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Chloroacetamid Spray Drift and Leaf Tatters in Hackberry

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CHLOROACETAMIDE SPRAY DRIFT AND LEAF TATTERS IN HACKBERRY

by

Ariana Miller

A Thesis

Presented to the Faculty of
The Graduate College at the University of Nebraska
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During the last decade, leaf tatters has been reported in white oak and hackberry across several Midwestern states. Herbicide spray drift studies have shown that chloroacetamides can induce leaf tatters. The objectives of this research were to: 1) identify vulnerable bud developmental stages in hackberry and 2) determine if different commercial chloroacetamides affect severity of leaf tatters. In 2008, a preliminary spray drift experiment was conducted on mature trees from a former hackberry provenance test stand. Acetochlor (Harness), S-metolachlor (Dual II Magnum), and dimethenamid (Outlook) were applied at concentrations approximating 27%, 54%, 81%, or 108% of the recommended field rate. Three developmental stages before bud burst were present on the selected trees. Leaf tatters did not develop on the selected hackberry trees. However, symptoms were observed on neighboring, non-target hackberry trees, which had been in the leaf unfolding and expanding stages at the time of spraying. In 2009, three year old hackberry seedlings were treated with 1%, 10%, and 100% of the recommended field rate of acetochlor (Harness), S-metolachlor (Dual II Magnum), and dimethenamid (Outlook). Folded buds and two unfolding leaf developmental stages were present on seedlings. Another spray study was conducted on 32 mature hackberry trees from the provenance stand. A solution of 5608 mg a.i./L dimethenamid (Outlook) was applied to trees in the unfolding and/or expanding leaf stage. Treated trees represented four provenances.
Image analysis was used to calculate seedling and mature tree leaf areas and estimate the seedling percentage of leaf tissue loss. Foliar damage was not significantly different between seedlings treated with water, 1%, or 10% of the field rate. Foliar damage was significantly different between seedlings treated with 1% or 100% of the field rate, and between seedlings treated with 10% or 100% of the field rate. Foliar damage in seedlings was not significantly different between the developmental stages. Additionally, symptoms of leaf tatters were observed on the treated mature hackberry. Future studies should focus on chloroacetamide concentrations above 10% of the recommended field rate.
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CHAPTER 1: REVIEW OF LEAF TATTERS AND HERBICIDE SPRAY DRIFT

Hackberry (*Celtis occidentalis*) is one of the five most common species in Midwestern urban forests (Iakovglou et al., 2002). It is a member of the Ulmaceae family. Its native range extends north into Quebec (Houle and Bouchard, 1990), south to Arkansas and Tennessee, and west into the Dakotas, Nebraska, and Kansas (Hibbs, 1976). The hackberry growing season varies between 120 to 250 days, depending on the location (Smalley, 1973). Hackberry has an indeterminate (free) growth pattern; new buds forming on the elongating stem will create new leaves throughout the summer. Leaves are elliptical or ovate, and the leaf margin is serrated. Hackberry reproductive buds are polygamomonoecious, and burst simultaneously to slightly before vegetative buds. Flowering occurs in early April along the southern part of hackberry’s range and in late May along the northern part of the range (Rosario, 1988). In Nebraska, hackberry flowers between mid-April and mid-May. Individuals usually live 100 to 140 years (Houle and Bouchard, 1990).

Hackberry is drought tolerant, but sensitive to fire damage and thus rare in communities that experience regular fire disturbance (Hartung and Brawn, 2006). Hackberry is among the trees suggested as an alternative to or a replacement for ash (*Fraxinus*) trees infested with Emerald Ash Borer, and in the 1960s Iowa planted hackberry to replace American elms (*Ulmus americana*) infected with Dutch Elm Disease (Hibbs, 1976).
In the last six years, homeowners in the towns of Wayne, Hartington, and Coleridge in northeast Nebraska have reported foliar damage to hackberry trees (Steve Rasmussen, personal communication 2007). In mild and moderate cases, leaves had ragged edges and/or irregular holes extending toward the center of the leaf. Trees with severe damage had leaves that were nothing more than veinal tissue – it looked as if a child had carefully cut away all tissue between the leaf veins. Affected trees had cupped leaves characteristic of herbicide injury. Sometimes cupped leaves also exhibited necrosis along the leaf edge and/or had irregular holes.

According to homeowners, abscission of damaged leaves may occur in early summer; one homeowner described raking hackberry leaves from his yard in June 2006 (Gary Howey, personal communication 2007). A second homeowner reported that a hackberry tree failed to fully leaf out until summer (Denelda Becker, personal communication 2007). Every homeowner interviewed lives near agricultural fields, employs a professional lawn care service, or has neighbors who employ a lawn care service. At least four private residents had lost eight mature trees by 2007 (Ron Brodersen, personal communication 2007) and the Christensen Well Company lost two trees by 2008 (Christensen Well Company, personal communication 2008). Although only a limited number of hackberry trees have died after exhibiting leaf tatters, residents expressed concern over losing their trees.

The reported symptoms in hackberry are the same as those of oak tatters, which was first reported in the 1980s in Iowa, Indiana, and Ohio. Reports of oak tatters in Minnesota and Wisconsin began in the mid 1990s (Hayes, 2005). Oak tatters primarily
affects white oak species, but red oak species may also be susceptible. The term leaf tatters is now applied to symptoms of oak tatters in any non-oak species. Leaf tatters has also been observed on prickly ash (*Zanthoxylum americanum*), black locust (*Robina pseudoacadia*) and some herbs (Bruce Blair; Iowa Department of Natural Resources forester, personal communication 2007).

Questions have arisen about the long term affect of leaf tatters on susceptible species. The logical assumption is that reducing leaf area on all or part of a tree’s canopy will reduce photosynthetic production during the growing season. Decreasing this year’s photosynthetic production decreases starch storage, which in turn limits sugars available next spring for leaf growth (John Vargo; University of Iowa Hygienic Laboratory environmental program manager, personal communication 2007). Leaf tatters may not be immediately fatal but it can progressively weaken a tree, thus increasing susceptibility to other biological or environmental stressors.

Herbicide spray and vapor drift are known to cause leaf tatters in oak and thought to cause leaf tatters in hackberry (Samtani et al., 2006; Hayes, 2005). Herbicide spray drift has been recognized as a threat to non-target plants and field boundary ecosystems for decades (Kleijn and Snoeijjing, 1997). Spray drift refers to small pesticide droplets that move off-target during application. Numerous factors influence the amount and range of movement of applied pesticide lost as spray or vapor drift including: spray equipment and technique, pesticide formulation, environmental conditions, soil type, and crop type (van den Berg et al., 1999). Reported drift measurements vary considerably due to the interactions of these factors.
The most important factors affecting spray drift are wind speed, boom height, and droplet size. Increasing wind speed and boom height increase spray drift. Decreasing droplet size increases spray drift. Increasing spray pressures and using certain types of spray nozzles decreases droplet size (Bernards et al., 2007).

One review states that usually 1 to 15% of ground-rig applied herbicide is lost as drift under normal conditions when measured 1 m from the last nozzle (Kleijn and Snoeijing, 1997). A review of spray studies involving low-flight, fixed-wing aerial applications reported that over 20% of the initial spray moves off the field. The median deposition of drift particles drops from ~5% at 30 m downwind of application to ~0.5% at 150 m (Bird et al., 1996).

At wind speeds of 7.1 km/h, pre-emergent application of tebuthiuron can result in 31% of the applied herbicide being lost as drift (Costa et al., 2005). Doubling wind speed increases drift 700% when measured 27 m downwind of the sprayer. Increasing boom height from 46 to 91 cm increased drift 350% when measured 27 m downwind. Doubling the distance downwind decreases the amount of drift five-fold (Bernards et al., 2007).

A field sprayer with a boom height of 40 cm, operating at a pressure of 250 kPa during a wind speed of 1.5 m/s resulted in 14% of the applied spray moving off target. A field sprayer with a boom height of 80 cm, operating at a pressure of 1000 kPa during a wind speed of 4 m/s resulted in 37% of the applied spray moving off target (Nordby and Skuterud, 1974).
A study of spray drift in Canada found that 35% of 2,4-D butyl ester applied to a field evaporated and drifted downwind. Initial droplet drift for ester and amine formulations of 2,4-D varied between 3% and 5% of the applied amount. Ground-rig applications of 2,4-D amine solutions at wind speeds varying from 5 to 35 km/hr resulted in <0.5% to 8% of the applied spray drifting 5 m downwind. Initial drift increased with wind speed at application time. Reported initial drift was also consistently higher when nozzle pressure increased from 100 kPa to 210 kPa. Depending upon the method of application (ground-rig or aircraft) and meteorological conditions, between 10% and 50% of initial vapor drift was still airborne 50 meters downwind (Maybank et al., 1978).

Commercial formulations of pesticides often contain adjuvants in addition to the active ingredient. Pesticide applicators may also add adjuvants to commercial products. Adjuvants do not act directly on the pest, but enhance performance of the pesticide by altering its application and increasing tissue penetration. For example, stickers are substances that make pesticides less prone to wash off by rain or irrigation. Safeners are substances that accelerate the metabolism of the herbicide in the crop plants but not the weeds. The addition of adjuvants can reduce the application rate required for an herbicide to achieve desired results (Stephenson and Solomon, 2007).

Research during the last six years supports the hypothesis that herbicide spray and vapor drift induce leaf tatters in oak and hackberry (Samtani et al., 2006; Hayes, 2005). A 2004 spray drift study examined the effects of 2,4-D ester, glyphosate, 2,4-D + glyphosate, dicamba, acetochlor + atrazine, and metolachlor on white oak seedlings. The herbicides were applied at 1%, 10% and 25% of the field rate. The seedlings were treated
at three different developmental stages: swollen bud, unfolding leaf, or expanded leaf. Only Harness Xtra (a.i. acetochlor+atrazine, field rate is 3.5 kg a.i./ha) and Dual Magnum (a.i. metolachlor, field rate is 2.0 kg a.i./ha) applied at 10% and 25% of the field rate to seedlings in the unfolding leaf stage induced leaf tatters (Samtani et al, 2005).

In 2005, white and red oak seedlings in the leaf unfolding stage were treated with acetochlor (Harness), metolachlor (Dual Magnum), and dimethenamid (Outlook) alone or in combination with atrazine (Aatrex). The field application rates are: 2 kg a.i./ha for acetochlor; 2.1 kg a.i./ha for metolachlor; 1.05 kg a.i./ha for dimethenamid; and 2.3 kg a.i./ha for atrazine. The herbicides were applied at 1%, 10%, and 25% concentrations of the field rate. Acetochlor, metolachlor, and dimethenamid applied alone or with atrazine induced leaf tatters in white and red oak seedlings (Samtani et al., 2006).

A spray drift study in hackberry seedlings followed the 2004 and 2005 spray drift studies in oak seedlings. Acetochlor (Harness) and metolachlor (Dual Magnum) were applied alone and in combination with atrazine (Aatrex) at 1%, 10%, and 25% of the standard field rate to hackberry seedlings in the unfolding leaf and expanded leaf stages. Leaf tatters was observed in seedlings treated during the unfolding leaf stage with 10% and 25% of the standard field rate. Atrazine did not contribute to the development of leaf tatters (Samtani, 2008).

The Iowa Department of Natural Resources and University Hygienic Laboratory conducted a pilot study to examine the link between chloroacetamide spray drift and oak tatters. Air, rain water, and leaf samples were collected near West Branch, Iowa and at White Pines Hollow Forest Preserve in Iowa. The site near West Branch is surrounded by
agricultural fields while the White Pines Hollow Forest Preserve is relatively isolated. The onset of oak tatters closely followed peaks in the amount of acetochlor present in air and rain water samples at both sites. Leaf tatters was routinely observed in leaves with higher tissue concentrations of acetochlor and metolachlor (Larabee-Zierath et al., 2006).

Acetochlor and metolachlor are chloroacetamides, a class of seedling shoot-inhibitors frequently used in corn and soybean systems. These herbicides are traditionally applied to the soil immediately after planting but before emergence of weeds (Bernards et al., 2007). The activity of chloroacetamides targets growing tissue, and therefore is considered phytotoxic only to emerging plants. Susceptible grasses and forbs usually fail to emerge. Plants which emerge may exhibit shortened midrib veins in leaves, which appear wrinkled or puckered. Uptake of chloroacetamide herbicides is through emerging shoots in grasses and forbs. Plants past the seedling stage can absorb chloroacetamides through roots and translocate chloroacetamides to vegetative structures (Vencill et al., 2002).

The primary action of chloroacetamides is inhibition of fatty-acid elongation to very long chain fatty acids (VLCFA) (Böger, 2003). Synthesis of 16-18 carbon chain fatty acids and fatty-acyl precursors occurs at the acyl-carrier protein in the chloroplast (Post-Beittenmiller, 1996). VLCFA synthesis involves extension of C18 fatty-acyl precursors in the endoplasmic reticulum and Golgi apparatus (Matthes and Böger, 2002). The VLCFA elongase complex includes four enzymes embedded in the ER membrane. The first enzyme initiates elongation by catalyzing the condensation reaction between malonyl CoA and the fatty-acyl precursor. Subsequent reduction, dehydration,
reduction steps add two carbons to the acyl chain. Chloroacetamides inhibit the condensation step of elongation by irreversibly binding to a cysteine in the reaction center (Böger, 2003).

Fatty acids are the building blocks of lipids, which are important for the formation of membranes, hormones, and waxes. Very long chain fatty acids stabilize plasma membranes. Membranes lacking very long chain fatty acids are less rigid and leaky. The reduction or absence of VCLFA impairs cell growth and division (Böger, 2003). Emerging seedlings and buds are affected by chloroacetamides because the herbicidal activity targets actively growing tissues.

The apparent increased susceptibility of hackberry and white oak is probably related to the timing of herbicide exposure. Chloroacetamide resistance or tolerance is unlikely to develop spontaneously, particularly in vascular plants. The condensing enzyme of the VLCFA elongase complex requires cysteine to function, thus tolerance of chloroacetamides is due to better metabolic detoxification or barriers to herbicide uptake (Böger, 2003). Foresters familiar with leaf tatters have suggested that hackberry and white oak susceptibility is due to phenology. Hackberry and white oak leaf out later than elm, ash, or other oak species. The time required for leaf development is also longer in white oak than red oak (Aron Flickinger; Iowa Department of Natural Resources forest health coordinator, personal communication 2007).

As of 2007, there was insufficient data to strongly support the hypothesis that chloroacetamides cause leaf tatters in hackberry. There was also limited data about the influence of phenology on the development of leaf tatters. The objectives of this research...
were to: 1) identify vulnerable bud and leaf developmental stages in hackberry and 2) determine if different commercial herbicides affect severity of leaf tatters symptoms.
CHAPTER 2: LEAF BUD CHARACTERIZATION

INTRODUCTION

Although phenology is suspected as a factor in leaf tatters, bud development is not well characterized in hackberry. There are no published data on the specific environmental factors (i.e. chilling hours, temperature) required for breaking dormancy in hackberry. The morphology of hackberry buds has never been described for the period between dormancy and bud breaking.

MATERIALS AND METHODS

Characterization of hackberry buds began in the winter of 2008. Cuttings were taken from dormant hackberry trees on the campus of the University of Nebraska, Lincoln, Nebraska on February 20, 2008 (DOY 51) and March 26, 2008 (DOY 86). Cuttings were dipped in 75% ethanol solution for 15 minutes and rinsed with distilled water for two minutes to reduce the possibility of microbial infection of the stems and buds. The cuttings were placed in GA7 tubes containing a forcing solution prepared using one packet (4.42 grams) of Chrysal Clear (Pokon and Chrysal USA) cut flower food dissolved in 0.5 L of water. Cuttings were placed in a growth chamber maintaining 24°C day and 18°C night temperatures under a 12- hour photoperiod. The photosynthetically active radiation (PAR) in the growth chamber was measured with an AccuPAR LP-80 ceptometer (Decagon, Pullman, WA). The average PAR was 544.4 Watts/m², with a standard deviation of 58.87 Watts/m².
The February cuttings were removed from the growth chamber on March 3, 2008 for examination (DOY 63) and on 5, 2008 (DOY 65) for dissection and examination. Cuttings collected on March 26 were dissected and examined on March 28 (DOY 88), April 1 (DOY 92), and April 7 (DOY 98). Buds were examined using a binocular dissecting scope. Pictures were taken with a Canon Power Shot S51S digital camera fitted onto the dissecting scope.

**OBSERVATIONS**

Three stages of bud development could be distinguished before actual bud burst occurred. During the dormant bud stage, the bud scales are tight and there is little swelling of the bud (Figure 2.1 and Figure 2.2). In the swelling bud stage, the scales separate but remain attached to a very swollen bud (Figure 2.3 and Figure 2.4). Slicing a swelling vegetative bud reveals tiny leaves (Figure 2.5). Bud scales are absent or barely present in folded vegetative buds and leaves are just starting to separate from each other (Figure 2.6). The margins of the outer leaves are not easily visible at the folded bud stage. Few bud scales remain on flower buds in the folded stage. The flowers are still closed and no leaves are visible without dissection (Figure 2.7).

Hackberry buds – even those located on the same stem – did not burst simultaneously. The reproductive and mixed buds (containing flowers and leaves) fully developed and burst after 12 days for the February cuttings, and after six days for the March cuttings. Dormant reproductive buds were observed at the time of the March 26th
collection and two days after. Reproductive buds of a stem collected in March progressed to the swelling and folded bud stages after three days in the growth chamber.

In contrast, vegetative buds developed more slowly than reproductive or mixed buds. Dormant vegetative buds were not observed on cuttings collected in February. There are a few possible reasons for this: the differences in physical appearance between flower and vegetative buds had not been noted at that time, and vegetative buds were simply overlooked; the stem cuttings did not produce vegetative buds that year; or vegetative buds do not respond as well to forcing as the reproductive or mixed buds. Dormant vegetative buds were observed at the time of collection in March and two days after. Swelling vegetative buds were observed after 12 days. Folded vegetative buds were observed after 12 days.
Figure 2.1. Dormant stage of hackberry reproductive or mixed bud, collected March 26, 2008. Cutting was photographed on the same day of collection.
Figure 2.2. Dormant stage of hackberry vegetative bud, collected March 26, 2008. Cutting was in growth chamber for two days.
Figure 2.3. Swelling stage of hackberry vegetative bud, collected March 26, 2008. Cutting was in growth chamber for 12 days.
Figure 2.4. Swelling stage of hackberry reproductive bud, collected on March 26, 2008. Cutting was in growth chamber for two days.
Figure 2.5. Longitudinal section of a hackberry vegetative bud at the swelling stage. Cutting was collected March 26, 2008, and kept in growth chamber for 12 days.
Figure 2.6. Folded stage of hackberry vegetative bud collected on March 26, 2008. Cutting was in growth chamber for 12 days.
Figure 2.7. Folded stage of hackberry mixed bud collected March 26, 2008. Cutting was in growth chamber for six days.
CHAPTER 3: PRELIMINARY SPRAY STUDY

INTRODUCTION

Previously published leaf tatters studies have not attempted to induce symptoms in mature hackberry trees. Hackberry seedlings have never been sprayed during three bud stages characterized for a leaf tatters study. Identifying the earliest vulnerable developmental stages will contribute to recommendations on managing for leaf tatters in hackberry. The goal was to induce leaf tatters symptoms in mature hackberry and identify potentially vulnerable bud stages.

MATERIALS AND METHODS

A preliminary spray drift experiment was conducted on 15 trees from a hackberry provenance test planted May 22 and 23, 1990. The provenances came from counties in Minnesota, North Dakota, South Dakota, Nebraska, Iowa, Kansas, Missouri, Oklahoma, and Arkansas. In the original stand, each provenance was represented by 20 half-sibling seedlings grouped in five replicates across 23 rows. Removal and death of trees in the intervening 18 years altered the number of rows and replicates.

The trees were selected on the basis of bud developmental stage without consideration to provenance. The day before spraying, developmental stage was marked with red (dormant bud), orange (swelling bud), or blue (folded bud) flagging.

Trees were sprayed on May 9, 2008 (DOY 130). Acetochlor (Harness), S-metolachlor (Dual II Magnum), or dimethenamid (Outlook) were applied at concentrations of 27%, 54%, 81%, or 108% of the maximum field rate recommended for agricultural soils in eastern Nebraska. The concentration of each active ingredient
present in solution at the recommended field rate is: 23 g/L (acetochlor), 33 g/L (S-metolachlor), and 56 g/L (dimethenamid). Herbicides were applied at 290 kPa using a 2 L capacity backpack sprayer fitted with a flat spray jet nozzle. Twelve trees were sprayed and three unsprayed trees acted as controls.

Leaves were collected on August 7, 2008 (DOY 220) for leaf area measurements. The 90-day wait between treatment and collection ensured that vegetative buds could progress to fully expanded leaves before destructive sampling. Pictures of treated buds documented leaf health until 43 days after treatment. Leaf area for each bud stage-herbicide-concentration combination was measured with a LI-3100 Area Meter (LI-COR Inc., Lincoln, Nebraska).

RESULTS

Damage of treated leaves could not be attributed solely to herbicides (data not shown). Analysis of variance could not be applied to leaf area due to the lack of replication of each treatment combination. All 15 trees exhibited damage by leaf chewing insects by the time of leaf collection, so leaf areas would not have provided information on foliar damage due to leaf tatters. Pictures of treated leaves showed little evidence of leaf tatters six (Figure 3.1) and nine days after treatment (DAT) (Figure 3.2, Figure 3.3, Figure 3.4, and Figure 3.5), and no unusual foliar damage was observed 22 DAT (Figure 3.6). Only two branches treated at the folded bud with 108% of a chloroacetamide showed any symptoms of tatters.
It is likely that the presence of bud scales at the early developmental stages prevented contact between tissue and herbicides. With the exception of the two aforementioned branches, the lack of leaf tatters symptoms among trees treated at 108% of the recommended field rate strongly suggests that mature hackberry trees are not vulnerable to chloroacetamide spray drift injury before bud burst.

Cupping and necrosis were observed on trees next to sprayed trees (Figure 3.7). The leaves of these non-target trees had been unfolding and expanding at the time of treatment. Tree buds progressed from dormant stage to unfolding in six to nine days. Swelling buds progressed to unfolding in six to eight days, and progressed to leaf unfolding in eight to 10 days. A few trees developed more rapidly, progressing from the swelling bud stage to leaf expansion within nine days. All buds on selected trees had progressed from the folded stage well into leaf unfolding within six days and leaf expansion within nine days. All selected trees had fully expanded leaves at 22 DAT.
Figure 3.1. Mature hackberry sprayed during the folded bud stage with dimethenamid (Outlook) at 30.3 g a.i./L (54% of the field rate). Leaves do not have symptoms of leaf tatters. Picture taken six DAT.
Figure 3.2. Mature hackberry sprayed during the swelling bud stage with S-metolachlor (Dual II Magnum) at 17.8 g a.i./L (54% of the field rate). Some leaves have lost tissue. Picture taken nine DAT.
Figure 3.3. Mature hackberry sprayed during the folded bud stage with S-metolachlor at 17.8 g a.i./L (54% of the field rate). Some symptoms of leaf tatters are apparent. Picture taken nine DAT.
Figure 3.4. Mature hackberry sprayed during the dormant bud stage with acetochlor (Harness) at 25.4 g a.i./L (108% of the field rate). Leaves exhibited no symptoms of leaf tatters. Picture taken nine DAT.
Figure 3.5. Mature hackberry sprayed during swelling bud stage with S-metolachlor at 8.9 g a.i./L (27% of the field rate). Expanding leaves appear wilted. Picture taken nine DAT.
Figure 3.6. Mature hackberry sprayed during folded bud stage with S-metolachlor at 17.8 g a.i./L (108% of the field rate). Leaves exhibited no symptoms of leaf tatters. Picture taken 22 DAT.
Figure 3.7. Mature hackberry directly west of a hackberry sprayed with dimethenamid at 60.6 g a.i./L (108% of the field rate). Leaves show symptoms of leaf tatters. Picture taken six DAT of selected trees.
CONCLUSIONS

Hackberry trees treated with chloroacetamide herbicides before the folded bud stage did not develop leaf tatters by 22 DAT. Leaf tatters was not induced even when the applied concentration of the herbicides’ active ingredients exceeded the recommended field rate. Leaf tatters was observed nine DAT on some branches treated during the folded bud stage. Non-target hackberry trees exposed to spray drift during the leaf unfolding and leaf expanding stages developed severe symptoms of leaf tatters.

This indicates that landowners can prevent leaf tatters in hackberry by altering herbicide application practices or selecting trees grown from non-local seed sources. Applicators of agricultural herbicides must apply chloroacetamides to benefit crop production. Preventing herbicide injury to non-target vegetation is unlikely to be a major consideration in planning herbicide application dates for farmers.

Community planners and residents who want hackberry trees in their community should consider trees from more southern or northern provenances. When selecting other provenances, buyers should inquire into when hackberry trees usually exhibit swollen buds and fully expanded leaves in that area. Buyers will also need to consider several years worth of data on the application times of chloroacetamides in their area. Ideally, there should be a window of 22 days between hackberry bud swelling and herbicide application for more southern seed sources. This would allow the hackberry leaves to reach full expansion and produce a cuticle. If a more northern seed source is desired then buyers should consider the latest dates of herbicide application in their own area. Buyers...
should look for provenances that will not enter the folded bud stage until at least a week after the latest application date.
CHAPTER 4: 2009 SPRAY STUDIES

INTRODUCTION

Following the results of the 2008 preliminary spray drift study, a study using hackberry seedlings and another study with the mature stand were conducted. Attention shifted to the folded bud stage and two unfolding bud stages as potentially vulnerable periods of developing hackberry leaves. The two unfolding bud stages were designated as unfolding1 and unfolding2. Buds in the unfolding1 stage were not fully open, but leaf separation was apparent (Figure 4.1 and Figure 4.2). The unfolding2 stage was assigned to buds that had at least one completely unfolded leaf. Buds in the unfolding2 stage usually had a combination of unfolding leaves and expanding leaves (Figure 4.3). The planned periods between treatment and sampling were dramatically shortened to reduce damage due to herbivory and capture symptomatic leaves before leaf abscission.

The method of measuring leaf area was also changed to accommodate the shorter periods between treatment and sampling. The usual method of quantifying injury in plants is through visual rankings of injury type or severity. Visual rankings are unlikely to be highly consistent between studies conducted by different researchers. The severity of leaf tatters induced by a treatment can be inferred from leaf area measurements; provided that the leaves were treated during the same developmental stage and develop at similar rates. The symptom primarily associated with leaf tatters is the presence of holes in the foliage. These holes result from the abscission of damaged leaf tissue, but chlorosis and necrosis develop prior to tissue abscission. The currently available leaf area meters known to the author do not differentiate between chlorotic, necrotic, and
healthy tissue. Digital analysis can be used to estimate healthy tissue area and/or injured tissue area.
Figure 4.1. Bud at the unfolding developmental stage. Leaves are just beginning to unfold.

Figure 4.2. Bud in the unfolding developmental stage. Oldest leaves have almost completely unfolded.
Figure 4.3. Bud in the unfolding developmental stage. The oldest leaves have completely unfolded and are expanding.
MATERIALS AND METHODS

Seedling Spray Drift Study

The herbicide drift study on seedlings was conducted at the University of Nebraska’s Agricultural Research and Development Center (ARDC) near Mead, NE. The on-site greenhouse has a north-south orientation and is partially shaded on the eastern end by an attached building. Greenhouse temperatures were controlled only by fans located on the north side and louvers located on the south side. Artificial lights supplemented natural daylight to maintain a 14-hour photoperiod. Twelve benches were arranged in three rows on the northern side of the greenhouse. HOBO Pro temperature and humidity data loggers (Onset Computer Corporation) with solar radiation shields were placed 0.92 m above the center of each bench (Figure 4.4). An outdoor spray rack was constructed consisting of two parallel 6.1 m rails erected on a gravel surface outside the greenhouse (Figure 4.5). The spray rack was located in an area protected from eastern, western, and northern winds by windbreaks and buildings.

Four hundred two-year-old hackberry seedlings were purchased from Charles E. Bessey Tree Nursery (Nebraska National Forest and Samuel R. McKelvie National Forest, Halsey, NE) in the winter of 2008 and arrived March 28, 2008 (DOY 88). The seedlings were potted on March 31 (DOY 91) in a 1:1:1 peat, perlite, and vermiculite (by volume) mixture in one gallon pots. Seedlings stayed in the ARDC greenhouse until May 8, 2008 (DOY 122), when they were moved to a shadehouse with approximately 50% shade and grown in pots for an additional year.
Figure 4.4. HOBO data loggers above greenhouse benches, ARDC near Mead, NE. Benches are on the northern side of the forestry greenhouse.
Figure 4.5. Outdoor spray rack outside the ARDC forestry greenhouse near Mead, NE.
Seedlings overwintered in the shadehouse on raised beds of sand. Seedlings in the shadehouse were numbered and labeled. The total height and main stem height of seedlings were recorded from November 22 (DOY 327) to November 30, 2008 (DOY 335). In February 2009, an initial 240 seedlings were selected as experimental units based on total and main stem heights; this selection process was intended to minimize height variation and avoid shrubby growth patterns.

The study was originally planned as a completely randomized split-plot design. Selected seedlings were randomly assigned to one of ten treatments: 235 mg/L, 2349 mg/L, or 23.5 g/L (1%, 10%, 100% field rate) acetochlor (Harness); 330 mg/L, 3295 mg/L, or 329 g/L (1%, 10%, 100% field rate) S-metolachlor (Dual II Magnum); 560 mg/L, 5608 mg/L, or 56 g/L (1%, 10%, 100% field rate) dimethenamid (Outlook); or water. Treatments were randomly assigned to 20 plots within each block. The combinations of herbicide type and concentration applied to seedlings were the whole plot treatments and the developmental stages were sub-plot treatments.

Experimental units were moved to the greenhouse on March 17, 2009 (DOY 76) to hasten breaking dormancy. All seedlings had rooted into the sand, and roots outside the pots were cut to remove the seedlings. On March 19 (DOY 78), the HOBO Pro sensors were set to record temperature and humidity data every 30 minutes. Seedlings were monitored two to three times a week for signs of bud breaking. Seedlings which broke dormancy were treated on April 17 (DOY 107), April 25 (DOY 115), or May 9 (DOY 129) according to progression of bud development.
Carpet squares (0.37 m\(^2\)) were placed upside-down around seedling bases prior to treatment to prevent herbicide adsorption by the soil mix. A three-nozzle spray boom with 48.3 cm spacing and TT11001VP spray tips (R and D Sprayers) delivering 18.6 ml/s at 330.9 kPa was used to apply the herbicides. The parallel rails of the spray rack kept the spray boom and nozzles at a constant height during application. The applicator used a metronome to ensure a constant speed of 0.36 m/s. The carpet squares were removed immediately after treatment.

Treated seedlings were allowed to dry outside before being returned to the greenhouse benches. Labels indicating bud development stage were affixed to selected branches. The progression of seedling health post-treatment was documented with a digital camera. Labeled buds and leaves were collected 15 DAT. The numbers of collected dead leaves, dead buds, and live buds were recorded for each seedling. Living leaves were scanned (Epson Expression 1600) at a resolution of 160 dots per inch (DPI).

Three blocks of seedlings were removed from the greenhouse prior to the May 9 spraying because they failed to break dormancy. On May 8, these seedlings were replaced with 30 shadehouse seedlings which exhibited buds between the folded and unfolding developmental stages. The replacement of 30 seedlings across three blocks was considered during analysis. Each bench containing replacement seedlings was treated as two blocks of ten seedlings instead of one block of twenty seedlings.

It was not unusual for an entire bud to die before its leaves completely unfolded. The indeterminate growth of hackberry stems makes it more difficult to predict how many potential leaves were lost for each bud death. A bud that died before its leaves
unfolded was counted as one dead leaf for the purposes of calculating average leaf area and estimating the percentage of tissue loss.

**Spray Drift in Mature Hackberry Stand**

A second spray drift study was conducted on the mature hackberry stand on May 21, 2009 (DOY 142). As with the preliminary experiment, the primary objective was to induce tatters in mature hackberry trees using a known concentration of herbicide. The secondary objective was to test for the influence of genetic variation (aside from phenology) on injury response.

A completely randomized design best characterizes the experimental design. Selection of experimental units occurred during the week prior to spraying. Selection was limited to the stand’s inner ten rows to minimize spray drift from surrounding agricultural fields. The selected 32 trees represented four provenances in five different rows, and the selected provenances were present in at least two different rows. The chosen provenances were from: Stafford county, Kansas; Crawford county, Kansas; Clark county, Kansas; and Ellis county, Kansas. All of the selected trees were in the unfolding1 and unfolding2 developmental stages.

A 10 L solution of dimethenamid (Outlook) at 5608 mg a.i./L (10% field rate) was foliage applied using a 15 L capacity backpack Sun Sprayer and a flat spray jet nozzle. The flow rate and pressure were variable; the flow rate was 15 ml/s at the maximum pressure (620 kPa). All provenance replicates were sprayed from the west except for one Ellis county replicate and one Crawford county replicate, which were
sprayed from the east. Leaves were sampled from the east and west sides of trees 8 DAT and scanned (Epson Expression 1600) at a resolution of 160 DPI. Leaves from the sprayed side of each tree were labeled ‘sprayed,’ and leaves from the side opposite the sprayed side were labeled ‘opposite.’

**Digital Image Analysis**

**Leaf Area**

Unlike previous leaf tatters studies, leaf area measurements were obtained using digital analysis instead of a leaf area meter. The digital images were processed using CVIPtools (Computer Vision and Image Processing Laboratory, Southern Illinois University at Edwardsville). Color bands (red, green, or blue) were extracted to create gray-scale images. Objects of interest (i.e. leaves) were isolated by converting gray-scale images to binary images with threshold limits.

Green band extraction and gray-level thresholding created a binary image of areas of necrotic tissue. Extraction of the red band (Figure 4.6.C) and subsequent gray-level thresholding created a binary image that excluded areas of chlorotic tissue (Figure 4.6.D). Binary images produced from the red and green bands were stacked to exclude all areas of injured tissue (Figure 4.6.E). Image segmentation removed leaf petioles (Figure 4.6.E); the resulting binary image represented healthy tissue area. All final binary images assigned white to the leaf area and black to the background for leaf area calculation (Figure 4.6.F).
The area (in pixels) of non-injured tissue was calculated from these binary images with the CVIPtools’ Features function. A 22.19 cm$^2$ disc used to calibrate leaf area meters was scanned at 160 DPI. The disc image was digitally processed to create a binary image from which pixel area could be calculated. Comparing the disc’s pixel area and actual metric area provided the conversion factors: 1 cm$^2$ = 3958.9 pixels and 1 mm$^2$ = 39.59 pixels. The calculated seedling and mature tree leaf areas were then converted to mm$^2$ (for seedling) or cm$^2$ (for mature trees).
Figure 4.6.A. Leaf from a seedling treated with Dual II Magnum (S-metolachlor) at a concentration of 329 g a.i./L (100% of the field rate). Picture taken 11 DAT and before sampling.

Figure 4.6.B. Same leaf after scanning at 160 DPI but before processing with CVIPtools.

Figure 4.6.C. Leaf after extraction of red band. Lighter gray tones after red band extraction correspond to higher levels of the red band in Figure 4.6.B. Darker gray tones after red band extraction correspond to lower levels of the red band in Figure 4.6.B.
Figure 4.6.D. Leaf after setting the gray-level threshold to 89 (out of a possible 255) to isolate leaf as a binary image. Chlorotic areas are not present in image, but necrotic tissue areas are present.

Figure 4.6.E. Binary image of leaf after stacking Figure 4.6.D. with the product of a green-band extraction and gray level thresholding of Figure 4.6.B. Additional segmentation removed leaf petiole.

Figure 4.6.F. Reversal of Figure 4.6.E’s binary code to prepare the image for area calculation with CVIPTool’s Features function.
QUANTIFYING LEAF TATTERS AS A PERCENTAGE OF TISSUE LOSS

Quantifying the percentage of tissue damage requires a reasonably confident estimation of total leaf area in the absence of any treatment. Estimation of total leaf area was complicated in cases of leaves that had abscised injured tissue before scanning. A second set of binary images was created to serve as models of total leaf area. Blue band extraction and gray-level thresholding created a binary image representing all leaf tissue (both injured and non-injured areas) present at time of sampling (Figure 4.7.B). The CVIPtools’ Segmentation function eliminated gaps and/or holes within leaves (Figure 4.7.C). The resulting images were later used to estimate the “whole” leaf areas – that is, what the total leaf area would have been in the absence of treatment.

For the first method of estimating “whole” leaf area, the least damaged leaves collected served as a model of “whole” leaf area for extremely damaged leaves. This method could be applied only if leaves met all of the following criteria: were from the same seedling; originated from the same bud; were at similar stages of expansion during the post treatment period. For example, Figure 4.8.A shows leaves identified as N, O, P, and Q in a seedling immediately after treatment. The same leaves can be identified in a later picture (Figure 4.8.B) and the scanned image (Figure 4.8.C). The leaves’ relative stages of expansion were determined from the library of digital pictures. From the examples shown (Figure 4.8.A to Figure 4.8.C), leaves N, O, and Q were of similar sizes and developmental stage. The area of the least damaged leaf was assumed to be equal to “whole” leaf area. Thus, the areas of leaves O and Q were assumed to estimate the “whole” leaf area of leaf N (Figure 4.8.C).
This imperfect method of estimation could not be applied to all leaves because there were gaps in the record of digital pictures. Some seedlings were unintentionally skipped during the process of photographing individual buds. Even though less than 50% of the seedlings were eligible for treatment, there were still a large number of seedlings – each with at least three stems labeled for bud stages - to photograph. If there was not an adequate record of digital pictures to ascertain the size/stage relationships between leaves, then the leaves were not included in analysis.
Figure 4.7.A. Scanned image of a leaf from a seedling treated with Dual II Magnum (S-metolachlor) at a concentration of 329 a.i./L (100% of the field rate).

Figure 4.7.B. Same leaf after extraction of blue band and gray-level thresholding to create a binary image. The image’s binary code was then reversed.

Figure 4.7.C. Segmentation of image in Figure 4.7.B. filled in gaps around leaf center and margins.
Figure 4.8.A. Individual unfolding2 stage leaves identified on a seedling immediately after treatment. Leaves N, O, and Q are of similar size and development.
Figure 4.8.B. The leaves N through Q identified on the same seedling 15 DAT. Leaf N has deteriorated more than leaves O or Q.
Figure 4.8.C. Leaves N through Q identified from the same seedling and stem after scanning. The “whole” area of leaf N could not have been estimated without the information in Figure 4.8.A.
SEEDLING LEAF MODELING

The second method of estimating total leaf area employed image modeling. Minimum bounding boxes were applied to the binary images of selected sample leaves (Chaudhuri and Samal, 2007). The length (in pixels) and width (in pixels) of a minimum bounding box is equal to the maximum length (in pixels) and width (in pixels) of the image. “Whole” leaf area was modeled as a percentage of the minimum bounding box area actually containing leaf area.

Reference leaves of various sizes and developmental stage were collected from untreated hackberry seedlings and trees. The leaves were scanned at 160 DPI (Epson Expression 1600) and processed with CVIPtools to create binary images. The actual area (in pixels) of each reference leaf was calculated using CVIPtools. A minimum bounding box was applied to each image. The aspect ratio was calculated from the length and width of each bounding box:

Equation 4.1

\[
\text{Aspect ratio} = \frac{\text{length}}{\text{width}}
\]

Each reference leaf was classified by developmental stage and shape. The developmental stages were applied to the individual leaves, not the buds from which the leaves originated. Developmental stages (partially-unfolding, expanding1, expanding2, and fully-expanded) were based upon length. Quartiles of the variable length were found with proc univariate in SAS (release 9.2; SAS Institute Inc., Cary, N.C.). The first, second, and third quartiles were assigned as maximum length values for the partially-unfolding, expanding1, and expanding2 developmental stages, respectively (Table 4.1).
Table 4.1. The ranges of lengths assigned to each developmental stage classification.

<table>
<thead>
<tr>
<th>Developmental Stage</th>
<th>Length (pixels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partially Unfolding</td>
<td>≤86.33</td>
</tr>
<tr>
<td>Expanding1</td>
<td>86.34 - 175.00</td>
</tr>
<tr>
<td>Expanding2</td>
<td>175.01 - 388.03</td>
</tr>
<tr>
<td>Fully Expanded</td>
<td>&gt;388.03</td>
</tr>
</tbody>
</table>
Figure 4.9.A. Example of the round leaf shape.

Figure 4.9.B. Example of the oval leaf shape.

Figure 4.9.C. Example of the tapered leaf shape.
Table 4.2. The aspect ratio ranges assigned to each shape. Aspect ratio is unitless.

<table>
<thead>
<tr>
<th>Shape</th>
<th>Aspect Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round</td>
<td>≤1.63</td>
</tr>
<tr>
<td>Oval</td>
<td>1.64 - 2.00</td>
</tr>
<tr>
<td>Tapered</td>
<td>&gt;2.00</td>
</tr>
</tbody>
</table>

Table 4.3. The percent of the minimum bounding box area actually occupied by leaf area for each combination of leaf developmental stage and shape.

<table>
<thead>
<tr>
<th>Developmental Stage</th>
<th>Partially Unfolding</th>
<th>Expanding1</th>
<th>Expanding2</th>
<th>Fully Expanded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Round</td>
<td>75%</td>
<td>73%</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Oval</td>
<td>72%</td>
<td>72%</td>
<td>65%</td>
<td>63%</td>
</tr>
<tr>
<td>Tapered</td>
<td>74%</td>
<td>71%</td>
<td>59%</td>
<td>58%</td>
</tr>
</tbody>
</table>
Initially, reference leaves were visually classified as round, oval, or tapered. Observed aspect ratio values were consistently lowest among round leaves (Figure 4.9.A), higher among oval leaves (Figure 4.9.B) and highest among tapered leaves (Figure 4.9.C). Each shape classification was then assigned to a specific range of aspect ratio values (Table 4.2). The actual pixel area for each reference leaf was converted to a percentage of the minimum bounding box area. The average percent-minimum-bounding box area was calculated for each developmental stage/shape classification (Table 4.3). “Whole” leaf area in the absence of herbicide treatment was estimated by:

Equation 4.2

\[ \text{Area}_t = x \times \text{Area}_{mbb} \]

where: \( x \) = average percent of the minimum bounding box filled with leaf, by developmental stage/shape,

\( \text{Area}_t \) = Estimated total leaf area, and

\( \text{Area}_{mbb} \) = Area of minimum bounding box

Sample leaves were assigned to a developmental stage/shape classification based upon minimum bounding box length and aspect ratio. The estimated “whole” leaf area was calculated using equation 2. As evident in table 4.3, there was not a percentage assigned to the minimum bounding boxes for leaves in the expanding2/round or fully-expanded/round developmental stages and shapes. This is because the reference seedling leaves in the later developmental stages tended to taper abruptly at the leaf tip.

The few sampled leaves classified as expanding2/round (five leaves) on the basis of length and aspect ratio were assumed to be expanding2/tapered leaves which had lost leaf tips to tatters damage. In these cases, the aspect ratio was adjusted to 2.00 and the
length of the minimum bounding box was re-calculated based upon the adjusted aspect ratio. The minimum bounding box area was re-calculated and the estimated leaf area was calculated as if the leaves were expanding tapered leaves. None of the sampled leaves were classified as fully-expanded.

The estimation method involving comparisons of heavily damaged leaves to less damaged leaves was applied alone and in conjunction with modeled leaf area. For example, if two leaves were from the same bud and developed at the same rate, then the area of the leaf with the most intact tissue was assumed to best approximate the “whole” leaf area for both leaves. A total of 110 leaves were still dropped from analysis, but this was less than 6% of the over two thousand leaves originally collected. In the absence of model generated estimates, 387 leaves - 18% of collected leaves - were excluded from analysis. Modeling was not used for leaves which still had all tissue (healthy and damaged) attached, as the percentage of tissue loss could be calculated directly from the total leaf area.
RESULTS

Hackberry Seedling Spray Drift Study

SEEDLING LEAF AREAS

Analysis of variance of seedling leaf areas (mm$^2$) was implemented with proc mixed in SAS (version 9.2). The residuals were checked for normality of data and homogeneity of variance. The sampled seedling leaf areas were not normally distributed. Transforming the areas with log10 approximated a normal distribution and all results apply to the transformed data. The plot of residuals versus the predicted means of transformed data was somewhat funnel shaped, indicating heterogeneity of variances.

The whole-plot treatment was defined as the combination of the herbicide type and application concentration. The interaction effect of whole-plot treatment and bud stage was not significant. The whole-plot treatment significantly affected leaf area at the $\alpha<0.05$ level (Table 4.4). The bud stage significantly affected leaf area at the $\alpha<0.05$ level (Table 4.4).

The least-squared means of the transformed leaf areas (Table 4.5.A) yielded a few surprises. Unexpectedly, among seedlings treated with acetochlor (Harness) or dimethenamid (Outlook), leaf area was smaller at 1% of the field rate than at 10% of the field rate. Among seedlings treated with dimethenamid, there was no difference in leaf area for seedlings treated at 1% of the field rate and seedlings treated at 100% of the field rate. The leaf area of control seedlings was smaller than the leaf area of seedlings treated with acetochlor or dimethenamid at 10% of the field rate. The low leaf area among control seedlings was surprising. Leaf area was smallest when treatment was applied
during the folded bud stage and largest when treatment was applied during the unfolding stage.
Table 4.4. Type 3 tests of fixed effects for transformed leaf area. ‘Treatment’ is the combined effect of herbicide x application applied to the seedlings. ‘Stage’ is the sub-plot effect of bud developmental stage.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>9</td>
<td>58</td>
<td>2.12</td>
<td>0.0423</td>
</tr>
<tr>
<td>Stage</td>
<td>2</td>
<td>82</td>
<td>4.76</td>
<td>0.0110</td>
</tr>
<tr>
<td>Treatment*Stage</td>
<td>18</td>
<td>82</td>
<td>0.66</td>
<td>0.8392</td>
</tr>
</tbody>
</table>
Table 4.5.A. The least square means estimates of transformed seedling leaf area. Least square means are provided for whole-plots (Treatment) and sub-plots (Stage).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Treatment</th>
<th>Estimate</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1% Dual II Magnum</td>
<td>0.5143</td>
<td>0.2728</td>
</tr>
<tr>
<td></td>
<td>10% Dual II Magnum</td>
<td>0.3884</td>
<td>0.2347</td>
</tr>
<tr>
<td></td>
<td>100% Dual II Magnum</td>
<td>0.1993</td>
<td>0.1887</td>
</tr>
<tr>
<td></td>
<td>1% Harness</td>
<td>0.7333</td>
<td>0.2091</td>
</tr>
<tr>
<td></td>
<td>10% Harness</td>
<td>0.9251</td>
<td>0.1884</td>
</tr>
<tr>
<td></td>
<td>100% Harness</td>
<td>0.1779</td>
<td>0.2004</td>
</tr>
<tr>
<td></td>
<td>1% Outlook</td>
<td>0.5147</td>
<td>0.2111</td>
</tr>
<tr>
<td></td>
<td>10% Outlook</td>
<td>1.0045</td>
<td>0.2034</td>
</tr>
<tr>
<td></td>
<td>100% Outlook</td>
<td>0.5414</td>
<td>0.1847</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0.8692</td>
<td>0.2033</td>
</tr>
<tr>
<td></td>
<td>Folded</td>
<td>0.4763</td>
<td>0.1066</td>
</tr>
<tr>
<td></td>
<td>Unfolding1</td>
<td>0.4957</td>
<td>0.0751</td>
</tr>
<tr>
<td></td>
<td>Unfolding2</td>
<td>0.7884</td>
<td>0.0921</td>
</tr>
</tbody>
</table>

Table 4.5.B. Average leaf area (mm$^2$) obtained from back transformation of LS means estimates. Average leaf areas are provided for whole-plots (Treatment) and sub-plots (Stage).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Treatment</th>
<th>Average Area (mm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1% Dual II Magnum</td>
<td>2.2681</td>
</tr>
<tr>
<td></td>
<td>10% Dual II Magnum</td>
<td>1.4457</td>
</tr>
<tr>
<td></td>
<td>100% Dual II Magnum</td>
<td>0.5823</td>
</tr>
<tr>
<td></td>
<td>1% Harness</td>
<td>4.4113</td>
</tr>
<tr>
<td></td>
<td>10% Harness</td>
<td>7.4159</td>
</tr>
<tr>
<td></td>
<td>100% Harness</td>
<td>0.5063</td>
</tr>
<tr>
<td></td>
<td>1% Outlook</td>
<td>2.2711</td>
</tr>
<tr>
<td></td>
<td>10% Outlook</td>
<td>9.1042</td>
</tr>
<tr>
<td></td>
<td>100% Outlook</td>
<td>2.4786</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>6.3995</td>
</tr>
<tr>
<td></td>
<td>Folded</td>
<td>1.9943</td>
</tr>
<tr>
<td></td>
<td>Unfolding1</td>
<td>2.1311</td>
</tr>
<tr>
<td></td>
<td>Unfolding2</td>
<td>5.1433</td>
</tr>
</tbody>
</table>
To separate the influences of herbicide type, herbicide application rate and developmental stage at treatment, contrasts were applied to transformed data (Table 4.6). As expected, leaf area was significantly larger among controls than seedlings treated at 100% of the field rate (P-value = 0.018). There was not a significant difference in leaf area between controls, seedlings treated at 1% of the field rate, and seedlings treated at 10% of the field rate. Leaf area was significantly higher among seedlings treated at 10% of the field rate compared to seedlings treated at 100% of the field rate (P-value = 0.006). Unusually, there was no difference in leaf area between seedlings treated at 1% of the field rate and 100% of the field rate.

It is suspected that the lack of a significant difference between the 1% field rate and 100% field rate levels was partly due to changes in blocking during the experiment. Compared to other treated seedlings, a greater percentage of seedlings treated at the 1% field rate level were replacement seedlings. General deterioration of health – minor wilting of unfolding leaves – was observed among all replacement seedlings after placement in the greenhouse and prior to any treatments.

Comparisons of the developmental stages indicate that leaf area was significantly larger among buds treated during the unfolding2 stage than buds treated during the folded bud (P-value = 0.017) or unfolding1 stage (P-value = 0.004) (Table 4.6). Leaf area was not significantly different between treatments at the folded bud stage or the unfolding1 stage. The differences in leaf area could indicate that earlier developmental stages are more sensitive to leaf tatters injury. However, the 15-day wait between treatment and sampling was not enough time for folded buds to develop fully expanded leaves. The
differences in leaf area seen between developmental stages may have no relation to leaf tatters damage.

The type of commercial herbicide applied did not have a significant effect on leaf area (Table 4.6). The selected brands of acetochlor, S-metolachlor, and dimethenamid formulations were equally detrimental to hackberry seedlings.
Table 4.6. Contrasts of different treatment effects levels. The effects of herbicide concentration (Concentration), developmental stage at time of treatment (Developmental stage), and type of herbicide are compared across other effects.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Contrasted Effect Levels</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>Control vs. 1%</td>
<td>1</td>
<td>58</td>
<td>1.33</td>
<td>0.2543</td>
</tr>
<tr>
<td>Concentration</td>
<td>Control vs. 10%</td>
<td>1</td>
<td>58</td>
<td>0.17</td>
<td>0.6857</td>
</tr>
<tr>
<td>Concentration</td>
<td>Control vs. 100%</td>
<td>1</td>
<td>58</td>
<td>5.88</td>
<td>0.0184</td>
</tr>
<tr>
<td>Concentration</td>
<td>1% vs. 10%</td>
<td>1</td>
<td>58</td>
<td>1.04</td>
<td>0.3116</td>
</tr>
<tr>
<td>Concentration</td>
<td>1% vs. 100%</td>
<td>1</td>
<td>58</td>
<td>2.60</td>
<td>0.1125</td>
</tr>
<tr>
<td>Concentration</td>
<td>10% vs. 100%</td>
<td>1</td>
<td>58</td>
<td>8.03</td>
<td>0.0063</td>
</tr>
<tr>
<td>Developmental stage</td>
<td>Unfolding2 vs. Folded and Unfolding1</td>
<td>1</td>
<td>82</td>
<td>8.96</td>
<td>0.0036</td>
</tr>
<tr>
<td>Developmental stage</td>
<td>Folded vs. Unfolding1 and Unfolding2</td>
<td>1</td>
<td>82</td>
<td>2.32</td>
<td>0.1313</td>
</tr>
<tr>
<td>Developmental stage</td>
<td>Folded vs. Unfolding1</td>
<td>1</td>
<td>82</td>
<td>0.03</td>
<td>0.8601</td>
</tr>
<tr>
<td>Developmental stage</td>
<td>Folded vs. Unfolding2</td>
<td>1</td>
<td>82</td>
<td>5.89</td>
<td>0.0174</td>
</tr>
<tr>
<td>Developmental stage</td>
<td>Unfolding1 vs. Unfolding2</td>
<td>1</td>
<td>82</td>
<td>8.64</td>
<td>0.0043</td>
</tr>
<tr>
<td>Type of herbicide</td>
<td>Dual II Magnum vs. Harness</td>
<td>1</td>
<td>58</td>
<td>1.88</td>
<td>0.1753</td>
</tr>
<tr>
<td>Type of herbicide</td>
<td>Dual II Magnum vs. Outlook</td>
<td>1</td>
<td>58</td>
<td>3.20</td>
<td>0.0789</td>
</tr>
<tr>
<td>Type of herbicide</td>
<td>Harness vs. Outlook</td>
<td>1</td>
<td>58</td>
<td>0.21</td>
<td>0.6493</td>
</tr>
</tbody>
</table>
ESTIMATED PERCENTAGE OF TISSUE LOSS

As stated earlier, the tissue loss as a percentage of area was estimated using two different methods, one of which included model-based leaf area estimates. Both sets of data had a normal distribution. The plot of the residuals versus the predicted mean was somewhat funnel shaped, indicating heterogeneity of variances, particularly for smaller predicted values.

Despite the difference in methods for estimating total leaf area in the absence of treatment, the analysis of variance results for percentage of tissue loss were the same. The interaction effect of treatment and bud stage was not significant. The effect of the whole-plot treatment was significant at $\alpha<0.05$ level (P-value = 0.02). The effect of bud stage was not significant (P-value = 0.44) (Table 4.7).

It was expected that the percentage of tissue loss would increase with increases in concentration for all tested herbicides. The least squared means indicated some departure from this expectation (Table 4.8). The percentage of tissue loss for seedlings treated with S-metolachlor (Dual II Magnum) was higher at 10% of the field rate than at 1% of the field rate; however, the percentage of tissue loss was the same at 10% of the field rate and 100% of the field rate. The percentage of tissue loss was consistently higher among seedlings treated with S-metolachlor (Dual II Magnum) at 1% and 10% of the field rate compared to seedlings treated with acetochlor (Harness) at 1% and 10% of the field rate. For seedlings treated with acetochlor (Harness), there was no difference in the percentage of tissue loss between treatments of 1% and 10% of the field rate. The same pattern occurred in seedlings treated with dimethenamid (Outlook).
Table 4.7. Type 3 test of fixed effects. Fixed effects are whole-plot (Treatment), sub-plot (Stage), and interaction of whole-plot and sub-plot effects.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>9</td>
<td>58</td>
<td>2.47</td>
<td>0.0185</td>
</tr>
<tr>
<td>Stage</td>
<td>2</td>
<td>80</td>
<td>0.83</td>
<td>0.4381</td>
</tr>
<tr>
<td>Treatment*Stage</td>
<td>18</td>
<td>80</td>
<td>0.40</td>
<td>0.9835</td>
</tr>
</tbody>
</table>
Table 4.8. Least squared mean estimates for whole-plot (Treatment) and sub-plot (Stage). ‘Treatment’ is the combination of herbicide type and herbicide concentration (as percent of field rate) applied to seedlings. ‘Stage’ is the developmental stage at time of treatment.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Treatment</th>
<th>Estimate</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1% Dual II Magnum</td>
<td>0.7603</td>
<td>0.1152</td>
</tr>
<tr>
<td></td>
<td>10% Dual II Magnum</td>
<td>0.9213</td>
<td>0.0981</td>
</tr>
<tr>
<td></td>
<td>100% Dual II Magnum</td>
<td>0.9258</td>
<td>0.0780</td>
</tr>
<tr>
<td></td>
<td>1% Harness</td>
<td>0.5930</td>
<td>0.0859</td>
</tr>
<tr>
<td></td>
<td>10% Harness</td>
<td>0.5991</td>
<td>0.0785</td>
</tr>
<tr>
<td></td>
<td>100% Harness</td>
<td>0.9340</td>
<td>0.0825</td>
</tr>
<tr>
<td></td>
<td>1% Outlook</td>
<td>0.7460</td>
<td>0.0877</td>
</tr>
<tr>
<td></td>
<td>10% Outlook</td>
<td>0.7734</td>
<td>0.0852</td>
</tr>
<tr>
<td></td>
<td>100% Outlook</td>
<td>0.8605</td>
<td>0.0770</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0.7049</td>
<td>0.0842</td>
</tr>
<tr>
<td></td>
<td>Folded</td>
<td>0.8008</td>
<td>0.0493</td>
</tr>
<tr>
<td></td>
<td>Unfolding1</td>
<td>0.7997</td>
<td>0.0371</td>
</tr>
<tr>
<td></td>
<td>Unfolding2</td>
<td>0.7450</td>
<td>0.0437</td>
</tr>
</tbody>
</table>
The percentage of tissue loss in seedlings with water (Table 4.7) was unexpected, but fits with recorded greenhouse observations. Out of the eight seedlings treated with water: five seedlings had leaf cupping; five had necrosis developing inward from leaf margins (one developed leaf tatters); and two seedlings suffered death at the growing points.

The different levels of herbicide concentration (as a percentage of the field rate) were compared across all herbicides (Table 4.9). As expected, the percentage of tissue loss was significantly higher among seedlings treated at 100% of the field rate than water (0% of the field rate) (P-value = 0.03). The percentage of tissue loss was significantly lower among seedlings treated at 1% of the field rate than seedlings treated at 100% of the field rate (P-value = 0.005). The percentage of tissue loss was significantly lower among seedlings treated at 10% of the field rate than seedlings treated at 100% field rate. There was no significant difference in the percentage of tissue loss between treatments of water, 1% of the field rate, and 10% field rate.

There was not a significant difference between S-metolachlor (Dual II Magnum) and dimethenamid (Outlook), or between acetochlor (Harness) and dimethenamid (Outlook) at α<0.05 level. The percentage of tissue loss was significantly higher among seedlings treated with S-metolachlor (Dual II Magnum) compared to seedlings treated with acetochlor (Harness) (Table 4.9).
Table 4.9. Contrasts of different treatment effects levels. The effects of herbicide concentration (Concentration), developmental stage at time of treatment (Developmental stage), and type of herbicide are compared across other effects. Upper and lower bounds define the 95% confidence interval.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Comparisons of Effect Levels</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>DF</th>
<th>t Value</th>
<th>P Value</th>
<th>Lower Bounds</th>
<th>Upper Bounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>Control vs. 1%</td>
<td>0.0052</td>
<td>0.0983</td>
<td>58</td>
<td>0.05</td>
<td>0.9582</td>
<td>-0.1915</td>
<td>0.2019</td>
</tr>
<tr>
<td>Concentration</td>
<td>Control vs. 10%</td>
<td>-0.0597</td>
<td>0.0860</td>
<td>58</td>
<td>-0.62</td>
<td>0.5365</td>
<td>-0.2517</td>
<td>0.1324</td>
</tr>
<tr>
<td>Concentration</td>
<td>Control vs. 100%</td>
<td>-0.2018</td>
<td>0.0533</td>
<td>58</td>
<td>-2.16</td>
<td>0.0347</td>
<td>-0.3886</td>
<td>-0.0151</td>
</tr>
<tr>
<td>Concentration</td>
<td>1% vs. 10%</td>
<td>-0.0648</td>
<td>0.0739</td>
<td>58</td>
<td>-0.88</td>
<td>0.3842</td>
<td>-0.2128</td>
<td>0.0832</td>
</tr>
<tr>
<td>Concentration</td>
<td>1% vs. 100%</td>
<td>-0.2070</td>
<td>0.0709</td>
<td>58</td>
<td>-2.92</td>
<td>0.0050</td>
<td>-0.3490</td>
<td>-0.0650</td>
</tr>
<tr>
<td>Concentration</td>
<td>10% vs. 100%</td>
<td>-0.1422</td>
<td>0.0662</td>
<td>58</td>
<td>-2.15</td>
<td>0.0360</td>
<td>-0.2747</td>
<td>-0.0096</td>
</tr>
<tr>
<td>Developmental stage</td>
<td>Folded vs. Unfolding1</td>
<td>0.0011</td>
<td>0.0487</td>
<td>80</td>
<td>0.02</td>
<td>0.9820</td>
<td>-0.0957</td>
<td>0.0979</td>
</tr>
<tr>
<td>Developmental stage</td>
<td>Folded vs. Unfolding2</td>
<td>0.0557</td>
<td>0.0565</td>
<td>80</td>
<td>0.99</td>
<td>0.3266</td>
<td>-0.0566</td>
<td>0.1681</td>
</tr>
<tr>
<td>Developmental stage</td>
<td>Unfolding1 vs. Unfolding2</td>
<td>0.0546</td>
<td>0.0441</td>
<td>80</td>
<td>1.24</td>
<td>0.2184</td>
<td>-0.0330</td>
<td>0.1423</td>
</tr>
<tr>
<td>Developmental stage</td>
<td>Folded vs. Unfolding1 and Unfolding2</td>
<td>0.0284</td>
<td>0.0479</td>
<td>80</td>
<td>0.59</td>
<td>0.5545</td>
<td>-0.0669</td>
<td>0.1237</td>
</tr>
<tr>
<td>Developmental stage</td>
<td>Unfolding2 vs. Folded and Unfolding1</td>
<td>-0.0552</td>
<td>0.0444</td>
<td>80</td>
<td>-1.24</td>
<td>0.2176</td>
<td>-0.1436</td>
<td>0.0332</td>
</tr>
<tr>
<td>Type of herbicide</td>
<td>Dual II Magnum vs. Harness</td>
<td>0.1604</td>
<td>0.0723</td>
<td>58</td>
<td>2.22</td>
<td>0.0305</td>
<td>0.0156</td>
<td>0.3052</td>
</tr>
<tr>
<td>Type of herbicide</td>
<td>Dual II Magnum vs. Outlook</td>
<td>0.0759</td>
<td>0.0726</td>
<td>58</td>
<td>1.05</td>
<td>0.3003</td>
<td>-0.0694</td>
<td>0.2212</td>
</tr>
<tr>
<td>Type of herbicide</td>
<td>Harness vs. Outlook</td>
<td>-0.0846</td>
<td>0.0662</td>
<td>58</td>
<td>-1.28</td>
<td>0.2063</td>
<td>-0.2170</td>
<td>0.0479</td>
</tr>
</tbody>
</table>
Mature Hackberry Stand Spray Study

Analysis of variance was conducted on leaf area data collected from the mature hackberry trees with proc mixed in SAS (version 9.2). The data had a normal distribution. The plot of residuals versus predicted means had no obvious pattern, indicating homogenous variances. The effects of provenance, the side (sprayed or opposite side) of the tree, and the interaction of effects were not significant at $\alpha<0.05$ level (Table 4.10). Spraying a solution of dimethenamid (Outlook) at 5608 mg a.i./L induced leaf tatters in mature hackberry trees.
Table 4.10. Type 3 test of fixed effects of mature hackberry tree leaf areas. Fixed effects tested are the provenance, side of tree from which leaf came, and interaction of provenance and side.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provenance</td>
<td>3</td>
<td>35</td>
<td>0.42</td>
<td>0.7425</td>
</tr>
<tr>
<td>Side</td>
<td>1</td>
<td>35</td>
<td>2.75</td>
<td>0.1064</td>
</tr>
<tr>
<td>Provenance*Side</td>
<td>3</td>
<td>35</td>
<td>0.38</td>
<td>0.7686</td>
</tr>
</tbody>
</table>
CONCLUSIONS

The seedling study did not provide results that can be acted upon by researchers, homeowners or community planners concerned about leaf tatters in hackberry. The chosen developmental stages did not significantly affect the severity of tatters. If there had been a significant difference, then suggestions to consumers on choosing hackberry provenances could have been refined. While there was a significant difference between herbicide concentrations of 1% or 10% of the field rate and 100% of the field rate, there was no significant difference between treatment with water and herbicide concentrations of 1% or 10% of the field rate. For researchers, the only new information was that the severity of leaf tatters was significantly higher among seedlings treated with S-metolachlor (Dual II Magnum) compared to seedlings treated with acetochlor (Harness).

The seedling study was plagued by unforeseen problems. Considering the trauma of being uprooted from sand and the low success of breaking dormancy, the seedlings were probably not at optimal health at the time of herbicide application. Logically, this would make them more vulnerable to any stressors. The development of leaf tatters’ symptoms in the controls indicate that some variable was overlooked when planning the spraying procedure. Perhaps control seedlings should have always been treated before the other seedlings to ensure that there were no herbicide particles lingering in the air around the spray rack. There is also the possibility that herbicides volatilized from leaf and bud tissues after seedlings were returned to the greenhouse. If herbicide volatilization was the culprit, then seedlings treated with the full field rate concentration
would have negatively affected the health of other, nearby seedlings (treated or untreated).

Future leaf tatters studies using hackberry should focus on a narrower range of herbicide concentrations, particularly above the 10% level. The definition of the second unfolding bud stage was very broad in this study, which may or may not have contributed to the lack of a significant difference between unfolding2 stage and folded bud or unfolding1 bud stage. Once unfolded leaves are observed, then developmental stage classification should focus on the individual leaves instead of attempting to classify the whole bud.

The method of determining leaf area in the 2009 spray studies was in itself experimental. There was a definite trade-off between time and the possibility of a more accurate measurement of area. If digital analysis is used in future studies, then one should heed the following recommendations: scan leaves at a DPI≥300; apply enough weight to prevent any shadowing behind sample leaves; save images in formats which lose less data (i.e. tifs); and take at least a week to familiarize oneself with the chosen digital analysis software and scanner prior to applying any treatments.

The methods used for estimating the percentage of tissue loss were inefficient and heavily dependent upon the library of digital images. The attempt to develop a model of leaf area solely from minimum bounding boxes was intrinsically flawed. The model became less accurate as the amount of actual damage increased, sometimes estimating “whole” leaf areas that were less than actual total non-abscised tissue areas.
CHAPTER 5: SUCCESS OF BREAKING DORMANCY IN SEEDLINGS

MATERIALS AND METHODS

By June 21, 2009, only 87 of the 210 originally selected seedlings had ever shown signs of breaking dormancy. The number of branches and stem diameter of all seedlings in the greenhouse were recorded on June 12, 17, 19, and 21. Seedlings originally selected for the study based upon height were classified as untreated or treated. The thirty seedlings selected for the study based upon bud staged were classified as specially-treated. Destructive measurements were performed on 20% of seedlings in each classification, and selection of seedlings was random.

Seedlings were removed from pots and placed on metal screens (Figure 5.1). The roots were rinsed with pressurized water. In cases of dense root mats, soil removal was sacrificed in favor of keeping root mats intact. The presence of any root or stem suckers was recorded. Seedling were examined to determine the extent of living tissue before separating live stems, dead stems, and roots (Figure 5.2 and Figure 5.3). The stems and roots were placed in paper bags and dried over-night in drying ovens before recording weights.
Figure 5.1. Photograph of root washing set-up. Screens on trestles prevented loss of roots during washing.
Figure 5.2. Lower part of seedling stem removed from roots. The lack of green tissue beneath the bark indicates that the cambium of the stem is dead.
Figure 5.3. Lower part of seedling stem removed from roots. Bark was scraped away to check for green (living) cambial tissue.
RESULTS AND DISCUSSION

The failure of so many seedlings to break dormancy in 2009 was puzzling because all seedlings broke dormancy in 2008. It was originally suspected that the greenhouse environment may have been responsible for the poor health of the seedlings in 2009. In the shade-house, 43% of the seedlings broke dormancy; 41% of the seedlings in the greenhouse broke dormancy. A simple visual comparison of the minimum and maximum temperatures in the greenhouse and shade-house reveals a dramatic difference in temperatures (Graph 5.1 and Graph 5.2). If differences in temperature affected seedlings’ success of breaking dormancy then there should be a greater difference between the percentages of greenhouse and shade-house seedlings successfully breaking dormancy. The greenhouse environment probably did not contribute to the success or failure of seedlings breaking dormancy.

Probability of success in breaking dormancy was tested for independence from blocks. Evidence of dependence was not found (P –value = 0.303). The average, minimum, and maximum temperatures of blocks began to differ after the seventh week (not shown). The temperature differences were caused by the louvers being open more often and for longer periods in May.

Logistic regression of success in breaking dormancy among greenhouse seedlings (excluding replacement seedlings) was implemented with proc glimmix in SAS (version 9.2). The replacement seedlings were excluded from analysis because they were placed in the greenhouse only after successfully breaking dormancy. The effects of diameter and number of branches on success of breaking dormancy were significant (P-value
<0.01) (Table 5.1). The effect of seedling height on the success of breaking dormancy was not significant. The intercept and slopes for each effect were estimated (Table 5.2). The success of dormancy can be modeled by:

Equation 5.1

\[ \text{logit}(y) = 0.285 + 0.5868x - 0.02668z \]

where: \( y \) = probability of breaking dormancy,
\( x \) = stem diameter (cm\(^2\)) at the base,
and \( z \) = number of branches

Decreasing stem diameter and increasing the number of branches decreases the probability that a seedling will successfully break dormancy. This trend should be considered in future studies which require that hackberry seedlings successfully break dormancy.

Seedling survival was defined according to the presence of live stems and suckers. Seedlings with any living cambial tissue or suckers were classified as alive. Seedlings without living cambial tissue and no suckers were classified as dead. Logistic regression of seedling survival was implemented with proc glimmix in SAS (version 9.2). The effects of stem diameter, root mass, stem mass (including branches), and treatment were significant at \( \alpha<0.1 \) level, but not \( \alpha<0.05 \) level (Table 5.3). The intercept and slopes for each effect were estimated (Table 5.4). The probability of seedling survival can be modeled by:

Equation 5.2

\[ \text{logit}(y) = -6.4270 + 8.5775w + 0.4912x - 1.1637z + t \]
Table 5.1. Type 3 test of fixed effects for the probability of seedlings breaking dormancy.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total height (cm)</td>
<td>1</td>
<td>195</td>
<td>0.01</td>
<td>0.9257</td>
</tr>
<tr>
<td>Stem diameter (cm)</td>
<td>1</td>
<td>195</td>
<td>10.18</td>
<td>0.0017</td>
</tr>
<tr>
<td>No. of branches</td>
<td>1</td>
<td>195</td>
<td>23.01</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 5.2. Estimates of the intercept and slopes for each variable describing seedling dormancy breaking. Only variables with a significant effect at \( \alpha<0.05 \) are included in the equation.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>DF</th>
<th>t Value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.2845</td>
<td>0.2214</td>
<td>11</td>
<td>1.29</td>
<td>0.2252</td>
</tr>
<tr>
<td>Total height (cm)</td>
<td>-0.0007</td>
<td>0.0069</td>
<td>195</td>
<td>-0.09</td>
<td>0.9257</td>
</tr>
<tr>
<td>Stem diameter (cm)</td>
<td>0.5868</td>
<td>0.1839</td>
<td>195</td>
<td>3.19</td>
<td>0.0017</td>
</tr>
<tr>
<td>No. of branches</td>
<td>-0.0267</td>
<td>0.0056</td>
<td>195</td>
<td>-4.80</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
where: \( y \) = the probability of survival,
\( w \) = stem diameter (cm\(^2\)) at base,
\( x \) = mass (g) of roots in pot,
\( z \) = mass (g) of stems

and \( t \) = effect of treatment

The effect of treatment was significantly different from zero only for the replacement (‘Special’ treatment) seedlings. Decreasing in-pot root mass, decreasing stem diameter, or increasing stem mass will negatively affect seedlings’ probability of survival. These trends conform to expectations; the root system determines a plant’s ability to take up water and necessary nutrients. The roots also provide energy storage in the form of starch. The stems are sugar sinks during leaf development.

As stated earlier, all seedlings were grown from seeds originating in New York. While the greenhouse environment did not affect the success of breaking dormancy, it is possible that the climate of eastern Nebraska affected the success of breaking dormancy. Historic monthly maximum and minimum temperatures for the Buffalo, New York area (ThreadEx station) were obtained through the National Oceanic and Atmospheric Administration (NOAA) Satellite and Information Service website. The daily maximum and minimum temperatures from May, 2008 to May, 2009 for Mead, Nebraska were provided by the High Plains Regional Climate Center, University of Nebraska, Lincoln (Mead station, Automated Weather Data Network) (Graph 5.3).

The monthly temperatures in the Buffalo area from 1971 to 2000 fell within a narrower range than the monthly temperatures at the Mead station from 2008 to 2009.
The shadehouse is in a more sheltered location than the Mead station, and the shadehouse has a southern exposure. The actual shadehouse temperatures were probably more moderate than those measured at the Mead station. It is possible that shadehouse temperatures were not low enough or were not consistently low long enough for seedlings to meet chilling requirements to break dormancy.
Table 5.3. Type 3 test of fixed effects for probability of seedling survival. Treatment refers to the spray treatments seedlings received during the simulated spray drift study.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total height (cm)</td>
<td>1</td>
<td>40</td>
<td>0.84</td>
<td>0.3636</td>
</tr>
<tr>
<td>Stem diameter (cm)</td>
<td>1</td>
<td>40</td>
<td>2.90</td>
<td>0.0964</td>
</tr>
<tr>
<td>No. of branches</td>
<td>1</td>
<td>40</td>
<td>0.19</td>
<td>0.6669</td>
</tr>
<tr>
<td>Roots (g)</td>
<td>1</td>
<td>40</td>
<td>3.69</td>
<td>0.0618</td>
</tr>
<tr>
<td>Stems (g)</td>
<td>1</td>
<td>40</td>
<td>3.61</td>
<td>0.0647</td>
</tr>
<tr>
<td>Treatment</td>
<td>2</td>
<td>40</td>
<td>2.93</td>
<td>0.0647</td>
</tr>
</tbody>
</table>
Table 5.4. Estimates of the intercept and slopes for each variable describing seedling survival. Only variables with a significant effect at \( \alpha<0.10 \) are included in the equation. ‘Special’ treatment refers to replacement seedlings. ‘Treated’ treatment refers to seedlings which broke dormancy and were sprayed. ‘Untreated’ treatment refers to seedlings which did not break dormancy and were not sprayed.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Treatment</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>DF</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>-6.4270</td>
<td>4.8131</td>
<td>40</td>
<td>-1.34</td>
<td>0.1893</td>
</tr>
<tr>
<td>Total height (cm)</td>
<td></td>
<td>-0.1403</td>
<td>0.1527</td>
<td>40</td>
<td>-0.92</td>
<td>0.3636</td>
</tr>
<tr>
<td>Stem diameter (cm)</td>
<td></td>
<td>8.5775</td>
<td>5.0381</td>
<td>40</td>
<td>1.70</td>
<td>0.0964</td>
</tr>
<tr>
<td>No. of branches</td>
<td></td>
<td>0.0501</td>
<td>0.1156</td>
<td>40</td>
<td>0.43</td>
<td>0.6669</td>
</tr>
<tr>
<td>Root (g)</td>
<td></td>
<td>0.4912</td>
<td>0.2556</td>
<td>40</td>
<td>1.92</td>
<td>0.0618</td>
</tr>
<tr>
<td>Stem (g)</td>
<td></td>
<td>-1.1636</td>
<td>0.6126</td>
<td>40</td>
<td>-1.90</td>
<td>0.0647</td>
</tr>
<tr>
<td>Treatment</td>
<td>Special</td>
<td>5.7614</td>
<td>2.3784</td>
<td>40</td>
<td>2.42</td>
<td>0.0200</td>
</tr>
<tr>
<td>Treatment</td>
<td>Treated</td>
<td>20.6457</td>
<td>2308.2100</td>
<td>40</td>
<td>0.01</td>
<td>0.9929</td>
</tr>
<tr>
<td>Treatment</td>
<td>Untreated</td>
<td>0</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>
Graph 5.1. Daily average temperatures in forestry greenhouse averaged across blocks.
Graph 5.2. Maximum and minimum daily temperatures during period of seedling spray drift study from High Plains Regional Climate Center, Mead station at ARDC forestry property.
Graph 5.3. Graph comparing the historic (1971-2000) average monthly temperatures for the Buffalo, NY area and the (2008-2009) average monthly temperatures for the High Plains Regional Climate Center Mead station.
WORKS CITED


Brodersen, Ron. Notes to visiting researcher. 26 Sept. 2007.


