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Mediation of Herbicide Effects by Hormone Interactions

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ABSTRACT

Chemical manipulation of the phytohormone system involves the use of herbicides for weed control in modern crop production. In the latter case, only compounds interacting with the auxin system have gained practical importance. Auxin herbicides mimic the overdose effects of indole-3-acetic acid (IAA), the principal natural auxin in higher plants. With their ability to control, particularly, dicotyledonous weeds in cereal crops, the synthetic auxins have been among the most successful herbicides used in agriculture. A newly discovered sequential hormone interaction plays a decisive role in their mode of action. The induction of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase in ethylene biosynthesis is the primary target process, following auxin herbicide signalling. Although the exact molecular target site has yet to be identified, it appears likely to be at the level of auxin receptor(s) for perception or signalling, leading ultimately to species- and organ-specific *de novo* enzyme synthesis. In sensitive dicots, ethylene causes epinastic growth and tissue swelling. Ethylene also triggers the biosynthesis of abscisic acid (ABA), mainly through the stimulated cleavage of xanthophylls to xanthoxal, catalyzed by 9-*cis*-epoxycarotenoid dioxygenase (NCED). ABA mediates stomatal closure which limits photosynthetic activity and biomass production, accompanied by an overproduction of reactive oxygen species. Growth inhibition, senescence and tissue decay are the consequences. Recent results suggest that ethylene-triggered ABA is not restricted to the action of auxin herbicides. It may function as a module in the signalling of a variety of stimuli leading to plant growth regulation. An additional phenomenon is caused by the auxin herbicide quinclorac which also controls grass weeds. Here, quinclorac induces the accumulation of phytotoxic levels of cyanide, a co-product of ethylene, which ultimately derives from herbicide-induced ACC synthase activity in the tissue. Phytotropins are a further group of hormone-related compounds which are used as herbicides. They inhibit polar auxin transport by interacting with a regulatory protein, the NPA-binding protein, of the auxin efflux carrier. This causes an abnormal accumulation of IAA and applied synthetic auxins in plant meristems. Growth inhibition, loss of tropic responses and, in combination with auxin herbicides, synergistic effects are the consequences.

Key words: Abscisic acid (ABA); 1-Aminocyclopropane-1-carboxylic acid (ACC) synthase; Auxin herbicides; Auxin transport; Cyanide; Ethylene; Indole-3-acetic acid (IAA); Phytotropins; Reactive oxygen species (ROS)

INTRODUCTION

Plant growth regulation is an interactive phenomenon in which phytohormones can act either

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synergistically or antagonistically to each other on the level of hormone signalling, concentration and physiological response. It is the balance between promoting and inhibiting agents in a network which ultimately governs the normal path of plant growth and development (Davies 1995). This conclusion can be drawn from a multitude of recent studies using transgenic plants, hormone mutants and synthetic compounds influencing hormone levels. Conversely, triggering an imbalance in hormone homeostasis and interactions through genetic, chemical or stress-related interferences usually leads to deregulation and phytotoxic inhibition of growth. This Janus-faced characteristic of physiological action finds particular expression in the biphasic dose-response curves that are typical of phytohormones. Consequently, chemical manipulation of the hormone system has gained practical importance, not only by the application of plant bioregulators for yield improvement (for review see Jung 1985; Grossmann 1990; Gianfagna 1995; Rademacher 2000), but also by the use of herbicides in weed control. In this context, synthetic compounds that interfere with the phytohormonal auxin system were among the first selective organic herbicides to be developed for agricultural and horticultural practice (for review see Cobb 1992; Devine and others 1993; Sterling and Hall 1997; Grossmann 2000a; 2003). These so-called growth regulator or auxin herbicides started a new era of weed control in modern crop production. They are translocated systemically in the plant and exert selective action, primarily against dicot weeds in cereal crops. Phytotropins are a further group of practically relevant, selective herbicides that affect the plant's auxin system (Hoffman and Smith 1949; Taiz and Zeiger 1998). Although to date, the exact molecular target sites of auxin herbicides and phytotropins have yet to be identified, considerable progress has recently been made in the elucidation of their herbicidal mode of action. Auxin herbicides basically act as synthetic mimics of indole-3-acetic acid (IAA) at high concentrations (Cobb 1992; Devine and others 1993; Sterling and Hall 1997; Grossmann 2000b, 2003). The herbicide syndrome is mediated through a newly discovered hormone interaction between sequentially induced ethylene and abscisic acid (ABA) biosynthesis. Phytotropins prevent polar auxin transport in sensitive plants and have the potential to synergize auxin herbicide action (Grossmann 2003). Although plant bioregulators have been recruited from nearly all of the classical five types of plant hormones (auxins, ethylene, gibberellins, ABA, and cytokinins) for commercial use, only those that affect the auxin system are practically relevant as herbicides. Thus, this review focuses particularly on these compounds and the elicited succeeding series of hormonal interactions which ultimately lead to plant death.

Auxin Herbicides

Auxin Overdose and the Phenomenon of Plant Growth Deregulation

Throughout the six decades since their initial discovery and application after World War II, auxin herbicides have been among the most successful herbicides used in agriculture (Sterling and Hall 1997). In 2000, auxin herbicides comprised approximately 9% ($US 1.3 billion) of the total sales, in fourth position after inhibitors of amino acid biosynthesis, photosynthesis and lipid biosynthesis (Phillips McDougall 2000). Over the years, a multitude of chemical classes of auxin herbicides with different weed spectra and types of selectivity have been synthesized and commercially introduced. They include phenoxycarboxylic acids such as 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 2,4-dichlorophenoxy acetic acid (2,4-D), which were the first auxin herbicides used in agriculture, benzoic acids, pyridine carboxylic acids, aromatic carboxymethyl derivatives and quinolinecarboxylic acids (Figure 1). During the past decade, studies on the mode of action have been particularly focused on the latest discovered family of auxin herbicides, the quinolinecarboxylic acids, exemplified by quinclorac and quinmerac (Grossmann 2000a). Both compounds have been used worldwide in agriculture since 1988. Unlike other auxin herbicides, quinmerac controls dicot weeds, such as cleavers (*Galium aparine*), in cereals and dicot crops, such as sugarbeet and oilseed rape, whereas quinclorac has herbicidal activity on dicot and grass weeds in rice and turf.

The general feature of auxin herbicides is that they mimic the action of the main auxin IAA (Cobb 1992; Devine and others 1993; Sterling and Hall 1997; Grossmann 2000a, 2003). As a structural requirement for this activity, a strong negative charge on the carboxyl group of the dissociated molecule, which is separated from a weaker positive charge on the planar aromatic ring with a distance of about 0.5 nm, has been proposed to be essential (Farrimond and others 1978). Depending on their concentration, auxins cause opposite effects in sensitive plant species (Morgan 1976; Taiz and Zeiger 1998). When present at low concentrations at the cellular sites of action, they stimulate growth and developmental

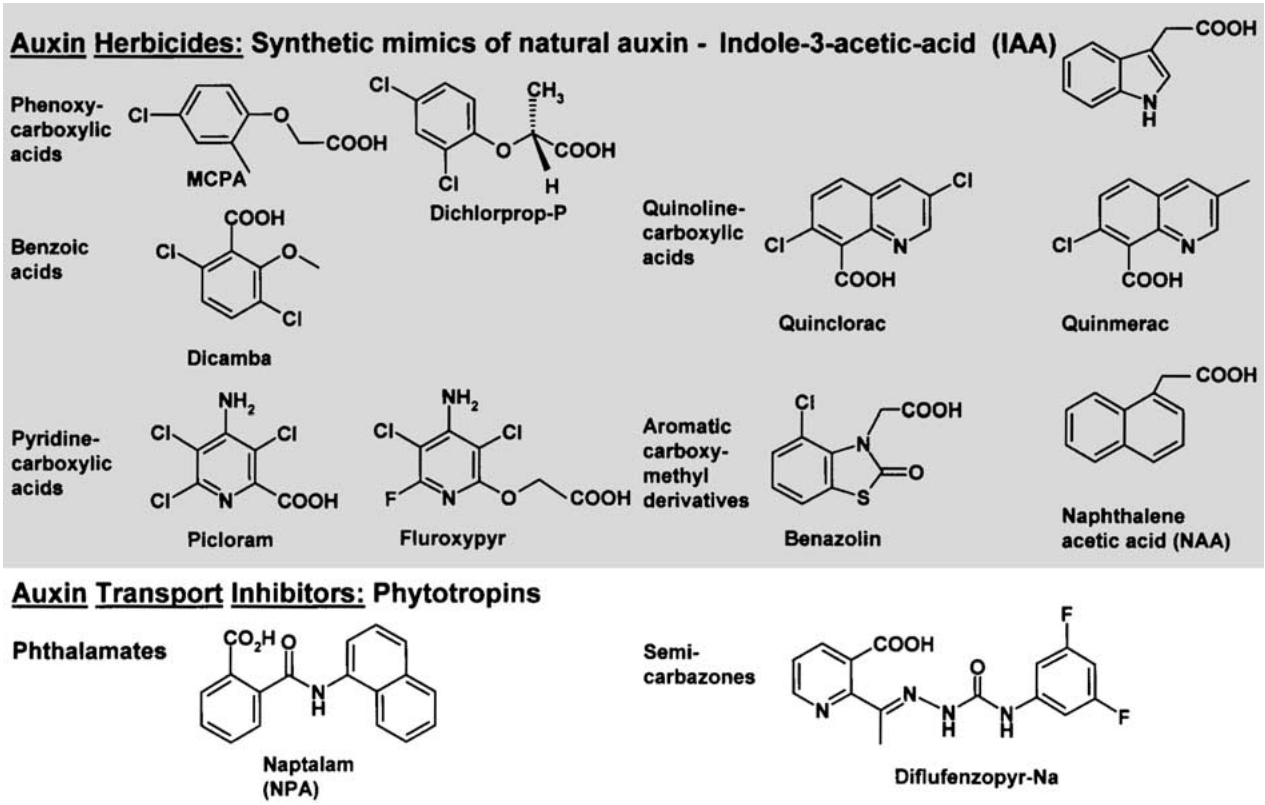

Figure 1. Structures of natural auxin, indole-3-acetic acid, and representatives of synthetic auxin herbicides and auxin transport inhibitors (phytotropins) belonging to the different chemical families.

processes, such as cell division and elongation, vascular differentiation, root formation, and apical dominance. With increasing concentration and auxin activity in the tissue, growth is disturbed and the plant is lethally damaged. Natural auxins like IAA are usually subjected to rapid inactivation through conjugation and degradation by multiple pathways in the plant (Ljung and others 2002). In contrast, applied synthetic auxins are long-lasting and more effective than IAA. They are stable and not inactivated by the plant as rapidly as the endogenous phytohormone. In addition to concentration effects, the spectrum of biological activities of auxins depends on tissue sensitivity, which is determined by the type of tissue, physiological stage and plant species and is probably mediated by differentially elicited signal transduction pathways (Cobb 1992; Sterling and Hall 1997; Grossmann 2000a; Zheng and Hall 2001).

When applied as herbicides, synthetic auxins cause the same morphogenetic symptoms on sensitive weeds as those elicited by IAA applied at high concentrations (Cobb 1992; Devine and others 1993) and those observed in transgenic, auxin-overproducing plants (Romano and others 1993). This phenomenon has been described as an auxin-overdose leading to an imbalance in auxin homeostasis and interactions with other hormones in the tissue (Cobb 1992). Since Gilbert's statement from 1946 that auxin herbicides cause susceptible plants ''to grow themselves to death,'' the hypothesis prevailing until recently mainly seized on the observed growth abnormalities. However, the ultimate cause of death remained open (Cobb 1992; Devine and others 1993; Sterling and Hall 1997). A continuous stimulation of plant metabolism, including nucleic acid and protein synthesis, was thought to elicit a deregulation of growth through distorted cell division and expansion, leading to the collapse of the correlating plant growth structure. Normal apical growth of shoot and root is inhibited. Abnormal growth is induced in other tissues at an inappropriate time and position, resulting in tissue swelling and epinastic deformations. Concomitantly, cell division and differentiation in meristematic and cambial cell regions are transiently initiated. These newly formed tissues were assumed to act as ''metabolic sinks'' leading to mobilization and attraction of carbohydrate and protein reserves at the expense of the functional tissues, which senesce to nourish it (Van Overbeek 1964; Cobb 1992). In contrast, a more specific mechanism of action

underlying growth inhibition and death is suggested by the high level of species selectivity of most auxin herbicides, combined with their rapid and in some cases stereoselective activity (for example, herbicidally active (+)-D-enantiomer as dichlorprop-P) (Figure 1) at low application rates.

Indeed, the time course of events induced by increasing concentrations of auxin herbicides or IAA in the tissue of sensitive dicot weeds can be divided into three phases (Cobb 1992; Devine and others 1993; Sterling and Hall 1997; Grossmann 2003). The first is a stimulation phase, which occurs within the first hours after application. This involves the activation of metabolic processes such as stimulation of ethylene biosynthesis through induction of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase in the shoot tissue (1–2 h), followed by symptoms of growth deformation (3–4 h), including initiation of stem curling, tissue swelling and leaf epinasty. Subsequently, ABA accumulates, initially detectable in the shoot tissue after 5–8 h. The second phase, which occurs within 24 h, includes growth inhibition of the root and, to a greater extent, of the shoot, with decreased internode elongation and leaf area, and intensified green leaf pigmentation. Concomitantly, reductions in stomatal aperture, transpiration, carbon assimilation and starch formation are observed. The third phase is the phase of senescence and tissue decay, which is marked by accelerated foliar senescence with chloroplast damage and progressive chlorosis and by the destruction of membrane and vascular system integrity, leading to desiccation, necrosis and finally to plant death.

Auxin Signalling and Gene Expression

From the molecular view, extensive affinity for auxin receptor(s) or subsequent signal transduction processes are assumed to be the primary mechanism of herbicide action as a consequence of an auxin overdose. Deduced from the multitude of developmental and morphological effects, it is suggested that one or several classes of auxin receptors and auxin signal transduction pathways exist. These receptors and pathways are thought to be heterogeneously distributed among different cell types and tissues and differentially affected by the different structural types of auxin herbicides (Sterling and Hall 1997; Grossmann 2000a; Zheng and Hall 2001). Auxin perception is thought to involve auxin binding to a plasma membrane-associated and/or soluble receptor at an internal site that targets the nucleus and regulates gene expression (Cobb 1992; Zheng and Hall 2001; Leyser 2001). However, no auxin-binding protein has yet been unambiguously identified and characterized as a specific receptor for IAA or synthetic auxin perception (Luschnig 1999). As a possible candidate for an auxin receptor, auxin binding protein 1 (ABP1) is currently discussed, which is assumed to function on the outer face of the plasma membrane in embryogenesis and auxin-induced cell expansion (for review see Jones and others 1998; Luschnig and Fink 1999; Timpte 2001). Characterization of the *ABP* gene family from auxin herbicide resistant and susceptible biotypes of wild mustard showed differences in the deduced amino acids (Zheng and Hall 2001).

According to a current model, after perception, auxin herbicide signalling triggers at least two major cascades of succeeding reactions (Sterling and Hall 1997; Grossmann 2000a). One of these centers on cell elongation and the cell wall as the site of action. Activation of membrane ion channels, particularly for calcium, and plasmalemma H^+-ATPases cause metabolic changes and impairment of cell wall stability and cell elongation (Devine and others 1993; Sterling and Hall 1997; Grossmann 2000a; Cosgrove 2000; Leyser 2001; Zheng and Hall 2001). The other concept focuses on the overexpression of early/primary auxin-response genes, which are thought to subsequently regulate late/secondary auxin-response genes, leading to aberrations in RNA and protein synthesis with consequent metabolic changes (Abel and Theologis 1996; Sterling and Hall 1997; Grossmann 2000a).

At present, five major classes of early auxin-response genes are known which are induced within a few minutes of auxin treatment, mostly independently of *de novo* protein synthesis (reviewed by Abel and Theologis 1996; Leyser 2001; Ward and Estelle 2001; Guilfoyle and Hagen 2001). Control of gene expression by auxin seems to be initiated by preexisting transcriptional activator proteins, the auxin response factors (ARFs). When cellular auxin concentrations are low, ARF proteins are blocked by forming heterodimers with repressor proteins, such as Aux/IAA (Guilfoyle and Hagen 2001). This prevents binding of ARF proteins to auxin-response elements in promoters of early auxin-response genes, thus repressing their transcription. When auxin concentrations are high, the repressor proteins are degraded, thus allowing the ARF proteins to dimerize and bind the auxin-response elements. This continuously activates transcription of early auxin-response genes as long as auxin concentrations remain high.

Because Aux/IAA repressors are derived from a family of early auxin-response genes (*Aux/IAA*), they regulate themselves through a negative feed-

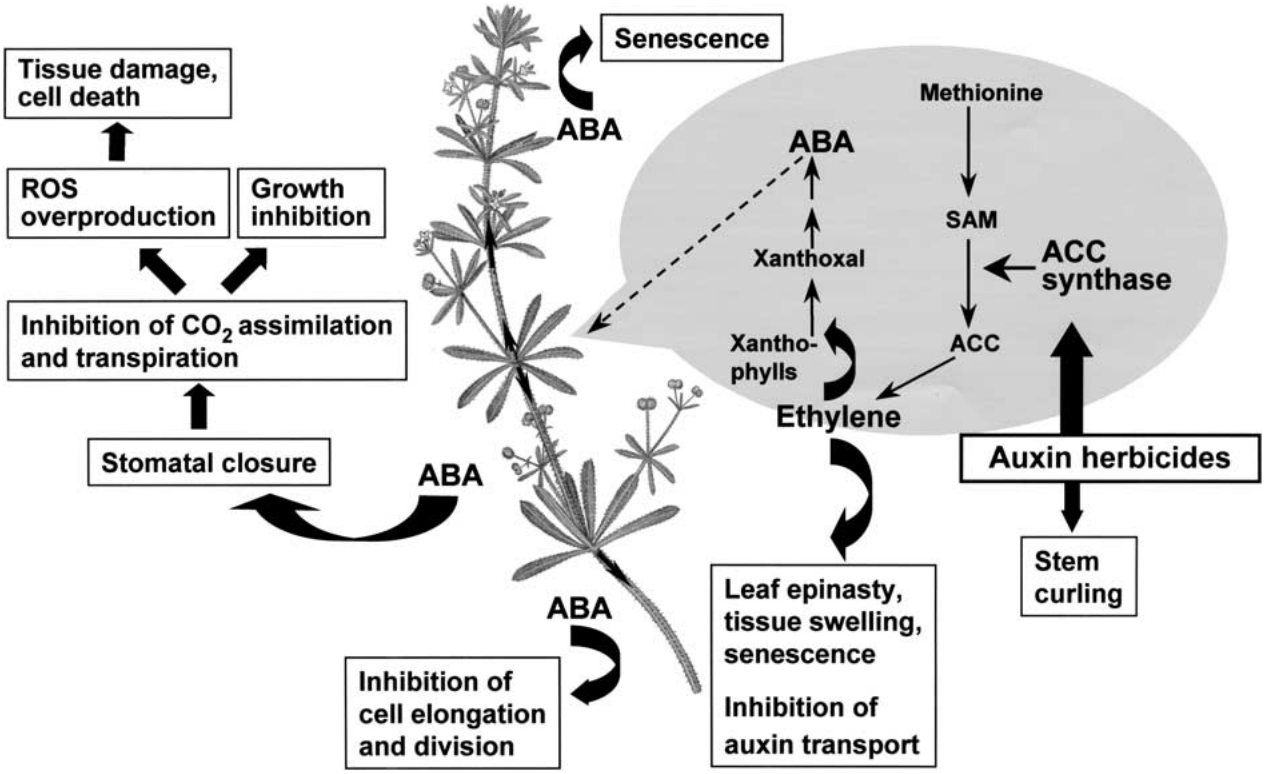

Figure 2. Proposed model of the mode of action of auxin herbicides in dicot plant species, as illustrated for cleavers (*Galium aparine*). Auxin herbicides lead to stem curling and induce ethylene biosynthesis through *de novo* enzyme synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase in shoot tissue. Produced ethylene elicits leaf epinasty and tissue swelling, and regulates auxin levels locally through inhibition of auxin transport. Ethylene also stimulates abscisic acid (ABA) biosynthesis by increasing xanthophyll cleavage to xanthoxal. ABA is distributed within the plant and mediates stomatal closure which limits transpiration and carbon assimilation, accompanied by an overproduction of reactive oxygen species (ROS). In addition, ABA directly inhibits cell division and expansion and promotes, together with ethylene, leaf senescence. Growth inhibition, tissue damage and cell and plant death are the consequences. SAM, S-adenosylmethionine. According to Grossmann (2003); reproduced with permission.

back loop. Auxin has been shown to promote degradation of the Aux/IAA repressor proteins by targeting them to a SCF-type ubiquitin-protein ligase (SCF^{TIR1}) in the ubiquitin-proteasome pathway (Gray and others 2001). It is speculated that auxin modifies Aux/IAA repressors, possibly by phosphorylation, for recognition by SCF^{TIR1}. Further early auxin-response genes include genes of, as yet, unknown function (*SAUR*, *GH3* gene families), those encoding glutathione S-transferase (*GH2/4*), and 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (*ACS*) in ethylene biosynthesis (Abel and Theologis 1996).

Auxin-Induced Ethylene Biosynthesis

Among the known hormone interactions in plant growth regulation, stimulation of ethylene production is a classical example. This early and ubiquitous response when auxins are applied to sensitive species (Abeles and others 1992) or overproduced in transgenic plants (Romano and others 1993) was discovered as early as 1935 by Zimmerman and Wilcoxon. Ethylene is a gaseous hormone which is involved in plant responses to stress and the regulation of senescence and plant growth (Abeles and others 1992; Kende and Zeevaart 1997). By reorienting microtubules from a transverse to a more longitudinal orientation, it promotes lateral cell expansion leading to swelling of stems and roots (Abeles and others 1992). In addition, induced ethylene mediates auxin effects such as leaf abscission and epinasty, and regulates auxin levels locally through ethylene-inhibited auxin transport (Abeles and others 1992). Consequently, auxin herbicides have direct and indirect effects (Figure 2). Although ethylene itself is not the primary agent by which auxin herbicides kill sensitive plants (Morgan 1976), former investigations have made it plausible that enhanced ethylene production is involved in

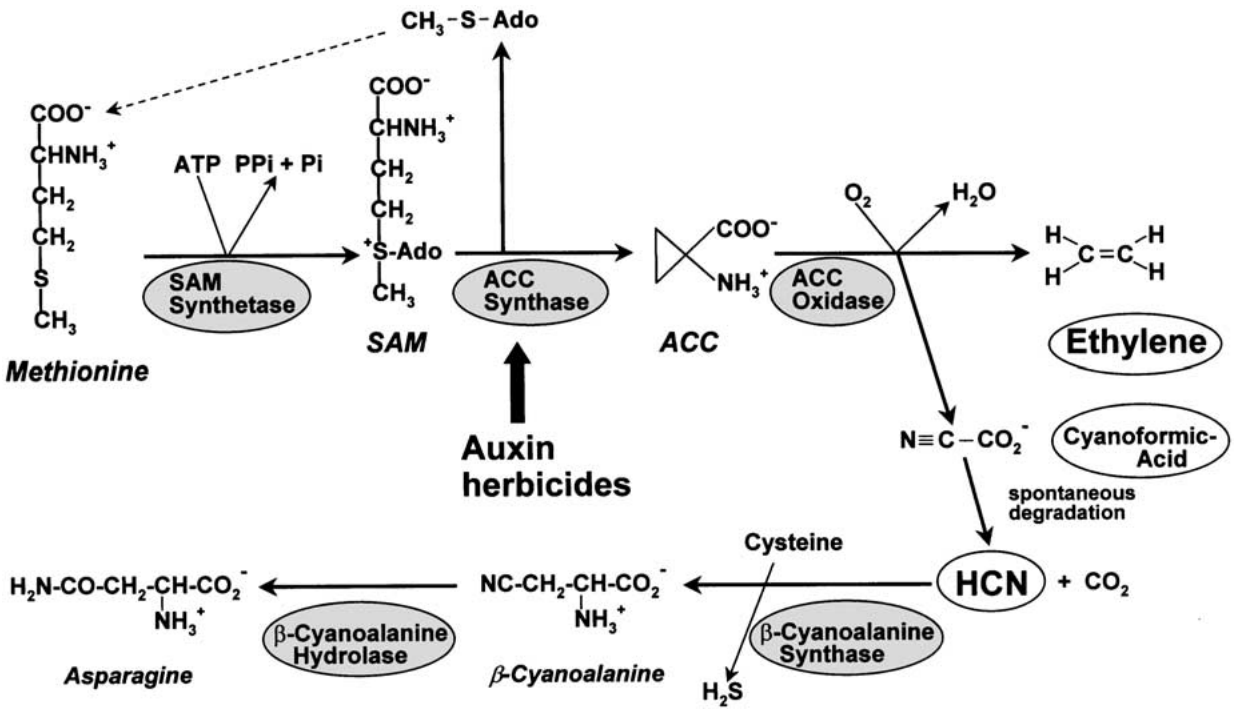

Figure 3. Biosynthetic pathway of ethylene and cyanide. Auxin herbicides stimulate the conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC), catalyzed by ACC synthase.Released methylthioadenosine (CH_3-S-Ado) is used for methionine synthesis. ACC is converted to ethylene, catalyzed by ACC oxidase, and, *via* cyanoformic acid, to CO_2 and hydrogen cyanide (HCN). HCN is detoxified by β-cyanoalanine synthase (β-CAS), which catalyzes the reaction of cysteine and cyanide to form hydrogen sulfide and β-cyanoalanine. β-Cyanoalanine ishydrolyzed to asparagine (Abeles and others 1992).

this process (for review see Cobb 1992; Sterling and Hall 1997; Grossmann 2000, 2003). In a multitude of dicot species and biotypes of differing sensitivity, stimulation of ethylene production by auxin herbicides has been found to correlate closely with the induced morphological effects, which could be lessened by adding inhibitors of ethylene biosynthesis (Morgan 1976; Hall and others 1985; Abeles and others 1992; Grossmann and Kwiatkowski 1993; Grossmann and Schmülling 1995; Hansen and Grossmann 2000). Accordingly, inactive auxin analogues did not promote ethylene formation (Sterling and Hall 1997).

However, conclusive evidence for a causal role of ethylene synthesis in auxin herbicide action has been presented only recently. This progress has been achieved with the identification of auxin-induced *de novo* synthesis of ACC synthase and plant mutants and transformants modified in ethylene biosynthesis, receptor and signalling (Romano and others 1993; Abel and Theologis 1996; Kende and Zeevaart 1997; Sterling and Hall 1997; Grossmann 2000a). The *ACS* gene family of early auxin-response genes encodes the key regulatory enzyme in ethylene biosynthesis that catalyzes the conversion of S-adenosylmethionine into ACC, the direct ethylene precursor (Figure 3). Isoforms of ACC synthase genes are differentially expressed or post-transcriptionally or -translationally regulated in different species and tissue types during senescence, in response to environmental stress conditions, and by auxins within a few minutes of treatment (Abel and Theologis 1996; Wei and others 2000; Wang and others 2002). Auxin-induced ACC synthase activity results in enhanced levels of ACC, closely paralleled by increased ethylene formation (Abeles and others 1992; Figure 3). Conversely, according to their biphasic behavior, low concentrations of auxins have been found to decrease ACC synthase activity and ACC and ethylene formation (Grossmann and Retzlaff 1997). In tomato, the *ACS* gene family contains at least nine genes, six of which are induced by auxin (Abel and Theologis 1996). In experiments using transgenic tomato plants containing the ACC synthase antisense gene *LE-ACS2* (Grossmann and Schmülling 1995), auxin-resistant or ethylene-insensitive lines of *Arabidopsis*, wild mustard (Wei and others 2000) and tomato

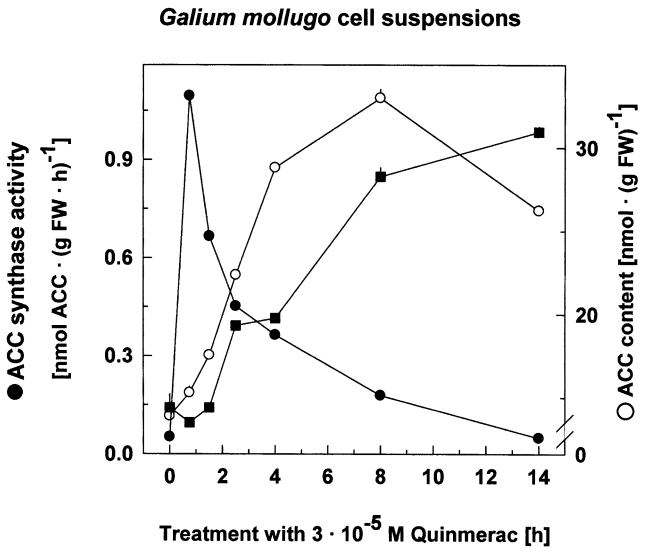

Figure 4. Time course of the sequential stimulation of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity and ACC and immunoreactive (+) abscisic acid (ABA) levels by the auxin herbicide quinmerac in heterotrophic cell suspensions of *Galium mollugo*. *Galium* cell suspensions were cultivated in Erlenmeyer flasks and treated with compound as described (Siefert and others 1994). ACC synthase activity, ACC and (+) ABA levels (related to fresh weight, FW) were determined in *Galium* cells according to Grossmann and others (2001). Unpublished result of F. Scheltrup and K. Grossmann.

(Hansen and Grossmann 2000), herbicide-induced morphological symptoms could be lessened or fully reversed.

Ethylene-Triggered Abscisic Acid Biosynthesis

Following auxin-induced ACC synthase activity and ethylene formation, massive accumulations of ABA in root and even more in shoot tissue were found using phytohormonal profiling of plant extracts (Scheltrup and Grossmann 1995). This was exemplified first for the dicot weed *Galium aparine* after root treatment with quinmerac. ABA accumulations reached 70 times the level in controls after 48 h, closely correlated with the inhibition of shoot and root growth. In contrast, the concentrations of other phytohormones including gibberellins, cytokinins, endogenous IAA (Scheltrup and Grossmann 1995) and jasmonic acid (J. Kwiatkowski, K. Grossmann; unpublished result) were only moderately changed. A slight increase in the levels of immunoreactive gibberellin A_1, but not more than 50%, might be explained by the known activity of auxin to promote gibberellin A_1 biosynthesis (Ross and others 2002). Overall, the hormonal balance was dramatically shifted in favor of the growth inhibitor ABA, as opposed to the growth-promoting gibberellins. Induction of ABA accumulation, which has been reported for the first time, was confirmed for auxin herbicides from the different chemical classes (Figure 1) and IAA at high concentrations (Grossmann and others 1996; Grossmann 2000a; Hansen and Grossmann 2000). This response has been observed in a variety of dicot species, including members of the Rubiaceae (*Galium aparine*), Solanaceae (*Lycopersicon esculentum*, *Solanum nigrum*), Umbelliferae (*Apium graveolens*, *Conium maculatum*), Fabaceae (*Medicago sativa*, *Trifolium* spp.), Scrophulariaceae (*Veronica* spp.) and Convolvulaceae (*Ipomoea* spp.) (Scheltrup and Grossmann 1995; Grossmann and others 1996; Hansen and Grossmann 2000; Grossmann 2000a). In contrast, crop species with natural tolerance to quinmerac, such as oilseed rape, sugarbeet and wheat, showed no stimulation of ACC synthase activity and ACC and ABA levels (Grossmann and Scheltrup 1998). This suggests selectivity based on target site, the induction of ACC synthase. ABA accumulation and concomitant epinastic effects and reduced shoot growth were also detected when *Galium* plants were exposed to ethylene-releasing ethephon (Scheltrup and Grossmann 1995; Grossmann 2000a). Studies with isolated plant organs demonstrated that auxin-induced increases in ethylene and ABA occur primarily in the shoot tissue. From there, ABA appears to be translocated to the roots (Grossmann 2000a, 2000b).

Quinmerac and IAA treatment stimulated ethylene biosynthesis through induced ACC synthase activity in *Galium* shoot tissue within 2 h (Grossmann and Scheltrup 1998; Hansen and Grossmann 2000). This induction was inhibited by cordycepin or cycloheximide, indicating *de novo* enzyme synthesis (Grossmann 2000a). Subsequently, increases in ABA and its biosynthetic precursor xanthoxal were found after 5–8 h (Hansen and Grossmann 2000). In *Galium* cell suspensions, ACC synthase activity and ABA levels increased as early as 1 h and 3 h, respectively, indicating a response directly following hormone signalling (Figure 4). The time course of ABA increase in plants closely correlated

with stomatal closure, with consequent inhibition of transpiration, carbon assimilation, plant growth and progressive foliar tissue damage (Scheltrup and Grossmann 1995; Grossmann and others 1996; Grossmann 2000b; Hansen and Grossmann 2000; Grossmann and others 2001; Figure 2). The latter effect was accompanied by an overproduction of reactive oxygen species (ROS) such as hydrogen peroxide (Grossmann and others 2001). This accumulation of hydrogen peroxide appears to be triggered by the decline of photosynthetic activity due to ABA-mediated stomatal closure which leads to higher leakage of electrons from the photosystems to O_2 in the chloroplasts (Grossmann and others 2001). In addition, the amounts of Cu/Zn- superoxide dismutase increased in the leaf tissue (S. Tresch, K. Grossmann; unpublished result) which further promotes hydrogen peroxide accumulation. Exogenously applied ABA mimicked auxin effects on these processes (Grossmann and others 1996; Grossmann and others 2001). This is in accord with the recognized hormonal role of ABA in stomatal closure, growth inhibition and promotion of leaf senescence (Kende and Zeevaart 1997). An accumulation of hydrogen peroxide is generally considered to contribute to oxidative tissue damage through membrane lipid peroxidation and probably process signalling in senescence (Dat and others 2000). In addition, a role of reactive oxygen species (ROS) in auxin-induced cell-wall loosening has been suggested (Schopfer 2001). Consequently, treatment of *Galium* shoots with hydrogen peroxide caused auxin-like phytotoxic symptoms including progressive leaf chlorosis, followed by wilting, tissue necrosis and plant death (K. Grossmann, unpublished result).

A causal relationship between these processes was established more firmly by molecular dissection of the target pathways of ethylene and ABA using inhibitors of biosynthesis and tomato mutants defective in ethylene perception or ABA synthesis (Hansen and Grossmann 2000). In addition, quantification of xanthophylls, xanthoxal, ABA and its catabolites revealed that auxin-induced ethylene stimulates ABA biosynthesis, mainly by increasing xanthophyll cleavage to xanthoxal (Grossmann 2000b; Hansen and Grossmann 2000; Figure 2). This step is catalyzed by the key regulatory enzyme 9-*cis*-epoxycarotenoid dioxygenase (NCED; Qin and Zeevaart 1999). The synthesis of this plastid enzyme is stimulated by drought (Iuchi and others 2001). Our results propose that the 9-*cis*-epoxycarotenoid dioxygenase is a target of auxin-induced ethylene.

In conclusion, ABA functions, together with ethylene, as a hormonal second messenger in the mode of action of auxin herbicides (Figure 2). The overproduction of ABA and consequently of hydrogen peroxide are the long-searched for missing links between induced ethylene synthesis and herbicide effects leading to growth inhibition, senescence and tissue decay (Figure 2).

Is ethylene-triggered ABA restricted to the action of auxin herbicides and IAA at high concentration or is it a principle in normal plant growth regulation? Although knowledge of interrelations among auxin, ethylene and ABA remains limited, correlative results gave rise to speculation that this hormone interaction also functions as a module in the signalling of other auxin-related processes, such as root gravireaction and growth inhibition of lateral buds in apical dominance (reviewed by Grossmann and Hansen 2001). However, in the latter case, recent studies using an assay with nodes from wild-type and hormone-signalling mutants of *Arabidopsis* suggested that ethylene is not a second messenger for auxin-mediated bud inhibition (Chatfield and others 2000). Ethylene and ethephon treatment have been shown to increase ABA levels in many plant tissues (Abeles and others 1992). In addition, the signal transduction chains of ethylene and ABA appear to be partly overlapping and interfering (Beaudoin and others 2000; Ghassemian and others 2000). Therefore, it is possible that ethylene-triggered ABA plays a role in phenomena which coincide with a strong stimulation of ethylene biosynthesis, such as senescence elicited under stress conditions and growth inhibition induced by cytokinins (reviewed by Grossmann and Hansen 2001). Cytokinin has been shown to stimulate ethylene biosynthesis through regulation of specific ACC synthase genes post-transcriptionally (Woeste and others 1999). Similar to auxin, cytokinin applied at high concentration or overproduced in mutant and transgenic plants inhibits shoot and root growth, accompanied by increased ethylene formation (Abeles and others 1992; Taiz and Zeiger 1998). It has been suggested that ethylene is involved in growth inhibition by cytokinin (Abeles and others 1992; Taiz and Zeiger 1998).

Moreover, recent experiments using *Galium* plants treated with benzyladenine revealed that stimulation of ethylene biosynthesis coincides with massive ABA accumulations and concomitant shoot growth inhibition (Grossmann and Hansen 2001).

Accordingly, the same regulatory principle appears to be involved in the development of *Agrobacterium tumefaciens*-induced plant tumors. Ethylene emission from tumors elicited by increased auxin and cytokinin levels caused an increase in ABA concentrations also in the host leaves, ac-

companied by stomatal closure (Veselov and others 2003). During fruit ripening in avocado, a rise in ethylene has been shown to be followed by an increase in ABA biosynthesis, which is regulated at the level of carotenoid cleavage (Chernys and Zeevaart 2000). However, there are also examples of opposite results from hormone interactions. In semiaquatic deepwater rice, stimulated ethylene leads to a decrease in endogenous ABA levels upon submergence (Kende and Zeevaart 1997). This shifts the hormonal balance in favor of the growth-promoting gibberellins, as opposed to the growth inhibitor ABA. Concomitantly, the responsiveness of the tissue to gibberellins increases. The hormone interaction has been suggested to stimulate internodal growth in deepwater rice (Kende and Zeevaart 1997). In addition, recent studies of several species indicated that one function of endogenous ABA may be to prevent the overproduction of ethylene and, as a result of this interaction, to maintain rather than inhibit shoot and root growth (Sharp 2002). Therefore, more detailed studies are required to establish causality and a broader range of species and mutants should be examined to determine whether the proposed hormonal interdependence is involved in normal plant growth regulation.

Role of Cyanide, Derived from Ethylene Biosynthesis

As previously mentioned, most auxin herbicides preferentially control dicot weeds. An exception to this phenomenon is the auxin herbicide quinclorac which also affects important grass weeds, such as *Echinochloa*, *Digitaria*, *Setaria* and *Brachiaria* spp. (reviewed by Grossmann 2000a). Growth inhibition with progressive leaf chlorosis, first on the growing areas of the youngest leaf blades, followed by wilting and necrosis of the entire shoot, are the predominant herbicidal symptoms. Quinclorac-induced increases of ABA and hydrogen peroxide alone were shown to be insufficient to elicit these effects (Grossmann 2000a, Grossmann 2003). In grasses, the accumulation of cyanide is crucial for phytotoxicity (reviewed by Grossmann 1996; Grossmann 2000a; Grossmann 2003).

Cyanide is formed as a coproduct of ethylene in the oxidation of ACC, catalyzed by ACC oxidase (Abeles and others 1992; Grossmann 1996; Figure 3). Cyanide is a well-known inhibitor of many important plant enzymes and proteins including cytochrome-c oxidase, ribulose-bisphosphate carboxylase, nitrate/nitrite reductase, catalase, Cu/Zn-superoxide dismutase, peroxidase and plastocyanin (reviewed by Grossmann 1996). The concentrations of endogenous cyanide required to cause 50% inhibition of these enzymes are mostly in the range of 5–10 μM (Grossmann 1996). Differential compartmentation of the intracellular site of cyanide release (ACC oxidase in the cytoplasm) and its detoxification (β-cyanoalanine synthase, the key enzyme for detoxifying hydrogen cyanide in plants, in the mitochondria; Figure 3) has been suggested to lead to transiently elevated, phytotoxic cyanide levels in cell compartments, such as cytoplasm, chloroplasts and peroxisomes (Grossmann 1996). The consequence is a multiplicity of metabolic effects that culminate in growth inhibition, tissue chlorosis and necrosis by cyanide (Grossmann 1996).

Quinclorac induced 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity organ-specifically in the root tissue of sensitive grass species, leading to subsequent increases in ACC as early as 1 h after treatment (Grossmann and Scheltrup 1997; Grossmann and Kwiatkowski 2000; Figure 5). The accumulated ACC appears to be transported from the root to the shoot, where it is converted to ethylene and cyanide by ACC oxidase (Grossmann and Kwiatkowski 1993; Grossmann and Scheltrup 1997; Grossmann 2000a). The process appears to be self-amplifying because ACC and its product cyanide induce ACC synthase activity in the shoot tissue. Treatment of isolated shoots of *Echinochloa crus-galli* (barnyard grass) with ACC *via* the vascular system stimulated ACC synthase activity and enhanced ethylene and cyanide production stoichiometrically (Grossmann and Kwiatkowski 1995; Grossmann and Scheltrup 1997; Grossmann 2000a). At 1 mM applied ACC, ACC synthase activity was stimulated 5-fold, relative to the control, after 6 h of treatment (Grossmann and Scheltrup 1997). Tissue cyanide concentrations went up from 5 μM in controls to 18 μM during 53 h of incubation (Grossmann and Kwiatkowski 1995). In addition, cyanide led to increased ACC synthase activity (Grossmann and Scheltrup 1997), probably through induction of ACC synthase gene transcription (McMahon and others 2000), whereas ethephon did not change enzyme activity. In susceptible grasses, cyanide levels in the shoot tissue accumulated according to the herbicide concentration and application time and closely correlated with herbicidal effects including growth inhibition, chlorophyll loss and reduction in carbon assimilation (Grossmann and Kwiatkowski 1993; Grossmann and Kwiatkowski 1995; Grossmann 2000a). Concomitantly, the β-cyanoalanine synthase was only slightly activated (Grossmann and Kwiatkowski 1995; Grossmann

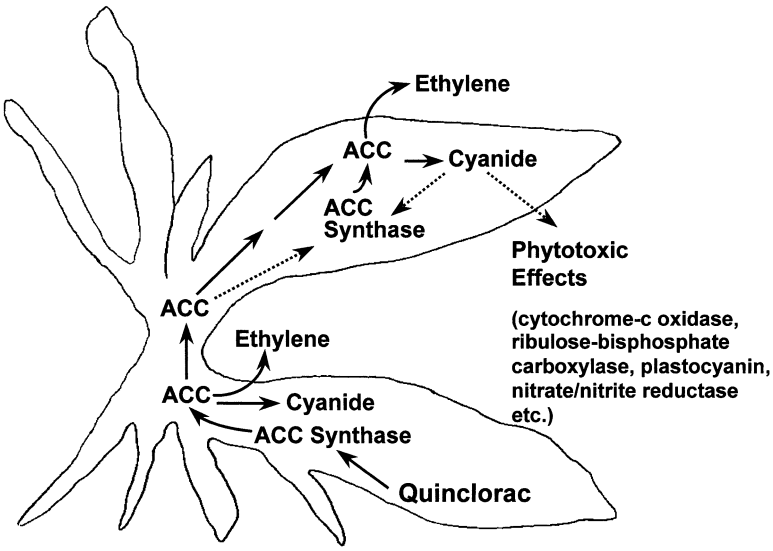

Figure 5. Proposed model of the mode of action of the auxin herbicide quinclorac in susceptible grasses. Quinclorac induces 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity primarily in the root. ACC and, to a lesser extent, ethylene and cyanide are produced. The accumulated ACC is transported from the root to the shoot, where it is converted to ethylene and cyanide by ACC oxidase. The process is self-amplifying because ACC and its product cyanide trigger ACC synthase activity in the shoot tissue. This explains why cyanide accumulation, and consequently phytotoxic effects of quinclorac, are most apparent in the shoots. Cyanide is an inhibitor of many important plant enzymes and proteins involved for example, in respiration, carbon and nitrogen assimilation and photosynthetic electron transport. Modified according to Grossmann and Scheltrup (1997) and Grossmann (2000a).

and Kwiatkowski 2000). Consequently, endogenous cyanide concentration increased, for example, 3-fold and 9-fold in the shoot tissue of *Echinochloa crus-galli* and *Digitaria sanguinalis* (large crabgrass), reaching a maximum of nearly 30 μM and 50 μM, respectively, within 96 h (Grossmann and Kwiatkowski 1995; Grossmann 2000a).

Applied KCN mimicked quinclorac effects at comparable endogenous cyanide concentrations (Grossmann and Kwiatkowski 1995). Auxin herbicides from other chemical classes, including dicamba and naphthalene acetic acid, caused lower ACC and cyanide levels and correlated with lower phytotoxicity (Grossmann and Kwiatkowski 2000; Grossmann 2000a). In contrast, tolerant rice and resistant grass weeds showed no notable changes in ACC synthase activity, ethylene and cyanide production (Grossmann and Kwiatkowski 2000). The selective induction of ACC synthase is decisive for the different response of grasses toward quinclorac. Contrary to susceptible grasses, unchanged cyanide levels were found in shoots of sensitive dicots, such as *Ipomoea* spp., *Trifolium* spp., *Veronica* spp., *Solanum nigrum* and *Galium aparine*, treated with quinclorac (Grossmann 2000a). As shown in *Galium*, only at the onset of senescence in treated plants, a 3-fold increase in HCN, released from the shoots into the gas phase, could be detected (Scheltrup and Grossmann 1995).

Therefore, we concluded that the accumulation of tissue cyanide, derived ultimately from quinclorac-stimulated ACC synthase activity in ethylene biosynthesis, plays a key role in mediating the herbicidal effects of quinclorac in sensitive grasses (Grossmann and Kwiatkowski 1995; Grossmann 2000a; Grossmann 2003; Figure 5). These results are in accordance with earlier findings reported by Tittle and others (1990) which had suggested for the first time a role of cyanide in the phytotoxic mode of action of the auxin herbicide 2,4-D in sensitive plants. Cyanide production derived from ethylene biosynthesis is thought to be a widespread phenomenon in plants, implicated in the induction of cell death under stress conditions, such as pathogen infection (Grossmann 1996). Accordingly, increases in cyanide levels have been observed during the formation of necrotic lesions in tobacco mosaic virus-infected tobacco leaves (Siefert and others 1995; Grossmann 1996).

Auxin Transport Inhibitors

According to a current model, IAA is synthesized primarily in growing shoot regions of plants, the apical shoot meristems and the young leaves (reviewed by Taiz and Zeiger 1998; Ljung and others 2002). From there, IAA is transported in a polar fashion through a parenchymatous cell-to-cell pathway towards basally located shoot tissues and the root (Muday and DeLong 2001). In roots, IAA moves acropetally towards the root apex *via* phloem and is redistributed basipetally, back from the root apex towards the base, through polar auxin transport in the outer layers of root cells (Muday and DeLong 2001). The latter pathway plays a role in gravity response and probably lateral root initiation.

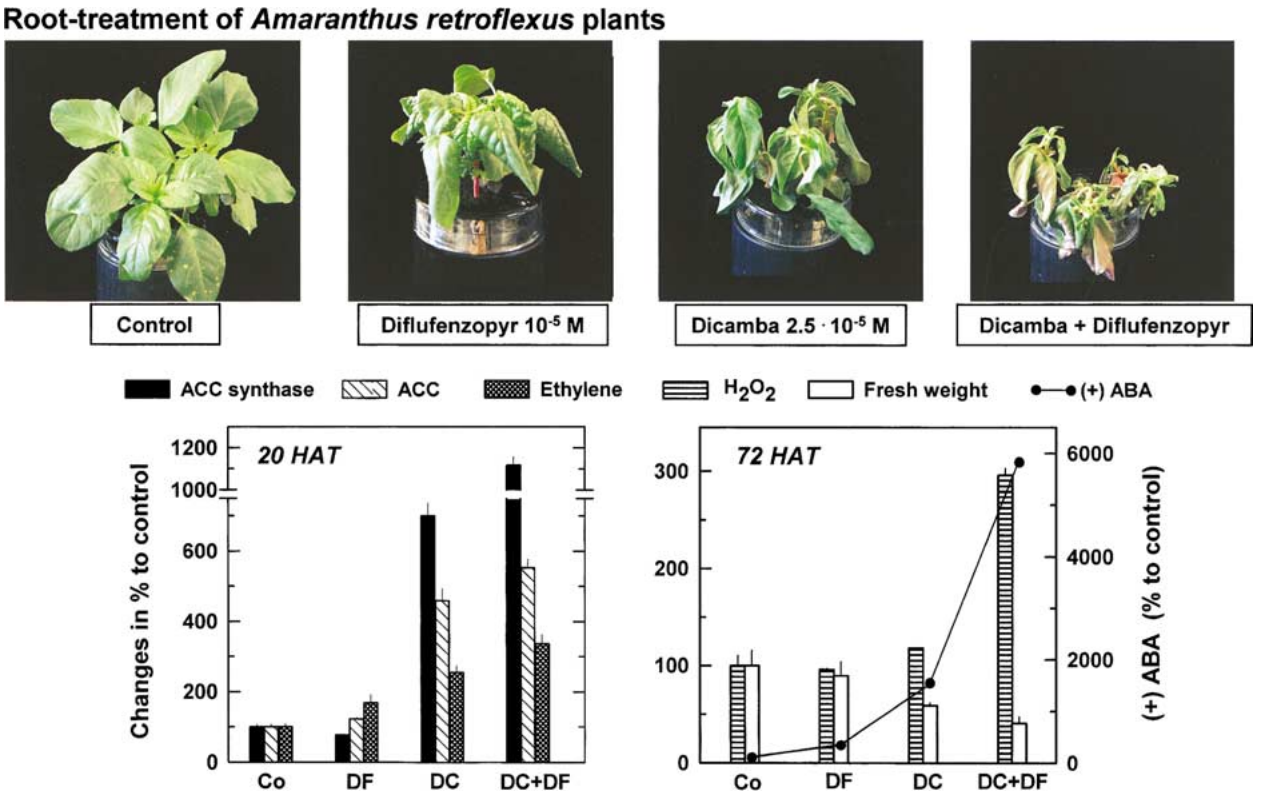

Figure 6. Effects of the auxin transport inhibitor diflufenzopyr (DF) and the auxin herbicide dicamba (DC) and combination of the compounds on young plants of the dicot weed waterhemp (*Amaranthus retroflexus*) after root treatment in hydroponics. Diflufenzopyr synergizes dicamba-induced 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity, and production of ACC and ethylene in shoot tissue (measured after 20 h). This is followed by accumulations of immunoreactive (+)-abscisic acid (ABA) and hydrogen peroxide (H_2O_2), and growth inhibition (measured after 72 h). Co, untreated control. Modified from Grossmann and others (2002).

Polar IAA transport out of cells is mediated by specific auxin efflux carriers which are thought to belong to the PIN protein family (Muday and DeLong 2001; Friml and others 2002). These carriers are asymmetrically localized in the plasma membrane to the basal side of cells. Polar auxin transport is ubiquitous among higher plant species and specific for IAA and synthetic auxins. It contributes to the formation of an auxin gradient from the shoot to the root, which choreographs plant growth and tropic responses, and development (Taiz and Zeiger 1998; Muday and DeLong 2001; Friml and others 2002).

Inhibition of polar auxin transport is the target process underlying the herbicidal effects caused by phytotropins (reviewed by Grossmann 2003). Phytotropins are members of a subset of auxin transport inhibitors which include natural (for example, curcumin, quercetin) and synthetic compounds, such as benzoic acids ortho-linked to an aromatic ring system (for example, naptalam, NPA) and a new class, the semicarbazones, exemplified by diflufenzopyr (Taiz and Zeiger 1998; Grossmann and others 2002) (Figure 1). Unlike auxins, they are not transported polarly (Taiz and Zeiger 1998). Phytotropins prevent the efflux of IAA and synthetic auxins from the cell and consequently their polar transport away from plant meristems. This causes an abnormal accumulation of endogenous IAA and exogenously applied auxin herbicides in the growing tissues of meristematic shoot and root regions (Grossmann 2003). Stunting or ''herbistatic'' suppression, particularly of emerged dicot weeds, accompanied by severe inhibition of root growth and loss of tropic responses, are the consequences. When applied in combination, diflufenzopyr interacts with auxin herbicides such as dicamba (Figure 1) and synergistically promotes their activity and the range of weed species affected (Grossmann and others 2002). Concomitantly, diflufenzopyr has been shown to enhance dicamba-induced ACC synthase activity and the succeeding cascade of effects including ethylene formation, ABA accumulation and hydrogen peroxide overproduction in

Galium aparine and waterhemp (*Amaranthus* sp.) (Grossmann and others 2002; Figure 6). This combination product was commercially introduced in 1999 in the USA and Canada (Bowe and others 1999). Diflufenzopyr also increased the activity of other synthetic auxin herbicides, including phenoxycarboxylic acids, pyridine carboxylic acids and quinclorac (Bowe and others 1999; Grossmann and others 2002). Although the precise mechanism by which they control auxin efflux is not known, phytotropins appear to interact with a plasma membrane and actin cytoskeleton associated protein, the so-called NPA-binding protein, which regulates the activity of the auxin efflux carrier (Muday and DeLong 2001). Recently, Geldner and others (2001) suggested that auxin transport inhibitors do not act auxin-specific but affect efflux by generally interfering with membrane-trafficking processes. However, the NPA-binding protein has not yet been identified. At present, the involvement of plasma membrane aminopeptidases in low-affinity NPA binding is discussed (Murphy and others 2002). Diflufenzopyr has a high affinity for the NPA-binding site, as shown by an auxin efflux inhibition value (I_{50}) of 19 nM and displacement of bound NPA (Subramanian and others 1997; Bowe and others 1999). Inhibition of the auxin efflux carrier blocks polar transport of both IAA and auxin herbicides.

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