Chapter 2

PHYSIOLOGY OF COTTON DEFOLIATION AND DESICCATION

J. Tom Cothren
Department of Soil and Crop Sciences
Texas A&M University System
College Station, Texas

C. Owen Gwathmey
Department of Plant and Soil Sciences
University of Tennessee
Jackson, Tennessee

Ron B. Ames
Crop Protection Research and Development
Uniroyal Chemical
Middlebury, Connecticut

INTRODUCTION

An overall understanding of cotton growth and development is necessary to fully appreciate the mechanism of the physiology of defoliation. Cotton is grown as an annual crop, but inherently is a deciduous perennial. The plant has a natural mechanism for shedding its mature leaves, although shedding is not necessarily synchronized with the most appropriate time for harvesting the lint. While leaves function to supply photosynthates to developing fruit during the growing season, their presence at harvest can lead to reductions in harvest efficiency and lint quality (Williford, 1992).

In addition, once the cotton bolls have opened, there is a potential for weight and fiber quality losses caused by weathering (Parvin, 1990). Therefore, leaf senescence and abscission in cotton usually are controlled with the use of harvest-aid chemicals. These products are applied to the crop prior to harvest to facilitate harvest of seed cotton. Harvest-aid programs, which include defoliants and desiccants, are used to insure optimal harvest timing and efficiency and are determined by several factors, including crop maturity and the production year. Defoliation refers to the accelerated leaf abscission brought about by chemicals, frost, or other factors, while desiccation refers to the accelerated drying of a leaf or plant part.

SENESCENCE

Plants develop from the time of germination until their death. The latter part of the developmental process, which leads from maturity to the ultimate loss of organization and function, is the process we call senescence. Senescence may be defined simply as those changes that eventually lead to the death of an organism or some part of it (Sexton and Woolhouse, 1984).

Some tend to equate the terms *aging* and *senescence*. Medawar (1957), however, offered the following distinction between the two processes: Aging is defined as those changes that occur in time, without reference to death as a consequence, and is not confined to living organisms. Senescence, on the other hand, is a highly ordered and genetically programmed process or series of processes within a living organism, leading to death. Senescence frequently is associated with leaf abscission.

A wide variety of factors and metabolic changes can trigger senescence. Determining which of the changes are central (primary) and which are peripheral (secondary) to senescence is difficult. Noodén *et al.* (1997) depicts senescence in three phases, with each phase being linked to initiators of senescence (e.g., temperature extremes, air pollution, and pathogen attack).

The initiation phase of senescence results in a potential shutdown of cell maintenance functions and is paralleled with an increase in key degradative enzymes. Several senescence-associated genes (SAG) that are involved in this phase have been identified (Hensel *et al.*, 1993; Lohman *et al.*, 1994). However, whether the SAG are causally linked to senescence initiation or to macromolecular turnover is not yet known.

The sequence similarity of SAG to cysteine proteases, which have been shown as a requirement for Programmed Cell Death (PCD) in the nematode, *Caenorhabditis elegans*, suggests that proteases may be good candidates for cell-death-initiation genes (Greenberg, 1996). PCD is the process by which individual cells activate an intrinsic senescence program. Ellis *et al.* (1991) indicated that PCD is a physiological cell death process involved in selective elimination of unwanted cells. The senescence process involves cell death on a large scale (Bleecker and Patterson, 1997; Pennell and Lamb, 1997).

Degeneration – The second phase, degeneration, is reflected as a disassembly of key metabolic processes that leads to the third and final stage of senescence, where a loss of homeostasis and cell membrane integrity eventually leads to cell death.

The maturity or senescence stage of development is not always related to the chronological age of the plant, but may reflect the conditions under which the crop is grown. Similar to many other genetically programmed developmental processes, the initiation of senescence is subject to regulation by environmental (external), as well as autonomous (internal), cues (Figure 1).

Plants senesce according to their growth habit. Some may senesce and die all at one time. Others may exhibit a progressive senescence, with some parts remaining active and in the juvenile stage, while older parts senesce and die. Juvenility refers to the early phase of growth during which flowering cannot be induced by any treatment (Thomas and Vince-Prue, 1984). Senescence in monocarpic plants, those plants that flower only once and then die, shows a close relationship to the processes of flowering and fruit growth. For example, senescence may be postponed if flowers or fruits are removed (Noodén and Guiamét, 1989).

Although it is a commonly held view that senescence represents a descent into chaos in terms of cellular and metabolic organization, it is in fact tightly controlled, with a highly ordered sequence of events (Sexton and Woolhouse, 1984). For example, in leaf senescence, some components of the chloroplast, such as the thylakoid membrane and chlorophyll, begin to degrade before other cellular components, such as the chloroplast envelope, mitochondria, and plasma membrane (Woolhouse and Jenkins, 1983).

Two senescence theories commonly are discussed: the nutrient diversion theory and the hormonal theory. The nutrient diversion theory refers to the competition among different parts of the plant for nutrition. Fruit or growing

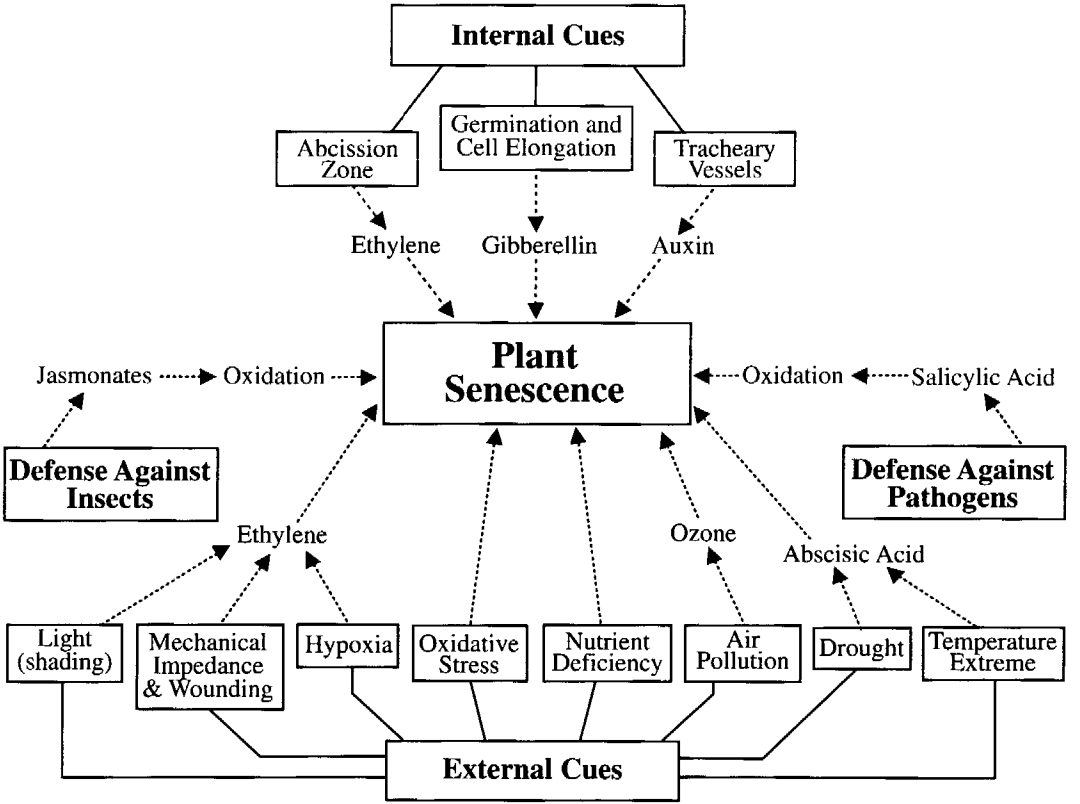


Figure 1. Mechanisms of senescence.

tips, for example, might constitute stronger sinks for translocation and thus accumulate nutrients to the point of starving older leaves. Sinclair and de Wit (1976) referred to a "self-destruct" theory in soybeans. Their theory states that the plant may relocate nutrients to meet immediate carbon demands at the cost of jeopardizing its continued survival. For example, when nutritional requirements for nitrogen exceed the ability of the plant to meet this demand, the plant remobilizes nitrogen contained in Fraction I protein (rubisco, the major CO_2-fixing enzyme) to provide the nutrient. As a consequence, the plant subsequently loses its ability to fix carbon dioxide and self-destructs through deterioration of chloroplast integrity.

A related senescence theory proposed by Kelly and Davies (1988) asserts that diversion of assimilates to developing fruit no longer is accepted by most researchers as the strongest regulator of senescence. Instead, they propose that development of the reproductive phase causes reproductive structures to become stronger sinks than their vegetative organs. The loss of sink strength in the root leads to reduced mineral nutrient and cytokinin transport from root to shoot, both of which are partly responsible for the initiation of leaf senescence. The loss of cytokinin transport to the shoot is important, as numerous experiments have shown cytokinins to delay senescence (Gan and Amasino, 1995; 1996).

The second theory is that senescence is hormonally controlled. Because of the decline of cytokinin levels in senescing leaves and the ability to delay senescence by external application of cytokinin (Gan and Amasino, 1995), the cytokinin class of hormones often is assigned a role in controlling leaf senescence. This has been further supported by the finding that expression of isopentenyl transferase (IPT), the enzyme that catalyzes the rate-limiting step in cytokinin biosynthesis, is suppressed with a senescence-specific promoter (Gan and Amasino, 1995). The involvement of cytokinins and other hormones in leaf senescence and abscission will be discussed later.

LEAF ABSCISSION

The term abscission is derived from the Latin *abscindere* – "to tear" – and therefore is an appropriate term for the process. Leaf abscission is a physiological process that involves an active separation of living tissue from the plant (Cathey, 1986) and usually occurs as a result of maturity,

senescence, or injury. Separation of the leaf from the plant occurs at the base of the leaf petiole in an area called the abscission zone (Figure 2) (Addicott, 1982; Sexton et al., 1985). This area is structurally distinguishable and is characterized by a structural line of weakness where abscission occurs. The abscission zone consists of one or more layers of thin-walled parenchyma cells resulting from anticlinal divisions across the petiole, except in the vascular bundle. The "abscission zone," or general region through which the fracture occurs, contains the same cell classes as adjacent tissues (Sexton and Woolhouse, 1984). However, wall breakdown usually is confined to a "separation layer" one to three cells wide in a zone that is five to 50 cells wide. Cells of the abscission zone generally are smaller than their counterparts in adjacent tissues. Toward the end of the senescence process, metabolic activity increases in the abscission layers as a result of alterations in the hormone levels of the leaf blade (Wilkins, 1984).

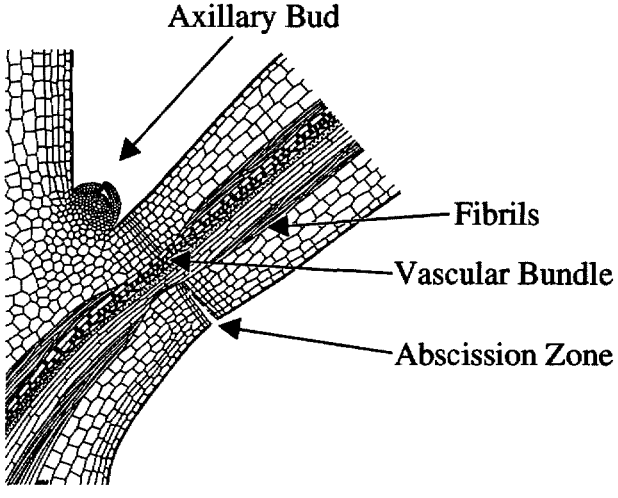


Figure 2. Abscission layer found within a leaf petiole. (Salisbury and Ross, 1992)

Prior to abscission, a variety of changes associated with senescence occur in the leaf. As an oxidative process, senescence involves a general deterioration of cellular metabolism (Pastori and del Rio, 1997). One of the more observable changes of senescence is a loss of chlorophyll (Noodén *et al.*, 1997), which sometimes is accompanied by a temporary build-up of anthocyanin (Matile,

1992). In addition, complex substances, such as proteins and carbohydrates, that have accumulated in the leaf are broken down to their constituent amino acids and sugars and translocated to other parts of the plant before the leaf abscises. Along with these breakdown products, significant amounts of mineral elements, such as nitrogen, phosphorus, potassium, and magnesium, also are translocated.

Other physiological changes prior to abscission include an increase in lipid peroxidation and membrane permeability (Thompson *et al.*, 1997), enhanced metabolism of activated oxygen species that produce severe cellular damage (Pastori and del Rio, 1997), and the loss of sufficient auxin levels to suppress the action of ethylene and abscisic acid (Morgan, 1984). Although ethylene and abscisic acid are present in leaves throughout their growth and development, higher levels of auxin tend to counter their activity. As senescence progresses, the auxin level subsides or its transport is inhibited, allowing ethylene and abscisic acid to enhance the senescent changes in the leaf blade, as well as the abscission process at the base of the petiole.

Such physiological changes include the increased activation of cell wall-degrading enzymes, such as cellulase and polygalacturonase, at the abscission layer. These enzymes degrade the pectic substances of the middle lamella and cell wall, and allow the leaves to fall from the plant. The significance of auxin in the abscission process is shown in the work of Abeles *et al.* (1992), who found that removal of the leaf blade promoted petiole abscission and that the process could be delayed by exogenous application of auxin to the petioles from which the leaf blades had been removed.

The primary regulator of the abscission process appears to be ethylene, while auxin acts as a suppressor of the ethylene effect. Morgan and Hall (1962), Hall and Morgan (1964), and Morgan *et al.* (1968) showed that ethylene stimulates IAA-oxidase activity and decarboxylation of IAA in cotton. Ethylene has been found to slow auxin transport (Morgan and Gausman, 1966; Morgan *et al.*, 1968; Beyer and Morgan, 1969; 1970; 1971) and, by inhibiting auxin transport, promotes abscission (Morgan and Durham, 1975). Because auxin prevents or delays abscission, both the destruction and the slowed transport of auxin to the abscission zone should promote abscission (Guinn, 1986).

Morgan (1984) describes the process of leaf abscission in three distinct sequential phases in his hormonal control model (Figure 3). The three phases include a leaf maintenance phase, a shedding induction phase, and a shedding phase. In the maintenance phase (I.), the leaf is healthy and fully functional.

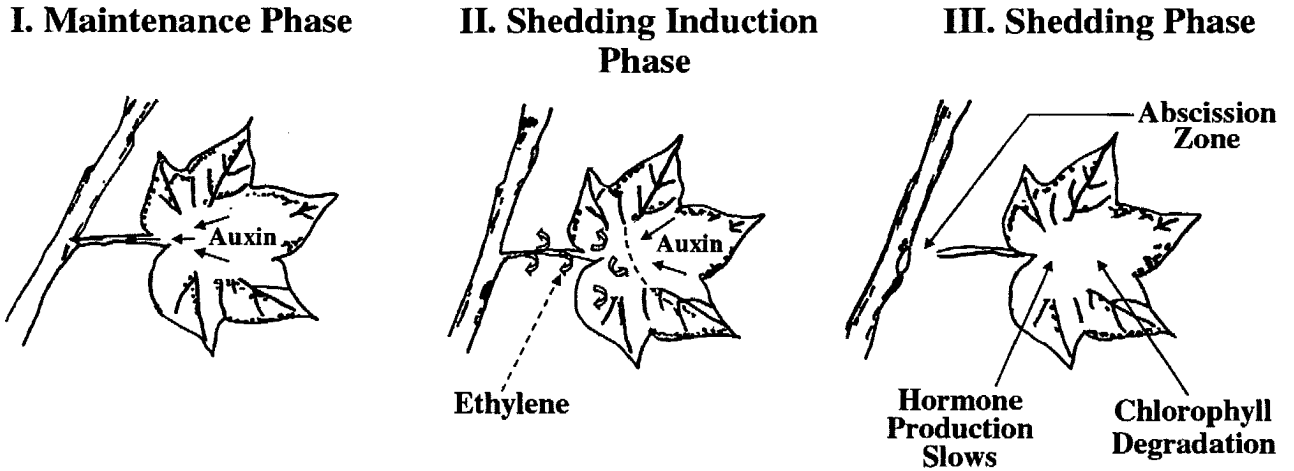


Figure 3. Three distinct sequential phases of the hormonal control of leaf abscission. (Morgan, 1984)

During this stage, high levels of auxin synthesis in the leaf prevent abscission by repressing the synthesis of the hydrolytic enzymes involved in abscission. During the shedding induction phase (II.), the auxin level decreases and the ethylene level increases. Ethylene may affect auxin activity by reducing its synthesis and transport, as well as by promoting its destruction (Sexton *et al.*, 1985; Kende and Zeevaart, 1997). After the enzymes associated with formation of the abscission layer increase and the concentration of ethylene increases relative to auxin, hormone production slows, chlorophyll degrades, and leaves drop in the shedding phase (III.).

HORMONES AND SENESCENCE

In higher plants, regulation and coordination of metabolism, growth, and morphogenesis often are dependent on signals from one part of the plant to another. Chemical messengers called hormones mediate this intercellular communication. Although theories regarding senescence differ as to the controlling force behind the process, the communication role of hormones is undisputed.

Until recently, five classes of plant hormones were recognized: auxins, gibberellins, abscisic acid, ethylene, and cytokinins. However, additional hormones, including jasmonic acid, salicylic acid, and brassinosteroids, have been proposed. Their functions in plant growth and development are not completely understood, but the brassinosteroids appear to have a more direct role in accelerating senescence, while the roles of salicylic acid and jasmonic acid are less direct. Hormones interact with specific proteins, called receptors, on the cell surface, causing the initiation of a cascade of enzyme activation steps. This cascade, often referred to as a *signal transduction pathway*, results in the production of "second messengers" that directly stimulate the responses and amplify the hormone signal.

One of the more common elements in the different signal transduction pathways is the participation of GTP-binding proteins (G proteins), which act as intermediates between the hormone-receptor complex and the enzyme systems that produce second messengers. The following outlines the role of G proteins in signal transduction in plants, as shown in Figure 4:

1) Binding of a hormone to its receptor on the cell surface causes activation of a G protein (i.e., guanosine triphosphate [GTP] exchanged for gua-nosine diphosphate [GDP]).
2) A series of molecular events is initiated by the activated G protein, including activation of phospholipase A_2 (PLA_2).
3) PLA_2 cleaves phospholipid (PL) to release lycophosphatidylcholine (LPC) and lycophosphatidylethanolamine (LPE).
4) LPC, LPE, and calcium activate a protein kinase (PK). Activated protein kinase will activate a phosphorylase and thereby activate an ATPase (the enzyme that hydrolyzes adenosine triphosphate [ATP]).
5) Ultimately, the ATPase hydrolyzes ATP and drives hydrogen ions across the plasma membrane.

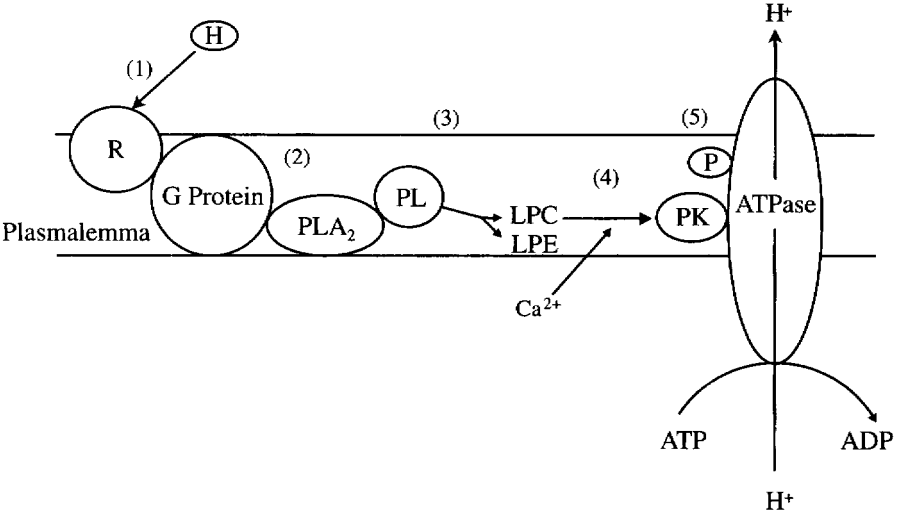


Figure 4. Proposed model for hormone-signal transduction in plant cells. (Adapted from Andre and Scherer, 1991) H - hormone; R - receptor; PLA2 - phospholipase A2; PL - phospholipase substrate; LPC - lycophosphatidylcholine; LPE - lycophosphatidylethanolamine; PK - protein kinase; P - high-energy phosphate; ATP - adenosine triphosphate; ADP - adenosine diphosphate; ATPase - the enzyme that hydrolyzes ATP.

In further support of the proposed model for signal transduction (Figure 4), evidence of G proteins, phospholipase A_2, and protein kinase have been identified in plant membranes (Blum *et al.*, 1988; Tate *et al.*, 1989; Millner and Causier, 1996). In addition, strong indications exist that inositol triphosphate (IP_3) causes the release of calcium from mitochondria and vacuoles in a manner similar to that in animals, indicating that it may serve as a second messenger in plant cells (Einspahr and Thompson, 1990). Perhaps the best-understood signal transduction pathway for plants is that of ethylene, as its hormone receptor has been identified, its gene sequenced (Hua *et al.*, 1997), and several steps in the pathway elucidated (Kieber, 1997).

Among the hormones and hormone-like compounds, most have some influence on regulation of senescence. Ethylene and abscisic acid generally accelerate senescence, while auxins and cytokinins delay it. In addition, some studies indicate that gibberellins may play a role in delaying senescence (Saks *et al.*, 1992; Jordi *et al.*, 1995; Kappers *et al.*, 1997; Zhu and Davies, 1997). The following is a brief discussion on the role of the hormones believed to play a significant role in senescence and abscission.

Ethylene – Studies have shown that ethylene increases the rate of chlorophyll, protein, and RNA degradation in leaf tissue. Rates of chlorophyll degradation decrease when inhibitors such as silver nitrate and aminoethoxyvinylglycine (AVG) block ethylene production. Although ethylene plays an important role as a signal to initiate senescence as plant tissue ages, the process can occur without it. Zacarias and Reid (1990) found that ethylene-insensitive mutants of *Arabidopsis thaliana* senesce at a slower rate than the wild type. In these situations it usually is best to think of ethylene as a senescence-promoter hormone, rather than as the cause of the senescence process.

Abscisic Acid – Although ethylene is recognized as the hormone that triggers abscission, ABA is involved in the process. For cotton, the ABA-induced abscission of fruits results from the ability of ABA to stimulate ethylene production. The effects of ABA on leaf senescence do not appear to be mediated by ethylene. While ethylene stimulates chlorophyll loss from wild-type *Arabidopsis*, it has no effect on ethylene-insensitive mutants. When both types were treated with ABA, chlorophyll loss was stimulated, indicating that promotion of senescence by ABA did not occur through its stimulation of ethylene production (Zacarias and Reid, 1990.)

Cytokinin – The ability of cytokinins to delay leaf senescence is widely known. When cytokinin is sprayed directly on a leaf, that leaf remains intact when other leaves of equal developmental age have yellowed and dropped off the plant. Cytokinins promote nutrient mobilization into areas that have been treated, which may occur because of the creation of a new source-sink relationship.

Auxin serves a dual role in abscission. The level of auxin progressively decreases from a high level in young leaves to a relatively low level in senescing leaves. At the onset of leaf abscission, addition of IAA inhibits leaf abscission, but, during the latter stages, IAA hastens the process, probably as a result of inducing ethylene synthesis. Younger leaves appear less sensitive to ethylene than older ones, which may be a reflection of the high level of auxin in the younger leaves.

Gibberellin – In addition to cytokinins and auxins, gibberellins also are considered promotive hormones that delay senescence (Whyte and Luckwill, 1966; Osborne, 1967).

HARVEST-AID CHEMICALS

Although cotton leaves senesce naturally and abscise, the use of chemical defoliation for more timely leaf removal is widely practiced. Harvest-aid chemicals can be used to control physiological processes such as growth, boll opening, and leaf drop. Through the control of these processes, more and better-quality lint is harvested, with less dry matter loss. The condition of the crop affects the response of the plant to harvest-aid chemicals. Generally, senescing cotton is more responsive to harvest-aid chemicals than less mature cotton, especially if the crop has a high sink strength through the presence of a heavy boll load.

A variety of commercially available harvest-aid chemicals exist (Table 1); however, two general categories are recognized: those with *herbicidal*, or contact, activity and those with *hormonal* or other growth regulant activity.

Herbicidal defoliants injure the plant, causing it to produce ethylene, which inhibits auxin and promotes abscission and, thus, leaf drop. Among the herbicidal defoliants are tribufos and endothall. Excessive rates of herbicidal defoliants cause rapid leaf death to occur before ethylene can be produced to cause formation of the abscission layer. As a result,

desiccation or leaf stick occurs instead of leaf drop. Dimethipin is known to alter water diffusion and, therefore, can be classified as a mild desiccant, although it does not interfere directly with senescence metabolism.

Hormonal harvest aids enhance ethylene production, which leads to increased activity of cell wall-degrading enzymes. The abscission zone forms more rapidly and promotes leaf drop. Thidiazuron and ethephon are examples of hormonal harvest aids, which are widely used for picker harvest and, in some instances, in combination with desiccants for stripper harvest.

Defoliants are less harsh treatments than desiccants, which are compounds that have the ability to disrupt membrane integrity. The loss of membrane integrity from application of these compounds leads to rapid loss of moisture and ultimately causes desiccation of the leaves.

Ethephon and other products can be used to accelerate boll opening and to enhance the activity of defoliants. Although harvest-aid chemicals promote leaf drop, they do so in a variety of ways. The following is a brief description of the mode of action of the harvest-aid chemicals listed in Table 1.

BOLL OPENERS/CONDITIONERS

Ethephon – The breakdown of ethephon to ethylene occurs primarily on the leaf surface (Beaudry and Kays, 1988). According to the abscission model, cell wall hydrolases are induced by ethylene into the separation layer of abscission zones to promote leaf shedding (Walhood and Addicott, 1968). The effectiveness of ethephon is increased by treating plants with formulations that are auxin transport inhibitors.

Ethephon + cyclanilide – Cyclanilide is an auxin transport inhibitor that, when combined with ethephon, enhances cellulase activity in abscission zones more than does ethephon alone (Pedersen *et al.*, 1997). Activity is enhanced at two different pH optima, suggesting that cyclanilide and ethephon may induce more than one type of cellular isozyme.

Ethephon + AMADS – Ethephon stimulates production of ethylene, and AMADS is an ethylene synergist.

DEFOLIANTS

Dimethipin causes an initial inhibition of protein synthesis that is responsible for the loss of stomatal control. Loss of stomatal control is associated with high rates of transpiration and loss of leaf turgor that leads to desiccation and, ultimately,

Table 1. Harvest-aid chemicals registered for use in cotton production as late as 2001.

Type	Common Name	Trade Name[1,2]	Manufacturer	Active Ingredient %	Active Ingredient lb per gal	Chemical Name
I. Boll Openers/ Conditioners	ethephon	Prep™ Super Boll® Ethephon 6 Boll'd	Aventis Group Griffin LLC Micro Flo Co. Agriliance LLC	55.4 55.4 55.4 55.4	6.0 6.0 6.0 6.0	(2-chloroethyl)phosphonic acid
	dimethipin	LintPlus™	Uniroyal Chemical	22.4	2.0	2,3-dihydro-5,6-dimethyl-1,4-dithiin-1,1,4,4-tetraoxide
II. Boll Openers/ Defoliants	ethephon + cyclanilide	Finish® Finish® 6	Aventis Group Aventis Group	32.1 + 4.3 51.4 + 6.4	4.0 + 0.5 6.0 + 0.75	(2-chloroethyl)phosphonic acid + 1-(2,4-dichloro-phenylaminocarbonyl)-cyclopropane carboxylic acid
	ethephon + AMADS	CottonQuik®	Griffin LLC	18.3 + 58.6	2.28	(2-chloroethyl)phosphonic acid + 1-aminomethanamide dihydrogen tetraoxosulfate
III. Defoliants	carfentrazone-ethyl	Aim™	FMC Corp.	40.0	40% w/w a.i. per lb[3]	ethyl *a*,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate
	dimethipin	Harvade®	Uniroyal Chemical	48.0	4.9	2,3-dihydro-5,6-dimethyl-1,4-dithiin-1,1,4,4-tetraoxide
	butifos, merphos, tribufos, tribufate (proposed)	Folex® Def®	Aventis Group Bayer AG, Germany	70.5 70.5	6.0 6.0	*S,S,S*,-tributyl phosphorotrithioate
	thidiazuron	Dropp® FreeFall™	Aventis Group Griffin LLC	50.0 50.0	50% w/w a.i. per lb[3]	N-phenyl-N'-1,2,3-thidizaol-5-ylurea
	thidiazuron + diuron	Ginstar®	Aventis Group	12.0 + 6.0	1.0 + 0.5	N-phenyl-N'-1,2,3-thidizaol-5-ylurea + 3-(3,4-dichlorophenyl)-1,1-dimethylurea
	sodium chlorate	Sodium chlorate	Several manufacturers	28.0 to 47.0	3.0 to 6.0	$NaClO_3$
	dimethipin + thidiazuron	Leafless™	Uniroyal Chemical	32.7 + 8.4	3.2 + 0.8	2,3-dihydro-5,6-dimethyl-1,4-dithiin-1,1,4,4-tetraoxide + N-phenyl-N'-1,2,3-thidizaol-5-ylurea
IV. Desiccants	paraquat	Cyclone® Max Gramoxone® Extra Gramoxone® Max Boa®	Syngenta Syngenta Syngenta Griffin LLC	43.8 37.0 43.8 37.0	3.0 2.5 3.0 2.5	1,1'-dimethyl-4,4'-bipyridinium dichloride
	sodium chlorate	Sodium chlorate	Several manufacturers	28.0 to 47.0	3.0 to 6.0	$NaClO_3$
V. Products with Other Applications	endothall	Accelerate®	Cerexagri	15.9	0.52	7-oxabicyclo[2,2,1]heptane-2,3-dicarboxylic acid (IUPAC) used as sodium, potassium, or amine salts
	glyphosate	Roundup Original™ Roundup Ultra® Roundup UltraMax™	Monsanto Co. Monsanto Co. Monsanto Co.	41.0 41.0 50.2	4.0 4.0 5.0	isopropylamine salt of N-(phosphonomethyl)glycine
	cacodylic acid	Quick Pick®	Platte Chemical Co.	31.0	3.1	hydroxydimethylarsine oxide or dimethylarsinic acid

[1] Partial list of trade names. Trade names listed are not intended as endorsement.
[2] Bolls Eye®, Cyclone®, Dropp® Ultra™, Ethrel®, Roundup®, Starfire®, and the original formulation of Prep™ have been discontinued or are no longer available under their original names.
[3] Dry formulation, measured in pounds.

abscission (Metzger and Keng, 1984). Labeling work with ^{14}C-leucine and ^{3}H-uridine suggests that dimethipin acts primarily on the processes associated with translation rather than transcription. Auxin synthesis and transport also are inhibited and ethylene synthesis is triggered, subsequently inducing cellulase production. With the induction of cellulase, digestion of cellulose occurs in the abscission zone of the petiole base. This activity weakens the abscission layer, and the leaf falls. The mode of action for dimethipin is summarized as an induced, slow disintegration of epidermal cell walls and a subsequent gradual water loss that triggers the release of ethylene and abscission. It is classified as a hormonal defoliant.

Butifos, merphos, tribufos, tribufate (proposed) – These are herbicidal defoliants that injure the palisade cells of leaves, causing release of ethylene and leaf abscission. These defoliants also cause an upsurge of stress-induced ethylene production through a mild leaf injury that stimulates the enzymes cellulase, pectinase, and polygalacturonase. These enzymes are involved in the hydrolysis of insoluble pectates and cellulose associated with the adherence of cells in the abscission zones. The juvenile plant hormones, auxin and gibberellin, which antagonize the abscission process, also appear to be impaired.

Thidiazuron enhances ethylene production; it also has been shown to disrupt the polar auxin transport system and is an excellent inhibitor of regrowth (Suttle, 1988). It is classified as a hormonal defoliant. Thidiazuron has been reported to have cytokine-like activity in sieva bean callus culture (Mok *et al.*, 1982).

Thidiazuron + diuron (DCMU) – Diuron is used to inhibit photosynthetic electron transport. The site of action for diuron is at the quinone acceptor complex in the electron transport chain between the two photosystems, PSI and PSII (Figure 5). Compounds such as diuron occupy the secondary quinone (Q_B) binding site of D1 and D2 proteins, two membrane proteins that make up the core of PSII reaction centers (Zer and Ohad, 1995). Because diuron is unable to accept electrons, the electron cannot leave Q_A, the first quinone acceptor. As such, diuron binding effectively blocks electron flow and inhibits photosynthesis. An ensuing chain reaction of lipid peroxidation results in leaky membranes, which cause cells to dry rapidly (Weed Science Society of America, 1994). The combination of both chemistries thus enhances the potential for defoliation.

Endothall is a post-emergence herbicide that can be mixed with certain defoliants or desiccants to enhance their performance. Its mode of action is not well understood.

Cacodylic acid is a nonselective herbicide; its mode of action is not well understood.

DESICCANTS

Paraquat (methyl viologen) acts by accepting electrons from the early acceptors of Photosystem I (between bound ferredoxin acceptors and NADP) (Figure 5). Paraquat then reacts with oxygen to form superoxide, O_2^-. Superoxide is a free radical that reacts nonspecifically with a wide range of molecules in the chloroplast, such as lipids, to reduce chloroplast activity (Scandalios, 1993). Production of the free radicals also causes disruption of membranes and a rapid moisture loss that leads to desiccation.

Sodium chlorate is a strong oxidizing agent in plants (WSSA, 1994). It is reduced to sodium chlorite by reaction with nitrate reductase. Sodium chlorite acts as a cotton desiccant and as a nonselective contact herbicide.

REGROWTH INHIBITORS

Glyphosate isopropylammonium – Evidence indicates that glyphosate blocks production of an enzymatic step in the shikimic acid pathway (Figure 6). It inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), the enzyme that condenses shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP)

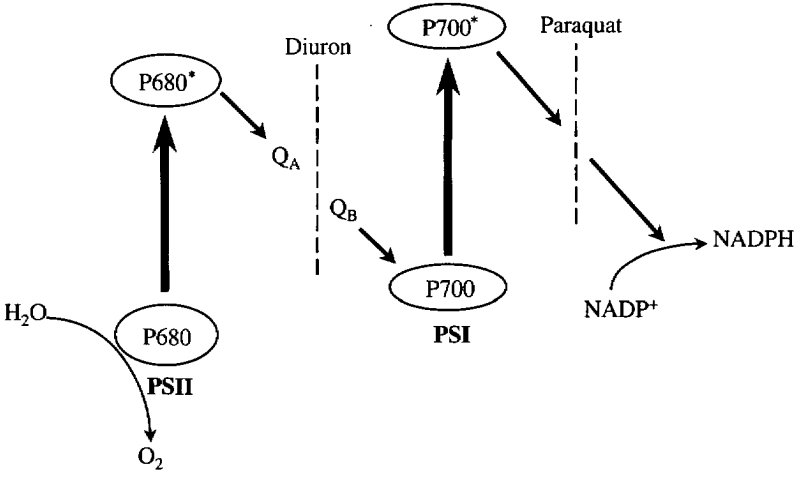


Figure 5. Z-scheme with location of diuron and paraquat action sites.

to yield EPSP and inorganic phosphate (Duke, 1988). As a result of this inhibition, production of three aromatic amino acids – phenylalanine, tyrosine, and tryptophan – is prevented, resulting in suppression of regrowth. Glyphosate has shown the ability to suppress regrowth in cotton when applied at various stages of boll opening (Landivar et al., 1994).

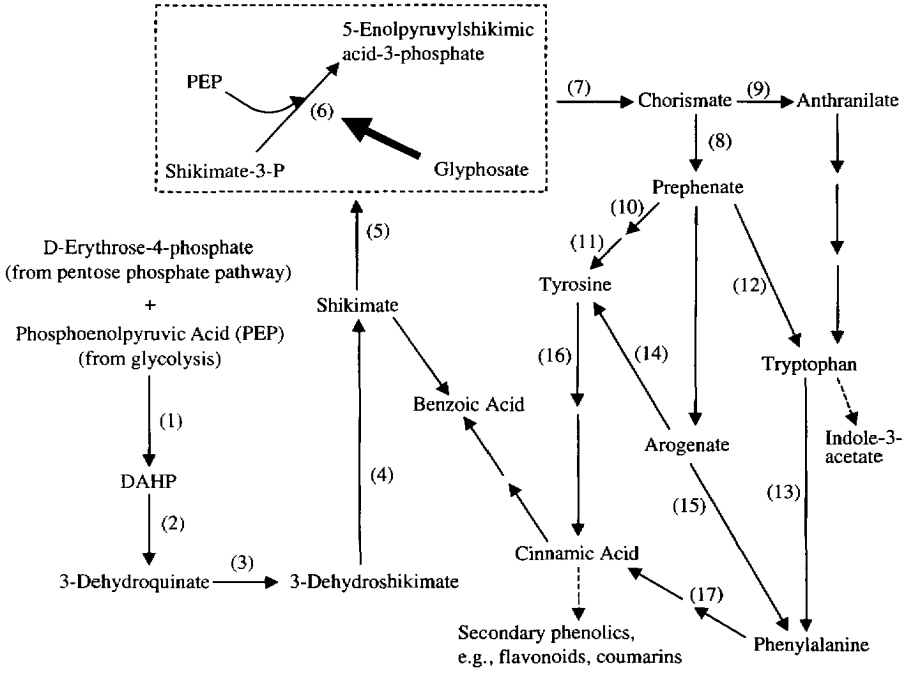


Figure 6. Glyphosate blocks production of an enzymatic step in the shikimic acid pathway. (1) 3-deoxy-*D*-arabino-heptulosonate-7-phosphate (DAHP) synthase; (2) 3-dehydroquinate synthase; (3) 3-dehydroquinate dehydratase; (4) shikimate dehydrogenase; (5) shikimate kinase, (6) 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS); (7) chorismate synthase; (8) chorismate mutase; (9) anthranilate synthase; (10) prephenate dehydrogenase; (11) tyrosine aminotransferase; (12) prephenate dehydratase; (13) phenylalanine aminotransferase; (14) arogenate dehydrogenase; (15) arogenate dehydratase; (16) tyrosine ammonia-lyase; (17) phenylalanine ammonia-lyase.

NEW AND EXPERIMENTAL COMPOUNDS

Carfentrazone-ethyl[1] induces inhibition of the enzyme protoporphyrinogen oxidase (Protox), which stops the formation of protoporphyrin IX, a precursor to chlorophyll biosynthesis. This results in the accumulation of reactive oxygen species inside the cell, which causes peroxidation of membrane lipids and leads to irreversible damage to cell membranes and functions. This mode of action is referred to as PPO inhibition. Carfentrazone-ethyl (Aim™) initially was registered as a corn, small grains, and soybean herbicide; the label was expanded in 2001 to include use as a defoliant in cotton.

Fluthiacet-methyl[2] has a similar mode of action (PPO inhibition), and the compound also has undergone evaluation as a potential cotton defoliant. Other PPO inhibitors, most of which are labeled for use as herbicides on other crops, also are being tested for efficacy as cotton harvest aids.

APPLICATION OF TANK MIXES

One of the more frustrating aspects of harvest aids is the lack of consistency achieved with individual compounds. After nearly 60 years of using defoliation compounds, many failures still are encountered each year; strategies that producers have employed successfully for many years can falter. Most failures are linked to either plant or environmental conditions that are not conducive to maximum plant response toward the chemicals being used.

The major factors that limit defoliation efficiency are condition of the plant and prevailing weather at time of application. Although an ample supply of moisture and nutrients is desired throughout the growing season to ensure uniform growth and development, the supply of each of these should be near depletion at defoliation time. Activity of harvest-aid chemicals is greatest when temperature, sunlight intensity, and relative humidity are high. An especially important factor is a night temperature above 16 C (61 F), as plant response to defoliants doubles for each 10-degree Celsius rise between 15 C and 35 C (59 F and 95 F) (Lane *et al.*, 1954).

---

[1] A product from FMC Corp. marketed as Aim™.

[2] A compound developed by Kumiai Chemical Industry Co. (KIH-9201) and tested as an experimental cotton desiccant/defoliant by Syngenta as CGA-248757, or Action™.

Unless the harvest-aid compound is formulated in a carrier that facilitates distribution across and penetration through a foliage surface, its potential biological activity may be negligible. Therefore, efforts to reduce the frequency of failures in harvest-aid strategies center on tank mixes consisting of harvest-aid chemicals and adjuvants, such as surfactants and wetting agents.

Adjuvants are materials that facilitate action of a herbicide or that facilitate or modify characteristics of herbicide formulations or spray solutions (McWhorter, 1982).

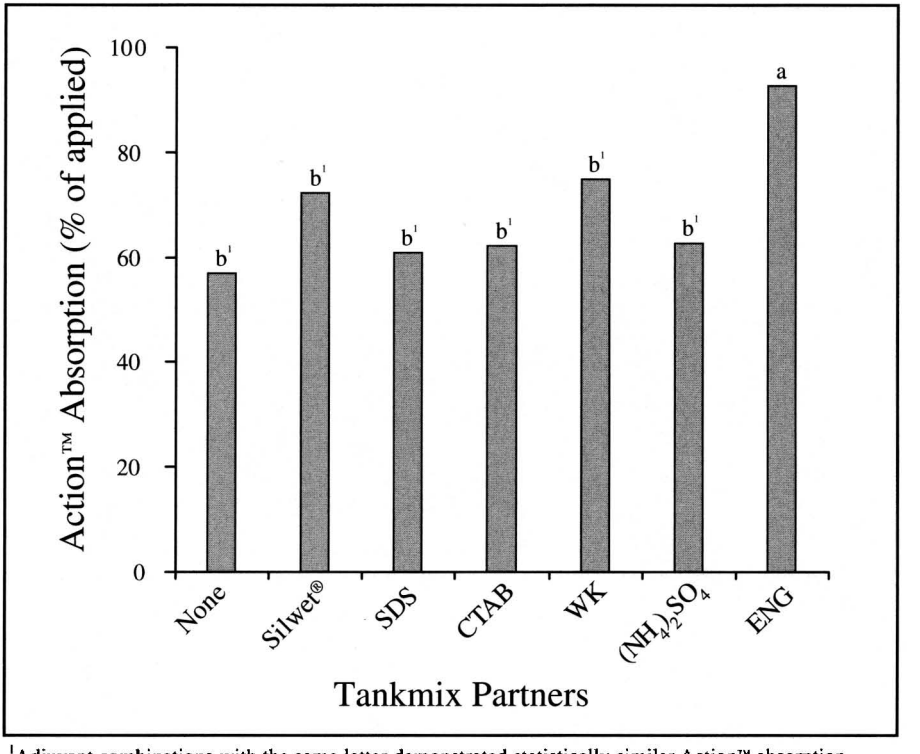
Surfactants – surface active agents – and wetting agents are two types of adjuvants. The Weed Science Society of America (1994) specifically defines surfactants as materials "that improve the emulsifying, dispersing, spreading, wetting, or other properties of a liquid by modifying its surface characteristics." They provide two definitions of wetting agents: 1) "a substance that serves to reduce the interfacial tensions and causes spray solutions or suspensions to make better contact with treated surfaces" and 2) "a substance in a wettable powder formulation that causes it to wet readily when added to water."

Adjuvants tend to concentrate on the surfaces of liquids in which they are dissolved. This translates to a situation in which the concentration of the surface-active agent is greater on the surface than in the bulk phase. Ordinarily, such molecules comprise two segments: lipophilic and hydrophilic. The lipophilic portion resembles a hydrocarbon and is relatively non-polar and water-insoluble. Adjuvant use improves the interface between the leaf surface and active ingredient, resulting in a greater degree of active ingredient available for biological activity. The hydrophilic portion is polar and more readily soluble in water. Surface active agents generally are classified by the polar portion of the molecule and, as such, usually are categorized as being anionic, cationic, non-ionic, or ampholytic.

The particular adjuvant selected for use in the tank mix also can influence rate of absorption. A comparison of a diverse group of adjuvants in a tank mix with Action™[1] showed enhanced absorption of this experimental desiccant/defoliant. Of the adjuvants tested, Eth-N-Gard® (ENG, an oil-based, non-polar adjuvant) showed the greatest absorption rates (Figure 7) (Stair *et al.*, 1998). In some cases, a combination of adjuvants is found to increase

[1] Action is Syngenta's name for CGA-248757, an experimental desiccant/defoliant product developed by Kumiai Chemical Industry Co. as KIH-9201.

absorption rates. For example, thidiazuron absorption was increased by approximately 30 percent with the addition of crop oil concentrate, approximately 10 percent with the addition of ammonium sulfate, and approximately 60 percent with the addition of both (Figure 8) (Snipes and Wills, 1994). As expected, defoliant absorption correlated positively to percentage of leaf drop. In addition, adjuvants are beneficial in cases where the leaf cuticle is relatively thick. See Chapter 3 for the effect of cuticular waxes on harvest-aid materials.

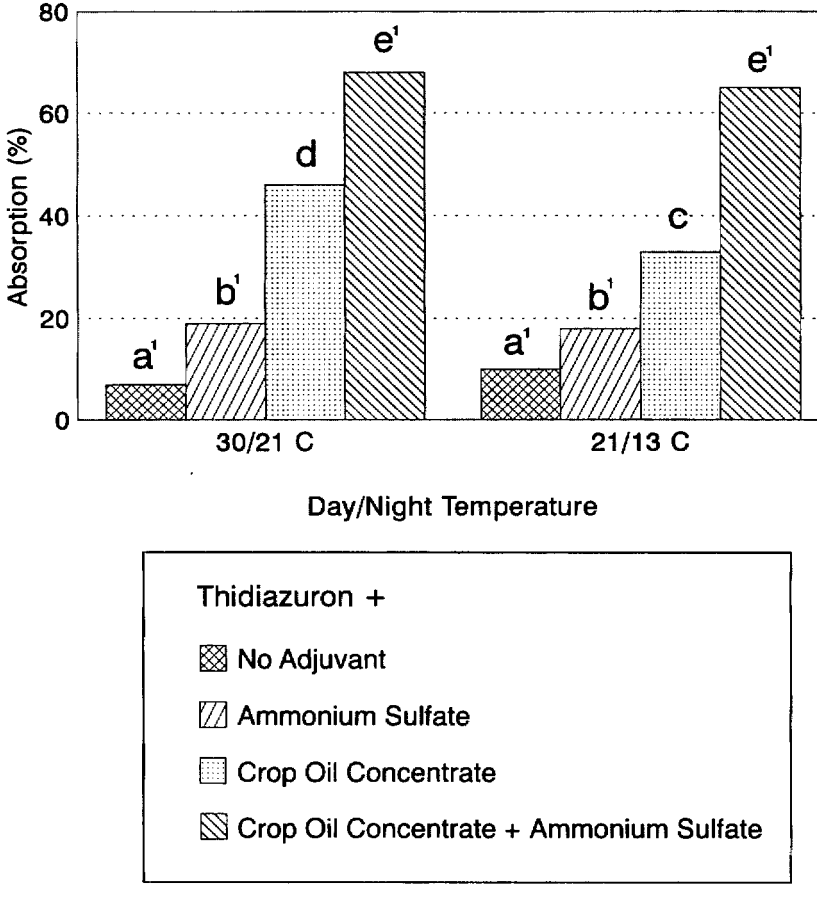


[1]Adjuvant combinations with the same letter demonstrated statistically similar Action™ absorption.

Figure 7. Effect of adjuvants on Action™ activity in tank mixes. (Stair *et al.*, 1998) Silwet® (Silicone), SDS (sodium dodecyl sulfate - anionic), CTAB (cetyl trimethyl ammomium bromate - cationic), WK (non-ionic), $(NH_4)_2SO_4$ (ion coupler), and ENG (Eth-N-Gard®, oil-based, non-polar).

SUMMARY

Growth and development of the cotton plant is genetically programmed and subject to regulation by many environmental (external) and autonomous (internal) factors. Programmed Cell Death is a term that refers to a process by which cells promote their own death through the activation of self-destruction



[1]Adjuvant combinations with the same letter demonstrated statistically similar thidiazuron absorption.

Figure 8. Effect of adjuvants on thidiazuron absorption in combinations. (Snipes and Wills, 1994)

systems. The plant developmental processes achieved through operation of PCD include senescence and the activation of the abscission zone.

Leaf senescence is the final stage of leaf development. High levels of auxin in juvenile or younger leaves prevent translocation of ethylene to the abscission zone and subdue formation of the abscission layer. During senescence, levels of leaf auxin decrease relative to the concentration of ethylene and abscisic acid. The increased levels of ethylene increase activity of the hydrolytic enzymes, cellulase and polygalacturonase, which are involved with cell wall degradation. These enzymes degrade the pectic substances of the middle lamella and cell wall and allow the leaves to fall from the plant.

Application of harvest-aid chemicals is accepted widely as a cultural practice to induce leaf abscission of cotton foliage. These compounds allow timely and efficient harvest of the lint to reduce harvest losses from weathering and to reduce leaf stain. Categories of harvest-aid chemicals include herbicidal and hormonal defoliants (including growth regulants), boll openers, and desiccants. Herbicidal defoliants injure leaf tissue, causing production of ethylene, which induces activation of enzymes associated with the formation of the abscission layer and subsequent leaf drop. This process also is induced by enhancing endogenous ethylene concentrations with hormonal defoliants. The boll openers are used to enhance ethylene production, which leads to quicker separation of the carpel walls. Desiccants are contact chemicals that cause disruption of membrane integrity, leading to rapid loss of moisture, which produces the desiccated leaf.

The previous growing conditions of the crop and prevailing weather conditions at time of application have great impact on performance of these chemicals. In addition, adjuvants commonly are used with harvest-aid compounds to enhance their uptake and activity. Consult Extension agents and specialists, farm consultants, and company representatives in your production area for available performance ratings in use of these compounds.

LITERATURE CITED

Abeles, F. B., P. W. Morgan, & M. E. Saltveit, Jr. (1992). *Ethylene in plant biology* (2nd ed.). New York: Academic Press.

Addicott, F. T. (1982). *Abscission.* Berkeley: University of California Press.

Andre, B., & G. F. E. Scherer. (1991). Stimulation by auxin of phospholipase A in membrane vesicles from an auxin-sensitive tissue is mediated by an auxin receptor. *Planta, 185,* 209-214.

Beaudry, R. M., & S. J. Kays. (1988). Flux of ethylene from leaves treated with a polar or non-polar ethylene-releasing compound. *Journal of American Society of Horticultural Science, 113,* 784-789.

Beyer, E. M., Jr., & P. W. Morgan. (1969). Ethylene modification of an auxin pulse in cotton stem sections. *Plant Physiology, 44,* 1690-1694.

Beyer, E. M., Jr., & P. W. Morgan. (1970). Effect of ethylene on the uptake, distribution, and metabolism of indoleacetic acid-1-^{14}C and 2-^{14}C and napthaleneacetic acid-1-^{14}C. *Plant Physiology, 46,* 157-162.

Beyer, E. M., Jr., & P. W. Morgan. (1971). Abscission: The role of ethylene modification of auxin transport. *Plant Physiology, 48,* 208-212.

Bleecker, A. B., & S. E. Patterson. (1997). Last exit: Senescence, abscission, and meristem arrest in *Arabidopsis*. *Plant Cell, 9,* 1169-1179.

Blum, W., K. D. Hinsch, G. Schulz, & E. W. Weiler. (1988). Identification of GTP-binding proteins in the plasma membrane of higher plants. *Biochemical and Biophysical Research Communications, 156,* 954-959.

Cathey, G. W. (1986). Physiology of defoliation in cotton production. In J. R. Mauney, & J. McD. Stewart (Eds.), *Cotton physiology, The Cotton Foundation Reference Book Series* (No. 1, pp. 143-154). Memphis, TN: The Cotton Foundation.

Duke, S. O. (1988). Glyphosate. In P. C. Kearney, & D. D. Kaufman (Eds.), *Herbicides-chemistry, degradation, and mode of action* (pp. 1-70). New York: Dekker Press.

Einspahr, K. J., & G. A. Thompson. (1990). Transmembrane signaling via phosphatidylinositol 4,5-bisphosphate hydrolysis in plants. *Plant Physiology, 93,* 361-366.

Ellis, R. E., J. Yuan, & H. R. Horvitz. (1991). Mechanisms and functions of cell death. *Annual Review of Cell and Developmental Biology, 7,* 663-698.

Gan, S., & R. M. Amasino. (1995). Inhibition of leaf senescence by autoregulated production of cytokinin. *Science, 270,* 1986-1988.

Gan, S., & R. M. Amasino. (1996). Cytokinins in plant senescence: From spray and pray to clone and play. *BioEssays, 18,* 557-565.

Greenberg, J. T. (1996). Programmed cell death: A way of life for plants. *Proceedings of the National Academy of Sciences of the United States of America, 93,* 12094-12097.

Guinn, G. (1986). Hormonal relations during reproduction. In J. R. Mauney, & J. McD. Stewart (Eds.), *Cotton physiology, The Cotton Foundation Reference Book Series* (No. 1, pp. 143-154). Memphis, TN: The Cotton Foundation.

Hall, W. C., & P. W. Morgan. (1964). Auxin-ethylene interrelationships. In J. P. Nitsch (Ed.), *Regulations naturéls de la croissance vegetable* (pp. 727-745). Paris: Centre National de la Recherché Scientifique.

Hensel, L. L., V. Grbic, D. A. Baumgarten, & A. B. Bleecker. (1993). Developmental and age-related processes that influence the longevity and senescence of photosynthetic tissues in *Arabidopsis*. *Plant Cell, 5,* 553-564.

Hua, J., H. Sakai, & E. M. Meyerowitz. (1997). The ethylene receptor gene family in *Arabidopsis*. In A. K. Kanellis, C. Chang, H. Kende, & D. Grierson (Eds.), *Biology and biotechnology of the plant hormone ethylene* (pp. 71-76). Boston: Kluwer Academic.

Jordi, W., G. M. Stoopen, K. Kelepouris, W. M. Krieken, & W. M. van Der. (1995). Gibberellin-induced delay of leaf senescence of *Alstroemeria* cut flowering stems is not caused by an increase in the endogenous cytokinin content. *Journal of Plant Growth Regulation, 14,* 121-127.

Kappers, I. F., W. Jordi, N. Tsesmetzis, F. M. Maas, & L. H. W. van der Plas. (1997). Gibberellins in leaves of *Alstroemeria* hydrids: Identification and quantification in relation to leaf age. *Journal of Plant Growth Regulation, 16,* 219-225.

Kelly, M. O., & P. J. Davies. (1988). The control of whole plant senescence. *CRC Reviews in Plant Sciences, 7,* 139-173.

Kende, H., & J. A. D. Zeevaart. (1997). The five 'classical' plant hormones. *Plant Cell, 9,* 1197-1210.

Kieber, J. J. (1997). The ethylene response pathway in *Arabidopsis. Annual Review of Plant Physiology and Plant Molecular Biology, 48,* 277-296.

Landivar, J. A., D. Locke, & D. Moseley. (1994). The effect of low rate of glyphosate on defoliation efficiency regrowth control, lint yield, and quality. *Proceedings of the Beltwide Cotton Conferences*, 1276-1278.

Lane, H. C., W. C. Hall, & S. P. Johnson. (1954). Effects of temperature on foliar abscission of cotton. *Proceedings of the Beltwide Cotton Defoliation Conference*, 8-48.

Lohman, K. N., S. Gan, M. C. John, & R. M. Amasino. (1994). Molecular analysis of natural leaf senescence in *Arabidopsis thaliana. Physiologia Plantarum, 92,* 322-328.

Matile, P. (1992). Chloroplast senescence. In N. Baker, & H. Thomas (Eds.), *Crop photosynthesis: Spatial and temporal determinants* (pp. 413-440). Amsterdam: Elsevier Press.

McWhorter, C. G. (1982). The use of adjuvants. In R. H. Hodgson (Ed.), *Adjuvants for herbicides* (pp. 10-25). Champaign, IL: Weed Science Society of America.

Medawar, P. B. (1957). *The uniqueness of the individual.* New York: Basic Books.

Metzger, J. D., & J. Keng. (1984). Effects of dimethipin, a defoliant and desiccant, on stomatal behavior and protein synthesis. *Journal of Plant Growth Regulation, 3,* 141-156.

Millner, P. A., & B. E. Causier. (1996). G-protein coupled receptors in plant cells. *Journal of Experimental Botany, 47,* 983-992.

Mok, M. C., D. W. S. Mok, D. J. Armstrong, K. Shudo, Y. Isogai, & T. Okamoto. (1982). Cytokinin activity of N-phenyl-N'-1,2,3-thiadiazol-5-5ylurea (thidiazuron). *Phytochem, 21,* 1509-1511.

Morgan, P. W. (1984). Is ethylene the natural regulator of abscission? In Y. Fuchs, & E. Chalutz (Eds.), *Ethylene: Biochemical, physiological, and applied aspects* (pp. 231-240). Boston: M. Nijhoff/Dr. W. Junk.

Morgan, P. W., E. Beyer, Jr., & H. W. Gausman. (1968). Ethylene effects on auxin physiology. In F. Wightman, & G. Setterfield (Eds.), *Biochemistry and physiology of plant growth substances* (pp. 1255-1273). Ottawa, Canada: The Runge Press.

Morgan, P. W., & J. J. Durham. (1975). Ethylene-induced leaf abscission is promoted by gibberellic acid. *Plant Physiology, 55,* 308-311.

Morgan, P. W., & H. W. Gausman. (1966). Effects of ethylene on auxin transport. *Plant Physiology, 41,* 45-52.

Morgan, P. W., & W. C. Hall. (1962). Effect of 2,4-dichlorophenoxyacetic acid on the production of ethylene by cotton and grain sorghum. *Physiologia Plantarum, 15,* 420-427.

Noodén, L. D., & J. J. Guiamét. (1989). Regulation of assimilation and senescence by the fruit in monocarpic plants. *Physiologia Plantarum, 77,* 267-274.

Noodén, L. D., J. J. Guiamét, & I. John. (1997). Senescence mechanisms. *Physiologia Plantarum, 101,* 746-753.

Osborne, D. J. (1967). Hormonal regulation of leaf senescence. *Symposia of the Society for Experimental Biology, 21,* 305-322.

Parvin, D. W., Jr. (1990). The importance of harvest initiation date. *Proceedings of the Beltwide Cotton Conferences*, 471-474.

Pastori, G. M., & L. A. del Rio. (1997). Natural senescence of pea leaves. *Plant Physiology, 113,* 411-418.

Pedersen, M. K., J. D. Burton, H. D. Coble, J. R. Collins, & D. Fritz. (1997). Efficacy of Finish and its mechanism of action. *Proceedings of the Beltwide Cotton Conferences*, 1363-1365.

Pennell, R. I., & C. Lamb. (1997). Programmed cell death in plants. *Plant Cell, 9,* 1157-1168.

Saks, Y., J. Van-Staden, & M. T. Smith. (1992). Effect of gibberellic acid on carnation flower senescence: Evidence that the delay of carnation flower senescence by gibberellic acid depends on the stage of flower development. *Plant Growth Regulation, 11*, 45-51.

Salisbury, F. B., & C. W. Ross. (1992). *Plant Physiology* (4th ed.). Belmont, CA: Wadsworth.

Scandalios, J. G. (1993). Oxygen stress and superoxide dismutases. *Plant Physiology, 101,* 7-12.

Sexton, R. L., L. N. Lewis, & P. Kelly. (1985). Ethylene and abscission. In J. A. Roberts, & G. A. Tucker (Eds.), *Ethylene and plant development* (pp.173-196). London: Butterworths.

Sexton, R., & H. W. Woolhouse. (1984). Senescence and abscission. In M. B. Wilkins (Ed.), *Advanced plant physiology* (pp. 469-497). London: Pitman.

Sinclair, T. R., & C. T. de Wit. (1976). Analysis of the carbon and nitrogen limitations to soybean yield. *Agronomy Journal, 68,* 319-324.

Snipes, C. E., & G. D. Wills. (1994). Influence of temperature and adjuvants on thidiazuron activity in cotton leaves. *Weed Science, 42,* 13-17.

Stair, D., L. Tarpley, & J. T. Cothren. (1998). The effect of adjuvant class on absorption of selected cotton harvest-aids, and resulting necrosis. *Proceedings of the Beltwide Cotton Conferences*, 1503.

Suttle, J. C. (1988). Disruption of the polar auxin transport system in cotton seedlings following treatment with the defoliant thidiazuron. *Plant Physiology, 86,* 241-245.

Tate, B. F., G. E. Schaller, M. R. Sussman, & R. C. Crain. (1989). Characterization of a polyphosphoinositide phospholipase C from the plasma membrane of *Avena sativa. Plant Physiology, 91,* 1275-1279.

Thomas, B., & D. Vince-Prue. (1984). Juvenility, photoperiodism, and vernalization. In M. B. Wilkins (Ed.), *Advanced plant physiology* (pp. 408-439). London: Pitman.

Thompson, J. E., C. D. Froese, Y. Hong, K. A. Hudat, & M. D. Smith. (1997). Membrane deterioration during senescence. *Canadian Journal of Botany, 75,* 867-877.

Walhood, V. T., & F. T. Addicott. (1968). Harvest-aid programs: Principles and practices. In F. C. Elliot, M. Hoover, & W. K. Porter, Jr. (Eds.), *Cotton: Principles and practices* (pp. 407-431). Ames: Iowa State University Press.

Weed Science Society of America (WSSA). (1994). *Herbicide handbook* (7th ed.). Champaign, IL: Weed Science Society of America.

Whyte, P., & L. C. Luckwill. (1966). Sensitive bioassays for gibberellins based upon retardation of leaf senescence in *Rumex obtusifolius. Nature, 210,* 1360.

Wilkins, M. B. (1984). *Advanced plant physiology*. London: Pitman.

Williford, J. R. (1992). Influence of harvest factors on cotton yield and quality. *Transactions of the American Society of Agricultural Engineers, 35,* 1103-1107.

Woolhouse, H. W., & G. I. Jenkins. (1983). Physiological responses, metabolic changes, and regulation during leaf senescence. In J. Dale, & F. L. Milthorpe (Eds.), *The growth and functioning of leaves* (pp. 449-487). Cambridge, England: Cambridge University Press.

Zacarias, L., & M. S. Reid. (1990). Role of growth regulators in the senescence of *Arabidopsis thaliana* leaves. *Physiologia Plantarum, 80,* 549-554.

Zer, H., & I. Ohad. (1995). Photoinactivation of photosystem II induces changes in the photochemical reaction center II abolishing the regulatory role of the QB site in the D1 protein degradation. *European Journal of Biochemistry, 231*, 448-453.

Zhu, Y. X., & P. J. Davies. (1997). The control of apical bud growth and senescence by auxin and gibberellin in genetic lines of peas. *Plant Physiology, 113,* 631-637.