

Mode of Action of the Growth Regulator Herbicides

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Early Discovery of IAA and 2,4-D

Although IAA (indole acetic acid) has been known to chemists as long ago as 1904, the first isolation of an active auxin occurred in 1931 by two Dutch biochemists. They isolated a compound called "auxin A" from 33 gallons of human urine. The first generally accepted report of IAA in a higher plant was published by A.J. Haagen-Smit and coworkers in 1946.

The discovery of 2,4-D, and related chemicals, occurred independently by four research groups in Britain and the U.S. during World War II. This discovery revolutionized modern agriculture.

Since the synthesis of 2,4-D, a number of other synthetic auxins have become commercially available. Although these products are referred to as growth regulators or phytohormones (previously known as plant hormones), they really represent only one group of growth regulators, the auxins. Auxins can be divided into six major groups; indole acids, naphthalene acids, phenoxy carboxylic acids, benzoic acids, picolinic acid derivatives (also called pyridine carboxylic acids) and the quinoline carboxylic acids. The first group contains the natural product IAA, and does not contain any herbicides. IAA is highly unstable in plants and metabolizes too fast to be an effective synthetic herbicide. The naphthalene acids (NAA) are used in research but are not commercially available as herbicides. The other groups contain many well known herbicides; phenoxy carboxylic acids (2,4-D, 2,4,5-T, 2,4-DB, dichlorprop, MCPA, MCPB, mecoprop), benzoic acids (dicamba, chloramben), picolinic acids (aminopyralid, clopyralid, picloram, triclopyr), quinoline carboxylic acids (quinclorac), and one yet to be named family (aminocyclopyrachlor). These compounds are often called auxinic herbicides. Quinclorac has also been shown to have growth regulator activity on broadleaf species, although it is not typically considered to be an auxinic herbicide on grasses.

Mode of Action of the Auxins

IAA influences nearly every aspect of plant growth and development, it is thought to act as a 'master hormone' in the complex network of interactions with other growth regulators. Shoot tips, including the young leaves, are the center of most abundant naturally occurring auxin synthesis in higher plants. Other rich sources are root tips, enlarging leaves, flowers, fruits and seeds. One of the difficulties in studying the mechanism of auxin action is the multitude of different kinds of physiological processes that they appear to control. Recent evidence indicated there are IAA receptor sites (auxin-binding proteins) which unleashes a cascade of events. Auxins seem to be involved in a number of developmental functions, including phototropism, apical dominance, senescence, cell growth and differentiation, and root formation.

The initial response of plants to auxin treatment can be categorized into three phases. First, there is a rapid response (within minutes), simulated by low pH and perhaps due to auxin stimulating the pumping of protons into the cell wall and loosening it. During this phase ethylene synthesis is also increased. The second phase of the response occurs 35-45 min after treatment, and involves the synthesis of nucleic acids. The third phase is when the plants senesce and tissue decay occurs. During this phase chloroplasts are damaged and chlorosis develops, membranes are destroyed and the plant loses its vascular system integrity which leads to wilting, necrosis and finally death.

Acid-growth hypothesis

According to the acid-growth hypothesis auxins initiate an acidification mechanism, possibly a membrane-bound H^+ pump (ATPase), with the result that proton efflux occurs and the pH of the solution in the matrix of the cell wall decreases. The resultant lowering of the pH of the solution bathing the cell walls has been suggested to activate enzymes, called expansins, capable of hydrolyzing wall polysaccharides, thereby softening the wall and allow cell extension. Movement of the sugar chains along the cellulose microfibrils occurred by a mechanism (enzymatic or non-enzymatic) which catalyzes breakage and reformation of the hydrogen bonds, allowing the glucan structures to creep inchworm-fashion along the cellulose microfibrils. The rate at which the sugar polymers moved increased at lower pH. This is due to a weakening of the hydrogen bonds. The loosening of the bonds decreased the resistance of the wall to turgor pressure. More water would move into the cell causing an increase in cell volume and irreversibly stretching the cell wall.

The acid-growth hypothesis was supported by evidence showing that an exogenous acid solution can induce short term growth, which could be stopped with the addition of more basic buffers. In addition, an inhibitor of acid-induced growth was also shown to inhibit auxin-induced growth. This suggests that the growth responses evoked by both auxin and acid involve some common step. It could also be argued that acidification is not just a result of growth, but is a necessary part of the growth phenomenon.

Cell elongation after 30 to 60 min does not involve acid-induced elongation, but is due to auxin turning on genes which help cells elongate by other mechanisms (i.e. synthesis of new cell wall material).

Nucleic acid metabolism

Plant tissues respond to auxin treatment by dramatically increasing nucleic acid and protein synthesis, and this effect is closely correlated to auxin-induced growth. However, this response may be independent of the cell wall loosening phenomenon, although this is by no means conclusive.

The action of auxin appears to involve specific gene activation at the transcriptional level. Auxin may interact with a binding protein and the auxin-protein complex then interacts with chromatin (filamentous complex of DNA, histones and other proteins constituting chromosomes) to cause an increase in DNA template available for transcription. The result of this action could be

altered DNA transcription and quantitative and qualitative changes in RNA synthesis. These RNAs would then serve as templates for the synthesis of the proteins required for the observed physiological responses.

The changes in DNA transcription in auxin-treated chromatin were shown to cause a substantially higher RNA polymerase activity than control chromatin. It was subsequently shown that the major influence of auxin was to increase the endogenous RNA polymerase of chromatin. The DNA-directed RNA polymerase functions in mRNA synthesis. It was demonstrated that auxins increased a specific set (at least 10) of translatable messenger RNAs that encode for a variety of proteins.

Auxinic Herbicides

The auxinic herbicides are still the most widely used herbicides in the world. They are used to selectively control broadleaf weeds in grass crops, including corn, wheat, barley, oat, sorghum, rice, sugarcane, pasture, rangeland, and turf. These compounds are all weak acid herbicides (see chapter on herbicide absorption) that are primarily applied postemergence and translocate via the phloem to the growing points and other sink regions in the plant.

At low doses, the growth regulator herbicides have a stimulatory effect on plant and cell growth similar to that of IAA. However, phytotoxic concentrations of the auxinic herbicides elicit a variety of symptoms in plants. Among these include, leaf chlorosis, altered stomatal function, stem tissue proliferation, root initiation in stem tissue, disintegration of root tissues, leaf cupping, stunted leaves, and abnormal apical growth. Many of these are secondary effects. In addition, auxin herbicides cause plugging of the phloem, growth inhibition, and tip and stem swelling.

The mechanism of action of these herbicides is thought to be the same as that of naturally occurring auxins. The primary effect of low levels of growth regulator herbicides on nucleic acid synthesis appears to be a stimulation of RNA polymerase followed by stimulation in RNA and protein synthesis. However, in meristematic tissues, high levels of auxins (typical of herbicidal concentrations) inhibit RNA synthesis and growth. In contrast, high auxin levels stimulate RNA and protein synthesis in mature tissues. This stimulation in the more mature stem regions causes parenchyma cells to divide in mature tissues. This often leads to uncontrolled growth and the production of callus tissue. Volume expansion of mature tissues is somewhat restricted by the presence of secondary cell walls and thickened cells, such as collenchyma and fibers.

Consequently, excessive cell division in these tissues can cause stem swelling and eventually cellular collapse. This occurs because the newly developed callus tissues crush the phloem and cortex, eventually resulting in rupturing of the epidermis of stem tissues. Symptoms normally appear within a few hours or days although death may not occur for several weeks or months.

A characteristic twisting symptom known as epinasty occurs following treatment with all of the auxin-like herbicides. This response is the result of an auxin-induced stimulation in ethylene production. It is thought that these herbicides stimulate ethylene production by promoting the

synthesis of RNA and the enzymes involved in ethylene synthesis. More specifically, auxin activates transcriptional genes that encode for the enzyme 1-aminocyclopropane-1-carboxylic acid synthase (ACS). Although ethylene is induced in most broadleaf species after exposure to auxinic herbicides, some broadleaf species (i.e. chrysanthemum, chickweed, tobacco, yellow starthistle) are tolerant to exogenous ethylene itself and phytotoxic symptoms induced by the herbicide are unaltered in the presence of ethylene biosynthesis inhibitors, suggesting ethylene plays no role in plant death. There are other broadleaf species (i.e. tomato) where auxin-induced ethylene induces the production of ABA (abscisic acid) and ABA which results in stomatal closure.

The characteristic symptoms of auxinic herbicides include rapid internode and petiole expansion due to the cell wall loosening response, and epinasty caused by the stimulation in ethylene. In addition, the inhibition in cell division in meristematic regions occurs at the same time as abnormal stimulation of cell division in mature tissues. Auxin-induced ethylene production leads to stimulation in ABA biosynthesis by up to 70 times the normal level. Together with ethylene, ABA functions as a hormonal second messenger in the mode of action of auxin herbicides. Increased ABA causes stomatal closure which photosynthesis and sugar production. In addition, ABA directly inhibits cell division and elongation and promotes, together with ethylene, leaf senescence with chloroplast damage and destruction of membrane and vascular system integrity. Another byproduct of the ethylene synthesis pathway is cyanide which injures sensitive grasses. Growth inhibition, tissue desiccation and decay and finally plant death are the consequences.

Phenoxy Carboxylic Acids

Phenoxy herbicides are formulated as either salts or esters. Esters are more volatile than salts and are more susceptible to vapor drift, particularly under warmer ambient conditions. However, ester formulations are more readily absorbed through the leaf cuticle and therefore, tend to be more active than salt formulations. This is especially true for waxy-leaved broadleaf species.

The phenoxy herbicides are widely used in many grass crops and in forestry and other non-crop areas. In California, phenoxy herbicides registered for use include 2,4-D, 2,4-DB, dichlorprop, MCPA, and mecoprop. 2,4-D is the oldest and most widely used of these compounds. MCPA is similar to 2,4-D, but is considered somewhat safer on grain crops and legumes. It is less effective on many weeds, such as borages (Boraginaceae), but may be more effective on some thistles and members of the carrot (Apiaceae) and buttercup (Ranunculaceae) families. Dichlorprop is primarily used for controlling brush. Mecoprop is generally used in combination with other auxinic herbicides for control of broadleaf weeds in turf. It is more effective than 2,4-D on chickweeds and clovers, and is safer on bentgrass turf. 2,4-DB is selective in legumes. It must be metabolically converted, through a oxidation reaction, to 2,4-D within the plant in order to be active. Many legumes crops, such as soybeans, peanuts, and seedling forage legumes (clover, alfalfa, and trefoil), as well as mints, metabolize 2,4-DB very slowly and, thus, are fairly tolerant to the herbicide.

Benzoic Acids

Among the benzoic acid herbicides, only dicamba is registered for use in California. It acts in plants the same way as the phenoxy herbicides and other auxins. The selectivity of dicamba is similar to 2,4-D, but it is generally considered to be more active than 2,4-D on perennial broadleaf weeds, legumes, and members of the smartweed (*Polygonaceae*) and pink (*Caryophyllaceae*) families. In contrast, it is less effective on mustards (*Brassicaceae*) and borages. Although the soil activity of dicamba is short, it does persist longer than 2,4-D. Dicamba is often used in combination with other phenoxy herbicides for control of turf and brush weeds.

Picolinic Acids

Four major herbicides belong to the picolinic acid group; aminopyralid, clopyralid, picloram, and triclopyr. Picloram is the only one that is not registered in California. Another herbicide that is very similar to aminopyralid is the new compound called aminocyclopyrachlor. It has not been classified in a chemical family as of yet. The action of these herbicides is similar to other auxinic herbicides. Triclopyr is very active on most shrub species, but also provides excellent control of most broadleaf species. It is one of the most important herbicides in non-crop areas. It has very little soil activity and tends to be somewhat weak on members of the mustard family (*Brassicaceae*). Aminopyralid and clopyralid are registered for use in rangelands, pastures, and wildlands. Aminocyclopyrachlor will also be registered in the same areas, but is likely to only be available as a premix with other sulfonylurea herbicides. These compounds are effective both post- and preemergence on susceptible species, but have a relatively narrow spectrum of selectivity. They are highly effective against plants in the *Asteraceae* (sunflower family), *Fabaceae* (pea family), *Solanaceae* (potato family), many members of the *Apiaceae* (carrot family) and *Polygonaceae* (smartweed family), and have activity on teasel (*Dipsacus* spp.). They are particularly effective for the control of thistles, including yellow starthistle, purple starthistle, Canada thistle. Aminocyclopyrachlor seems to also have good activity on a number of invasive shrubs.

Quinoline Carboxylic Acid

Quinclorac can stimulate ethylene production and cause symptoms in sensitive broadleaf species very similar to that of other auxinic herbicides. However, it is also selective for control of many grasses by a mechanism that appears to involve inhibition in cell wall synthesis. Thus, it is possible that this herbicide possesses two different mechanisms of action in plants.

Auxinic Herbicide Selectivity between Broadleaf and Grass Species

It is thought that no single aspect of herbicide behavior could completely explain auxin herbicide selectivity between broadleaf (dicotyledon) and grass (monocotyledon) species. Although a number of factors may be involved in selectivity, there is no evidence for differences in the target auxin binding sites between monocotyledons and dicotyledons. This may account for resistance in some dicot species.

Some of the possible explanations include:

1. The arrangement of the vascular tissue in bundles surrounded by protective tissue in monocotyledons seems to prevent the destruction of the phloem by the disorganized

growth caused by the herbicides. Furthermore, there is no auxin-sensitive layer of cells capable of cell division in the vascular bundles of monocotyledons.

2. Translocation of foliar-applied auxins from the site of application is less in monocotyledons than in susceptible dicotyledons. Differences in translocation also exist among species of dicotyledons.
3. There are differences in metabolism between monocotyledons and dicotyledons that could also contribute to selectivity. Differences in metabolism can also account for selectivity among dicotyledons. It has even been suggested that cucumbers compartmentalize 2,4-D in the vacuoles and this affords the species a greater degree of tolerance.

Although grass crops are tolerant to auxinic herbicides, they can be injured if these herbicides are applied during rapid cell division (tillering or flowering) or during rapid growth (high temperatures and high soil moisture). Corn and sorghum stems may become brittle after auxinic herbicide application. Wheat and rice may exhibit buggy-whipping and malformed seed heads after 2,4-D treatment.

Herbicide Resistance

A total of 28 weed species in 15 countries have developed resistance to the auxinic herbicides, with the first case appearing in 1957. In the United States and Canada, the dicot species yellow starthistle (*Centaurea solstitialis*), spreading dayflower (*Commelina diffusa*), field bindweed (*Convolvulus arvensis*), wild carrot (*Daucus carota*), kochia (*Kochia scoparia*), prickly lettuce (*Lactuca serriola*), and wild mustard (*Sinapis arvensis*) have been reported to be resistant to one or more of the auxinic herbicides (weedsociety.org). The mechanism of resistance has not been identified in most cases, but may be due to either differential binding to the target receptor site, as appears to be the case with wild mustard, or enhanced metabolism of the herbicide to non-phytotoxic metabolites.

Relevant references

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