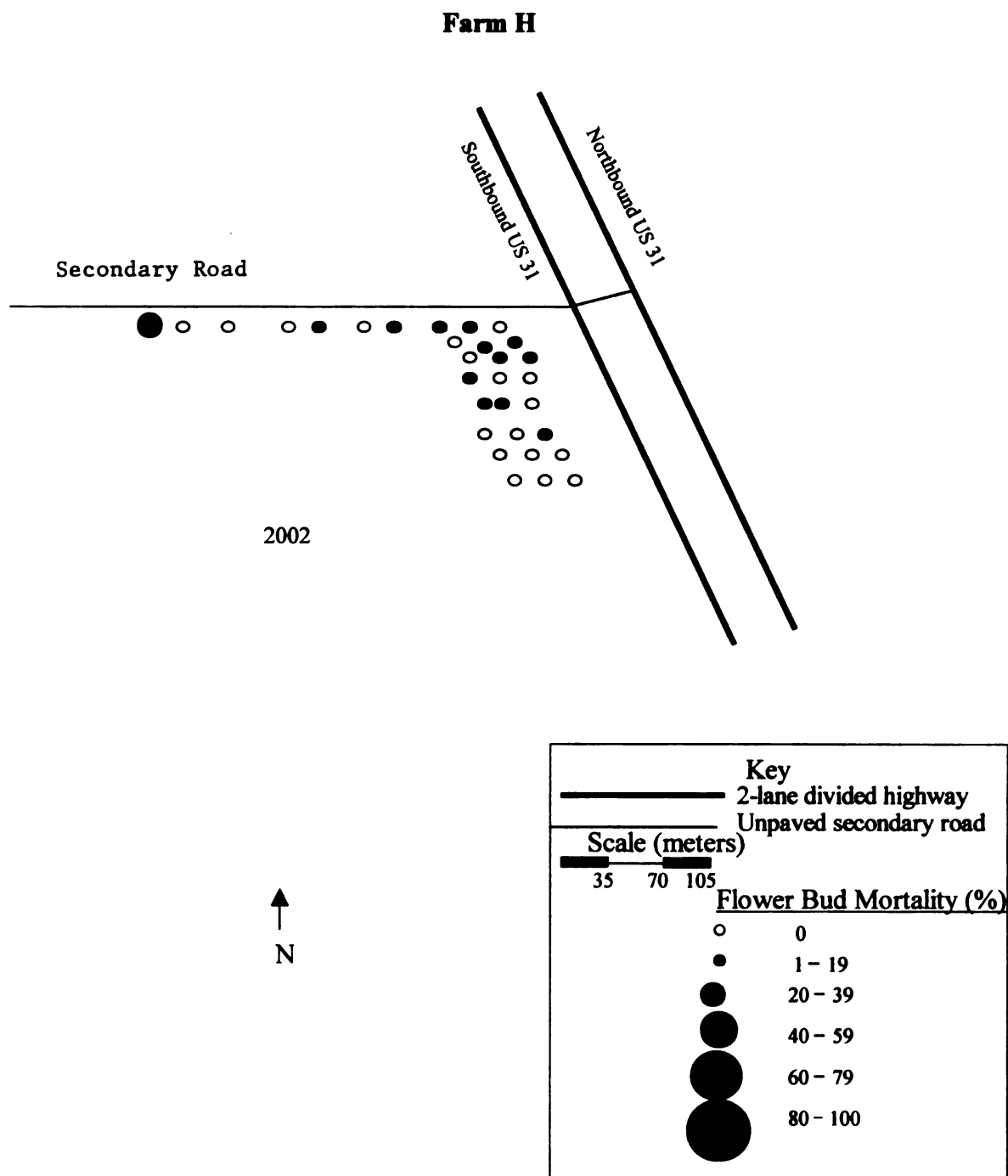


Figure 5. Percent flower bud mortality on farm 'H' in May 2002 illustrating field locations relative to the adjacent roads.

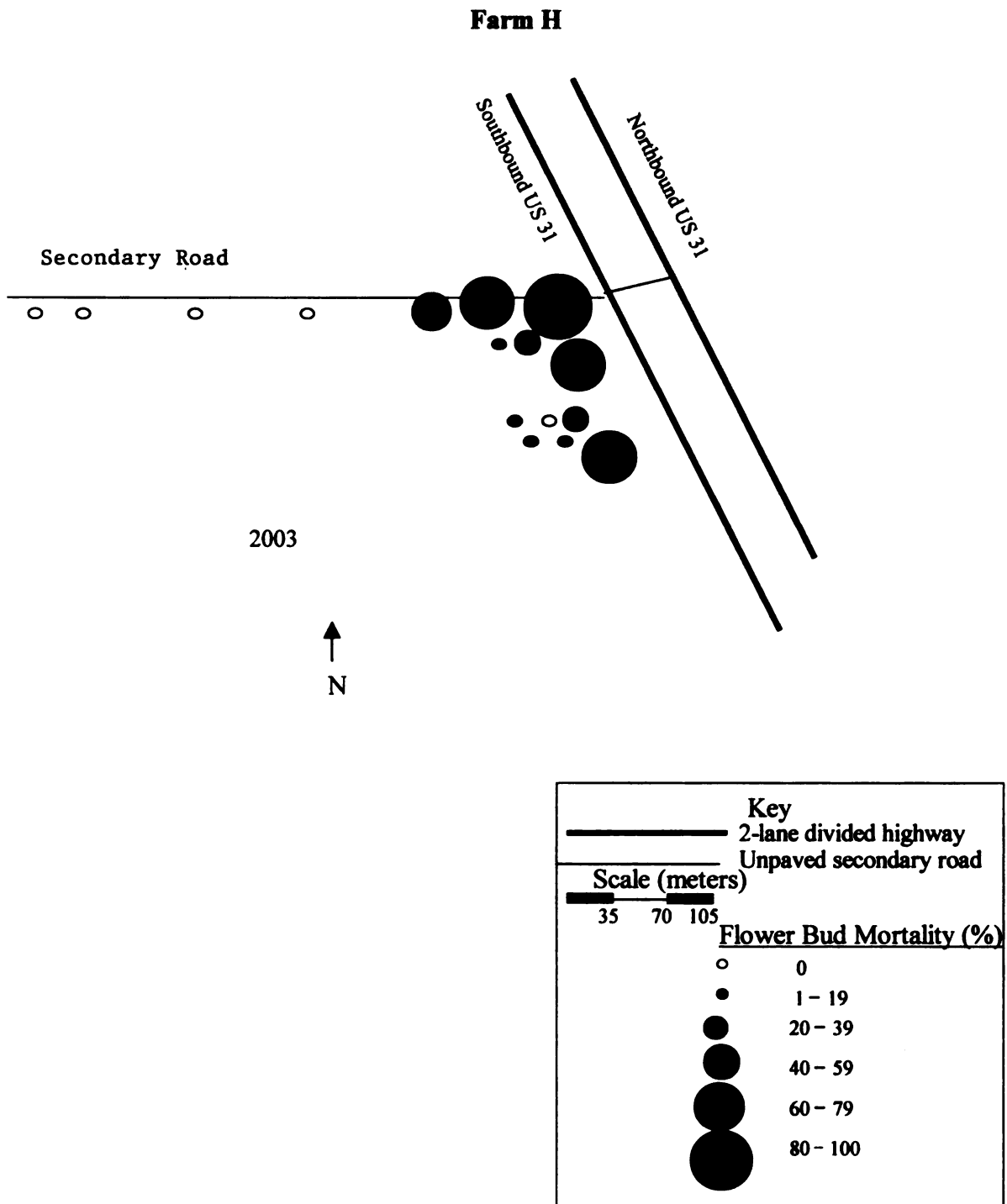


Figure 6. Percent flower bud mortality on farm 'H' in May 2003 illustrating field locations relative to the adjacent roads.



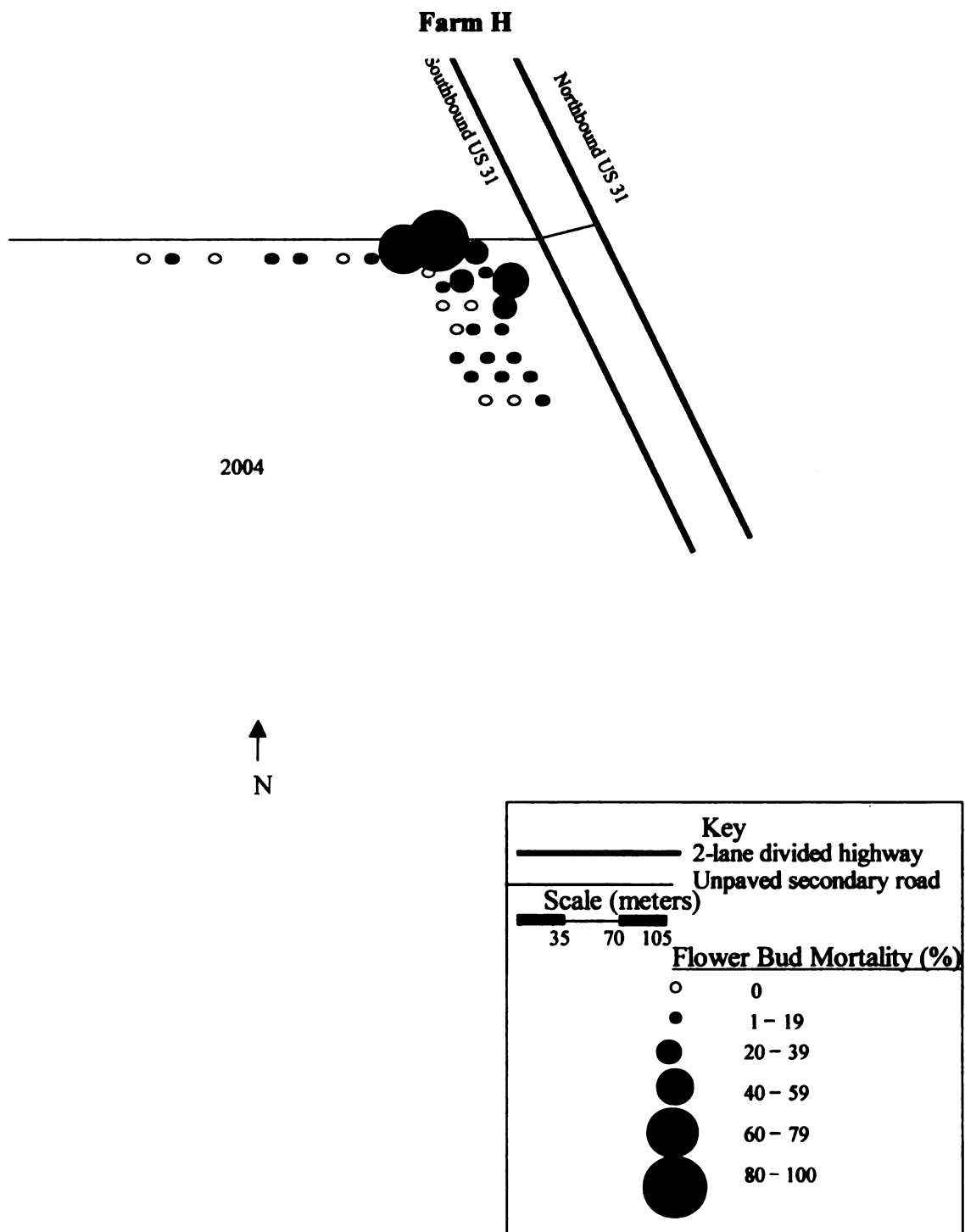


Figure 7. Percent flower bud mortality on farm ‘H’ in May 2004 illustrating field locations relative to the adjacent roads.

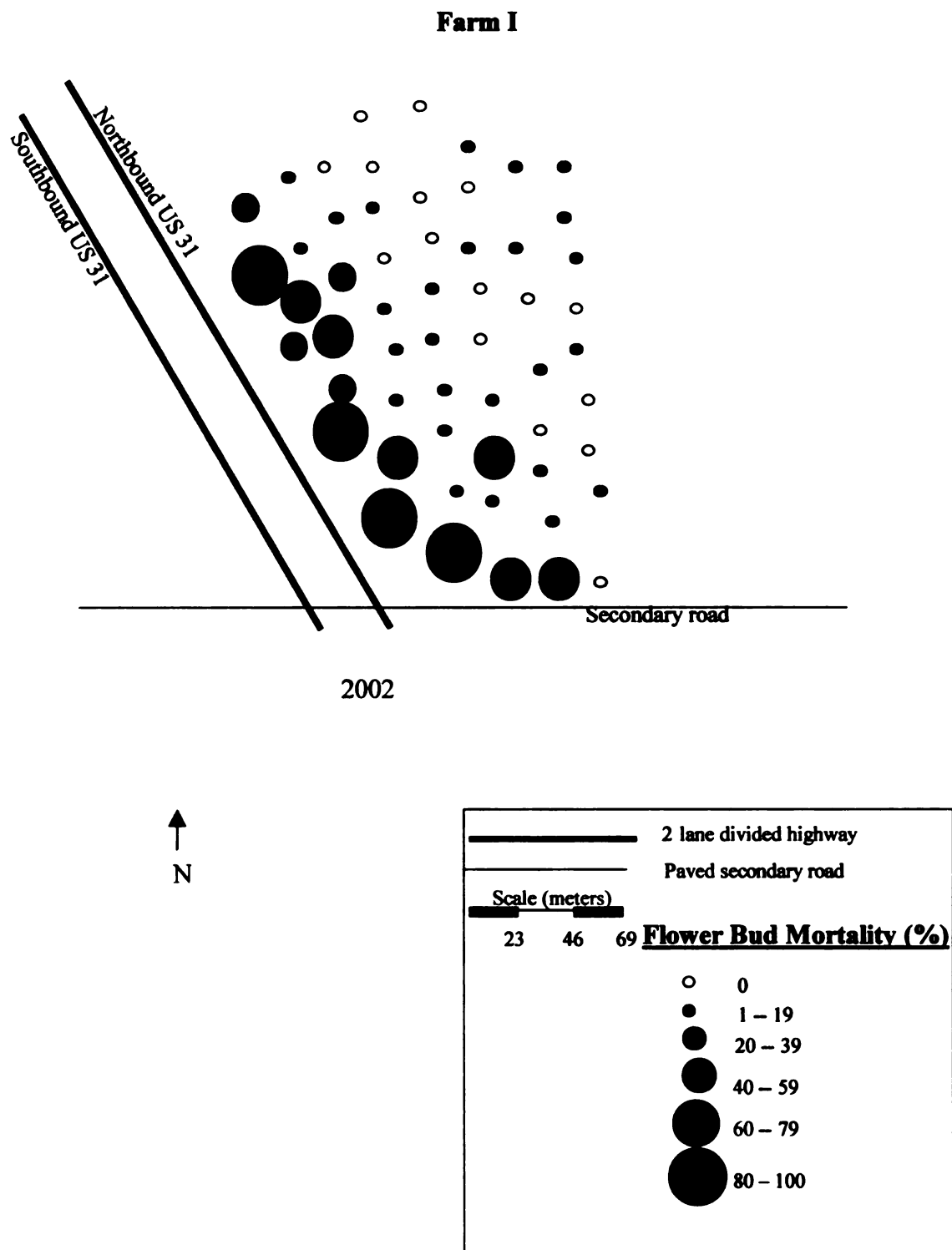


Figure 8. Percent flower bud mortality on farm 'I' in May 2002 illustrating field locations relative to the adjacent roads.

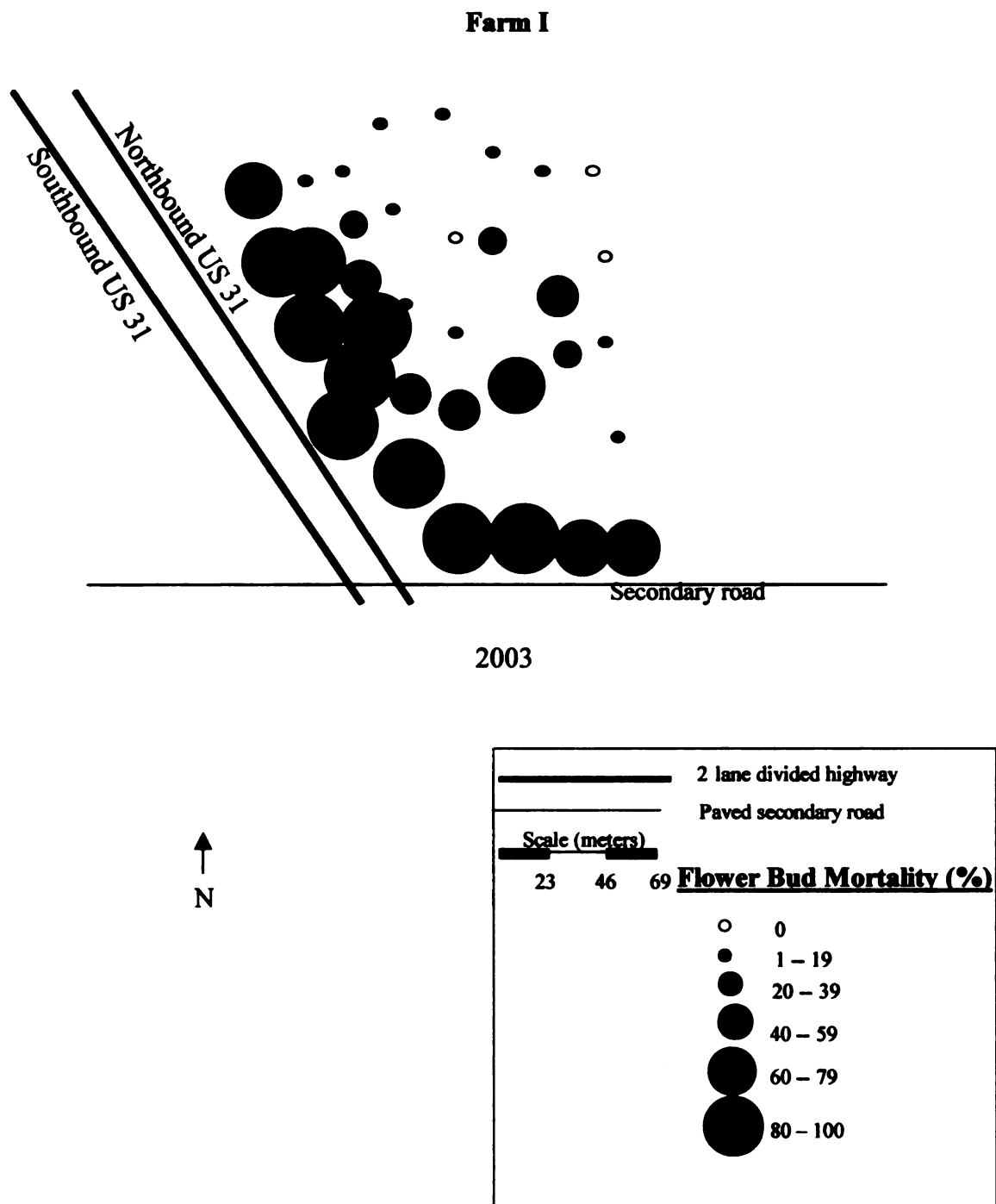


Figure 9. Percent flower bud mortality on farm 'I' in May 2003 illustrating field locations relative to the adjacent roads.

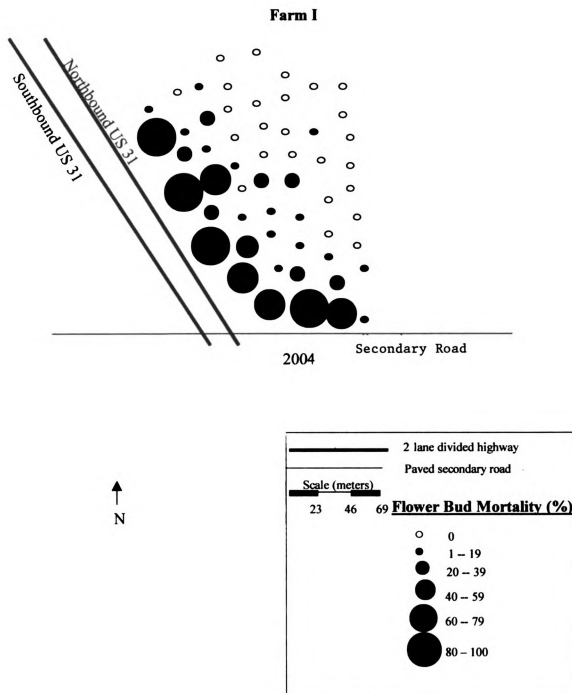


Figure 10. Percent flower bud mortality on farm 'I' in May 2004 illustrating field locations relative to the adjacent roads.

# Farm K

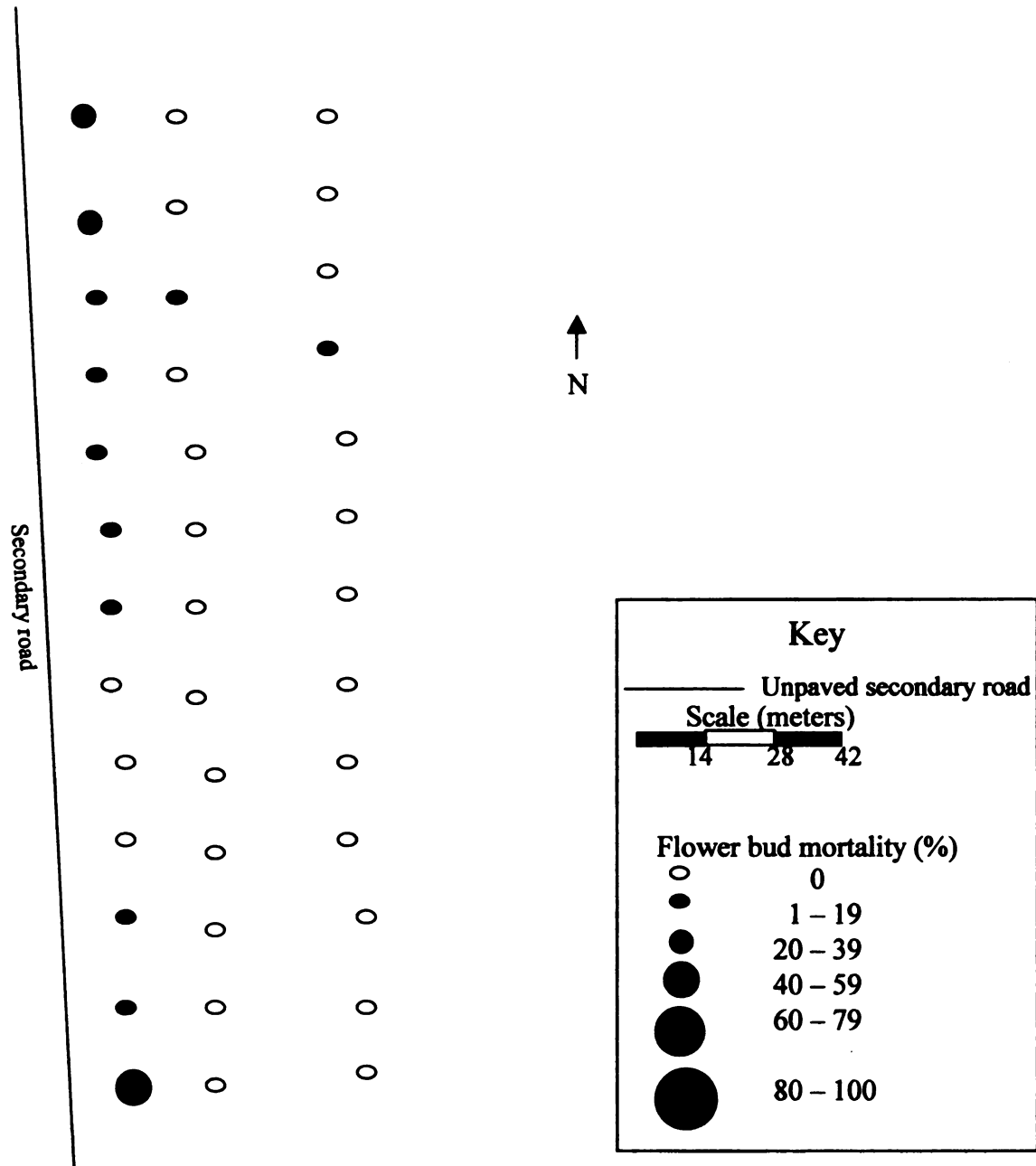


Figure 11. Percent flower bud mortality on farm ‘K’ in May 2002 illustrating field locations relative to the adjacent road.

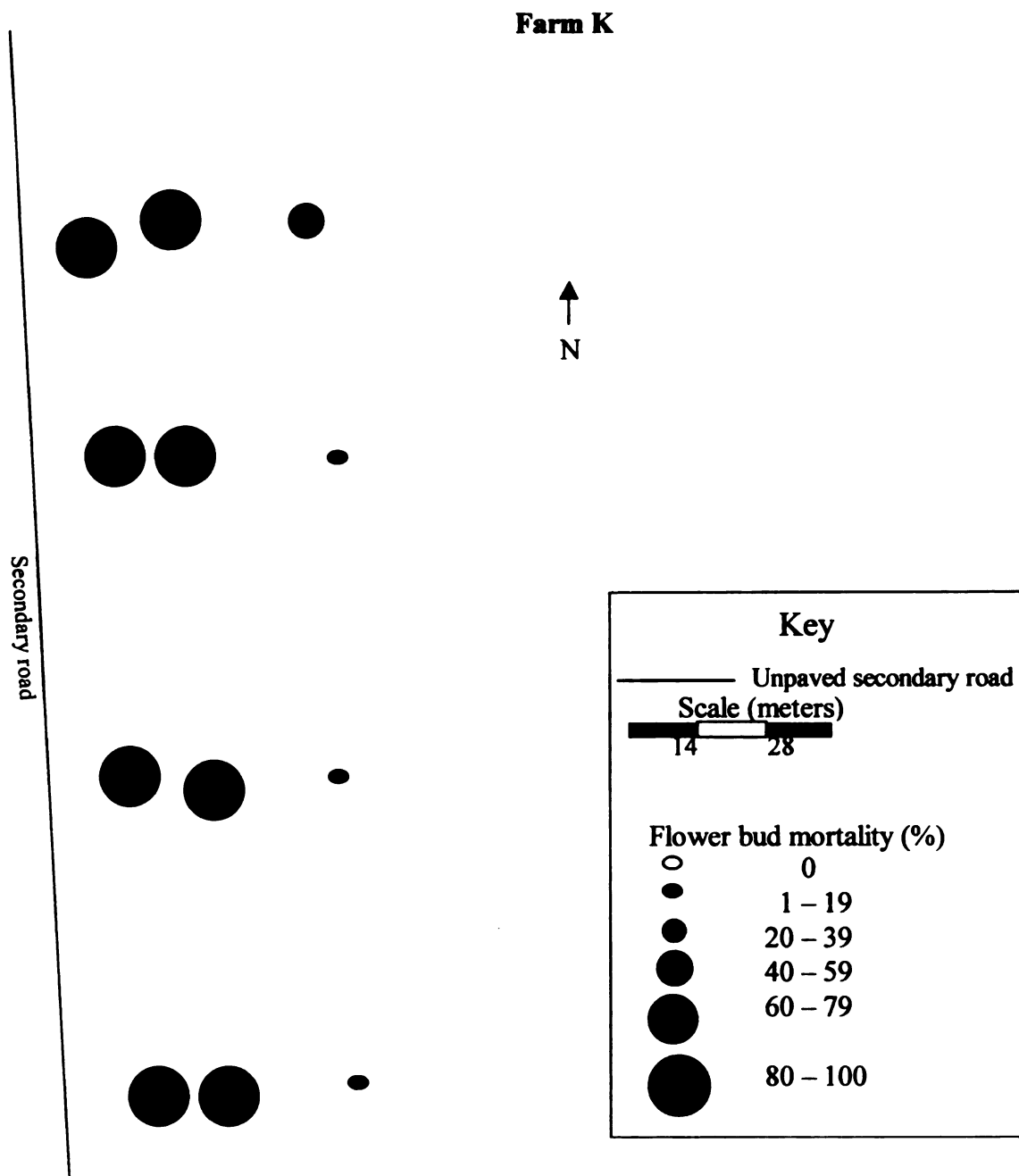


Figure 12. Percent flower bud mortality on farm 'K' in May 2003 illustrating field locations relative to the adjacent road.

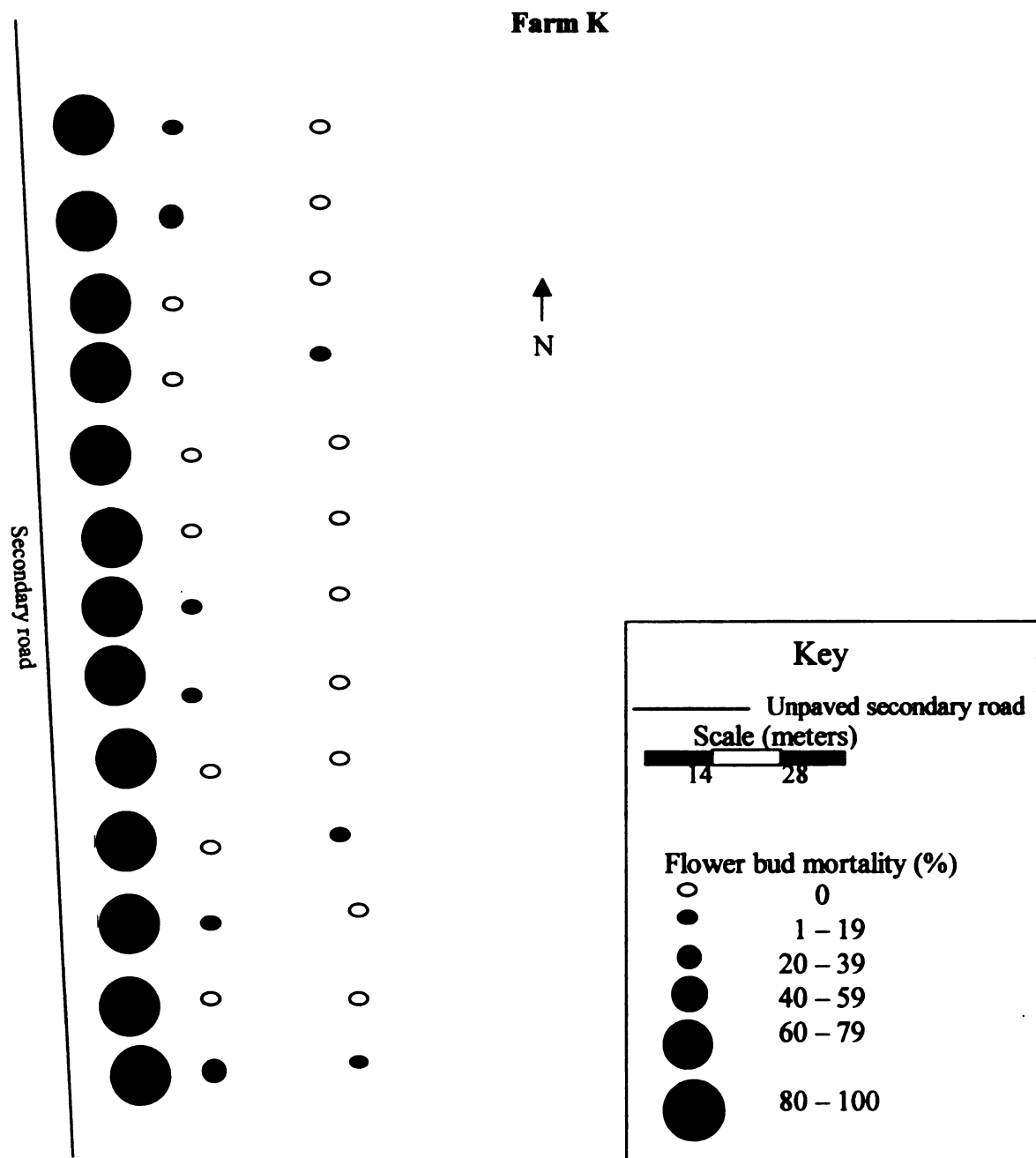


Figure 13. Percent flower bud mortality on farm 'K' in May 2004 illustrating field locations relative to the adjacent road.

**Dormant twig sampling.** Flower bud mortality and salt deposition levels were recorded monthly from four farms. In 2003, bud mortality increased through the winter on plants closest to the two-lane highway at farm 'B', but that trend was not observed on plants further away from the road (Figure 14A). Salt deposition on dormant shoots was dynamic, but the greatest amount of salts rinsed from the shoots were from plants closest to the two-lane highway (Figure 14B).

Bud mortality increased generally through the winter at farm 'C', and was always greater on plants closest to the two-lane highway (Figure 15A). Two general distances to the road were used for comparison at this farm ('close' and 'far'), encompassing a narrow range of actual distances (35 – 50 and 85 – 105 m, respectively). Salt deposition levels also varied with distance to the road, and shoots sampled from plants closer to M-45 always had higher more salt deposited on the plants (Figure 15B), which conforms to expectations. But as in the case of farm 'B' (due north of this farm), salt deposition levels were dynamic and not additive through the winter.

Bud mortality levels at farm 'I' always increased through the winter (Figure 16A). Salt deposition levels were dynamic, as seen at the other farms, but the highest salt deposition level ( $38 \mu\text{g cm}^{-1}$  twig tissue) was measured from shoots from this farm (Figure 16B). Mean bud mortality and salt deposition were averaged across the whole farm, as the orientation of this farm to US 31 made it difficult to sample twigs at different distances to this divided highway.

Plants at farm 'K' were evaluated only in 2003 for flower bud injury and salt residues at different distances to the nearest road (Figure 17). Bud mortality was initially high on plants closest to the road, and increased to 100% in Feb. and Mar. (Figure 17A).



Plants at further distances to the road always sustained less bud injury than the closest plants, but always increased in mortality through the winter. Salt deposition was always greatest on shoots collected closest to the road, but did not seem to accumulate through the sampling period. Lesser amounts of salts were deposited on plants further from the road, with salt levels that seemed to remain constant through the winter (Figure 17B).

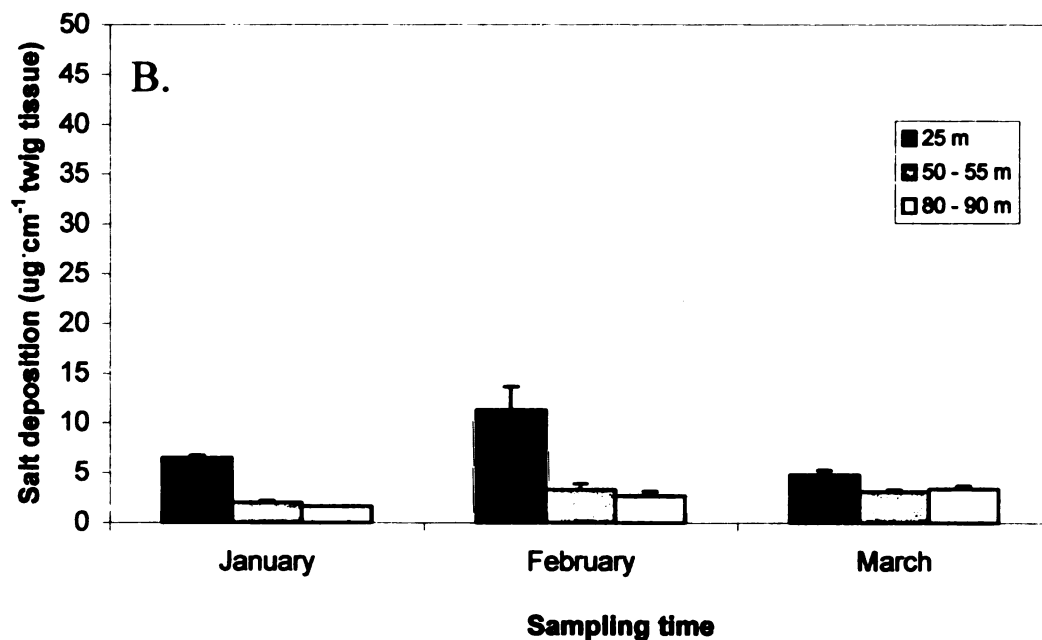
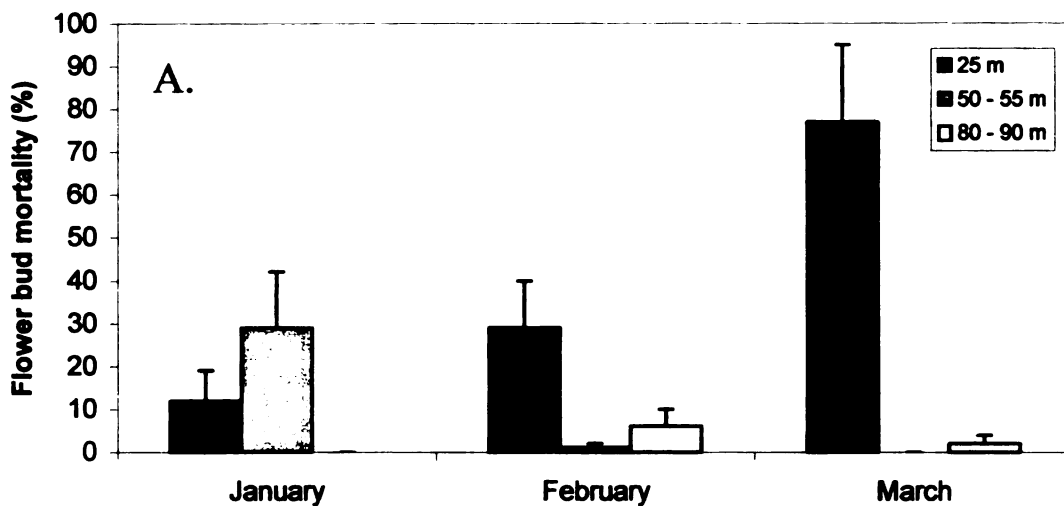


Figure 14: Percent flower bud mortality (A) and salt deposition (B), by month, at farm 'B', Ottawa County, Mich., 2003. Bars represent standard error. Legend indicates distance to road.

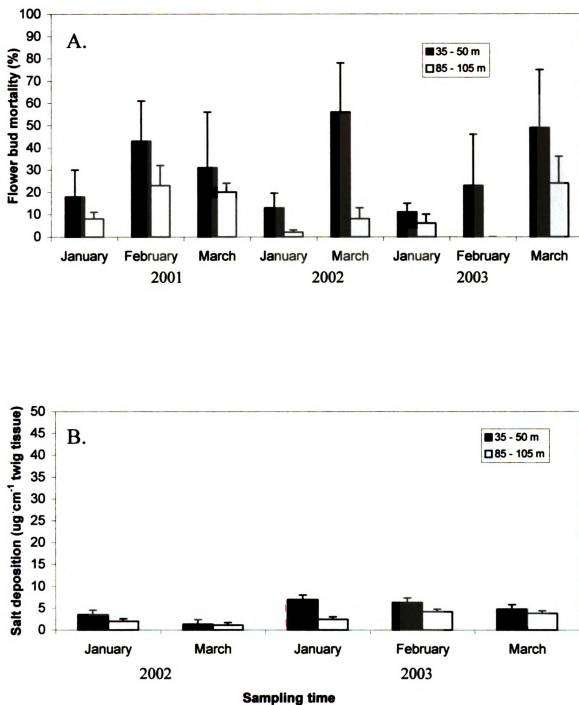


Figure 15: Percent flower bud mortality (A) and salt deposition (B), by month, at farm 'C', Ottawa County, Mich., 2001 - 2003. Bars represent standard error. Legend indicates distance to road.

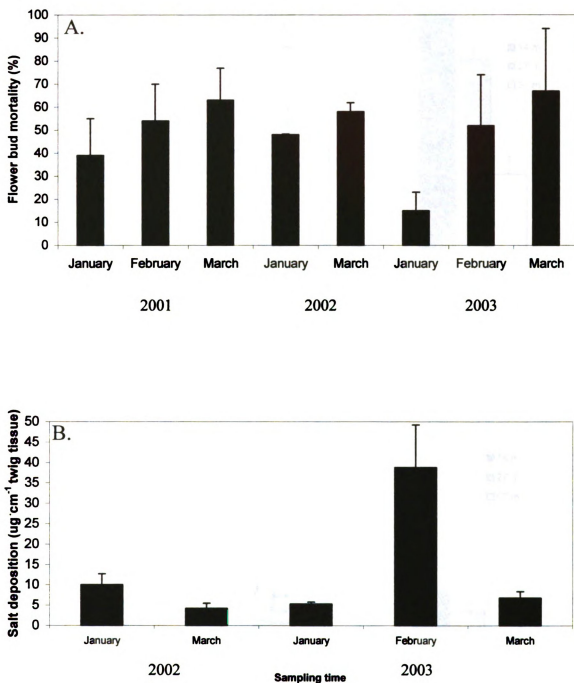


Figure 16: Percent flower bud mortality (A) and salt deposition (B), by month, at farm 'I', Ottawa County, Mich., 2001 - 2003. Bars represent standard error.

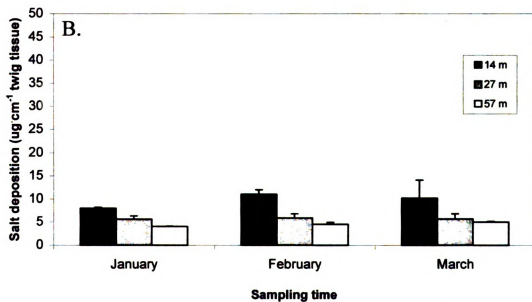
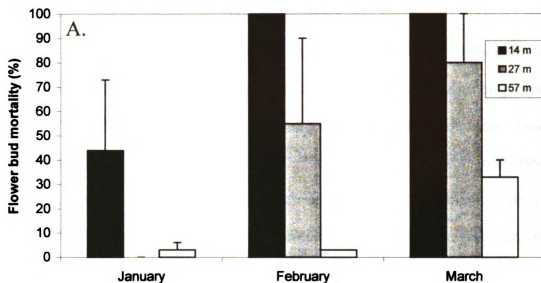


Figure 17: Percent flower bud mortality (A) and salt deposition (B), by month, at farm 'K', Ottawa County, Mich., 2003. Bars represent standard error. Legend indicates distance to road.

## **DISCUSSION**

These field observations indicate that deicing salts are responsible for much of the injury in blueberry fields next to Michigan roads. This conclusion is based on general patterns of mortality within fields as well as salt levels measured on twig tissue. Deicing salt has been shown to damage lowbush blueberries in Nova Scotia (Eaton et al., 1999), as well as other woody roadside plants (Hofstra and Hall, 1970; Sucoff, 1975; Lumis et al., 1976; Thompson and Rudder, 1986; Pedersen et al., 2000; Bryson and Barker, 2002).

While wind direction was not monitored as part of these studies, general observations are that prevailing winds move eastward in this region. Patterns of injury in farms were generally consistent with expected movement of salts off of roads. For example, the injury on farms east or downwind of a divided highway consistently were greatest closest to the road (Figures 2, and 11 – 13), whereas there was no consistent pattern of injury on farms west of the highway (Figures 3, and 8 – 10). Farms ‘H’, ‘I’, ‘J’ also bordered secondary roads, which may have contributed to mortality, since there was higher mortality along secondary roads (Figure 5 – 10). Salt injury to plants is usually greater downwind of roads (MDOT, 1993). Similar patterns of injury were observed at Farm ‘K’, which is situated east (downwind) of a secondary road. Injury was consistently higher closest to the road (Figures 11 – 13).

Farms north or south of salted roads showed clear mortality patterns some years but not others. Injury on farm ‘C’, which is south of a two-lane highway, was consistently highest close to the road (Table 5). In contrast, farms that were north of the same highway (farm ‘B’), and north of a secondary road (farms ‘D’, ‘E’), showed similar patterns in one season, but inconsistent patterns in two other seasons (Table 5). Although

prevailing wind direction is generally from the west, winds out of the northwest or southwest would have different effects on salt deposition levels on farms north or south of the two-lane highway.

Traffic volume and salting rates can influence damage to roadside vegetation (Sucoff, 1975). Farms with low levels of bud mortality were usually west of the divided highway (farms 'FG', and 'H'), and north and south of the two-lane highway (farms 'B', 'C', respectively), and north of the secondary road (farms 'D' and 'E'). Deicing application rates for Ottawa County were not available, so it is not possible to make comparisons of salt application by road type. Hence, it is not known if the low bud mortality incurred by farms 'D' and 'E' were a result of less salt being applied to these types of roads, lower ADT, prevailing wind direction, or a combination of all factors. Average daily traffic data for Ottawa County were provided by Michigan Department of Transportation, and from this it is clear that ADT was higher on the divided highway than on the other road types (data not shown).

Some surveyed farms were bordered by more than one road (Figures 5 – 10), affecting the pattern of bud injury. For example, farm 'I' was bordered by US 31 to the west, and a secondary road to the south (Figures 8 – 10). It appears that plants close to either road sustain more bud injury. Bud mortality at farm 'H' was somewhat evenly distributed throughout the field, but also found along the secondary road to the north (Figure 5). The secondary road to the north of farm 'H' also appeared to contribute to injury (Figures 5 – 7).

Roadside trees and shrubs may shield plants from salt spray and affect flower bud injury and salt deposition levels (Eaton et al., 2004) (Appendices 14 – 22) Although

some farms in this survey had varying amounts of vegetation borders, there was no clear indication that this affected patterns of injury on these farms. In virtually all cases, vegetation consists of mixed deciduous and coniferous species at varying heights.

Flower bud mortality on four farms was monitored monthly, and tended to increase during the course of the winter. Mortality was apparent early in the winter on plants closest to the road, and increased with time (Figures 14A, 15A, 17A). Mortality on plants furthest from the road tended to be minimal early in the winter, and did not always increase with time (Figures 14A, 15A, 17A). This suggests that injury associated with salt is cumulative, and not related to a single weather event. For example, injury on plants close to the road at farms 'B' and 'K' increased as the season progressed in 2003 (Figures 14A and 17A), whereas injury to plants farther from the road did not always increase through the winter.

Salt levels on twigs were usually highest close to the road (Figure 14A, 15A, 17A). Salt levels did not consistently increase or decrease from month to month. At some farms, salt levels seemed to increase (Figure 17B), at other farms salt levels seemed to decrease (Figure 16B). Variability of salt levels on twigs could be the result of precipitation events. However, winter precipitation can occur as rain or snow, and rain would be expected to remove more salt than snow.

Salt levels in this survey were derived from electrical conductivity and ranged from 1 to 39  $\mu\text{g cm}^{-1}$  shoot tissue. It is not known whether all of this salt was derived from road salt since Na and Cl were not specifically measured. Eaton et al. (1999) reported a linear decrease in NaCl residues on lowbush blueberry twigs from a salted highway by analyzing for chloride specifically.



Deicing salt was more damaging to flower buds during colder winters. Bud injury was greatest in 2003 and 2004 than in 2002 (Table 4). The 2001 – 2002 winter was relatively mild, with temperatures falling below  $-15^{\circ}\text{C}$  only once (Appendix 3). Average bud mortality the following May was low (Table 4). Temperatures fell below  $-15^{\circ}\text{C}$  5 times during the 2002 – 2003 winter, (Appendix 5), and below  $-20^{\circ}\text{C}$  three times during the 2003 – 2004 winter (Appendix 7). Flower bud mortality levels were considerably higher after the cold 2002 – 2003 and 2003 – 2004 winters, averaging 41% in 2003 and 24% in 2004. Salt has been shown to reduce the cold hardiness of plants (Sucoff, 1975; Sucoff et al., 1976; Sucoff et al., 1976A).

Sucoff et al. (1976, 1976a) showed that NaCl sprays decreased the cold hardiness of the species tested, although the mechanism of injury remained elusive. Exposing blueberry flower buds to deicing salt spray may cause injury by disabling the cold tolerance mechanism within the flower buds, but this needs to be fully investigated. That deicing salts deposited onto dormant blueberry twigs are somehow affecting water relations within the flower buds should also be investigated, as water content of blueberry flower buds is associated with cold tolerance (Bittenbender and Howell, 1975). Flower buds are recognized as critically sensitive to freeze injury (Bittenbender and Howell, 1976), and may be more susceptible than shoots to cold injury, as they lose heat readily due to a lower heat capacity and more surface area than shoots (Andrews et al., 1986). It is possible that several mechanisms resulting in flower primordia death begin occurring simultaneously soon after exposure to salts in general.

Throughout the course of these farm surveys, it was apparent that deicing salt injury symptoms (e.g., shoot dieback, flower bud mortality) resemble ‘classic’ winter

injury symptoms. These symptoms are even more extreme after severe winters. The difference between these two types of injury are the relationship with distance to the road; i.e., winter injury can usually be expected to uniformly affect any given field, but especially low-lying areas due to the lack of cold air drainage, but deicing salt spray usually only affects vegetation adjacent to treated roads.

Long-term exposure to deicing salt spray may result in severe plant stress. Plants in the southern part of farm 'A' nearest to US 31 (Appendix 14) were apparently severely stressed, and several had been pruned to the ground just prior to the 2004 farm survey. One additional observation during 2003 and 2004 was that many plants in the rows closest to US 31 at farms 'A' and 'I' had severe dieback and appeared near death, with these plants all showing signs of possible saprophytic fungal pathogen infections. This was diagnosed by the presence of necrotic regions directly surrounding vegetative and floral buds on shoots; bleached-white cankers on shoots and canes; and the presence of pycnidia on last year's shoot growth. It is possible that salt spray stress may offer certain pathogenic fungi an entry court into the necrotic tissues, thus further weakening the plants (Shaw et al., 1982).

Deicing salts appear to have been responsible for flower bud injury and twig dieback in blueberry fields adjacent to Michigan highways. This conclusion is based on the following observations: 1.) Patterns of bud injury are consistent with proximity to roads and prevailing wind direction; 2.) Elevated salt levels are apparent on twigs closest to salted roads; 3.) Deicing salts have been shown to injure lowbush blueberries and other woody plants.

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### **CHAPTER III**

## **DEICING SALTS REDUCE COLD HARDINESS AND INCREASE FLOWER BUD MORTALITY OF Highbush BLUEBERRY**

## ABSTRACT

### DEICING SALTS REDUCE COLD HARDINESS AND INCREASE FLOWER BUD MORTALITY OF Highbush Blueberry (*Vaccinium corymbosum* L.)

Injury to highbush blueberries growing along major and minor roads in Ottawa County, Mich., has been observed since the early to mid-1990s. Symptoms include shoot dieback, reduced flowering and yields. To determine if this injury was the result of deicing salts applied to roads, salt (NaCl) spray was applied to potted blueberry plants, and to the plant root zones. Bushes sprayed 6 times during the winter with NaCl solutions (0, 2, 4, 8, 16 and 32 g L<sup>-1</sup>) developed the same injury symptoms observed in roadside fields, and injury severity was proportional to the concentration applied. The root media of other potted plants was saturated with NaCl solutions (0, 1, 3, 9 and 27 g L<sup>-1</sup>) in Mar. 2002. Pots were then rinsed with fresh well water when growth began in April to determine if soil salt caused similar damage. The highest soil salt levels killed most above ground growth, and damage diminished with decreasing salt levels. Twigs were also excised from branches sprayed twice with NaCl solutions or water and frozen incrementally to measure the temperature resulting in 50% flower bud mortality (LT<sub>50</sub>). Salt exposure reduced the LT<sub>50</sub> of flower buds, by as much as 11.0 °C, relative to the control, even within 7 hours of treatment. Additional studies with chloride salts (NaCl,

KCl, CaCl<sub>2</sub>, MgCl<sub>2</sub>) and sodium salts (NaCl, NaAC, Na<sub>2</sub>SO<sub>4</sub>) indicated that most salts reduced the cold tolerance of blueberry flower buds to some degree.



## **INTRODUCTION**

Sodium chloride is the most widely used deicing material applied to roads in the north central and Eastern United States (MDOT, 1993). Unfortunately, salt damages deciduous and coniferous vegetation growing along treated roads (Lumis et al., 1975; Bowers and Hesterberg, 1976; MDOT, 1993; Bryson and Barker, 2002). Symptoms include shoot/branch dieback, browning of evergreen needles in late winter/early spring, reduced flowering, delayed foliar growth, witch's broom growth (branch ends), early fall coloration, and plant death in extreme cases (Sucoff, 1975). Species vary (within and between) in sensitivity to aerial salt spray deposited directly on plants and soil salt inundation resulting from melt water runoff (Bryson and Barker, 2002).

Highbush blueberry plantings along salted roads in western Michigan commonly exhibit twig dieback and flower bud mortality. The degree of injury is influenced by distance to the nearest salted road, orientation of the farm to the nearest salted road (in this instance, east or west of a divided highway, or north or south of a two-lane highway), and severity of the winter (Chapter 2). Flower mortality and salt deposition on twigs decreased with distance from the road, and generally increased as the winter progressed. However, although no work on highbush blueberry had been done, some work describing the effects of deicing salt on yield and flowering in lowbush blueberry (*Vaccinium angustifolium*) was performed in Nova Scotia in the late 1990s (Eaton et al., 1999).

Most of the studies dealing with deicing salt damage to woody plants focused on the phytotoxicity of NaCl. However, various chemicals other than NaCl are used for deicing purposes. The phytotoxicity of some of these alternative deicing materials (e.g., MgCl<sub>2</sub>, KCl, CaCl<sub>2</sub>, Na<sup>+</sup>-acetate) is not known. Exposure to NaCl decreases the cold

hardiness of certain woody species (Sucoff, 1975; Sucoff and Hong, 1976; Sucoff and Hong, 1976a; MDOT, 1993).

How NaCl decreases the cold tolerance of susceptible species is unknown. Salt residues on tissue surfaces may cause injury by desiccating cells (Sucoff, 1975). Salt may also penetrate tissues and disrupt cellular membranes (Zobel and Nighswander, 1990). Sucoff (1975) proposed salt enters dormant shoots via leaf traces.

The objectives of this research were to determine if the injury symptoms observed on roadside blueberries could be induced by spraying young plants, or treating soil with NaCl, and determine how NaCl and other salts affect the cold tolerance of blueberry.

## **MATERIALS AND METHODS**

**Salt Spray Simulation.** Thirty two-gallon 'Bluecrop' blueberry plants approximately five years-old and one meter tall were heeled into the ground in Dec. 2001 in a lathouse in East Lansing, Mich. Plants were sprayed to run-off on 7 Jan. 2002 with NaCl solutions of 0, 2, 4, 8, 16, or 32 gL<sup>-1</sup> using a backpack sprayer. Statistical design was a completely randomized design, with five whole plant replicates per treatment.

Aluminum foil was placed around the base of pots to prevent solutions from entering the soil. A screen was used to shield adjacent plants. Sprays were repeated five times at two-week intervals thereafter.

Salt deposition on twig surfaces was measured before each application by collecting one twig from each plant and placing twigs in 50 mL centrifuge tubes containing 20 mL of double deionized water. Tubes were capped and inverted ten times to rinse off surface salts. The electrical conductivity of the rinsate was measured with an EC Meter (Orion Research, Boston, MA). The linear length of twigs was measured and salt deposition was expressed per unit of twig length. When plants reached full bloom (20 May), the numbers of live and dead flower buds were counted on five randomly sampled twigs per bush. Data were analyzed using the PROC GLM command (SAS Institute, Cary, NC), and linear and quadratic regression analyses were performed using MINITAB (Release 13.2, State College, PA).

**Soil Salt Inundation.** In Dec. 2001, 25 'Jersey' blueberry plants in one-gallon containers were heeled into the ground in a lathouse so that the tops of the pots were 5 cm above the soil. Plants were arranged in a completely randomized design, and five replicate plants were treated with 1 liter of NaCl solutions of 0, 1, 3, 9, or 27 gL<sup>-1</sup> on 1

Mar. 2002. Solutions were poured into the pots so that they percolated throughout the media. Four weeks later, at bud swell, each pot was flushed with approximately 4 L of water. Plants were kept in the lathouse until one month after full bloom (June 20), when new shoot growth, leaves, flowers and fruit were removed from each plant. Tissues were dried at 70 °C for 10 d and weighed. Dried tissue samples were analyzed for Na<sup>+</sup> (Thomas, 1982) and Cl<sup>-</sup> (Hipp and Langdale, 1971) by the MSU Soil and Plant Nutrient laboratory. Data were subjected to ANOVA using the PROC GLM command (SAS Institute, Cary, NC), and linear and quadratic regression analyses were performed using MINITAB (Release 13.2, State College, PA).

**Cold Hardiness Study 1.** To study the effect of salt spray on the hardiness of blueberry flower buds, five branches from mature 'Jersey' bushes in East Lansing, Mich. were sprayed to runoff with NaCl solutions of 0 (deionized water), 16, or 64 g L<sup>-1</sup> on 21 and 22 Dec. 2002. On 23 Dec. 2002, fifteen twigs at least 10 cm long and possessing three or more flower buds were removed from each branch. Twigs from the five branches sprayed with the same salt treatment were combined in a plastic bag containing a moist paper towel, and transported in an ice-filled cooler to the laboratory.

Within 24 hours, the twigs were prepared for controlled temperature freezing as described previously (Howell and Weiser, 1970; Stergios and Howell, 1973). Three twigs from each salt treatment were bundled together in moist cotton cheese cloth wrapped in aluminum foil. Bundles were placed in an Ultralow Freezer (Scientemp, Adrian, Mich.) programmed to cool at a rate of 3 °C per hour. Three bundles were placed in a cold room at 3 °C, to serve as a control.

Three bundles (replicates) were removed when the temperature reached -17, -20, -23, -26, -29, and -32 °C. Bundles were held at 3 °C for 1 d, and 20 °C and 100% relative humidity for 5 d. Buds were cross-sectioned and observed for oxidative browning using a dissecting microscope to assess flower bud viability. Brown primordia were rated as dead. The temperature resulting in approximately 50% flower primordia mortality ( $LT_{50}$ ) was calculated using the modified Spearman-Kärber method (Bittenbender and Howell, 1974).  $LT_{50} = T_1 - (1/2 d) + d (\text{sum } b_i) / n$ , where  $LT_{50}$  = temperature at which 50% of samples are killed;  $T_1$  = highest lethal temperature;  $d$  = temperature interval between treatments;  $b_i$  = total number of dead samples in the  $i^{\text{th}}$  treatment; and  $n$  = number of samples per treatment. Linear regression analyses were performed on  $LT_{50}$  values using MINITAB (Release 13.2, State College, PA).

**Cold Hardiness Study 2.** To determine the effects of road salt exposure on hardiness of blueberry flower buds, 10 cm twigs bearing at least three contiguous flower buds were excised on 13 Feb. 2003 from mature 'Bluecrop' plants from a farm in Ottawa County, Mich. This farm was east of a major divided highway, and had experienced high levels of flower bud mortality. Sampled plants were approximately 65 m and 200 m from the highway, and twigs were placed in plastic bags, and transported back to East Lansing, Mich., in an ice-filled cooler for freeze-testing as described previously. Bundles containing 3 twigs from each field position were removed at 3 °C/hour temperature intervals from -18 °C to -33 °C. The statistical model used was a split-split-plot design, with the bundle forming the main plot, twig was a sub-plot, and bud position the sub-sub-plots. Data were analyzed for ANOVA using the PROC GLM command (SAS Institute,

Cary, NC), and linear and quadratic regression analyses were performed using MINITAB (Release 13.2, State College, PA).

**Cold Hardiness Study 3.** To study the effects of lower spray concentrations of NaCl on cold hardiness, branches of 20 year-old 'Bluecrop' plants at a small commercial blueberry field in Haslett, Mich. were treated with 0, 4, 8, or 16 g L<sup>-1</sup> on 27 and 28 Feb. 2003. Twigs were excised on 1 Mar. 2003, placed in plastic bags in an ice-filled cooler, and returned to the laboratory for controlled freezing. Twigs were prepared, frozen, and evaluated as described previously, but for this study, three bundle replicates were removed when temperatures reached -15, -18, -21, -24, -27, -30, and -33 °C.

**Cold Hardiness Study 4.** The goal of this study was to determine how rapidly NaCl exposure affects the cold hardiness of blueberry flower buds. Branches of mature 'Jersey' blueberry plants in East Lansing, Mich. were sprayed to run-off with NaCl at 0 or 64 g L<sup>-1</sup> on 13 Jan. 2004. Treated branches bore a minimum of 15 twigs, 10 cm long, and three contiguous flower buds. Twigs were excised 1 hour after treatment, transported back to Michigan State University in plastic bags, and prepared within 7 h for cold hardiness studies. For this test, three bundle replicates were removed every two hours, at 2 °C/hour decrements between -17 °C and -29 °C. Post-freezing handling and evaluation of flower primordia were the same as described previously. Statistical methods were the same as in Study 3.

**Cold Hardiness Study 5.** This study compared the effects of four Cl<sup>-</sup> salts on the cold hardiness of blueberry flower buds. On 12 and 13 Jan. 2004, branches of mature 'Jersey' blueberry plants were sprayed with deionized water, or solutions of potassium chloride (KCl), magnesium chloride (MgCl<sub>2</sub>), NaCl, and calcium chloride (CaCl<sub>2</sub>). All solutions

contained the same  $\text{Cl}^-$  concentration as that in NaCl at  $64 \text{ g L}^{-1}$ ,  $0.6\text{M Cl}^-$ , and were applied by a hand sprayer to drip. Four bundle replicates were removed every hour at  $3^\circ\text{C}$  decrements, from  $-16^\circ\text{C}$  to  $-31^\circ\text{C}$ . Post-freezing handling and evaluation of flower primordia were handled as in the previous cold hardiness studies.

**Cold Hardiness Study 6.** This study tested the effects of  $\text{Na}^+$  salts on the cold hardiness of blueberry flower buds. On 15 and 16 Jan. 2004, branches of 12 year-old 'Jersey' blueberry plants were sprayed with deionized water, or solutions of sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), NaCl, or sodium acetate (NaAC). Solutions were standardized to the same  $\text{Na}^+$  concentration as that in NaCl at  $64 \text{ g L}^{-1}$ ,  $0.39\text{M Na}^+$ , and applied by a hand sprayer to drip. Three bundle replicates were removed every hour, at  $3^\circ\text{C}$  decrements between  $-16^\circ\text{C}$  to  $-31^\circ\text{C}$ . Post-freezing handling and evaluation of flower primordia were handled as in previous studies.

## **RESULTS**

**Salt Spray Study .** Flower bud mortality increased linearly with increasing NaCl spray concentration (Figure 15), from 2% (control) to 48% (32 g·L<sup>-1</sup> NaCl). Spraying branches over 7 weeks showed salt deposition levels from 3.1 to 4.1 µg·cm<sup>-1</sup> NaCl after the first spray, and deposition levels were lower after the final spray application on 19 Mar. 2002 than after the first spray application (Figure 16). Highest NaCl treatment (32 g·L<sup>-1</sup> NaCl) always had higher amounts of NaCl deposited on the twigs, but the lower levels did not always have the lowest levels. Salt rinsed from sprayed twigs ranged from 2.8 to 4.1 µg·cm<sup>-1</sup> stem length. Treatments did not significantly affect salt residues until the later sampling dates, when the higher spray concentrations resulted in higher salt residues.

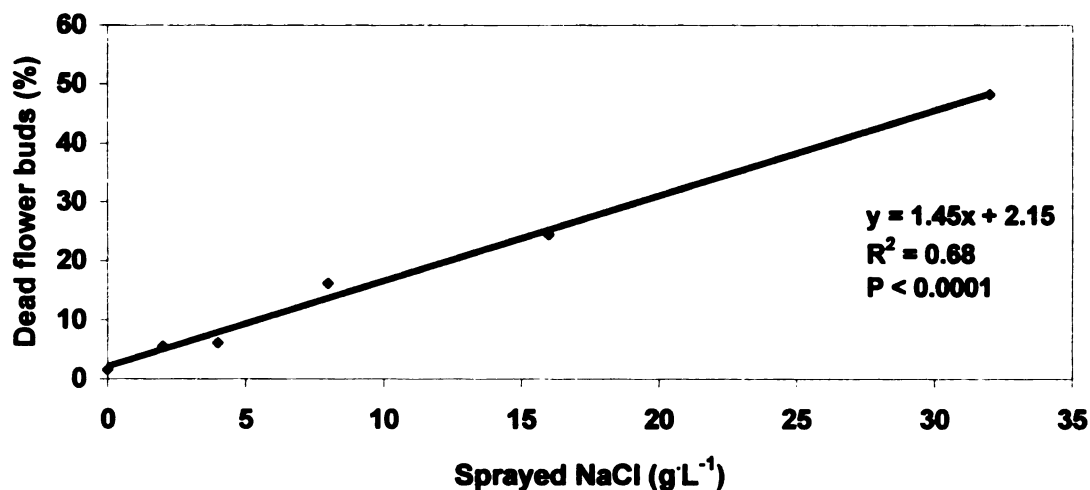


Figure 18: Percent dead flower buds of 'Bluecrop' blueberry bushes treated with NaCl sprays on 19 Jan., 4 Feb., 18 Feb., 1 Mar., and 19 Mar. 2002, in May 2002.



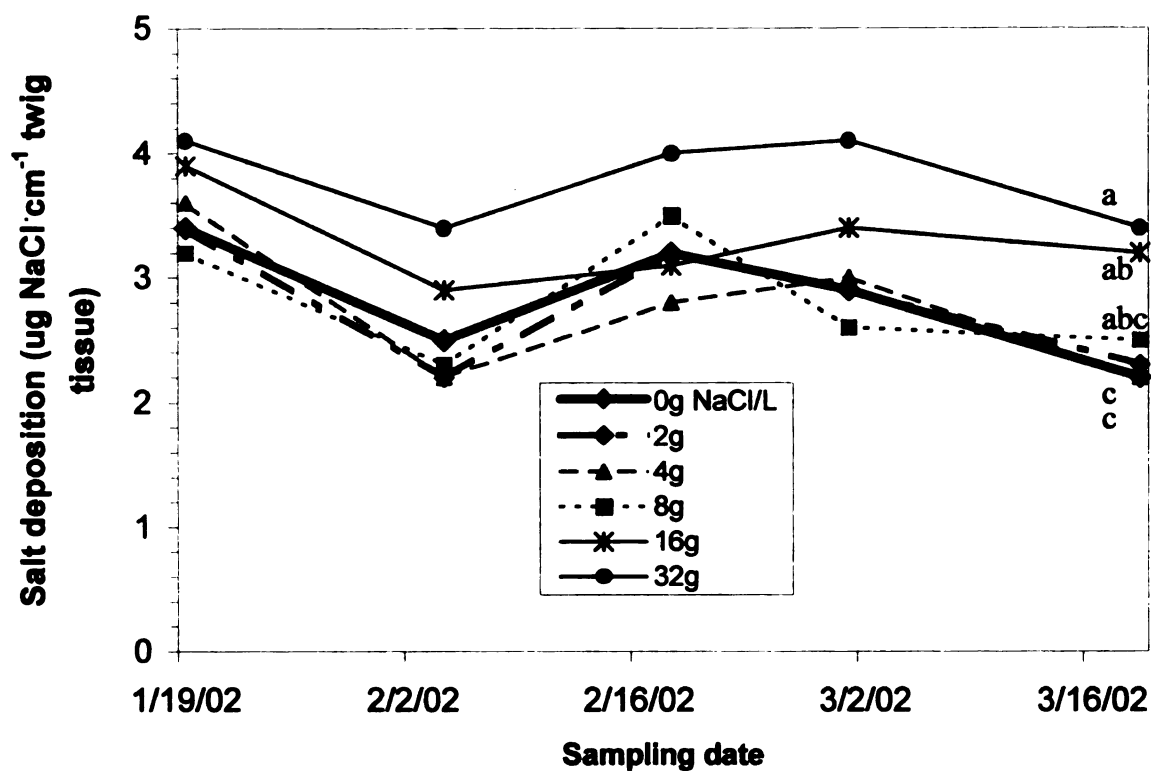


Figure 19: Salt rinsed from twig surfaces of dormant 'Bluecrop' highbush blueberry branches treated with NaCl sprays on 19 Jan., 4 Feb., 18 Feb., 1 Mar., and 19 Mar. 2002. Letters indicated significant differences between treatment means ( $P \leq 0.05$ ). No letters indicated insignificant differences.

**Soil Salt Inundation.** Increasing salt levels applied to soil in March resulted in a linear increase in flower bud mortality by May (Figure 17), and a decrease in dry matter production by June (Table 12). Plants treated with the higher salt levels contained a considerable amount of dead branches, and vegetative buds were either killed or developed slowly. Even though the soil volume was leached with water in April, soil  $\text{Na}^+$  and  $\text{Cl}^-$  levels in May increased linearly with application rate (Table 12).

Table 5: Soil  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations and new dry matter production (leaves, shoots, fruit) by 20 June 2002 of potted 'Bluecrop' blueberry plants treated with NaCl solutions applied to the rootzone on 1 Mar. 2002.

| NaCl applied<br>( $\text{g L}^{-1}$ ) | Leaf, shoot and<br>fruit dry weight<br>(g) | Soil salt ( $\text{mg kg}^{-1}$ ) |      |
|---------------------------------------|--|-----------------------------------|------|
|                                       |  | Na                                | Cl   |
| 0                                     | 11.0                                       | 95                                | 270  |
| 1                                     | 11.9                                       | 404                               | 481  |
| 3                                     | 11.4                                       | 777                               | 801  |
| 9                                     | 8.7  | 1810                              | 1970 |
| 27                                    | 1.3  | 2602                              | 2990 |
| Significance                          |  |                                   |      |
| Linear                                | ***  | ***                               | ***  |
| Quadratic                             | ***  | ***                               | ***  |

\*\*\* Significant main effects at  $P \leq 0.0001$ , for NaCl treatments partitioned into linear or quadratic orthogonal contrasts.

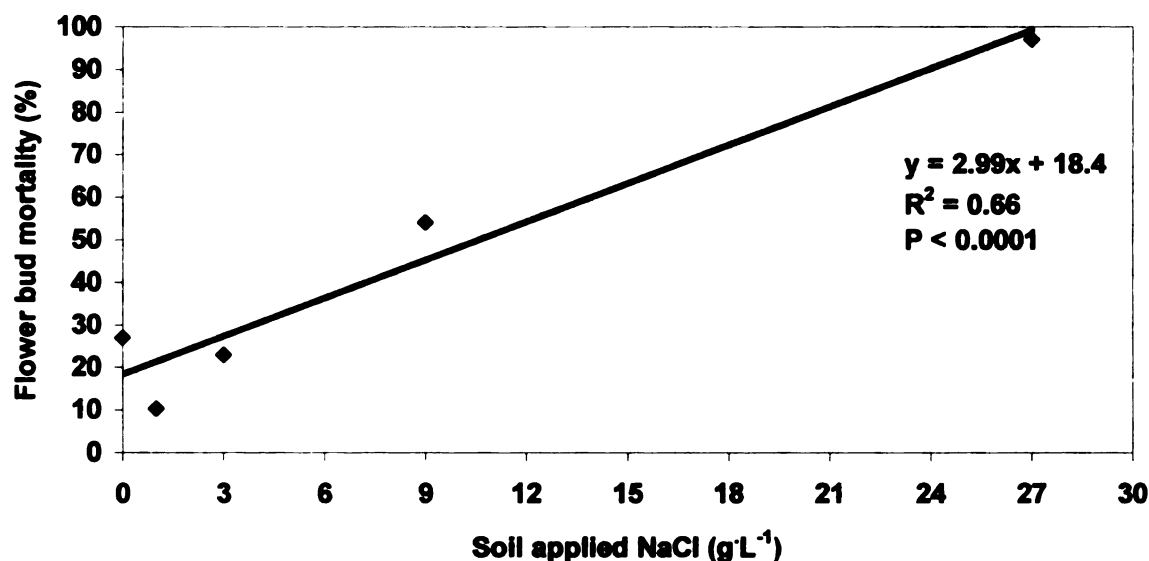


Figure 20: Relationship between rate of NaCl applied to soil in March and flower bud mortality in potted 'Jersey' blueberry bushes in May 2002.

**Cold Hardiness Study 1.** NaCl sprays applied to 'Jersey' branches for two consecutive days in January resulted in a linear reduction in flower bud hardiness (Table 13). Sprays of 16 and 64 g·L<sup>-1</sup> NaCl reduced the LT<sub>50</sub> of buds by 7.5 °C and 11.0 °C, respectively, compared to controls.

Table 13: Effect of NaCl sprays applied to branches of 'Bluecrop' highbush blueberry on 21 and 22 Dec. 2002, on cold hardiness of flower buds measured on 2 Jan. 2003.

| NaCl applied<br>(g·L <sup>-1</sup> )              | LT <sub>50</sub> (°C) |
|---|-----------------------|
| 0   | -28.0                 |
| 16  | -20.5                 |
| 64  | -17.0                 |
| Significance <sup>z</sup>                         |                       |
| Linear  | ***                   |
| *** significant linear response, $P \leq 0.001$ . |                       |

**Cold Hardiness Study 2.** Flower buds sampled in Feb. from bushes 65 m from a major divided highway in West Mich. were significantly ( $P \leq 0.0001$ ) less hardy ( $LT_{50} = -13.0$  °C) than buds from bushes 200 m from the highway ( $LT_{50} = -31.0$  °C).

**Cold Hardiness Study 3.** ‘Bluecrop’ branches sprayed for 2 consecutive days in February with NaCl ranging from 0 to 16 g·L<sup>-1</sup> also exhibited a linear decrease in flower bud cold hardiness measured 5 days later (Table 14). The highest NaCl concentration reduced the  $LT_{50}$  by 6.0 °C, compared to the control.

Table 14: Cold hardiness of flower buds measured on 9 Mar. 2003 treated with NaCl sprays applied to branches of ‘Bluecrop’ highbush blueberry on 27 and 28 Feb. 2003.

| NaCl applied (g·L <sup>-1</sup> ) | $LT_{50}$ (°C) |
|-----------------------------------|----------------|
| 0                                 | -22.5          |
| 4                                 | -18.0          |
| 8                                 | -19.5          |
| 16                                | -16.5          |
| Significance                      |                |
| Linear                            | ***            |
| Quadratic                         | ***            |

\*\*\* significant at  $P \leq 0.001$ .

**Cold Hardiness Study 4.** ‘Jersey’ branches sprayed with NaCl at 64 g·L<sup>-1</sup>, then sampled an hour later and tested for hardiness within 7 h also had a significantly ( $P = 0.012$ ) lower flower bud hardiness ( $LT_{50} = -24.0$  °C) than control ( $LT_{50} = -28.5$  °C).

**Cold Hardiness Study 5.** Sprays of chloride salt solutions (NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub>, KCl) supplying equivalent Cl<sup>-</sup> concentrations all reduced the hardiness of ‘Jersey’ flower buds

(Table 15). Potassium chloride resulted in the smallest reduction in cold hardiness (5.0 °C).

| Table 15. Cold hardiness of flower buds measured on 21 Jan., 2004 treated with Cl <sup>-</sup> salt solutions applied to branches of 'Jersey' highbush blueberry on 13 and 14 Jan. 2004. |                       |
|--|-----------------------|
| Treatment  | LT <sub>50</sub> (°C) |
| Control  | -26.0a <sup>z</sup>   |
| KCl  | -21.5b                |
| MgCl <sub>2</sub>  | -18.0c                |
| CaCl <sub>2</sub>  | -17.5c                |
| NaCl   | -16.0c                |
| P-value  | ≤ 0.0001              |
| <sup>z</sup> means within columns followed by a different letter are significantly different by Tukey's HSD (P ≤ 0.05).  |                       |

**Cold Hardiness Study 6.** Spraying 'Jersey' branches for two consecutive days in January with NaCl, NaAC, Na<sub>2</sub>SO<sub>4</sub> (supplying equivalent Na<sup>+</sup> concentrations) and freeze-testing twigs for hardiness on the third day resulted in a linear reduction of hardiness of flower buds of 5.0 °C, 4.5 °C, respectively, compared to deionized water (Table 16).

| Table 16: Cold hardiness of flower buds measured on 23 Jan. 2004 treated with selected Na <sup>+</sup> -salts applied to branches of 'Jersey' highbush blueberry on 15 and 16 Jan. 2004. |                       |
|--|-----------------------|
| Treatment  | LT <sub>50</sub> (°C) |
| Control  | -20.0a <sup>z</sup>   |
| Na <sub>2</sub> SO <sub>4</sub>  | -19.0a                |
| NaAC   | -15.5b                |
| NaCl   | -15.0b                |
| P-value  | ≤0.0001               |
| <sup>z</sup> means within columns followed by a different letter are significantly different by Tukey's HSD (P=0.05).  |                       |

## **DISCUSSION**

Multiple sprays of NaCl applied to blueberries during the winter resulted in similar injury symptoms (shoot dieback, flower bud mortality) observed in blueberry fields adjacent to salted roads in west Michigan (Chapter 2), indicating that deicing salt spray is predominantly responsible for the flower bud loss on blueberry farms in Ottawa county.

Consistent with Thompson and Rudder (1986), multiple sprays of salt to the above-ground portions of plants did not kill plants, whereas the highest rates of soil-applied NaCl resulted in considerable plant damage evident by early May (at full-bloom). Injury symptoms in the field, as much as complete loss of flower buds, are consistent with those from the soil salt inundation experiment (mean 97% flower bud loss), but the degree of shoot and cane dieback from the soil salt inundation study was much more severe than that seen at all but the most severely affected farms ('A' and 'I'). Excessive soil salt cannot be considered as a possible explanation for the flower bud loss at the most exposed sites where high levels of flower bud mortality were observed until more thorough soil sampling has been performed at those sites, as well as tissue sampling for internal  $\text{Na}^+$  and  $\text{Cl}^-$ .

Salt residues on twigs as a result of sprays (Figure 16) were similar to those observed on twigs adjacent to salted roads (Chapter 2). The fact that similar levels of salt accumulation, and the range of flower primordia injury, were observed on twigs sampled during the salt spray trial and from field-grown plants (Chapter 2) provides confidence that deicing salt spray was most likely responsible for injury to blueberry plants in Ottawa County, Mich. Deicing salt spray also had negative effects on lowbush

blueberries adjacent to major Canadian highways (Eaton et al., 1999). These workers reported the highest concentration of deicing salts on shoots from plants nearest the highway, and yields as well as number of living flower buds were inversely correlated with salt concentration on the shoots. Plastic shelters placed over plants reduced salt levels on shoots and increased numbers of live flower buds and yields, suggesting this type of barrier is effective at mitigating the damaging effect of deicing salts on lowbush blueberry.

High levels of  $\text{Na}^+$  and  $\text{Cl}^-$  remained in the soil samples after flushing with four liters of fresh water. Control pots contained surprisingly high levels of  $\text{Na}^+$  (95 ppm) and  $\text{Cl}^-$  (270 ppm). Soil chloride levels higher than 200 ppm may cause toxicity to glycophytic and halophytic plant species (Salinity Lab. Staff, 1954). Again, these are the levels from the soil cores taken just before destructive harvesting of the plants in late June, after uptake and flushing (due to irrigations) of these ions must have been occurring. This means that either the soil and/or the irrigation water used by the nursery contained high  $\text{Na}^+$  and  $\text{Cl}^-$  levels, or the irrigation (well) water used to maintain the plants contained high levels of these ions. Since the plants receiving the three lowest salt levels exhibited less than 30% of the flower bud mortality as the plants receiving the highest salt level (Figure 17), this could indicate that highbush blueberry is moderately tolerant of salt in the root zone. Highbush blueberry is considered somewhat drought-tolerant (Gough, 1994), and this feature may play a role in a plant's ability to tolerate salinity (Tal et al., 1979). Also, the root system of highbush blueberry is shallow (Gough, 1994), so plants may not be exposed to any salts that leach below depths of 20 – 25 cm.

Pedersen et al. (2000) measured annual accumulations of Na<sup>+</sup> and Cl<sup>-</sup> in soils adjacent to salted roads and found a gradual accumulation of salts in soils from one year to the next, and others have addressed the question of whether deicing salts accumulate in roadside soils over years (Button, 1964; Westing, 1969; Sucoff, 1975; Lumis et al., 1976; Bryson and Barker, 2002). Soil sampling in salt-affected fields might help determine whether soil salt is contributing to the injury in Ottawa County blueberry fields. It is possible that plants closest to US 31 at farms 'A' and 'I', and plants closest to M-45 at farms 'B' and 'C', are experiencing a gradual accumulation in shoot and cane tissues that are contributing to high levels of flower bud mortality. Farms 'A' and 'I' appear to be suffering the highest levels of flower bud loss and reduced yields.

Deicing salts decrease the cold hardiness of highbush blueberry flower buds; further, it is probable that decreases in cold hardiness from deicing salts are greater under field conditions than those measured under controlled circumstances. In the case of cold hardiness study #2, it is likely that field conditions are such that very high levels of deicing salt spray are being generated following a snow event for widely varying periods of time, resulting in a dynamic environment. These extreme conditions probably result in a situation where known trends relating to plant cold hardiness responses are not easily predicted under these specific circumstances.

Bud position did not affect flower primordia mortality, except in salt spray study #2 (data not shown). This is not consistent with observations on highbush blueberry (Biermann et al., 1979; Hancock et al., 1987), or *Vitis labruscana* 'Concord' (Stergios and Howell, 1977), where bud hardiness was significantly affected by bud position. The reason for this could be that treated shoots were usually vertical or nearly so, meaning the



salt treatments, applied to the point of drip, would drip down the length of the shoot due to gravity, concentrating greater quantities of salt around the lower flower buds. This might mean that their cold tolerance was affected to a greater degree than the terminal flower buds due to greater salt exposure, negating their greater cold hardiness. Salt spray applications for the cold hardiness evaluations were conducted during the mid-winter months when all flower buds are expected to be at a more or less equal stage of cold tolerance, which would likely obscure the effect of bud position (development) on cold tolerance. Differences in hardiness by bud position might be expected to appear if these same studies were conducted in late fall / early winter, or late winter / early spring.

Salt reduced the cold tolerance of blueberry flower buds almost immediately (study #4). The consistent effect of NaCl in decreasing the cold hardiness of flower buds agrees with Sucoff (1975), Sucoff et al. (1976), and Sucoff and Hong (1976a). How salt exposure compromises hardiness is unknown. The reduction in flower bud hardiness resulting from salt exposure could be related to desiccation, or perhaps  $\text{Na}^+$  or  $\text{Cl}^-$  toxicity (Sucoff, 1975). However, Northover (1987) found no evidence of crystallized NaCl deposits inducing desiccation in peach shoots; it was found that NaCl toxicity only occurred when present in solution. The data from experiments 5 and 6 do not indicate a specific ion toxic effect, as  $\text{Na}^+$  and  $\text{Cl}^-$  deicing salts both reduced the cold tolerance of flower buds, except for  $\text{Na}_2\text{SO}_4$ . Results from experiment 6 seem to suggest that the toxic effects of  $\text{Na}^+$  can be somewhat mitigated by sulfate ( $\text{SO}_4$ ), which might explain the lack of effect on cold tolerance by  $\text{Na}_2\text{SO}_4$ . This could also mean that  $\text{Na}_2\text{SO}_4$  is not an effective deicer for this same reason (i.e.,  $\text{SO}_4$  mitigates the depression on the freezing point of water by  $\text{Na}^+$ ).

One way  $\text{Na}^+$  in particular can desiccate flower buds relates to the hydration effect on the ion itself. The radius of  $\text{Na}^+$  is 0.98 Angstroms (Å) in the dehydrated state and 7.90 Å in the hydrated state (Foth, 1978). Compare this to  $\text{Li}^+$  (0.78 Å dehydrated, 10.03 Å hydrated), and  $\text{K}^+$  (1.33 Å dehydrated, 5.32 Å hydrated) (Foth, 1978). Clearly,  $\text{Na}^+$  has a high affinity for water. Regarding the deicing materials tested in this study, NaCl and NaAC would likely have a stronger desiccating potential based on the  $\text{Na}^+$  content of these materials, as opposed to  $\text{Ca}^{2+}$  ( $\text{CaCl}_2$ ),  $\text{Mg}^{2+}$  ( $\text{MgCl}_2$ ), and  $\text{K}^+$  (KCl).

The reduction in cold tolerance by salt exposure in highbush blueberry flower buds has never been quantified or described in any way prior to these studies. Also, the decreases in cold tolerance occurred at temperatures that usually occur in an average winter in West Michigan. This means that these observed responses by way of reduced cold tolerance are reflective of real-world observations, hence, significant to blueberry farms at-risk of experiencing deicing salt injury.

Cold hardness was affected most severely by NaCl compared to  $\text{Na}_2\text{SO}_4$ , NaAC,  $\text{MgCl}_2$ ,  $\text{CaCl}_2$  and KCl. Based on  $\text{LT}_{50}$  levels, it would seem that KCl might be a promising candidate for reducing injury along US 31 and M-45; at least on stretches of those highways adjacent to the most seriously affected farms in Ottawa County, Michigan. It is not known at this time where any of these alternative deicers are being used for deicing purposes in Michigan. Potassium chloride is effective down to  $-11.0^\circ\text{C}$  (MDOT, 1993). The lowest effective temperature of KCl is lower than that of NaCl, so this should not exclude KCl from use as a possible deicing alternative to NaCl. Information regarding  $\text{Na}_2\text{SO}_4$  as a deicing material could not be found.

Sodium toxicity is reportedly not as widespread as  $\text{Cl}^-$  toxicity, and is mainly related to low  $\text{Ca}^{2+}$  levels in saline substrates, or high  $\text{Ca}^{2+}/\text{Na}^+$  ratios in combination with poor soil aeration (Marschner, 1990). Experiments with many species showed that  $\text{Cl}^-$  damage was more severe than  $\text{Na}^+$ , and work with *Rubus* spp. treated with NaCl demonstrated that  $\text{Cl}^-$  accumulated in the shoots faster than  $\text{Na}^+$ , and the severe necrosis was attributed primarily to  $\text{Cl}^-$  (Wright et al., 1992). In spite of these findings and many others related to salt injury to plants, there does not seem to be uniform agreement in the literature on how salt damages dormant plants.

Short days, low ambient relative humidity, and extracellular ice formation during freezing temperatures, all contribute to cell dehydration. The movement of cellular water to extracellular ice crystals is an especially important strategy for cold tolerant plants (Kuroda et al., 2003). However, excessive dehydration is lethal to cells (Kuroda et al., 2003).

Deep supercooling is a freeze-avoidance mechanism for some plant tissues (i.e., blueberry flower buds, and xylem ray parenchyma cells [XRPCs] in most temperate zone trees) that seems to suggest that the cold acclimation process involves reduction or elimination of ice nucleating sites in such tissue cells, development of effective barriers to ice nucleation events, or both (Burke et al., 1976). In XRPCs that undergo deep supercooling, a low temperature exotherm is produced below  $-20\text{ }^{\circ}\text{C}$  (Kuroda et al., 2003). Supercooling is also a freeze-avoidance mechanism that occurs in flower buds, XRPCs, and some other tissues, and occurs at higher temperatures than deep supercooling.



Since blueberry flower buds tolerate winter cold by deep supercooling (Quamme, 1991), salt exposure may reduce bud hardiness by disrupting this process. One way this might occur is by desiccating, or depressing the freezing point of the flower bud scales, which are the first tissues to freeze when flower buds freeze (Ishikawa and Sakai, 1981). This would then allow the flower primordia axis to desiccate and/or freeze, destroying the freeze avoidance mechanism(s) that may already have occurred. The flower primordia would logically suffer cold injury. Another explanation might be that NaCl damages the bud scales, reducing the insulating and anti-desiccating protection provided by the bud scales. When flower buds were dissected following these controlled freezing studies, the bud scales of salt-treated twigs often appeared tattered and loosely connected to the flower buds (personal observation), giving rise to this theory. Exposure to NaCl might compromise the integrity of the individual bundle fibers to the point that strong wind gusts loosen the bud scales. This could make the ice nucleation event easier. Flower and leaf buds are important organs for reproduction and vegetative proliferation in the following growing season, but they are often also more susceptible to freezing than are twig tissues (Ishikawa et al., 1997).

The highest soil salt level (i.e.,  $27\text{g}\cdot\text{L}^{-1}$  NaCl) had a more severe effect on flower bud mortality in highbush blueberry than the highest salt level in the salt spray trial ( $32\text{g}\cdot\text{L}^{-1}$  NaCl) (Figures 15 and 18), indicating that salt spray is less harmful to the survival of flower buds than soil borne salts. However, the overall level of injury to highbush blueberry plants in the fields as assessed in the spring farm surveys suggests that salt spray is the probable factor. All salts with known deicing properties decreased the cold tolerance of highbush blueberries in this study, although NaCl appeared to be the

most harmful (Tables 13 through 16). Sodium sulfate did not decrease cold tolerance, but it is unknown whether it possesses deicing properties.

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## **CHAPTER IV**

## **CONCLUSIONS**

## **CONCLUSIONS**

These studies indicate that twig dieback and flower bud mortality in blueberry fields along highways in Michigan were the result of windborne salt spray deposited on twigs. This conclusion is based on the fact that salt deposition and flower bud mortality were greatest on plants closest to the road. Flower bud mortality was generally greatest on farms east of a divided major highway, which was downwind of the prevailing wind direction. Secondly, treating plants with salt sprays induced twig dieback and flower bud mortality, that appeared identical to the injury on plants next to roads. Salt sprays resulted in similar salt levels on twigs as observed on twigs of bushes next to roads, although salt deposition levels on field-grown plants were probably reduced by precipitation events.

Soil salt accumulation may also contribute to plant injury close to roads, but only at the most severely affected sites (farms 'A' and 'I'). Intermediate levels of NaCl applied to the soil of potted plants resulted in flower bud mortality and twig dieback similar to plants next to salted roads. Highest rates resulted in severe injury, including complete loss of flower buds and negligible growth. Some plants closest to the highway also exhibited chronic dieback and poor growth. Because soil salt levels were not measured at varying distances from the road, the influence of soil salts on plant injury cannot be determined.

One observation made at severely affected farms is that many plants set few or no flower buds. High soil and/or tissue levels might be contributing to this aberrant growth. Elevated soil salt levels could be responsible for increasing plant tissue salt levels to the

extent that flower bud development is suppressed, but not toxic to the plant. This could be studied in the future by detailed soil and tissue sampling.

Salt exposure decreased the cold hardiness of 'Jersey' and 'Bluecrop' highbush blueberry flower buds. Flower bud mortality in fields adjacent to major roads in west Michigan may result from reduced hardiness and subsequent winter injury since typical winter injury symptoms are similar to those induced by salt exposure. The results of the cold hardiness spray study #2 suggests that NaCl decreases cold hardiness to a greater extent in the field than that which was measured under controlled conditions. This makes sense, as it is not possible to duplicate the variables influencing salt spray injury in the field under controlled conditions, meaning that it is likely that NaCl causes more serious injury in the field than observed after controlled salt treatments. This would translate into greater yield losses for the grower than that which the regression models described (Chapter 2).

How salt reduces hardiness is not clear. However, all salts tested, except Na<sub>2</sub>SO<sub>4</sub>, decreased cold hardiness, indicating that the effect of salt on hardiness is not a specific response to Na<sup>+</sup> or Cl<sup>-</sup>. This hints at a physical effect, since differences in ionic radii between the hydrated and dehydrated state for Na<sup>+</sup> indicate a high affinity for water. Differences between the hydrated and dehydrated ionic radii for Cl<sup>-</sup> were not found in the literature, but if Cl<sup>-</sup> can penetrate cellular membranes passively with Na<sup>+</sup> in the case of NaCl exposure, this would explain why NaCl always decreased cold hardiness greater than the other salts tested. Sulfate may partially mitigate the toxic effects of Na<sup>+</sup>, as evidenced by the fact that Na<sub>2</sub>SO<sub>4</sub> did not affect the cold tolerance of flower buds.

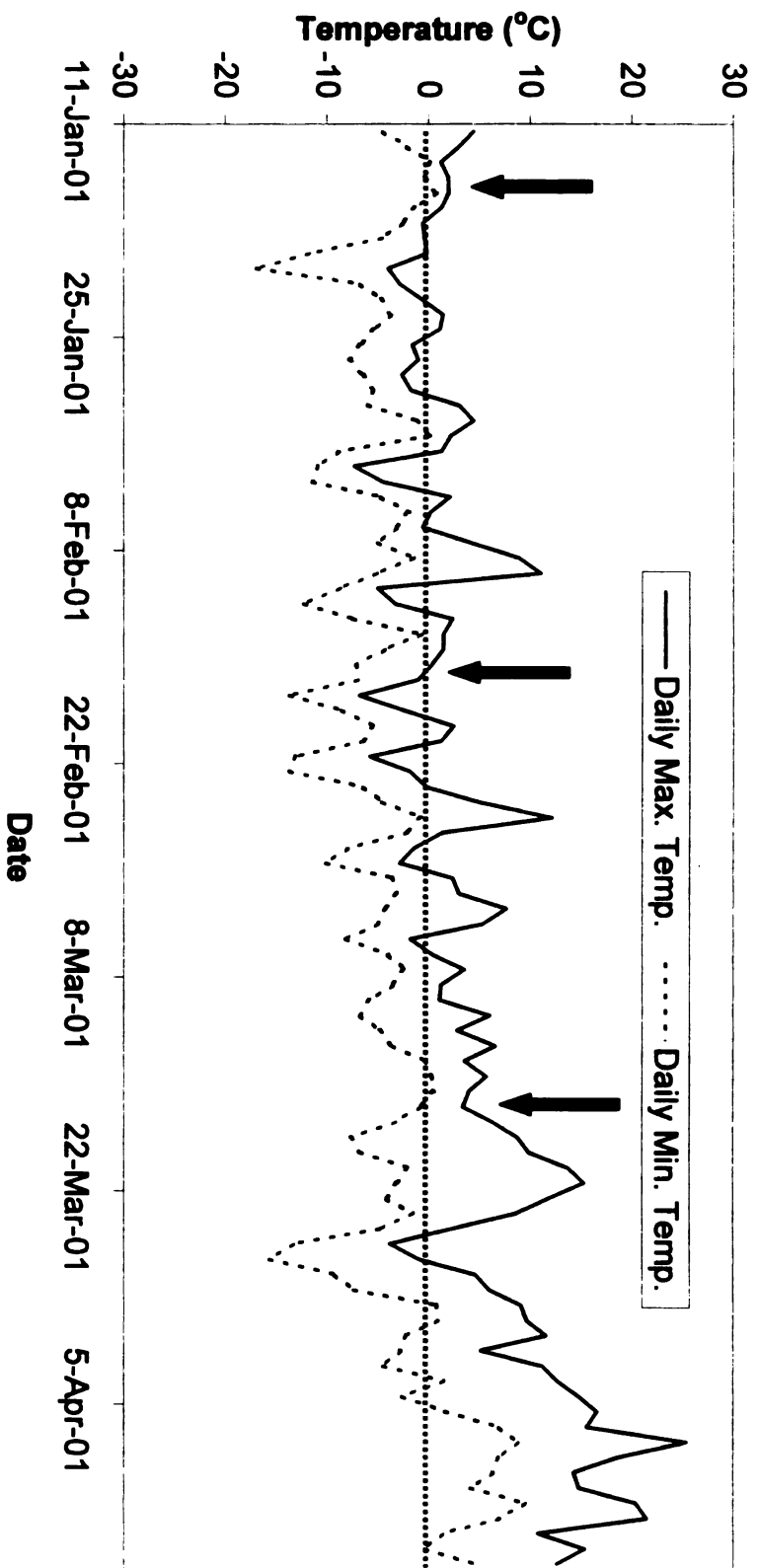
More needs to be known about how NaCl is affecting other woody vegetation, groundwater supplies, and the soil structure in areas where deicing materials are utilized. Since deicing salts will continue to be applied during the winter months throughout Michigan for the foreseeable future, surveying other fruit and nursery crops would give a better idea of other plant species that are sensitive (or tolerant), as well as revealing additional economic losses.

Deicing salts have been in use for about 60 years, and injury has been reported occurring to plants surveyed as part of this work for about the last 10 years. Given the apparent increasing decline in the general health of blueberry plants at some of the most severely affected farms as seen during the May farm surveys, it is reasonable to predict that plant mortality will soon be reported to blueberries growing along US 31, especially if deicing salt usage increases. Usage will likely increase if Census Bureau predictions, that population growth in Ottawa county will increase 20% over the next 10 years (US Census Bureau, 2000), are accurate, as this will result in an increase in vehicular traffic on US 31 and M-45. Vehicular traffic on US 31 has steadily increased within the last 8 years (Tim Croze, MDOT, pers. comm.) Average daily traffic (ADT) on US 31 north of M-45 increased by 20% from 1997 to 2001 (Tim Croze, MDOT, pers. comm.) This may be because the population of Ottawa County increased nearly 27% during the 1990s, and is projected to increase by 30% or greater by the year 2025 (U.S. Census Bureau, 2000). An increase in traffic volume will require more timely NaCl applications, perhaps with increased frequency, and with more salt spray being whipped-up by the escalating vehicular traffic, which could potentially result in increased plant injury due to deicing salt.

The use of sprayable materials to protect against salt spray injury remains elusive. An attempt was made to find a sprayable material that might protect the plants from deicing salt spray injury. After a preliminary trial to test the products for phytotoxicity, spray trials were performed under controlled conditions and in the field. No material protected flower buds (Appendix 23).

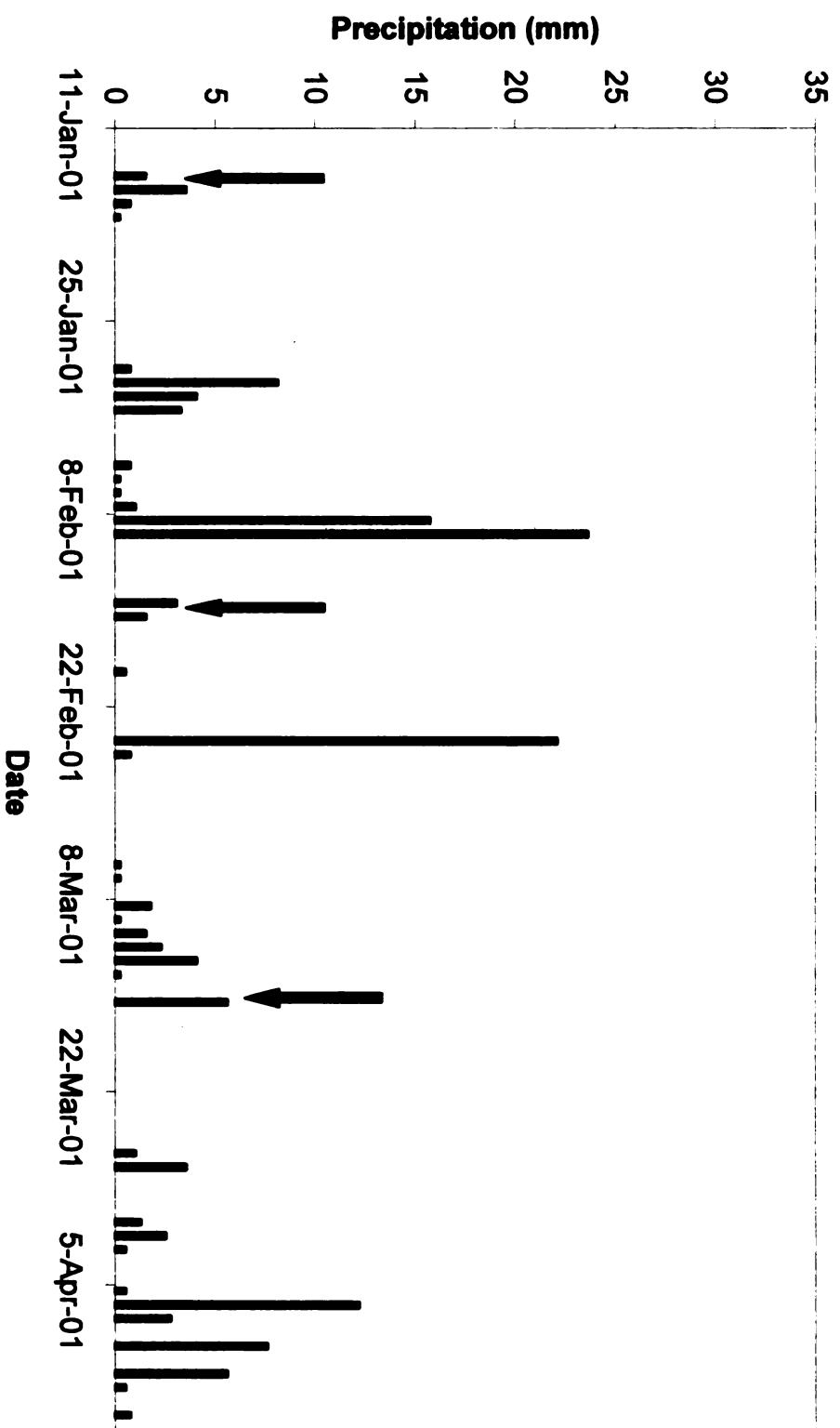
Injury to blueberries in west Michigan might be reduced by applying less phytotoxic deicing salts along stretches of highway adjacent to blueberry plantings, applying just enough deicing material to achieve the desired result, using precision application equipment, or planting salt-tolerant coniferous tree species as a screen against wind-blown salt spray. New blueberry plantings should not be planted within 500 feet of any road receiving deicing salt applications in the winter months.

## **APPENDICES**

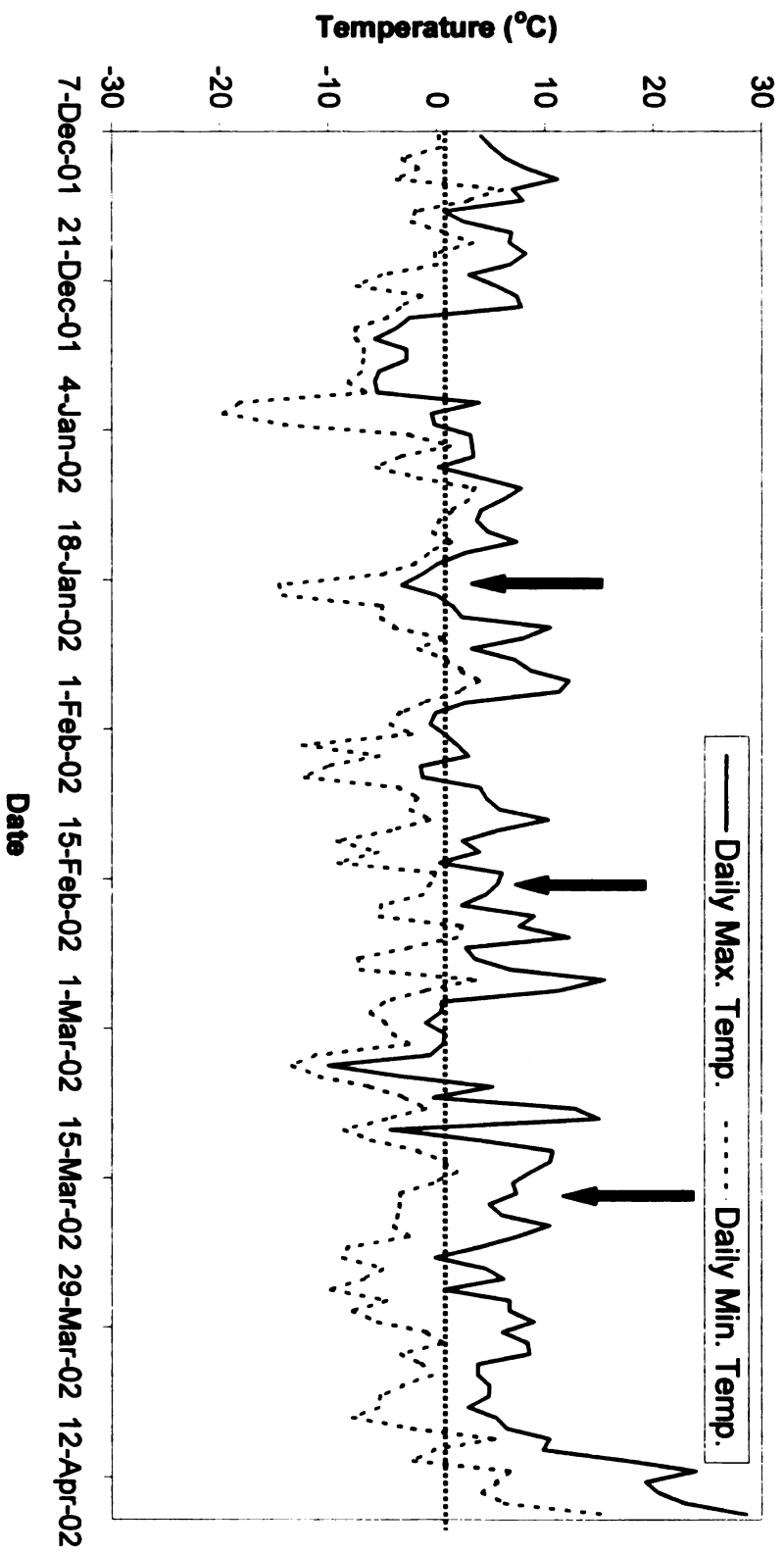


Appendix 1: Maximum and minimum daily temperatures during twig sampling, 2001, for Ottawa County, Mich. Twigs were excised 15 Jan. 01, 15 Feb. 01, and 15 Mar. 01 (arrows).

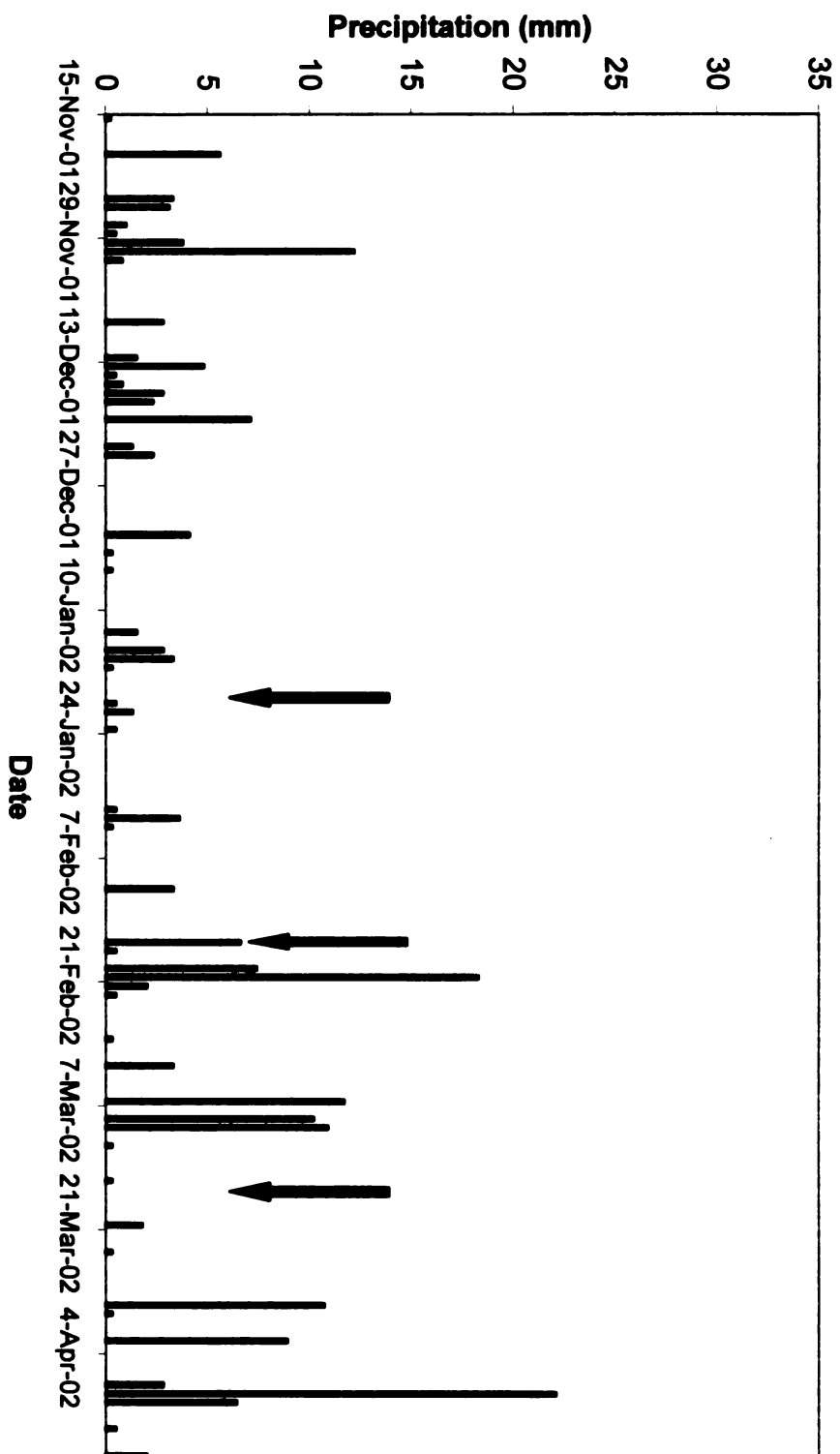




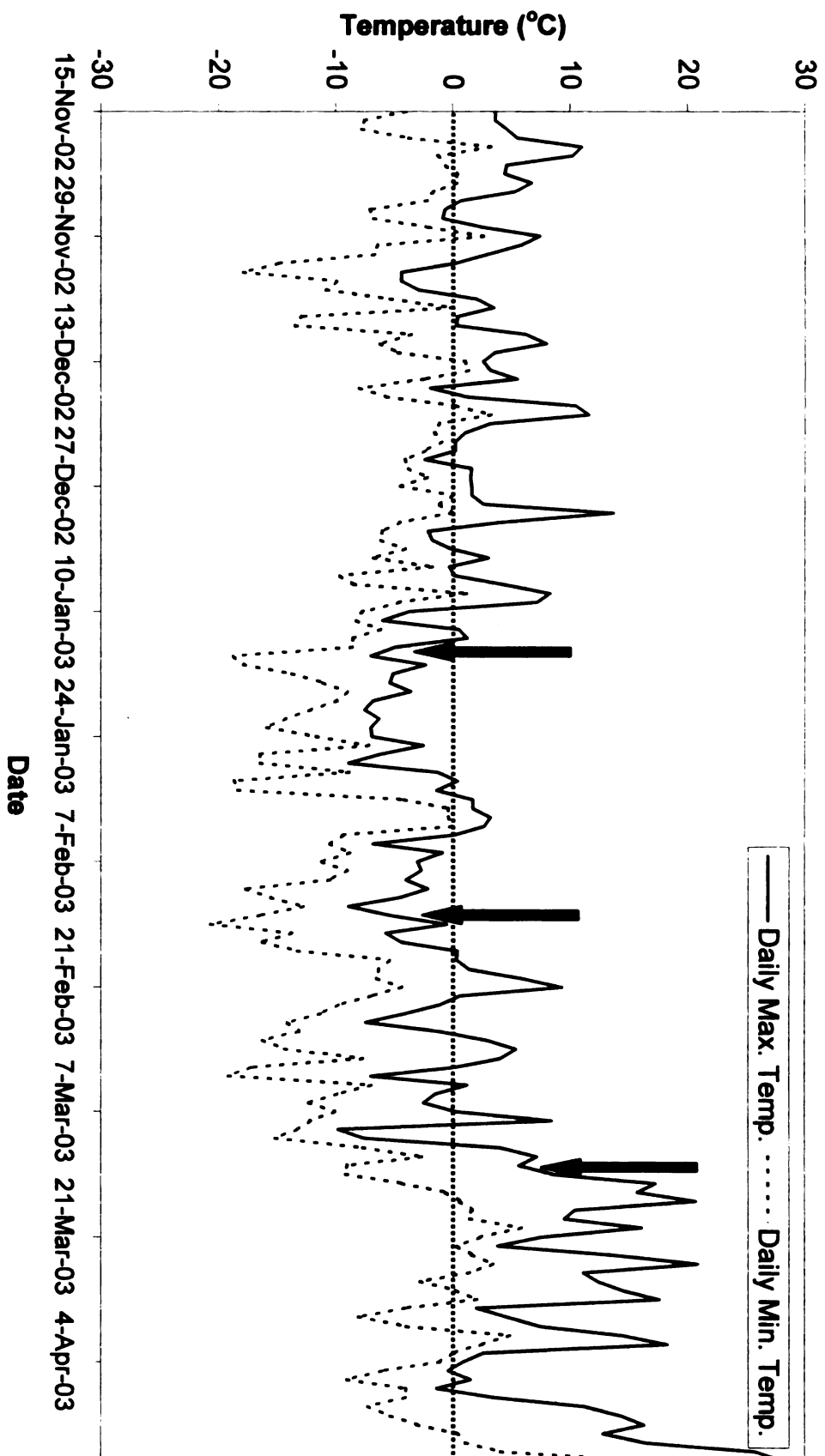
Appendix 2: Precipitation events during twig sampling, 2001, for Ottawa County, Mich. Twigs for study excised 15 Jan. 01, 15 Feb. 01, and 15 Mar. 01 (arrows).



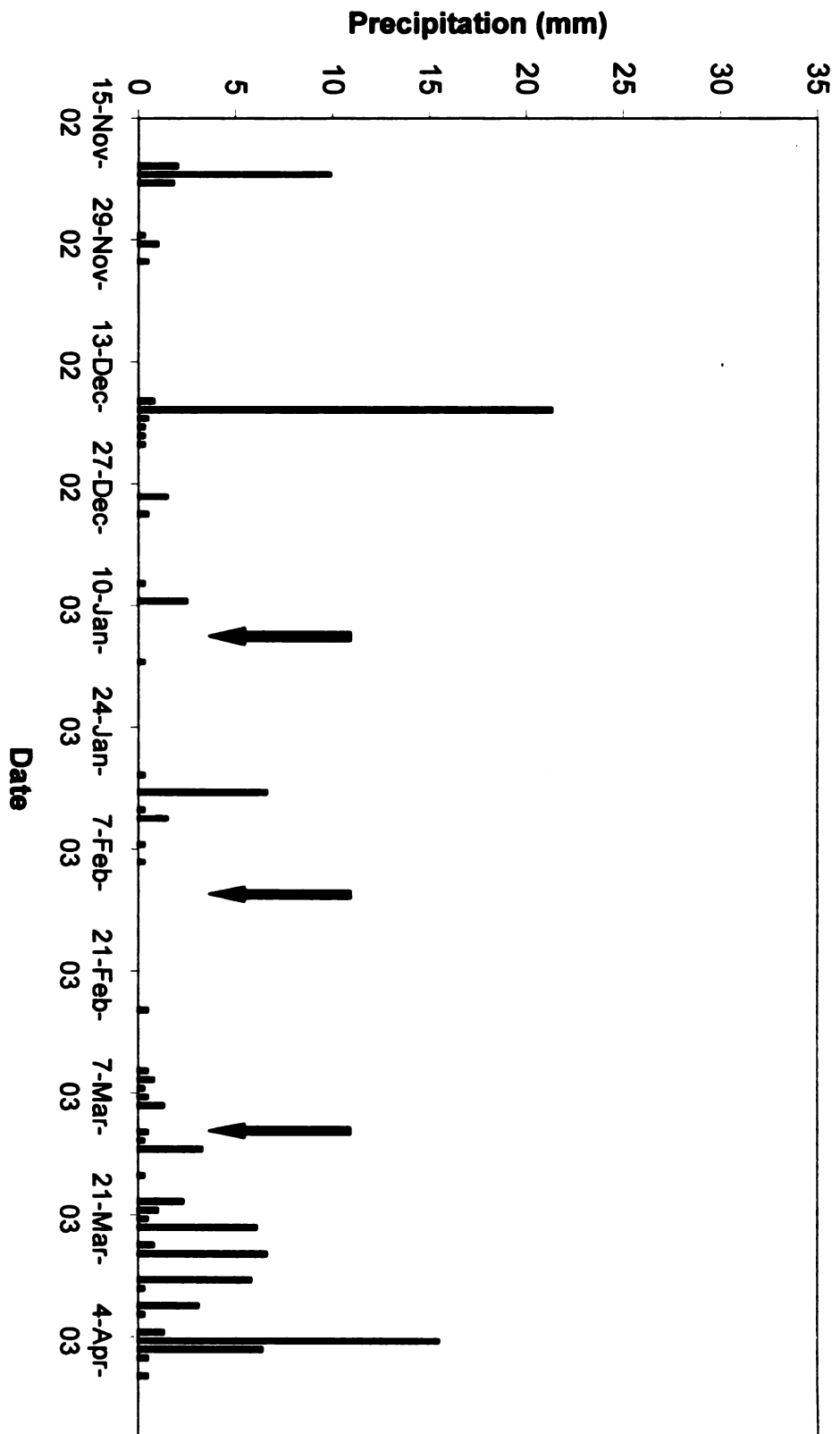
Appendix 3: Maximum and minimum daily temperatures during twig sampling, 2001 – 2002, for Ottawa County, Mich. Twigs were excised 19 Jan. 02, 16 Feb. 02, and 16 Mar. 02 (arrows).



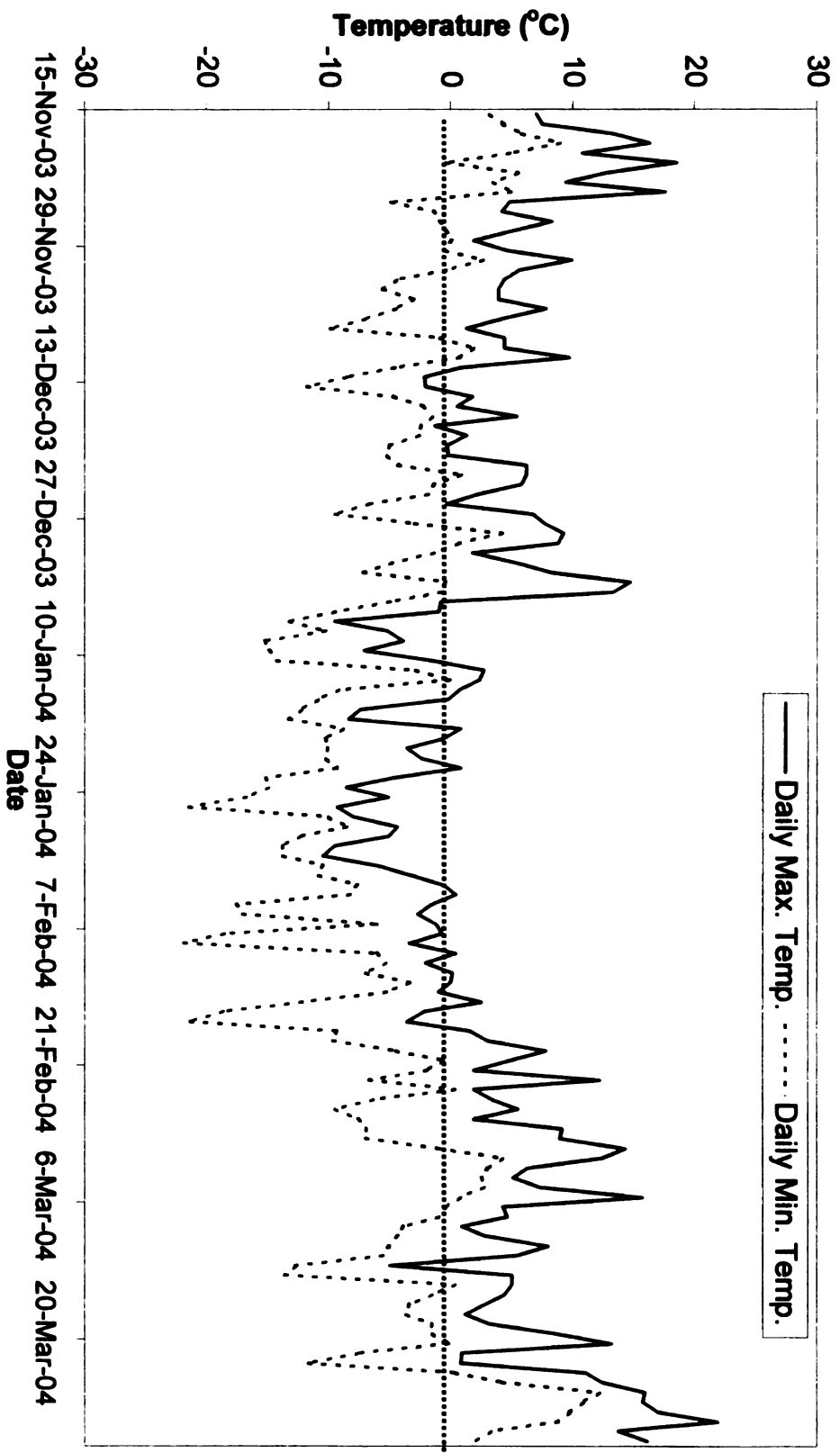
Appendix 4: Precipitation events during twig sampling, 2001 – 2002, for Ottawa County, Mich. Twigs were excised 19 Jan. 02, 16 Feb. 02, and 16 Mar. 02 (arrows).



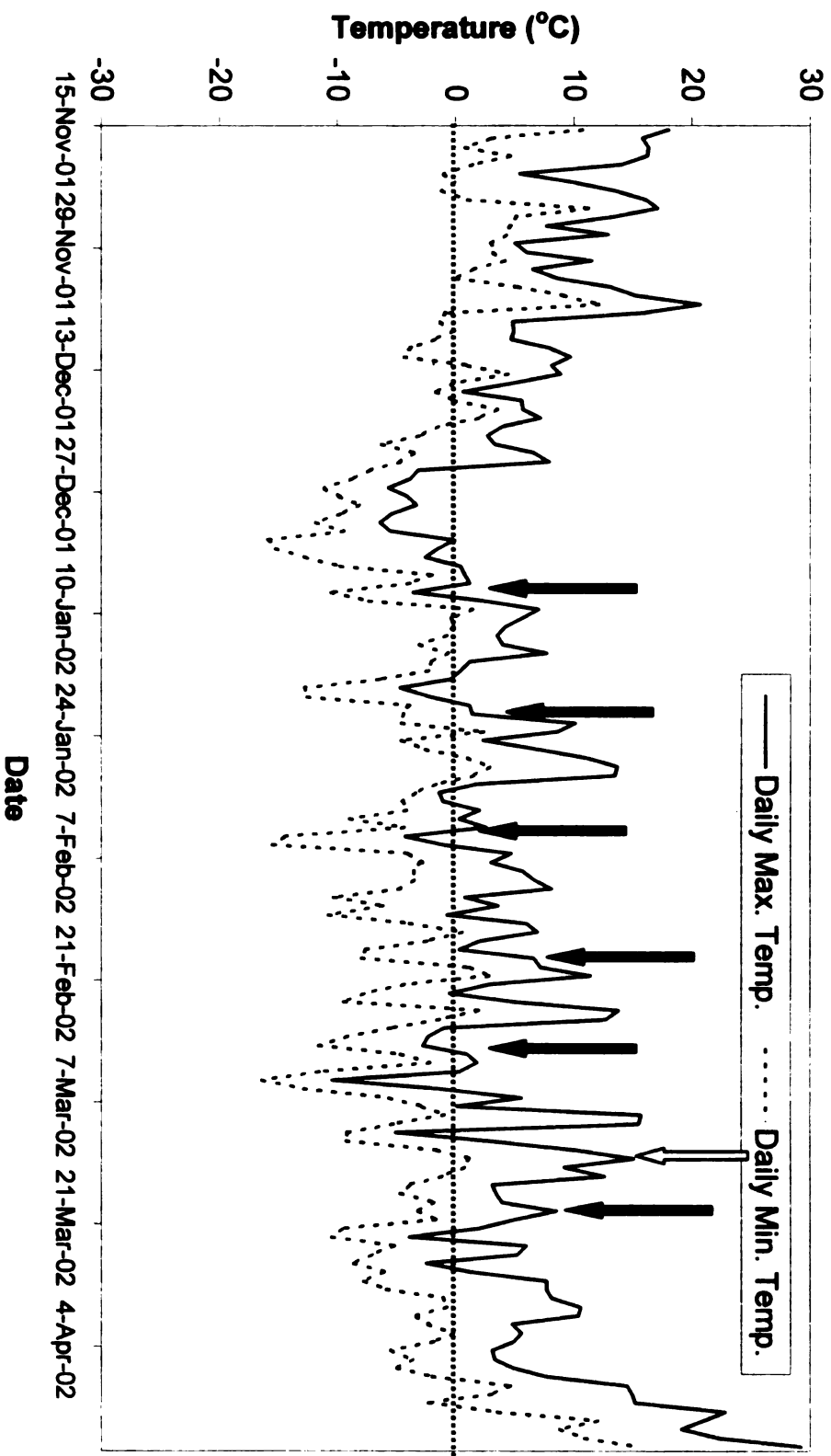
Appendix 5: Maximum and minimum temperatures during twig sampling, and prior to cold hardiness salt spray study #2, 2002 – 2003, Ottawa County, Mich. Twigs excised on 14 Jan. 03, 13 Feb. 03, and 13 Mar. 03. Twigs for salt spray study #2 excised on 13 Feb. 03.



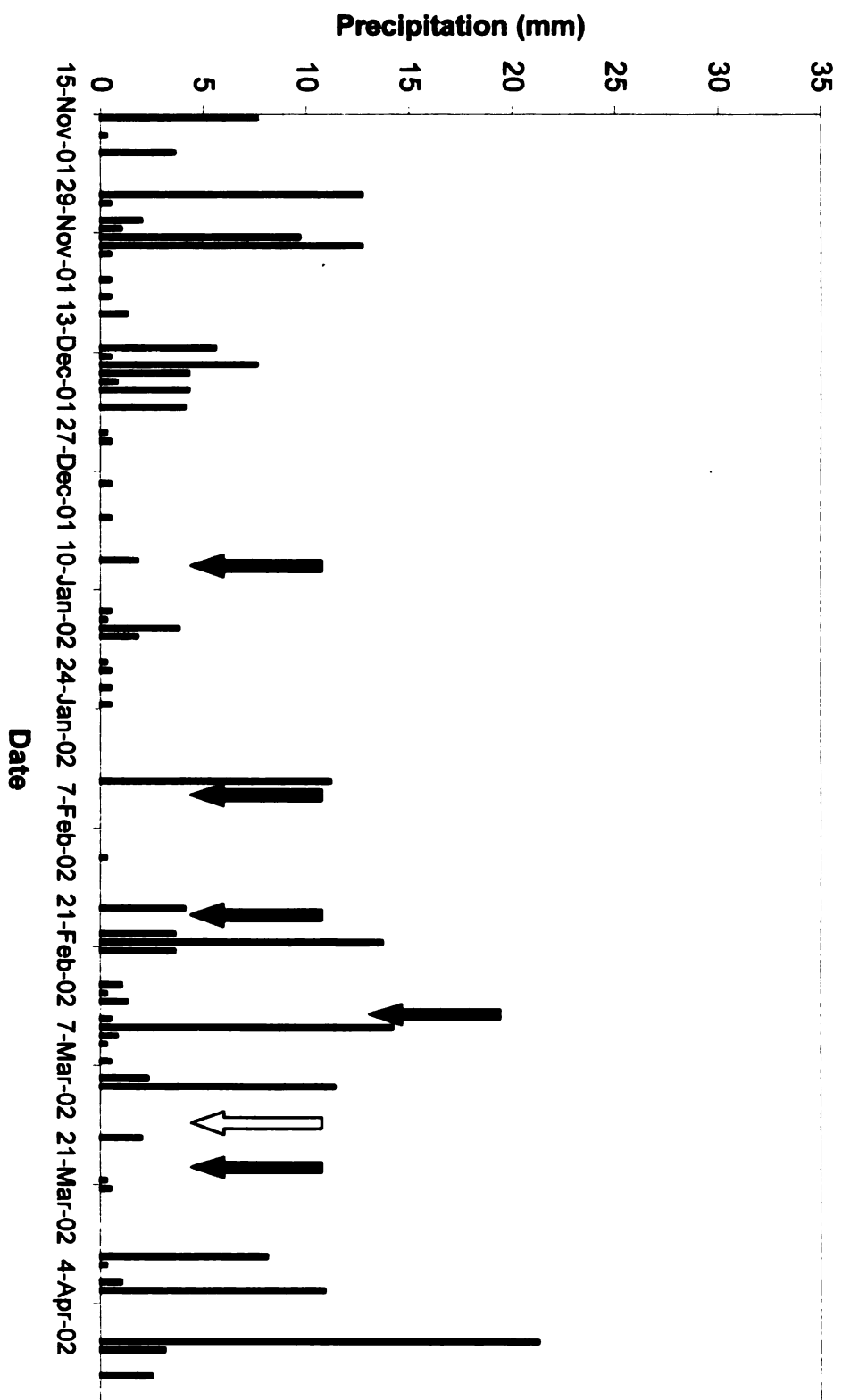
Appendix 6: Precipitation events during twig sampling, and prior to study 6, 2002 – 2003, Ottawa County, Mich. Twigs excised on 14 Jan. 03, 13 Feb. 03, and 13 Mar. 03. Twigs for salt spray study #2 excised on 13 Feb. 03.



Appendix 7: Maximum and minimum daily temperatures prior to May 2004 farm surveys, Ottawa County, Mich.

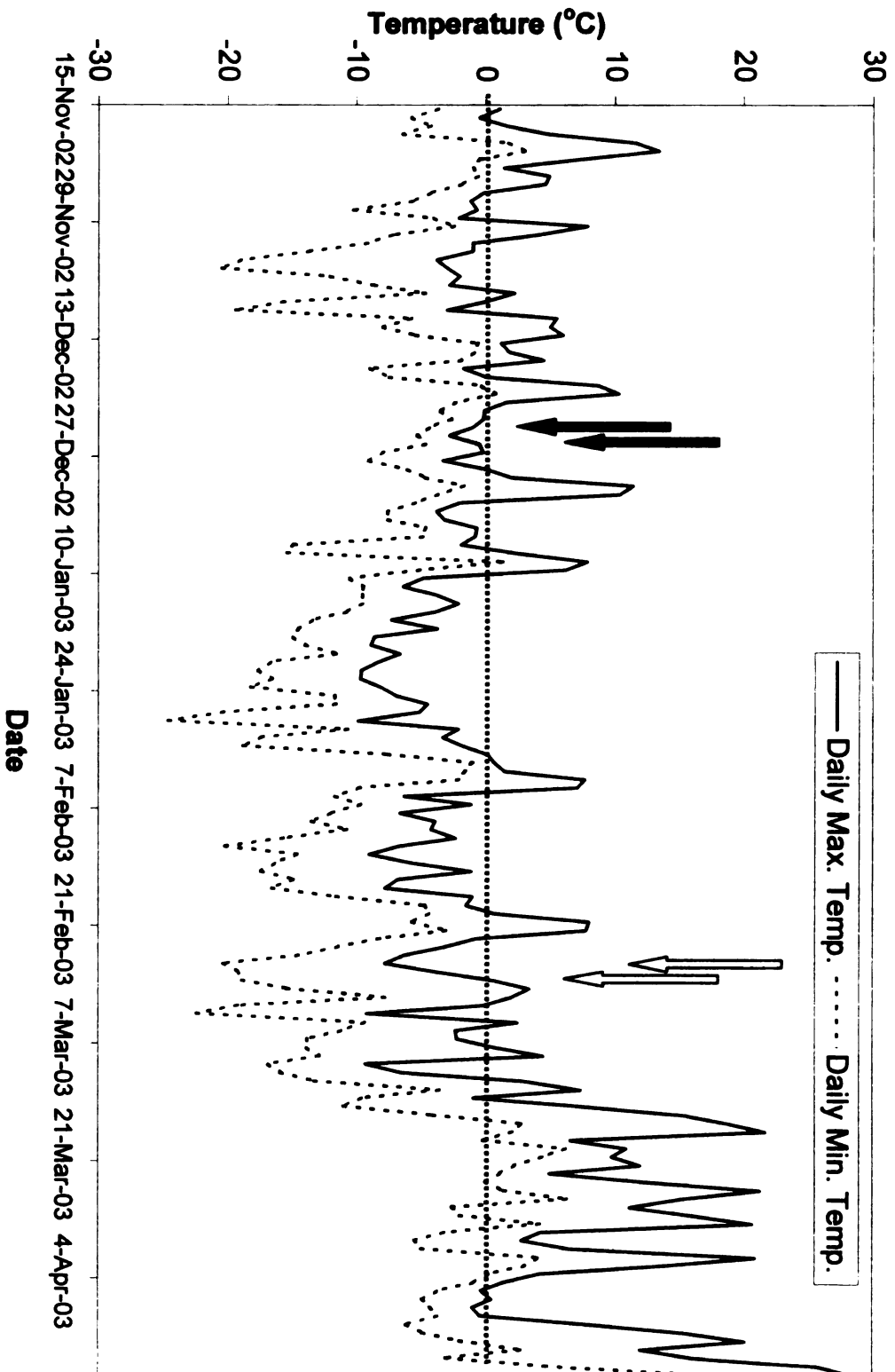


Appendix 8: Maximum and minimum daily temperatures prior to salt spray and soil salt inundation studies, 2001 – 2002, at HTRC, Ingham County, Mich. Controlled salt spray applications applied on 7 Jan. 02; 21 Jan. 02; 4 Feb. 02; 18 Feb. 02; 1 Mar. 02; and 19 Mar. 02 (solid arrows, study 2). Salts applied to soil for soil salt inundation (study 3) on 13 Mar. 02 (unfilled arrow).

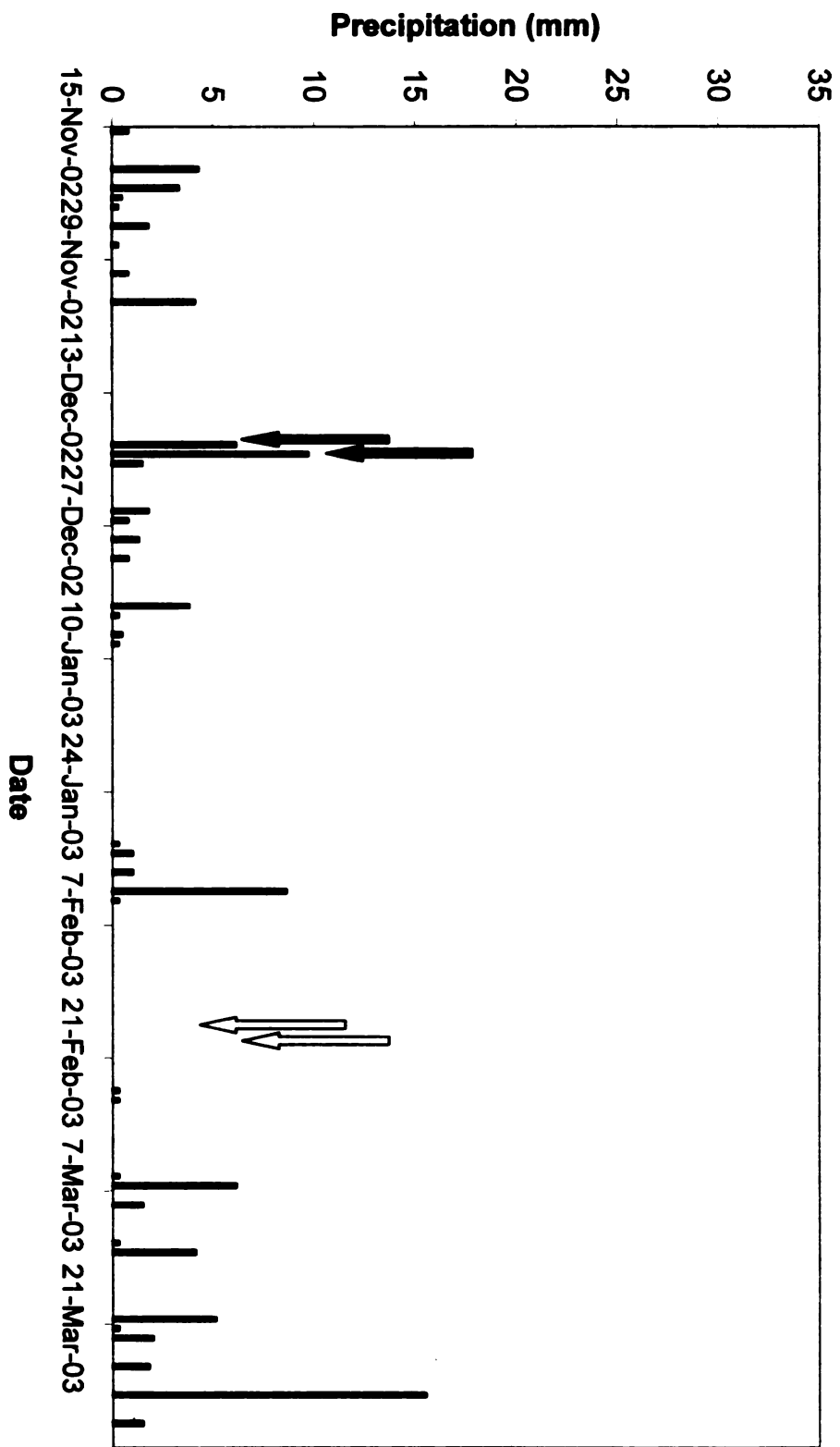


Appendix 9: Precipitation events prior to salt spray study (solid arrows) and soil salt inundation study (unfilled arrow), HTRC, 2001 – 2002, Ingham County, Mich.

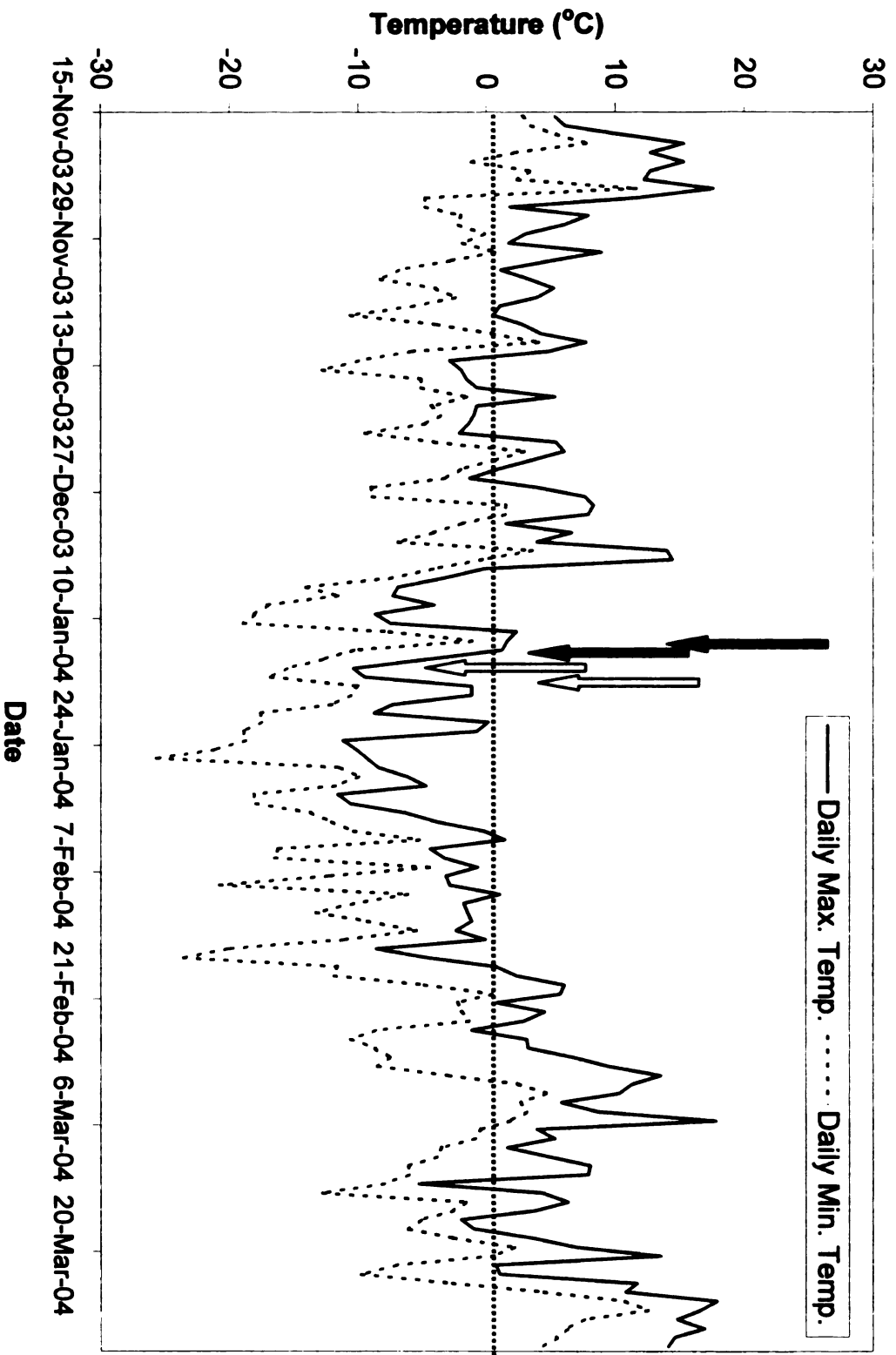




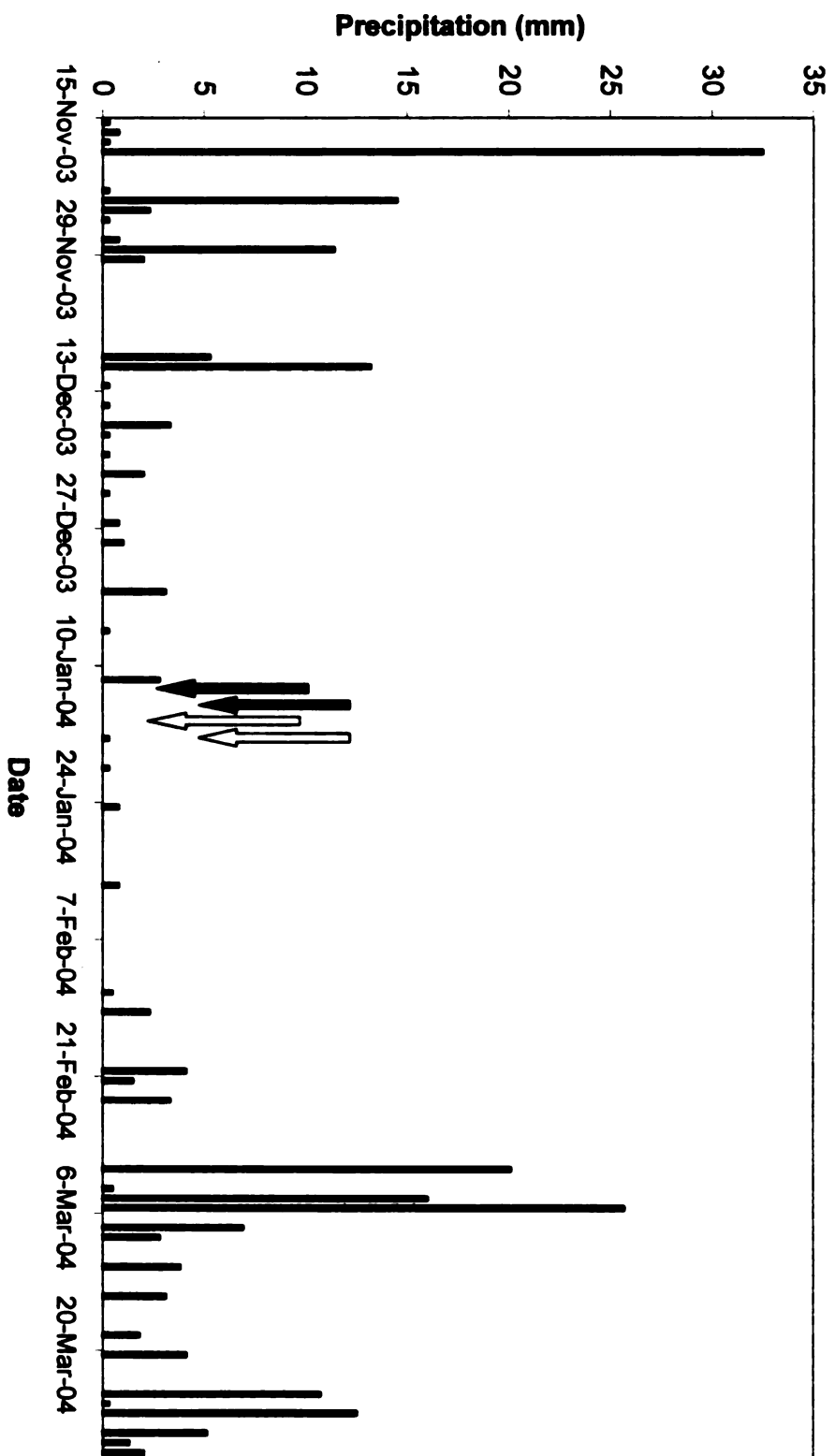
Appendix 10: Maximum and minimum daily temperatures during cold hardiness salt spray study 1 (solid arrows), and study 3 (unfilled arrows), Ingham County, Mich., 2002 – 2003.



Appendix 11: Precipitation events during cold hardiness salt spray study 1 (solid arrows), and study 3 (unfilled arrows), Ingham County, Mich., 2002 – 2003.

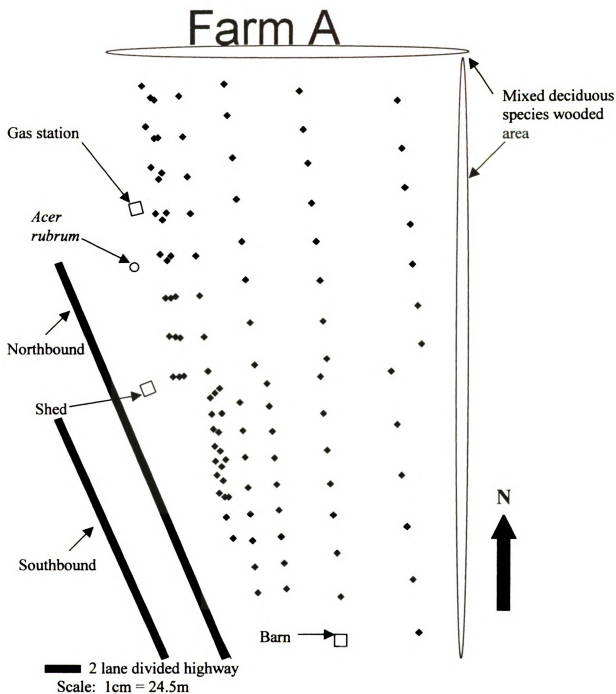


Appendix 12: Maximum and minimum daily temperatures during cold hardiness salt spray studies 4 and 5 (solid arrows), and study 6 (unfilled arrows), HTRC, Ingham County, Mich., 2003 – 2004.

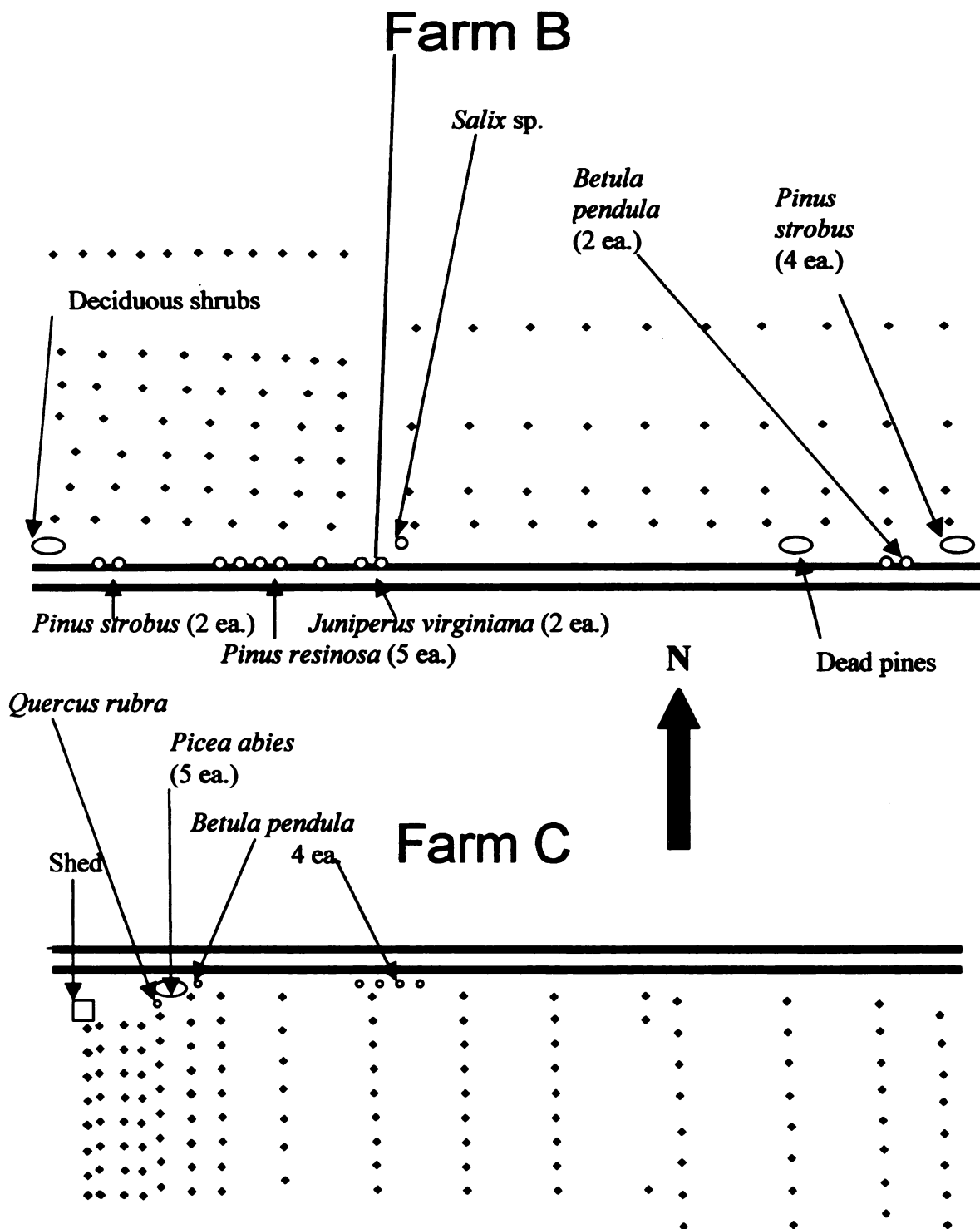


Appendix 13: Precipitation events during cold hardiness salt spray studies 4 and 5 (solid arrows), and study 6 (unfilled arrows), HTRC, Ingham County, Mich., 2003 – 2004.





Appendix 14: Schematic map of farm 'A', Ottawa County, Mich., showing surveyed plant locations, structures, and vegetation.

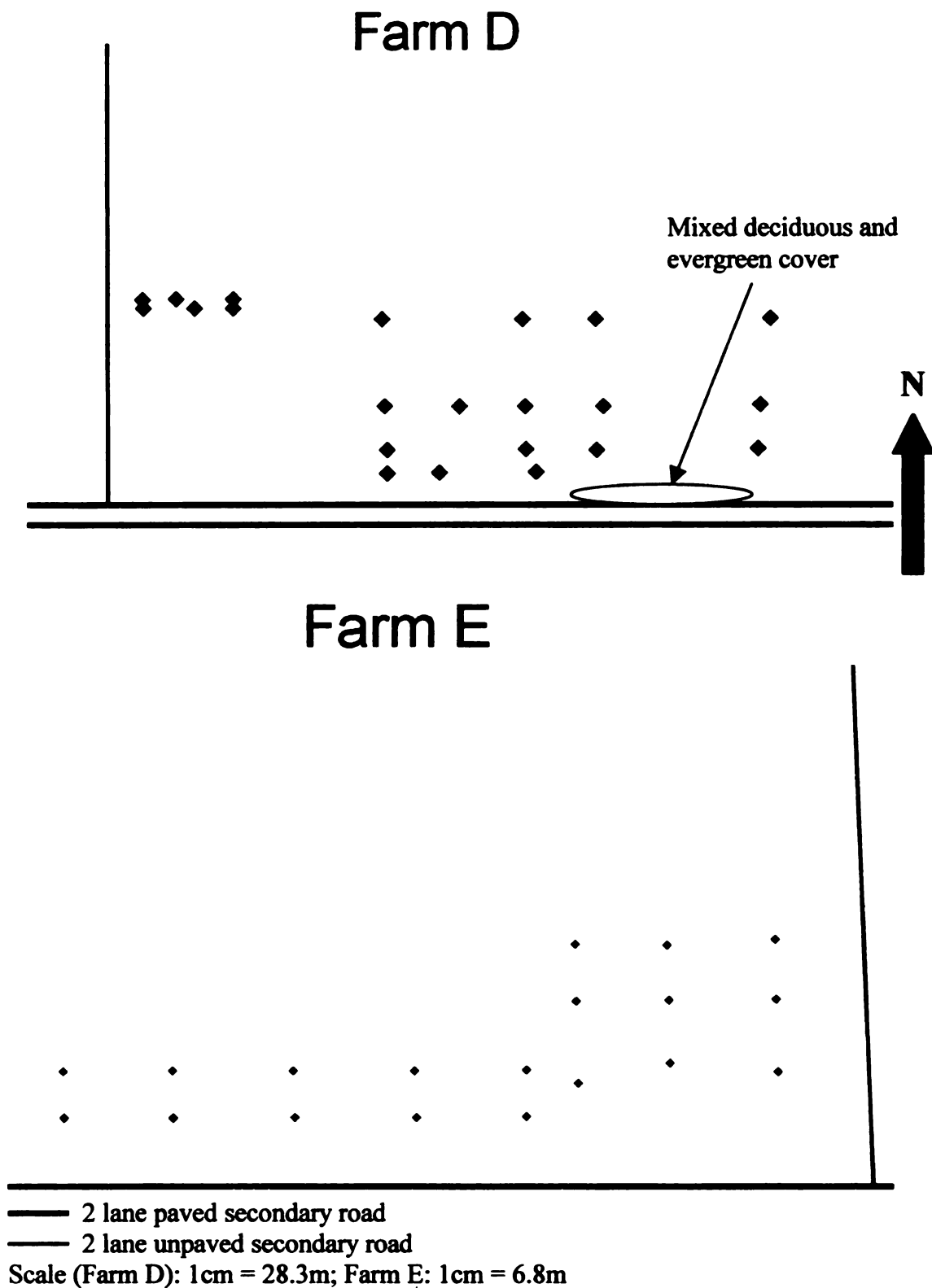


— 2 Lane undivided highway

— Farm access road

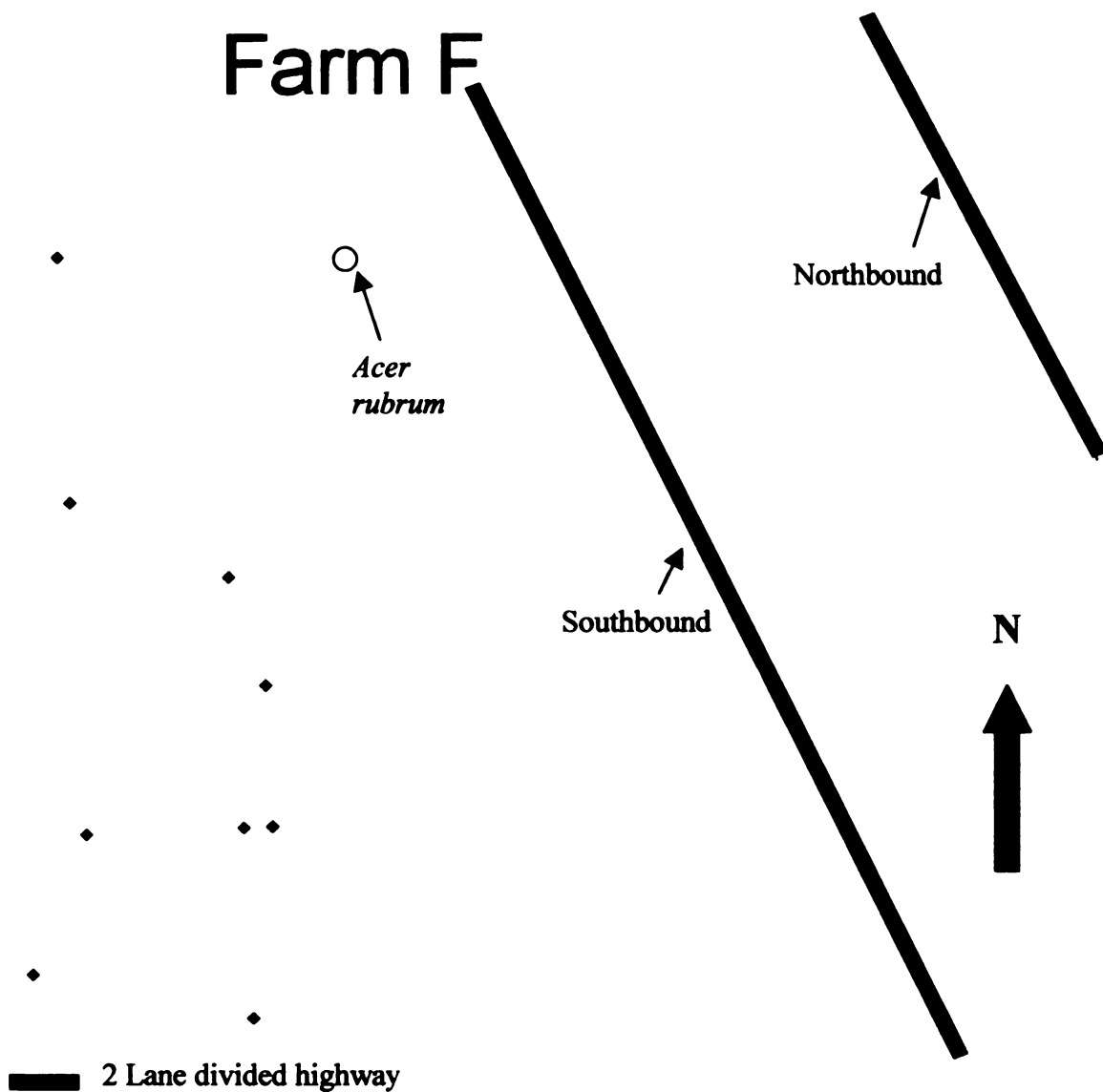
Scale (Farm B): 1cm = 28m; Farm C: 1cm = 38.6m

Appendix 15: Schematic map of farm 'B' (north of M-45 -- top), and farm 'C' (south of M-45 -- bottom), Ottawa County, Mich., showing surveyed plant locations, structures, and vegetation.



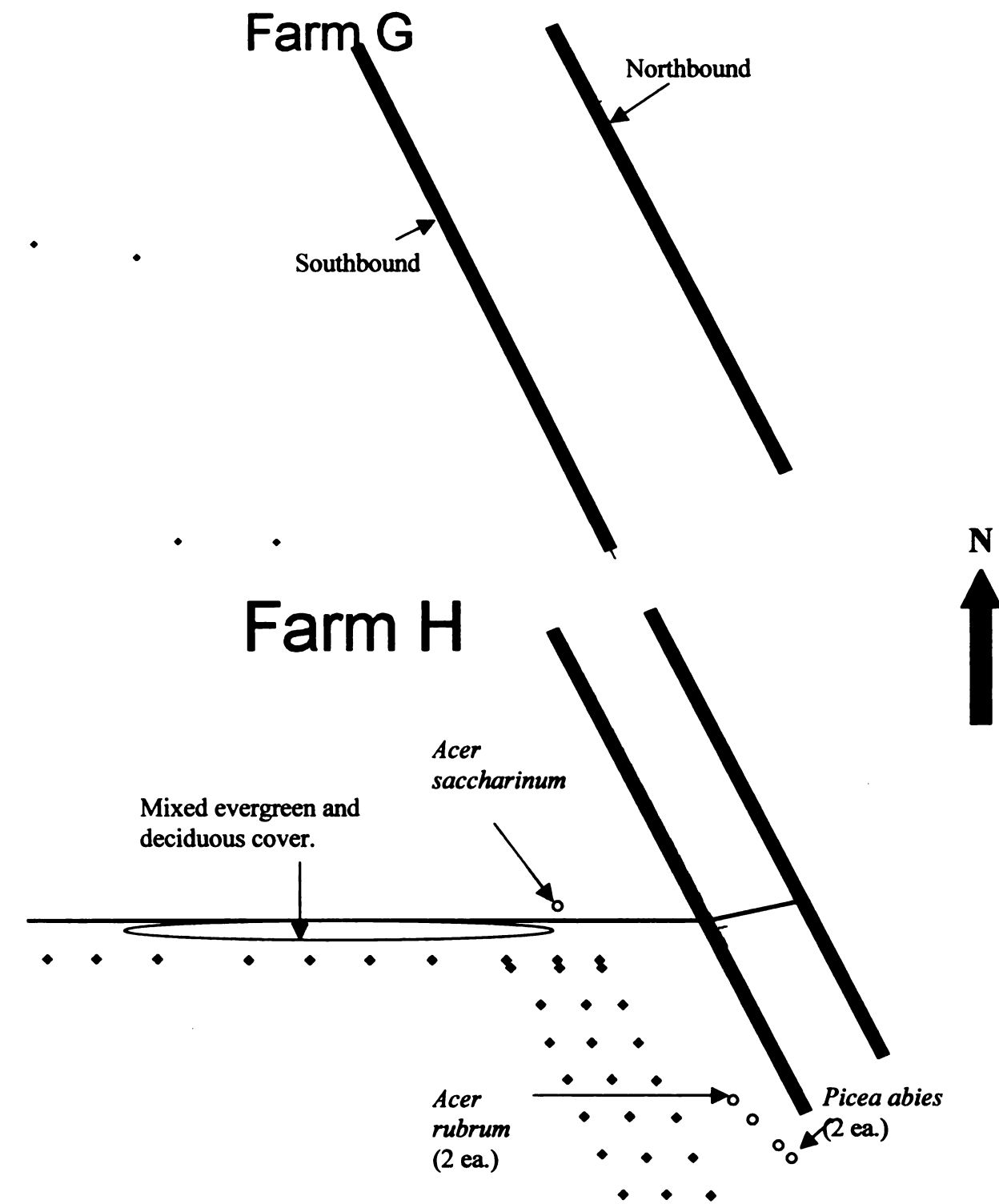
Appendix 16: Schematic map of farms 'D' (east of 144<sup>th</sup> Avenue – top), and farm 'E' (west of 144<sup>th</sup> Avenue – bottom), Ottawa County, Mich., showing plant locations, structures, and vegetation.



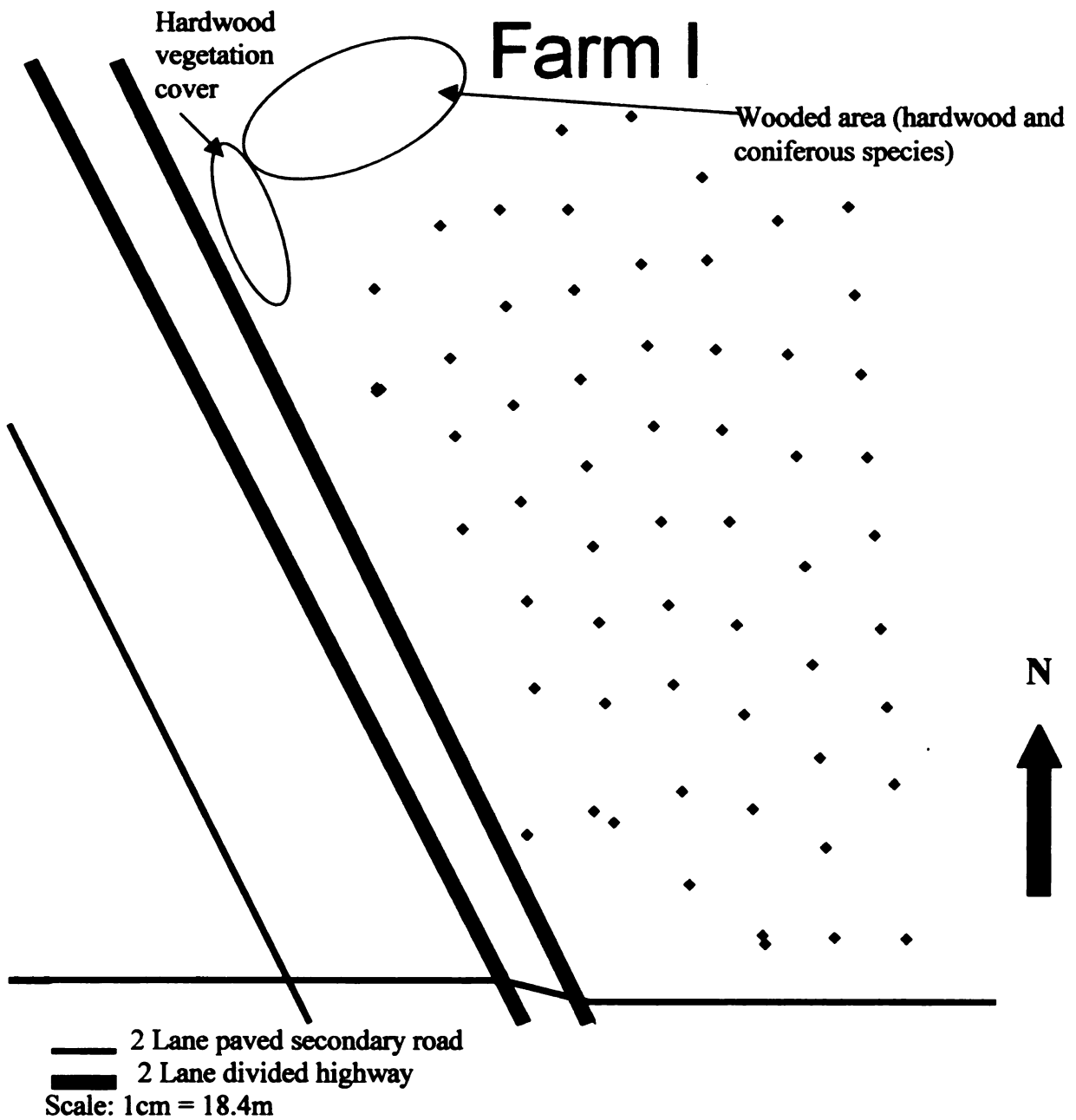


Scale: 1cm = 4.9m

Appendix 17: Schematic map of farm 'F', Ottawa County, Mich., showing surveyed plant locations, structures, and vegetation.

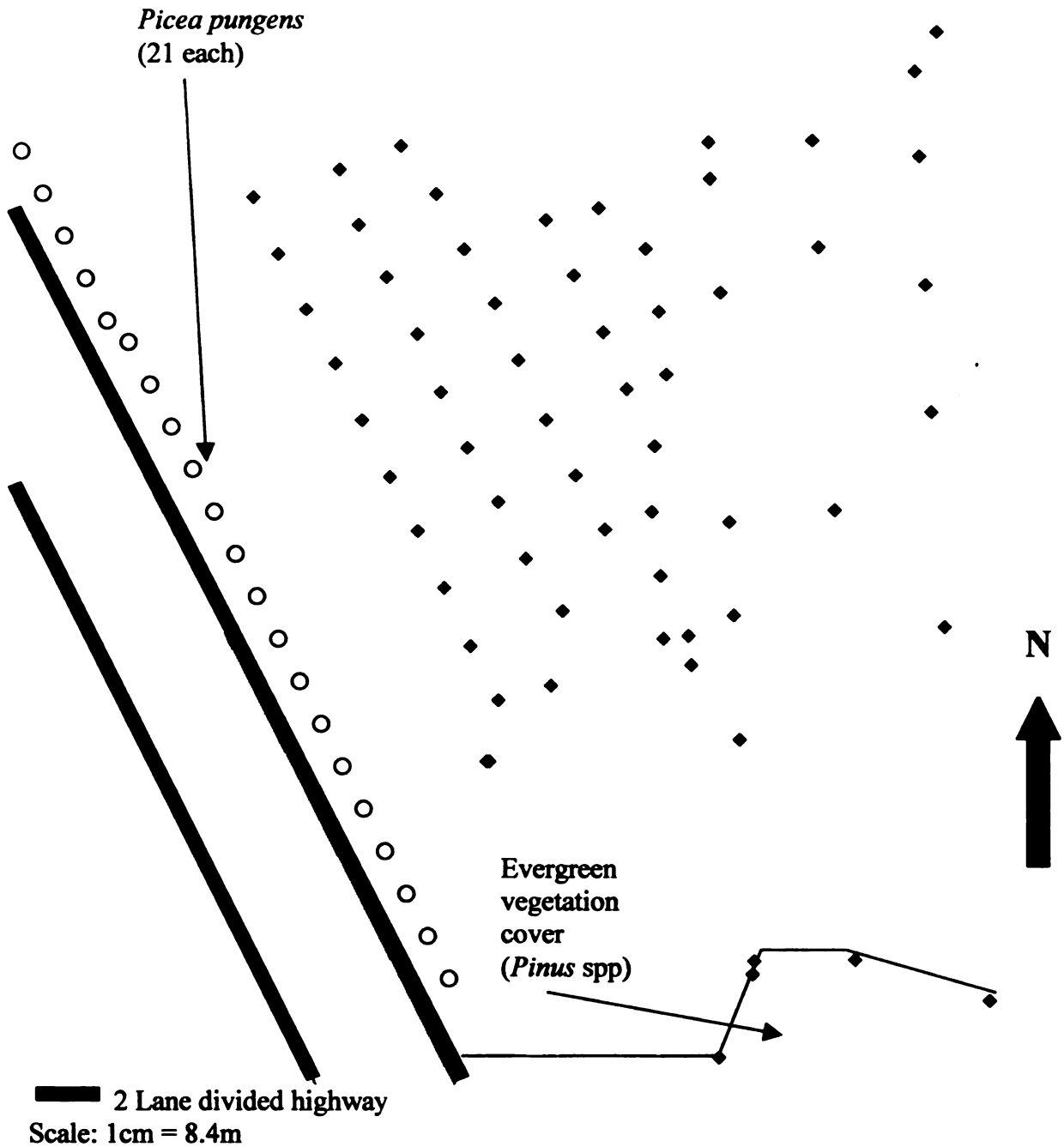


Appendix 18: Schematic map of farms 'G' (west of US 31 – top), and farm 'H' (west of US 31 – bottom), Ottawa County, Mich., showing surveyed plant locations, and vegetation.



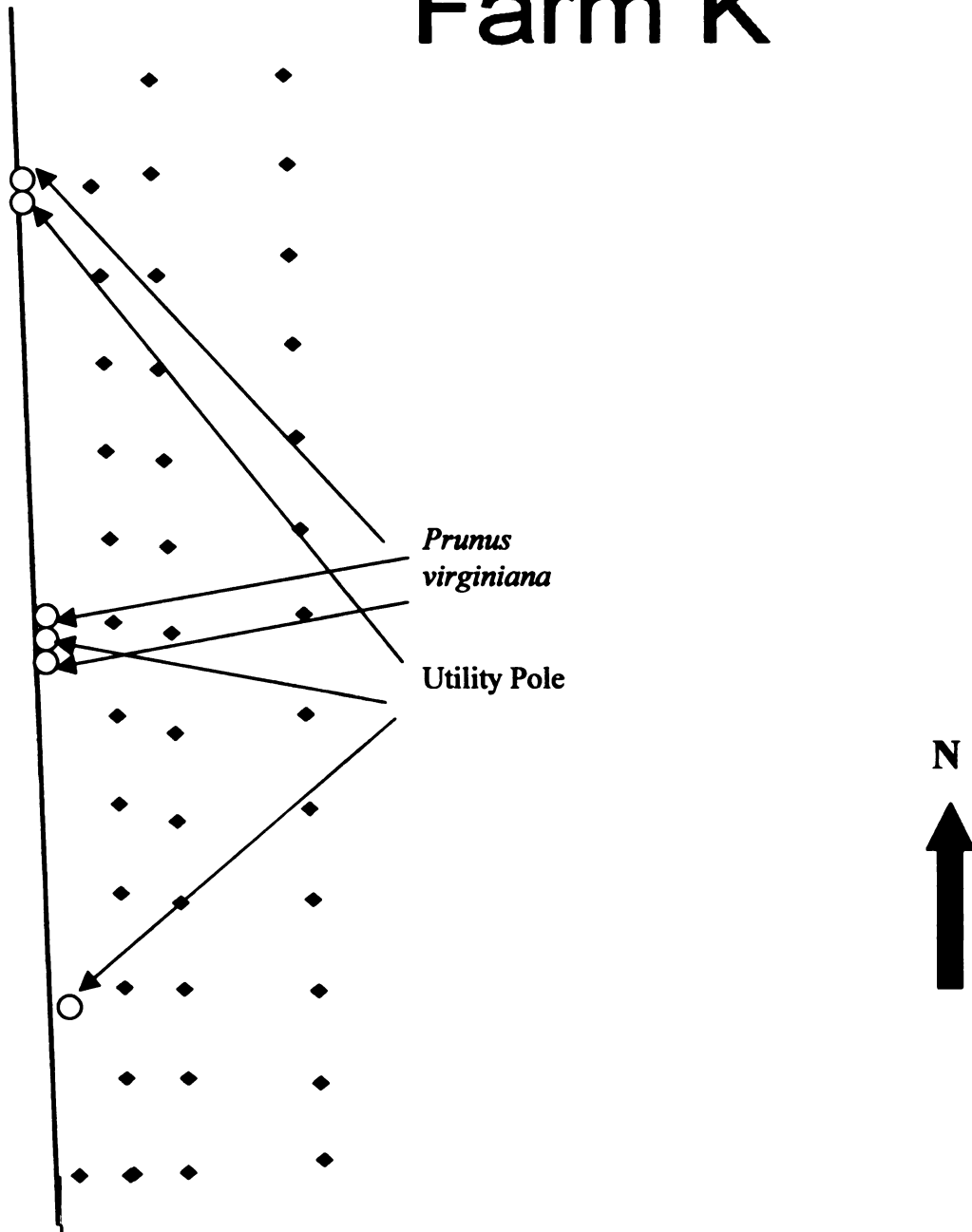
Appendix 19: Schematic map of farm 'I' (east of US 31), Ottawa County, Mich., showing plant locations and vegetation.

# Farm J



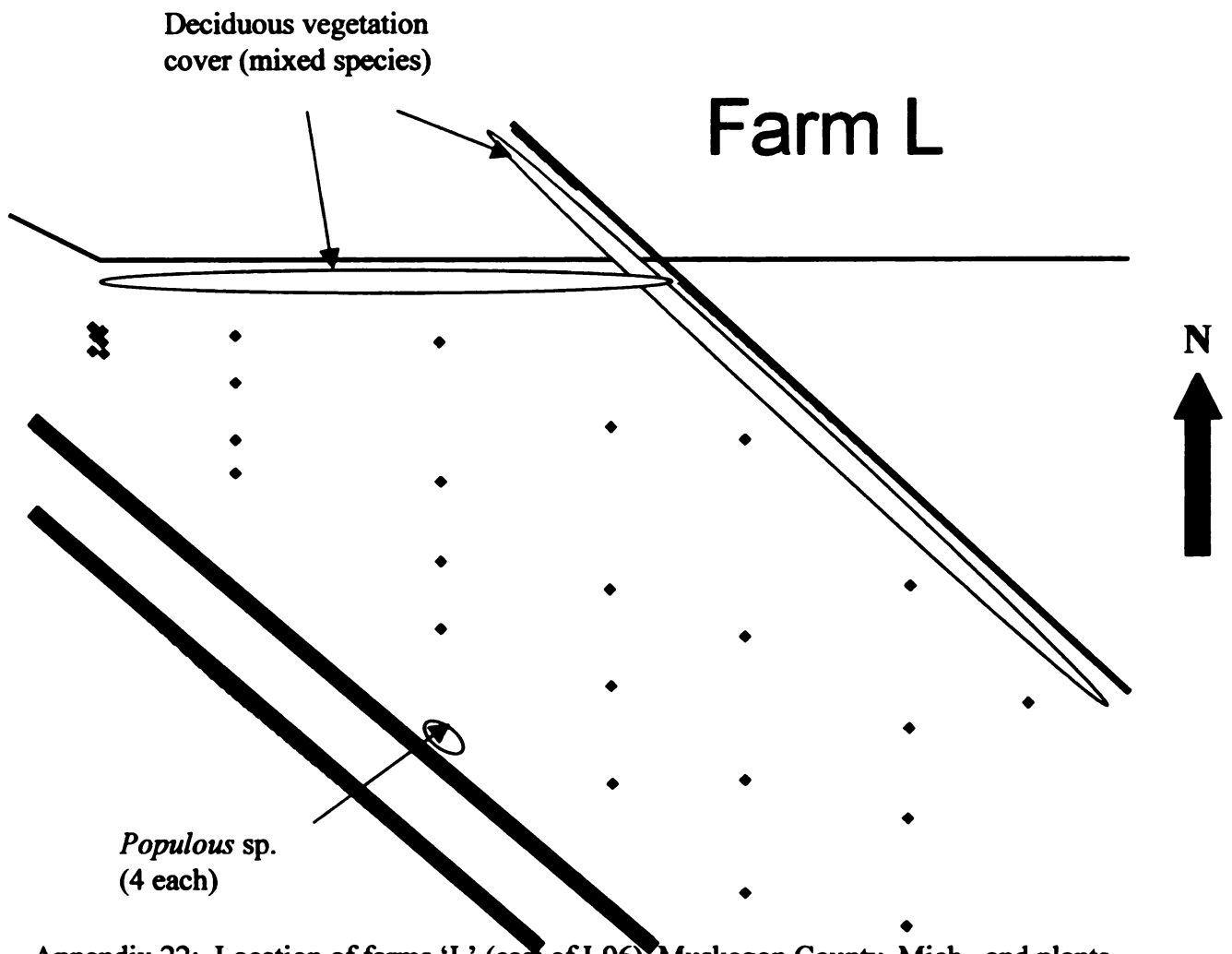
Appendix 20: Schematic map of farm 'J' (east of US 31 and south of farm 'I'), Ottawa County, Mich., showing surveyed plant locations and vegetation.

# Farm K



Appendix 21: Location of farms 'K' (east of 120<sup>th</sup> Avenue – top), Ottawa County, Mich., and plants from Study 1, 2002 and 2004.

— 2 lane unpaved road  
Scale: 1cm = 28.6m



Appendix 22: Location of farms 'L' (east of I-96), Muskegon County, Mich., and plants from Study 1, 2002 and 2004.

- 2 Lane unpaved road
- 2 Lane paved road
- 2 Lane divided highway

Scale: 1 cm = 27.5m

## Appendix 23

Several materials were tested to see if protection from salt spray could be achieved using sprayable materials. Two trials were set-up at farm 'J' in November (trial #1) and early December (trial #2) 2003 in a randomized complete block design. Each block was at a different distance to the nearest salted road (US 31). Data were collected at full-bloom, coinciding with the May 2004 farm surveys, and consisted of counting the number of living and dead flower buds on ten randomly selected twigs.

Table 1: Mean flower bud mortality of various hypothetical protective materials applied to blueberry flower buds, farm 'J', Ottawa, County, Mich., 2003 – 2004 (materials applied on 25 Nov. 2003 [trial #1] and 4 Dec. 2003 [trial #2], and flower bud counts performed on 5 May 04). Materials tested to investigate possible protection from ambient salt spray (US 31).

### Protective Coating Trial #1

| <u>Material Used / Qty. Applied (L)</u> | <u>Mean Flower Loss (%)</u> |
|---|-----------------------------|
| Sunspray 6E / 22.7                      | 36a <sup>z</sup>            |
| Kaolin Clay / 15.1                      | 51a                         |
| Control (DI H2O) / 11.3                 | 22a                         |
| Cerenat – 1% / 15.1                     | 42a                         |
| Moisturin / 22.7                        | 33a                         |
| Unsprayed Control                       | 51a                         |
| P-value                                 | 0.49                        |

<sup>z</sup> means within columns followed by a different letter are significantly different by Tukey's HSD (P=0.05).

### Protective Coating Trial #2:

| <u>Material Used / Qty. Applied (L)</u> | <u>Mean Flower Loss (%)</u> |
|---|-----------------------------|
| Whitewash (10%) / 15.1                  | 32b <sup>z</sup>            |
| Control (DI H2O) / 11.3                 | 45ba                        |
| Sunspray UF (2x) / 9.5                  | 65a                         |
| P-value                                 | 0.10                        |

<sup>z</sup> means within columns followed by a different letter are significantly different by Tukey's HSD (P=0.05).

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