The Effects of 2-Chloroethylphosphonic Acid on Bud Vascularization in Sorghum Halapense, L.

Robert Allen Peterson

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THE EFFECTS OF 2-CHLOROETHYLPHOSPHONIC ACID ON BUD VASCULARIZATION IN SORGHUM HALAPENSE, L.

BY

ROBERT ALLEN PETERSON

A thesis submitted in partial fulfillment of the requirements for the degree Master of Science, Major in Botany, South Dakota State University

1970

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ACKNOWLEDGEMENTS

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THE EFFECTS OF 2-CHLOROETHYLPHOSPHONIC ACID ON BUD VASCULARIZATION IN SORGHUM HALAPENSE, L.

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser      Date

Head, Botany-Biology Department      Date
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Review of the Literature</td>
<td>1</td>
</tr>
<tr>
<td>Purpose of the Study</td>
<td>12</td>
</tr>
<tr>
<td>METHODS AND MATERIALS</td>
<td>13</td>
</tr>
<tr>
<td>Preparation of Experimental Material</td>
<td>13</td>
</tr>
<tr>
<td>Preparation of Tissue for Sectioning</td>
<td>14</td>
</tr>
<tr>
<td>Selection of Material for Anatomical Investigation</td>
<td>17</td>
</tr>
<tr>
<td>RESULTS</td>
<td>19</td>
</tr>
<tr>
<td>Morphological Observations</td>
<td>19</td>
</tr>
<tr>
<td>Anatomical Observations</td>
<td>23</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>47</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>51</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>52</td>
</tr>
</tbody>
</table>
LIST OF TABLES

1. Peterson method for dehydration of johnsongrass stem tissue ................................................. 15

2. Triarch quadruple stain flow diagram ......................................................................................... 16

3. Relative stages of development of xylem and phloem in treated and untreated plants at 3, 6, 9, 12, and 15 days after treatment ................................................................. 45
LIST OF FIGURES

1. Nodal positions at the apex of a johnsongrass shoot ........... 18
2. Effects of ethrel concentrations on plant height in johnsongrass ................................................. 20
3. Effects of ethrel concentrations on crown bud growth in johnsongrass ........................................ 21
4. Plant height and bud growth as a function of ethrel concentrations in johnsongrass ......................... 22
5. Diagram illustrating the relationship of the axillary bud to the internodal tissue in johnsongrass ........ 24
6. Nodal and internodal vascular complex of the third node of johnsongrass aerial stems ...................... 25
7. Young cauline bundles of untreated johnsongrass .......................................................... 28
8. Intermediate cauline bundles of untreated johnsongrass .................................................. 29
9. Mature cauline bundles of untreated johnsongrass .......................................................... 30
10. Untreated johnsongrass 3, 6, 9, 12, and 15 days after treatment ............................................... 33-35
11. Ethrel treated johnsongrass (3,000 ppm) 3, 6, 9, 12, and 15 days after treatment .......................... 37-39
12. Ethrel treated johnsongrass (30,000 ppm) 3, 6, 9, 12, and 15 days after treatment ..................... 41-43
13. Bud vascularization patterns .......................................................... 46
INTRODUCTION

Review of the Literature

*Sorghum halapense* L., better known as johnsongrass is described as having spikelets in pairs, one sessile and fertile, the other pedicellate, sterile but well developed, usually staminate, the terminal sessile spikelet with two pedicellate spikelets. The plant is a tall perennial with flat blades and terminal panicles of 1 to 5 jointed, tardily disarticulating racemes. The ligule is round and firm, 1-3 millimeters long. Culms arise from extensively creeping rhizome, with blades mostly less than 2 centimeters long. The sessile spikelet is 4.5 to 5.5 millimeters long, ovate oppressed-silky. The readily deciduous awn is 1 to 1.5 centimeters long geniculate, twisted below, pedicellate spikelet 5 to 7 millimeters long and lanceolate (Beal 1896, Hitchcock 1950, and Gleason and Cronquist 1963).

Johnsongrass is usually found in open ground, fields, and waste land. Being native to the Mediterranean region, it is found in the tropical and warmer regions of both hemispheres. In the United States it is located from Massachusetts to Iowa and Kansas; south to Florida and Texas, west to Southern California.

After being introduced into the United States in the early 17th century, it has spread throughout the southern United States. It now infects rich bottomland in the lower corn belt and cotton fields of the South and rich farmland of the Southwest. There have been many attempts
to stop this encroachment such as summer fallow, herbicides and burning to name a few (Arceneaux 1943, Leonard and Harris 1950, Rea 1952, Rea 1954, and Stamper 1952). For example, it would take eight successive flamings two weeks apart to establish an expected kill (Arceneaux 1943).

Johnsongrass excludes almost all other competitors in its invasion by rhizome mats. The young shoots grow rapidly in warm, moist soil, appearing above ground in about five days. Early growth is slow, but by six weeks the plants are two and one half to three feet tall with rhizome growth being horizontal with vertical shoots as opposed to only horizontal growth in the earlier stages. Eight week old plants are four to five feet tall. The rhizomes seem to be concentrated at eight inches, but may go as deep as thirty inches (Schmerbauch and Slife 1958).

Many perennial weeds, of which johnsongrass is a ranking member, are difficult to eradicate because they reproduce vegetatively by prolific production of axillary buds on rhizomes. The concern here is with the release of the buds from rest due to decapitation of the top growth. Herbicides, cutting, or burning may kill only the top growth but does nothing more than release from rest the rhizome lateral buds. A translocating herbicide apparently bypasses the buds that are not actually growing and they escape the toxic effects of the herbicide. Thus while only one shoot of a plant is killed, the buds
will grow into a new stand. If one of these rhizomes, with its buds were grown in culture, apical bud meristem activity proceeds without lateral bud growth. This is caused by apical dominance of the rhizomal bud apex. In one of his experiments, Beasley (1967) cut a rhizome of johnsongrass into nodal sections to see to what extent apical dominance was present, and found that without exception average data from each experiment indicated the following trend: increasing lateral shoot extension with increasing distance from the cut proximal end of multi-nodal sections.

Torrey (1967) explained apical dominance in this way: Auxin, such as indoleacetic acid, appears to be synthesized in the terminal bud of the shoot and has the highest concentration in the apex and the youngest leaves. Removal of the terminal bud stops cell elongation. Lateral buds have been shown to produce almost no auxin and remain undeveloped if the terminal bud remains active. Upon removal of the terminal bud, axillary buds began to elongate. If one applies synthetic indoleacetic acid in place of the decapitated bud, the axillary buds usually remain inhibited. Thus, the auxin from the terminal bud inhibits lateral bud auxin production and subsequent development.

Naylor and Skoog (cf Torrey 1967) showed that DNA synthesis is completely suppressed in the cells of the meristem of lateral buds in the presence of the terminal bud or when artificially applied auxin is placed at the surface of the decapitated tip. As soon as the tip
is removed, DNA synthesis proceeds in the cell of the meristem of the axillary bud, nuclei divide within two or three days, and the buds elongate. Recently it has also been shown that, even in the presence of the terminal bud, direct application of a cytokinin to the lateral bud apex will overcome the auxin inhibition effect and elongation will occur. Here, there seems to be clear evidence that auxin interacts with cytokinin to control shoot development.

As a result of work on the effect of gravity on the growth of lateral buds, Smith and Wareing (cf Moorby 1968) have suggested that some unknown factor moves from roots to the physically highest part of the stem where it promotes the growth of lateral buds. This possibility raises the question of whether the growth substances found in an organ are produced in that organ and the extent to which growth substances move over long distances through the plant (Moorby 1968).

Mitchell and Brown (1946) reported that 2,4-D will travel from the treated leaf into the stem and from the stem into the roots. They found that the translocation from the leaves is confined mainly to the living tissue of phloem and parenchyma. The translocation from the roots is by way of the xylem.

Weaver and DeRose (1946) found that the 2,4-D translocation was influenced by the photosynthetic activity of the plant. This would influence the velocity of downward movement in which the phloem is probably the important tissue involved.
Growth substances can move considerable distance within the vascular tissue of the plants (Moorby 1968). Unfortunately, however, the factor controlling this movement, its effect on the movement of other substances, and its significance in plant growth regulation have not, as yet, been investigated (Moorby 1968).

Auxin has been shown to play an essential role in vascular tissue differentiation and may be the limiting factor in its formation (Jacobs 1952). Van Overbeek (1938) suggested that auxins may impede the flow of nutrients to the buds by causing some kind of blockage of the vascular supply. Gregory and Veale (1957) found in flax that nitrogen and carbohydrate levels were important factors influencing apical dominance. McIntyre (1964) also found that the levels of nitrogen controlled apical dominance in *Agropyron repens* L. The higher levels released apical dominance while the lower inhibited bud growth but was induced by decapitation.

Pate *et al.* (1964) worked with Dichlobenil and Dicamba on alligatorweed in an attempt to show how vascularization between the bud and stem is affected by different treatments. They found the destruction of phloem, cambium, and assorted parenchyma above and within nodes in sections of plants treated with the two chemicals. The most recently differentiated phloem of Dichlobenil treated stems and the end walls of the young sieve tubes had collapsed twenty-four hours after treatment. Four days after treatment, destroyed tissue was
observed within and above the nodes. However, inactive buds generally did not appear to be injured. Bud tissue destruction was noted in a few older buds. Destruction was invariably associated with phloem tissue. The vascular connections between inactive buds and the main stem were not differentiated, which appeared to prevent expression of herbicidal activities within these inactive buds. This indicated that restricted translocation between stem and inactive axillary buds may prevent the herbicidal destruction of these buds until differentiation occurs.

Ball (1953) studied the effects of certain growth regulating substances on the rhizome of *Aegopodium*. Parker (1965) observed the action of soil applied herbicides. Meyer and Buchholtz (1962) noted the effects of chemicals on buds of quack grass. Beasley (1969) studied the effects of chemical stimulants on johnsongrass and nutsedge.

In the grasses, the lack of developmental relationships between the bud and the subtending (axillary) leaf is particularly clear (Esau 1965 and Sharman 1942). Although one usually associates a bud more closely with the leaf subtending it, anatomically and physiologically it has been clearly shown to be more closely related to the leaf above (Etter, cf Fisher 1964).

This same concept is given by Evans (1940) in his "phytonic unit" theory. In 1879, Gray (cf Evans 1940) employed the term "phytomere" to designate one of these phytonic units or structures which, when produced
in a series, make a plant of the higher grade. More recently, Weatherwax (cf Evans 1940) has amplified this definition:

"An internode together with the leaf at its upper end, and the bud at its lower end constitute a phytomer, the unit of structure of the shoot." He later added (1930): "A single leaf arising from the upper end of each internode subtends a bud, or the primordium of one, which is borne in the embryonic region of the lower end of the internode next above it." The leaf and bud of the same phytomer are therefore on opposite sides of the axis.

Sorghum and Zea have a short type of apex, bearing only one to perhaps three primordia (Sharman 1947). With the buds arising from three to four phytomer units behind the growing point, the tissues of the very young phytomers organizing just below the apical meristem, remain for a time in a highly meristematic condition. It is during this young vegetative condition that a bud may organize from the intercalary meristem (Evans 1940).

The bud draws its nutrients from the cortical parenchyma of the parent shoot, since at this early stage there is no vascular connection between the nascent bud and the rhizomal stele; and indeed, in some buds there may never be. The developing bud at first grows out as a mound of tissue, with a distal but incompletely organized meristem, below which differentiation of a vascular strand of incipient vascular tissue begins (Wardlaw 1968). Prior to the development of procambium,
such regions are composed only of essentially isodiametric meristematic cells (Garrison 1949).

This small-celled tissue is quite distinct from the surrounding large-celled cortical parenchyma, in which cell division has also been induced. As the bud elongates, its vascular system, traversing the cortex of the parent rhizome, becomes increasingly important in the translocation of materials to its actively growing apex (Wardlaw 1968).

Kumazawa (cf Esau 1965) found that the vegetative axillary buds in maize are initiated far from the shoot apex and are connected to the main axis by basipetal differentiation of procambium. Hershey (1930) found the differentiation of the vascular system of sorghum parallels that of maize in the sequence of cellular development in the bundle, the order of differentiation of bundles in a stem, and in the gross aspects of nodal anatomy. But Esau (1965) feels that because the distance between a bud and the vascular region of the shoot is so short, it is often difficult to recognize whether the differentiation is acropetal or basipetal. She also thought that due to the similar appearance of both young and mature phloem, this identification would be very difficult.

Garrison (1965) and Kundu and Rao (1955) found in early vascularization of axillary buds of dicots that, phloem differentiates earlier than xylem and precedes xylem by five plastochrons. Sharman (1942) also agreed that phloem differentiation is followed by xylem in the monocotyledon Zea Mays L.
As growth proceeds, the bud apex becomes more highly organized; the first leaf is formed and others follow in a regular phyllotactic sequence. The final development of the bud represents the last activity of a phase of growth which started on one side of the apex at the point where the leaf was initiated and passed down the leaf internode unit and then reached its conclusion in the formation of a bud on the other side of the stem. When the provascular strand differentiates from the bud into the axis, it differentiates in the base of the internode and not in tissue associated with the insertion of the leaf below, in the axil of which the bud formed (Sharman 1942). At every stage of bud development we have growth and a whole system of reacting and interacting factors. Bud development is thus essentially an epigenetic one, each phase being affected by the antecedent phases (Wardlaw 1968).

There was little information found concerning the nodal anatomy of monocotyledons. Literature was especially lacking on the anatomical relationship between a bud and its corresponding node.

Hayward (1938) found in wheat that at each node some 25 to 35 bundles enter the stem from the leaf sheath. Approximately half of these pass through the node into the outer hypodermal ring, while the remaining, somewhat larger bundles penetrate more deeply and become part of the inner vascular ring.

The node of corn is woody and rigid and its vascular anatomy is very intricate, owing to the fact that at each node there is a large
number of vascular bundles entering the stem which penetrate the nodal zone horizontally for various distances before they incline downward. In addition to this, although many of the bundles of the internode above pass directly through the node, a certain proportion of them terminate at each node by anastomosing with other bundles (Evans 1928).

The vascular bundles "seldom pass through more than two or three nodes without branching." The nodal complex results from numerous small branchings that arise from the vascular bundles as they enter the node, and from small peripheral bundles. The division, subdivision, and anastomosing of these bundles accounts for the vascular meshwork which constitutes the nodal plate (Evans 1928).

The procambial tissue gives rise to both xylem and phloem. It is this differentiation that will be considered in this study. The immature vascular strands, i.e. procambium of the bud, are initiated in the intercalary or residual meristem region. The procambium cells first appear denser than the more vacuolated cells of the ground tissue (Esau 1965).

"The provascular strand seems to result from divisions of a single chain of cells as most of the cells forming the strand have coincident end-walls. Later the strand is increased mainly by division of the more central cells in both tangential and radial longitudinal directions, so that even if not everywhere originally derived from one cell chain, the strand now has a stratified appearance when seen in sections cut in longitudinal sections" (Sharman 1942).
As procambium differentiation progresses, the cells are elongated and the nuclei dispersed throughout the cells. There is loss of continuity between the cell end walls caused by longitudinal growth of the cells. The cells become highly vacuolated. This early growth is usually at the expense of the cells surrounding those that began dividing first. Longitudinal growth increases with overlapping of cells and the beginning of differentiation of vascular elements (Esau 1965).

As the sieve tube elements begin to form, the companion cell and its nucleus can be easily found. The sieve tube element nucleus will degenerate with sieve areas and sieve plates formed. The sieve tube element is now called a sieve tube member. The sieve tube members will together form the sieve tube which is characteristic of mature phloem (Cheadle 1941).

The xylem matures later than the phloem but follows much the same sequence as phloem. Both differentiate from the same tissue. The young xylem and phloem are very difficult to distinguish from one another until sieve areas are found in phloem or until secondary walls are formed in the xylem. The xylem will usually lay down annular secondary wall thickenings first. This is followed ontogenetically by pitted secondary walls. Also characteristic of mature xylem is vessel members which are perforated elements aggregated longitudinally into rows of cells connected through the pores or perforations (Esau 1965).
The Purpose of the Study

An assumption can be postulated that the auxin produced in the stem apex may inhibit bud development by preventing differentiation of vascular connections between the cauline bundle and the bud primordia. This would deprive the primordia of nutrients and growth substances being translocated in the mainstream vascular elements. The assumption is that prior to decapitation, vascularization is incomplete to the laterals in johnsongrass and that after decapitation, vascularization becomes complete. Dr. C. A. Beasley has found that plants treated with ethrel will react the same as decapitated plants. This study was designed to examine ethrel treated plants and examine anatomically the vascularization of lateral bud procambial strands, to determine if ethrel will increase differentiation of vascular tissue, thus making the bud physiologically receptive to herbicides.
METHODS AND MATERIALS

Preparation of Experimental Materials

Johnsongrass rhizomes were exhumed from a mature stand formerly established as a clone. Single-node rhizome segments were excised by means of double-bladed knives. Segments were placed vertically on stainless-steel nails embedded in plastic strips. Axillary buds were oriented above the nodes. The segment-holding strips then were placed in mist chambers. Nine days after the beginning of mist chamber culture, those segments bearing shoots falling within the limits of desired uniformity (80mm to 160mm) were removed and transplanted to 0.8 liter of fertilized soil per each 6-inch plastic pot. Dry perlite (0.8 liter) was added as support for the developing shoots. These pots were placed in a greenhouse where they were allowed to grow for 31 days, after which the perlite was poured off and the new rhizome system was examined. Plants again were selected for uniformity of development and only those plants having shoots from 600mm to 1000mm and one or two developing rhizome buds (1mm to 5mm) were selected for testing. Four groups of plants were treated, respectively, with 30,000, 3,000, 300, and zero ppm ethrel. At 3, 6, 9, 12 and 15 days after treatment, eight plants from the four groups were selected as test plants. At each harvesting date, plant height and bud growth were recorded. The foregoing experimental design was devised and treatments were made by Dr. Charles A. Beasley at Standford Research Institute, South Pasadena, California.
To control possible effects of shipping on live plants from California to South Dakota, the experimental materials were collected, aspirated, killed and fixed at Stanford Research Institute. They were then shipped in killing and fixing solution to our laboratory in South Dakota.

Preparation of Tissue for Sectioning

Killing and Fixing. Several methods for killing and fixing johnsongrass tissue were used in the experimental stages of this project. Formalin-acetic-alcohol (Sass 1958) and a modification of the technique with increased concentrations of glacial acetic acid were used in the early stages of this investigation. Fixing in FAA followed by soaking in hydrofluoric acid did not yield satisfactory results. Extending dehydration times in ethyl-butyl series was not fruitful. Soaking the paraffin blocks in detergent water before each sectioning session was not satisfactory. The killing and fixing agent that yielded best results and was used for the remainder of this study was the Navaschin type (Craf V) chrome acetic acid-formaldehyde solution (Sass 1958).

Dehydration. Early attempts at dehydrating the tissues preparatory to imbedding using the classical ethyl-butyl alcohol method (Sass 1958) failed because of the toughness of the tissue and the hardening effect of the ethyl alcohol in the early stages of the dehydrating process. Taking into consideration the softening effects of glycerol, the
penetrating effects of ether and the miscibility of ethanol and methanol to xylol, the following procedure was constructed and was used effectively throughout this study (Table 1).

Table 1. Peterson method for dehydration of johnsongrass stem tissue.

<table>
<thead>
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<th>Steps</th>
<th>Treatment</th>
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<th>Time Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>glycerol-ethanol</td>
<td>50-50</td>
<td>2 weeks</td>
</tr>
<tr>
<td>2</td>
<td>ether-methanol</td>
<td>50-50</td>
<td>1 week</td>
</tr>
<tr>
<td>3</td>
<td>xylol-ethanol</td>
<td>25-75</td>
<td>2 hours</td>
</tr>
<tr>
<td>4</td>
<td>xylol-ethanol</td>
<td>50-50</td>
<td>2 hours</td>
</tr>
<tr>
<td>5</td>
<td>xylol-ethanol</td>
<td>75-25</td>
<td>2 hours</td>
</tr>
<tr>
<td>6</td>
<td>xylol</td>
<td>100</td>
<td>2 hours</td>
</tr>
</tbody>
</table>

Infiltration and Imbedding. Conventional imbedding procedures were used throughout this experiment (Sass 1958).

Microtome Sectioning. All serial sectioning of johnsongrass stem material was made on the rotary microtome. When a series was not required the vibratome was used. All paraffin sections were cut ten microns thick and mounted on slides preparatory to staining.

Staining and Dehydrating. All sections used in this study were stained by the Triarch quadruple stain technique using safranin, crystal violet, fast green and orange G. Dehydration was obtained with ethanol, clove oil and xylol. The staining and dehydration procedures are recorded in Table 2.
Table 2. Triarch quadruple stain flow diagram.

100% Xylo1
2-5 minutes

50-50 Xylo1
& Ethanol
2 minutes

100% Ethanol
2 minutes

95% Ethanol
2 minutes

85% Ethanol
2 minutes

70% Ethanol
2 minutes

55% Ethanol
2 minutes

Safranin
45-60 minutes

Water rinse
10 seconds

Water rinse
20 seconds

100% Xylo1
5 minutes

Orange G Clove oil
15 minutes

Orange G Clove oil
5 minutes

Orange G Clove oil
5 minutes

Fast green
10 seconds

100% Ethanol
10 seconds

100% Ethanol
10 seconds

100% Ethanol
10 seconds

100% Ethanol
10 seconds

100% Ethanol
10 seconds

Water rinse
10 seconds

Water rinse
10 seconds

Crystal violet
15 seconds
Selection of Material for Anatomical Investigation

Because lateral buds on above ground shoots of johnsongrass make their earliest appearance in the second or third nodal segments, transverse and longitudinal sections of these nodes and adjacent internodes were selected for study. Since the vascular system in the youngest laterals would be procambial in nature in untreated stems, it was hypothesized that subsequent effects of treatment on previously undifferentiated vascular tissue would be easily recognized.

One aspect of this study was purposely omitted. A second control, consisting of decapitated, untreated plants harvested at 3, 6, 9, 12, and 15 days after decapitation, was an obvious consideration for this study. Thus a comparison of treated plants with both untreated intact and the decapitated plants could have been made. However, the first three nodes in a young shoot totaled about 5 to 7 millimeters in length and were buried under 5 to 7 leaf sheaths (Figures 1A and 1B). Trying to remove the leaf sheaths and then to remove only the meristematic tip without injury to the lower nodes would have been impossible with our facilities.
Fig. 1. Nodal positions at the apex of a johnsongrass shoot. 
A. Young shoot showing position of the apex (½ natural size). 
B. Longitudinal section of the apex taken at X (Fig. 1 A). 
Magnification X250.
RESULTS

Morphological Observations

The visible effects of 2-chloroethylphosphonic acid on shoot development in johnsongrass were easily recognized. The plants treated with 30,000 ppm ethrel exhibited much less vertical growth than those treated with lower concentrations (Fig. 2). Fifteen days after foliar treatment with ethrel, the average shoot height increase for each experimental group was recorded as follows: 30,000 ppm, 6 cm; 3,000 ppm, 26 cm; 300 ppm, 37 cm; untreated, 44 cm.

Axillary and crown bud growth was affected quite differently with ethrel treatment. The following measurements represent the average length of the first five buds below the crown: treatment with 30,000 ppm ethrel resulted in an average bud length of 18.2 mm; 3,000 ppm, 17.8 mm; 300 ppm, 15.2 mm; untreated, 12.8 mm. The treatment with ethrel not only increased bud elongation below the crown but it also released from rest the lateral buds located on the aerial stem (Fig. 3). The untreated plants had no visible buds on the stem. Figure 4 graphically represents the effects of bud growth and shoot growth as a function of treatments with varying ethrel concentrations.
Fig. 2. Effects of ethrel concentrations on plant height in johnsongrass. From left to right: 30,000, 3,000, and 300 ppm of ethrel, foliarly applied 15 days prior to harvest (photographing). Major portions of leaf blades were removed prior to photographing.
Fig. 3. Effects of ethrel concentrations on crown bud growth in johnsongrass. From left to right: 30,000, 3,000, and 300 ppm of ethrel, foliarly applied 15 days prior to harvest (photographing). Note axillary bud development, at higher nodes, where 30,000 ppm ethrel was used.
Fig. 4. Plant height and bud growth as a function of ethrel concentrations in johnsongrass.
Anatomical Observations

The phytonic unit was evident in johnsongrass as it has been recognized in other grasses. Sharman (1942) described this as a disc of insertion. The upper half of the disc gave rise to the node with the leaf primordia and the lower half of the disc gave rise to the internode with its lateral bud (Fig. 5).

Two separate plate-like areas of vascularization were found at the nodal region of johnsongrass aerial shoots. The lower plate consisted of a complex of vascular bundles, some of which traversed the node to the leaf sheaths. Others extended into the internodes as cauline bundles (Fig. 6A). The bundles immediately above the nodal plate in internodal tissue are a complex of primordial bud traces (Fig. 6B). The procambial strands of provascular bud traces were clearly recognizable when compared to the mature vascular strands in the nodal plate. The bud procambial complex is low in the internode and bears little relationship to the nodal arrangement of the subtending leaf.

The two halves of the disc of insertion were easily recognizable during microtome sectioning. Paraffin embedded specimens cut easily, forming excellent ribbons through the internodal base with its bud procambia (shown in Fig. 6B). Upon reaching the nodal complex, (as illustrated in 6A) the ribbons would not form and the section shredded considerably.
Fig. 5. Diagram illustrating the relationship of the axillary bud to the internodal tissue in johnsongrass.
Fig. 6. Nodal and internodal vascular complex of the third node of johnsongrass aerial stems. A. Nodal complex showing mature leaf traces (longitudinal and oblique views) and cauline bundles (transverse views). B. Bud complex of procambial strands (longitudinal view). Large mature transverse bundles in the stem center are internodal cauline bundles. Magnification X100.
This study does not involve the effects of ethrel treatment on differentiation of leaf traces and cauline bundles. However, an illustrated description of mature and maturing cauline bundles, both in longitudinal and transverse view, serves as a standard for comparison with lateral bud vascular differentiation patterns as effected by ethrel treatment.

First signs of delimitation of the procambium were recognizable by differential staining of elongated derivatives of the apical meristem. They were also characterized by the alignment of their nuclei and their coincident end walls.

Cauline bundle; transverse course of differentiation. Planes of division of strands within the phloic segment of the young procambium were variously oriented, while in the xylary procambium, they were predominately radially seriated (Fig. 7A).

As procambium differentiated, the cells became more vacuolated, losing their dense appearance, but retaining their short transverse diameters. Endarch protoxylem maturation occurred in xylary procambium within the first two nodes of the stem apex, and was recognizable by differential staining and thickened secondary walls. Protoxylem was usually one cell but occasionally contained as many as three or four cells. Metaxylem differentiated in a radial seriation pattern due to the restriction of late procambial divisions to a predominately tangential plane (Fig. 8A). Three to four metaxylem vessels and
several tracheids and parenchyma cells comprised the mature cauline bundle (Fig. 9A).

Phloem matured centripetally in cauline bundles and protophloem sieve tube elements were the first cells of the phloic procambium to mature. Protophloem first appeared as a cluster of 10 to 15 thick-walled cells. Early metaphloem, lying centripetal to the protophloem, had immature walls (Fig. 7A). As differentiation occurred the protophloem and the metaphloem became difficult to distinguish from one another (Fig. 8A). As the vascular bundle matured the early protophloem was crushed. The residue was easily found centrifugally to the later developed protophloem, which was found in a clustered arrangement of sieve tube elements and companion cells. Metaphloem was fully developed with thickened primary walls (Fig. 9A).

Cauline bundle; longitudinal course of differentiation. Inter-nodal xylem differentiated centripetally but maturation was centrifugal so the annular and helically thickened, secondary-walled protoxylem tracheids were endarch (Fig. 7B). The protoxylem was first to be found very close to the growing point in an immature vascular bundle. These were characterized by having annular or helically thickened walls (Fig. 8B). Mature bundle tracheary elements contained scalariform and pitted secondary wall thickenings (Fig. 9B).

First phloem elements to differentiate were metaphloem sieve mother cells adjacent to the xylary procambium. Differentiation was
Fig. 7. Young cauline bundles of untreated johnsongrass. A. Transverse section of a young bundle. B. Longitudinal section comparable to A. Magnification X250.
Fig. 8. Intermediate cauline bundle of untreated johnsongrass.
A. Transverse section of cauline bundle. B. Longitudinal section comparable to A. Magnification X250.
Fig. 9. Mature cauline bundle of untreated johnsongrass. A. Transverse section of cauline bundle. B. Longitudinal section comparable to A. Magnification X250.
acropetal (Fig. 7B). However, mature phloem sieve tube elements were formed at the periphery of the bundles as a cluster of thick-walled cells that were recognizable by their differential affinity for orange G (Fig. 8B). Sieve areas were common on side walls while end walls with sieve plates were not easily distinguishable. The bundles near the center of the stem had a full complement of protoxylem and protophloem. Bundles located closer to the stem periphery had smaller protoxylem lacunae and a smaller amount of crushed protophloem. The outermost and smallest bundles had only metaphloem and metaxylem tissues and show no evidence of disruption of vascular elements (Fig. 9B). The effects of ethrel on the bud vascularization will now be described.

Untreated.

The untreated plants had very little vascularization occurring. The vascular tissue development of these plants collected 15 days after treatment was essentially the same as the plants collected three days after treatment. Only undifferentiated procambial tissue was found in the controls.

After three days, the cells of the procambial tissue were stratified with coincident end walls. The procambial cells were denser than the surrounding ground tissue with their nuclei aligned parallel to the end walls. At this early stage of development it was impossible to distinguish xylem from phloem. There appeared to be little longitudinal growth (Fig. 10A).
After six days little change in cell differentiation was noticeable. The tissue was definitely stratified with coincident end walls present. There was some vacuolation evident but the nuclei were still found in rows. No longitudinal growth was evident (Fig. 10B).

Nine days after treatment some longitudinal growth had taken place. The cells had not lost their stratified appearance and coincident end walls were still found. No increased vacuolation was found in the cells, and nuclei were still aligned. Development is not far enough along to distinguish phloem from xylem (Fig. 10C).

After 12 days, more longitudinal growth was evident but no differentiation. The cell walls and nuclei were still aligned and we find little vacuolation (Fig. 10D). Those harvested 15 days after treatment have a procambial pattern almost identical to those of 12 days (Fig. 10E).

**Ethrel treatment: 3,000 ppm.**

Bud procambium of the third node proximal from the stem tip showed considerable effects from 3,000 ppm ethrel treatment when compared to untreated plants. Plants harvested three days after treatment exemplified essentially the same level of development as the 15 day, untreated plants. Some longitudinal growth occurred but no differentiation. The cells were stratified with coincident end walls. The nuclei were aligned and some vacuolation had taken place (Fig. 11A).
Fig. 10. Untreated johnsongrass 3, 6, 9, 12, and 15 days after treatment. Magnification X950. A. Three days after treatment. B. Six days after treatment.
Fig. 10. (Continued). C. Nine days after treatment. D. Twelve days after treatment.
Fig. 10. (Continued). E. Fifteen days after treatment.
At six days substantial vascular differentiation had occurred. Longitudinal growth had increased with overlapping of cell end walls. Coincident end walls and nuclear alignment, characteristic of earlier procambium was disrupted. Vacuolation became more apparent. The protoplast took on a mottled appearance. Slime plugs were seen associated with end walls of several phloem sieve tube elements (Fig. 11B).

At nine days after treatment phloem differentiation was evidenced by sieve areas on metaphloem sieve mother cells. Vacuolation increased but no xylem differentiation was apparent. Longitudinal growth was progressive and companion cells were recognizable in a few strands (Fig. 11C).

At 12 days more longitudinal growth had taken place with the phloem companion cells clearly evident in the sections studied. Sieve areas were present on most sieve tube elements. Protophloem had taken on the appearance of maturity. Deeper stained walls of the protophloem set it apart from adjacent procambium and the immature metaphloem. The nuclei were still present in the sieve tube elements at this stage of differentiation but were showing some signs of degeneration. Xylem differentiation was still lacking (Fig. 11D).

Fifteen days after treatment protophloem maturation was recognizable with metaphloem sieve tube elements well differentiated. Companion cells were present with obvious nuclei. The metaphloem sieve tube nuclei have degenerated somewhat. Metaxylem vessel element differentiation has occurred but no maturation of xylem is evident (Fig. 11E).
Fig. 11. Ethrel treated johnsongrass (3,000 ppm) 3, 6, 9, 12, and 15 days after treatment. Magnification X950. A. Three days after treatment. B. Six days after treatment.
Fig. 11. (Continued). C. Nine days after treatment. D. Twelve days after treatment.
Fig. 11. (Continued). E. Fifteen days after treatment.
Ethrel treatment: 30,000 ppm.

The effects of 30,000 ppm ethrel on bud procambial vascularization were obvious from first observations. The plants harvested three days after treatment were more advanced than the 3,000 ppm treatment after three days. It was more advanced than the untreated approximately 15 days after treatment (Fig. 12A).

After six days rapid development of the procambium was evident. The cells showed much longitudinal growth with vacuolation occurring. Loss of coincident end walls was apparent and the stratified appearance was beginning to disappear. Although no maturation was recognizable, changes were characteristic of xylem differentiation (Fig. 12B).

At nine days, primary pit fields were present in differentiated but immature xylem. Reticulated and pitted secondary walls were common and indicative of secondarily thickened, mature xylem. Phloem was not as well differentiated here at nine days as in the 3,000 ppm treated after six days (Fig. 12C).

After 12 days more pitted tracheids were present in the vascular bundles than in those harvested at nine days. No further growth was evident in tracheary cells at 12 days. Phloem differentiation after 12 days corresponds with differentiation at six days in 3,000 ppm treatment. First signs of sieve areas were recognizable (Fig. 12D).

At 15 days much the same results were noted, xylem tracheids in abundance with phloem differentiation at the level of sieve tube
Fig. 12. Ethrel treated johnsongrass (30,000 ppm) 3, 6, 9, 12, and 15 days after treatment. Magnification X950. A. Three days after treatment. B. Six days after treatment.
Fig. 12. (Continued). C. Nine days after treatment. D. Twelve days after treatment.
Fig. 12. (Continued). E. Fifteen days after treatment.
elements with young sieve areas visible (Fig. 12E). The preceding observations were tabulated and appear in Table 3.

**Anastomosis of bud and stem vascularization.**

Lateral bud vascular differentiation was not the only mature vascular tissue showing effects of ethrel treatment. Those plants treated with ethrel had lateral bud bundles connecting with the cauline bundles. This anastomosing was found to occur between the phloem of the cauline bundle and the phloic portion of the bud procambial strand. Those that occurred in the 3,000 ppm ethrel treatment were between very young bud procambial strands (Fig. 13B), while those found in the 30,000 ppm treated plants had xylem present in the bud bundles (Fig. 13C). The anastomosing of the bud trace bundles to the cauline bundle was never found in the untreated plants (Fig. 13A).
Table 3. Relative stages of development of xylem and phloem in treated and untreated plants at 3, 6, 9, 12, and 15 days after treatment.

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0 = procambial level of development
X = degree of differentiation
XX = elongation-vacuolation
XXX = thickening of walls of first maturing cells
XXXX = most cells relatively well differentiated
XXXXX = complete differentiation of most cells, maturation of early cells
XXXXXX = completely mature elements


DISCUSSION

This study has shown that the phytonic unit (Evans 1940) or, as Sharman (1942) described it, the disc of insertion (Fig. 5) was recognized in johnsongrass. The axillary bud of johnsongrass had its origin in the intercalary meristem at the base of the internode and not in the node itself. Esau (1965) and Sharman (1942) agreed that the bud is more closely associated with the leaf above it than the leaf subtending it. This anatomical arrangement results in two plate-like complexes of vascular tissue. The upper plate is composed of residual meristem tissue containing procambial bud traces located at the base of the internode just above the actual node. The lower plate-like area included the vascular complex of cauline bundles which either became leaf traces or extended unbranched through the nodal complex into the next internode. Thus recognition of the bud vascularization was easily distinguished from the foliar and the cauline bundles.

Untreated.

The effects of ethrel on the bud vascularization in johnsongrass was easily recognized. The control plants had very little differentiation and no maturation of bud traces. There was elongation of the cells by apical intrusive growth but no change in nuclear position or cell width. Lateral buds were in a resting condition in the control plants.
Plants treated with 3,000 ppm ethrel had undergone more vascular differentiation after six days than the untreated plants 15 days after treatment. At six days in 3,000 ppm ethrel treated plants, gliding growth was apparent in bud procambial differentiation and nuclei were no longer aligned. The cells were narrower with more overlapping of end walls evident. These differentiated cells after nine days were identified as phloem sieve mother cells. Several stages of sieve element differentiation were evident after nine days because (a) no companion cells were associated with some mother cells, (b) companion cells were associated with some sieve elements and (c) the nucleus had not degenerated in the sieve elements with associated companion cells.

After twelve days, the protophloem was maturing. Sieve areas were found on most sieve tube elements with companion cells clearly evident. It was now possible to distinguish between the deeper stained (orange G) protophloem and the immature metaphloem.

There was no xylem differentiation apparent after 12 days. This was not surprising since, at least in the stem, phloem differentiates first in grasses, exceeding xylem by five plastochrons (Garrison 1965 and Kundu and Rao 1955). Mitchell and Brown (1946) found that 2,4-D was translocated from treated leaves via phloem and parenchyma to the stem. Assuming ethrel used the same pathway, one can hypothesize that there would be more ethrel concentrated in the phloem tissue than in
the xylem, thus resulting in phloem differentiation before xylem. The translocation pathway of dichlobenil was found by Pate et. al. (1964) to occur in the phloem in alligatorweed.

Xylem differentiation was noted at 15 days after treatment but no maturation was found. The protophloem maturation was recognized here. As compared to the control that had very little procambial differentiation, it was found that with 3,000 ppm ethrel treatment, there was some xylem differentiation, considerable phloem differentiation, and some phloem maturation. Bud vascularization in johnsongrass was definitely stimulated at 3,000 ppm ethrel.

Phloem in bud procambium of 3,000 ppm ethrel treated plants matured earlier than the xylem. This indicated a stimulatory effect of 3,000 ppm ethrel on phloem differentiation and maturation and slight effects on xylem differentiation.

**Treatment: 30,000 ppm ethrel.**

Those plants treated with 30,000 ppm ethrel had procambial differentiation at three days that was more advanced than the 3,000 ppm treatment after three days and the controls after 15 days.

After six days, rapid differentiation of the procambium occurred, and although no maturation was recognizable, the anatomical changes and position of tissue in the strands were characteristic of xylem. Three days later primary pit fields were present in well-differentiated but as yet immature metaphloem. Phloem differentiation was well behind that of the 3,000 ppm at nine days. The same trend was found after
12 days. After 15 days mature xylem tracheary tissue appeared in abundance, complete with reticulated and pitted secondary walls, and some phloem sieve tube elements were present with sieve areas barely visible.

The xylem in bud procambia of the 30,000 ppm ethrel treated plant matured earlier than the phloem. This suggested that ethrel acted as a stimulant to xylem differentiation and maturation at 30,000 ppm while repressing phloem differentiation.

**Anastomosis of bud and stem vascularization.**

A significant observation was made in bud vascularization patterns in 3,000 and 30,000 ppm treated plants. Joining of mature vascular tissue of the lateral bud with mature bundles of the stem was accomplished by acropetal and basipetal maturation of xylem and acropetal maturation of the phloem of bud procambia. No such anastomosing was ever observed in control plants. All connections of bud vascularization terminated in the metaphloem of cauline bundles. Thus better translocation pathways would be available to the buds. This may explain the decreased rate of plant growth and the stimulation of bud growth with higher concentrations of ethrel (Fig. 4). Translocation flow would not only be longitudinal now but also horizontal into the buds.

The significance of these observations should not be underestimated. Completion of mature vascular tissue from cauline bundles into the bud was accomplished and a translocating herbicide could be readily available to the lateral bud.
SUMMARY

1. The phytonic unit was recognized in johnsongrass.
2. Ethrel treatment of johnsongrass released buds from their resting condition and resulted in decelerated growth of the plant.
3. Untreated johnsongrass stems had almost no differentiation of bud procambial tissues after 15 days.
4. Johnsongrass treated with 3,000 ppm ethrel had rapid lateral bud phloem differentiation and maturation and slow xylem differentiation.
5. Johnsongrass treated with 30,000 ppm ethrel had rapid lateral bud xylem differentiation and maturation and slower phloem differentiation.
6. Plants treated with ethrel, both 3,000 ppm and 30,000 ppm ethrel, contained several lateral bud bundles anastomosing with cauline bundles while no such anastomosing was found in the untreated stems.
LITERATURE CITED


Parker, C., "The Importance of Shoot Entry in the Action of Herbicides Applied to the Soil", Journal Paper No. 2528, Purdue University Agricultural Experiment Station, 1965.


