

2,4-D Past, Present, and Future: A Review

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Since its discovery and initial commercialization in the 1940s, 2,4-D has been an important tool for weed control in a wide variety of crop and noncrop uses. Work studying its chemistry, physiology, mode of action, toxicology, environmental behavior, and efficacy has not only helped elucidate the characteristics of 2,4-D but also provided basic methods that have been used to investigate the properties of hundreds of herbicides that followed it. Much of the information published by researchers over 60 yr ago is still pertinent to understanding the performance of 2,4-D today. Further, new studies continue to be published, especially regarding the mechanisms of 2,4-D action at the molecular level. New uses for 2,4-D, sometimes enabled by biotechnology, continue to be developed. This review strives to provide an overall understanding of 2,4-D activity in plants, plant sensitivity to 2,4-D, toxicological impacts, and current and future uses.

Desde su descubrimiento y su comercialización inicial en los años 1940s, el 2,4-D ha sido una herramienta importante para el control de malezas en una amplia variedad de cultivos y para usos no agrícolas. Trabajos estudiando su química, fisiología, modo de acción, toxicología, comportamiento en el ambiente, y eficacia han ayudado no solamente a elucidar las características del 2,4-D, pero también han brindado métodos básicos que han sido usados para investigar las propiedades de cientos de herbicidas que lo han seguido. La mayoría de la información publicada por investigadores durante los últimos 60 años es todavía pertinente para el entendimiento del desempeño de 2,4-D, hoy en día. Además, nuevos estudios continúan siendo publicados, especialmente en relación a los mecanismos de acción del 2,4-D a nivel molecular. Nuevos usos para el 2,4-D, algunas veces producto de la biotecnología, continúan siendo desarrollados. Esta revisión busca brindar un entendimiento general de la actividad de 2,4-D en las plantas, la sensibilidad de las plantas al 2,4-D, los impactos toxicológicos, y sus usos presentes y futuros.

2,4-D has been a part of agriculture for over 70 yr and still has an important place in weed control programs around the world. The “discovery” of 2,4-D appears to have occurred through a series of multiple, independent experiments by both British and American researchers that took place in the early 1940s (Kirby 1980; Peterson 1967; Troyer 2001). Since these activities were happening in the midst of World War II, often under the control of the military, early publications and patents related to the chlorophenoxyacetic acids did not always reflect the actual sequence of events (Troyer 2001). Although it can be debated as to where credit for discovery lies, the commercialization of 2,4-D in 1945 revolutionized weed control and gave rise to

the discipline of Weed Science as well as an entire industry. Other phenoxy-carboxylic acids (MCPA, 2,4,5-T, 2,4-DB, dichlorprop, etc.) were developed in a similar time frame, but 2,4-D has consistently been the most widely used member of the chlorophenoxy chemical family. Though it has been extensively researched, new advances related to 2,4-D continue to be made. This review is intended to examine the evolution of 2,4-D as a herbicide from discovery to current uses and projections for its future applications. It will also examine various aspects of auxin activity in plants and questions related to weed resistance, as well as an examination of public perceptions and a review of recent studies that address these perceptions. Finally, information on new technologies related to 2,4-D will be presented.

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Forms and Chemical Characteristics

In its pure form, 2,4-D acid is a relatively nonvolatile dry crystalline solid. It is only slightly soluble in water (44,558 mg L⁻¹) (Gervais et al.

Table 1. Predominant forms of 2,4-D and their chemical properties (Gervais et al. 2008; WSSA 2014).

Form	CAS no.	Molecular weight	Vapor pressure	Solubility	Log K_{OW}
				mg L ⁻¹ @ 25 C	
Acid	94-75-7	221	1.4×10^{-7} mm Hg @ 25 C	44,558	0.177
DMA ^a salt	2008-39-1	266.13	Dissociates to acid	729,397	Dissociates to acid
Choline salt	1048373-72-3	324.7	Dissociates to acid	768,000	Dissociates to acid
EHE	1928-43-4	333.26	3.6×10^{-6} mm Hg @ 25 C	0.0867	5.78

^a Abbreviations: CAS, Chemical Abstracts Service; DMA, dimethyl amine; EHE, 2-ethylhexyl ester.

2008). Therefore, it must be modified and changed into a preparation that readily disperses and forms a suitable mixture with water. Table 1 provides a comparison of the relative properties of various 2,4-D forms. There are two basic types of formulations, amine salts and esters, that have gained widespread acceptance in the marketplace, and a third, 2,4-D choline, that has more recently been introduced (WSSA 2014).

Amine Salts. When the acid of 2,4-D is reacted with an amine, the salt of 2,4-D is formed. The salt-based formulation renders the 2,4-D acid active ingredient water soluble. The amine salt formulations of 2,4-D include isopropylamine, triisopropylamine, diethanolamine, and dimethylamine. The latter is the most widely used (2,4-D Industry Task Force II, personal communication). Amine formulations are readily soluble in water (greater than 50% by weight) and form a true solution. The amine salts have gradually replaced mineral salts (lithium, potassium, sodium, and ammonium) because the amine salts are more readily dissolved in water.

When placed in water, these salt formulations dissociate, or separate, into the acid part, which carries a negative charge, and the amine part, which carries a positive charge. In hard water, which is high in calcium and magnesium ions (Ca^{2+} and Mg^{2+}), these and other cations can associate with the negatively charged 2,4-D acid part of the molecule and form insoluble salts that can precipitate, reducing the amount of herbicide in solution and plugging line screens and nozzle body screens on a sprayer. For this reason, sequestering agents are included in most amine formulations to reduce or eliminate this problem. Most amine salts of 2,4-D form a clear solution when dissolved in water; however, the addition of sequestering agents to the formulation can impart a darker, amber color. This change in color has no impact on the biological

activity or crop tolerance of the formulation. Most amine salts are not soluble in petroleum oils. Salts can be formulated as liquid or dry preparations.

Choline Salt. Most recently a choline salt of 2,4-D has been developed by Dow AgroSciences (Anonymous 2015a; Li et al. 2013). This salt of 2,4-D has greater stability and lower opportunity for volatilization than other forms of 2,4-D (Eytcheson et al. 2012; Sosnoskie et al. 2015). A combination of 2,4-D choline plus glyphosate has been recently registered in the United States (USEPA 2014). Other formulations of 2,4-D choline are in development.

Esters. Reaction of 2,4-D acid with an alcohol forms an ester. Some ester forms of 2,4-D include butoxyethyl ester (BEE), 2-ethylhexyl ester (2-EHE, previously known as iso-octyl ester), propylene glycol butyl ether ester, methyl ester, isopropyl ester, and butyl ester. BEE and 2-EHE have gradually replaced the other esters mentioned and are the only technical esters being supported for reregistration, with the exception of special use for the isopropyl ester on citrus. Esters made from alcohols with an alkyl chain of four carbons or fewer are considered highly volatile. This group includes the methyl, isopropyl, and butyl esters. Removal of these formulations for most uses from the commercial channels in the early 1980s significantly reduced off-target injury to sensitive plants caused by vapor drift (Steve McMaster, Industry Task Force II on 2,4-D, personal communication). On the other hand, esters of 2,4-D made from alcohols with an alkyl chain of more than four carbons are classified as low-volatile esters. It is important to remember that the longer the carbon chain the lower the volatility. The 2,4-D esters made from long-chain alcohols that are classified as low-volatile esters include BEE and 2-EHE (which has a chain of eight carbons). Esters are readily soluble in petroleum oils but are insoluble in water. For this reason they are

formulated as emulsifiable concentrates for application in either water or oils. When an emulsifiable concentrate formulation of 2,4-D ester is mixed with water, the emulsifying agent keeps the tiny oil-like droplets suspended and dispersed, and the ester is held in an oil-in-water type of emulsion. The 2,4-D emulsion appears milky. If allowed to stand, the oil droplets may separate; however, mixing will reform the emulsion.

In general, ester forms of 2,4-D can have greater activity than salt formulations under some circumstances, such as dry conditions, but may also cause greater crop injury because of faster uptake, which may temporarily overwhelm the plants' detoxification mechanisms. Ester formations are considered more rain fast than salt formulations. Once applied, ester formulations quickly penetrate into the leaf surface (cuticle) and are converted to the acid. Ester formulations do not dissociate or ionize when added to water like amine formulations, and thus, do not react with cations in hard water to form insoluble precipitates.

Other Formulations. Over the years, many different formulations of 2,4-D have been produced (Anonymous 2007). Acid formulations, both dry and liquid, have been marketed, as well as salts such as lithium, sodium, trolamine, and others. These formulations have seen limited use and in some cases are no longer commercially available.

Uptake and Translocation

The uptake and translocation of 2,4-D is representative of many phloem-mobile herbicides and was extensively studied from the 1950s until the 1970s. Previous reviews have discussed the results of this early research (Currier and Dybing 1959; Franke 1967; Pillmoor and Gaunt 1981; Richardson 1977; Robertson and Kirkwood 1969, 1970; Sargent 1965), but more recent research has not been reviewed.

The objectives of this section are to summarize observations and results on factors influencing 2,4-D uptake and translocation. Many environmental, plant, and application factors can influence 2,4-D uptake, but translocation is less frequently altered. The factors influencing uptake will be discussed, including any potential effects on translocation.

Environmental Factors. *Light.* Light intensity and quality affect herbicide uptake and translocation by dictating photosynthesis, photoassimilate transport, and cuticle characteristics. Limited research has been conducted regarding the effect of light on 2,4-D uptake and translocation. Uptake of 2,4-D is consistently increased in the presence of light as compared with dark, but the effect of light intensity appears to vary by species (Sargent and Blackman 1972; Schultz and Burnside 1980). However, light intensity may influence translocation as demonstrated by Schultz and Burnside (1980) in hemp dogbane (*Apocynum cannabinum* L.), where increasing light intensity from 50 to 75 flux resulted in 14% greater movement out of the treated leaf.

Temperature. Temperature affects herbicide uptake and translocation by influencing photosynthetic rates and altering the cuticle and many other plant-related processes. Higher temperatures, within limits, result in greater photosynthesis and photoassimilate production, greater enzyme activity, and greater phloem loading, potentially increasing herbicide translocation. The physical state of the plant cuticle and stomatal openings can be altered through increased temperatures, which can influence herbicide uptake (Kirkwood 1999). In general, 2,4-D uptake is greater at higher temperatures but translocation is not altered (Pallas 1960; Schultz and Burnside 1980; Sharma and Vandeborn 1970).

Humidity. The relative humidity during a herbicide application influences herbicide uptake by influencing stomatal conductance, herbicide droplet drying time, and other factors, but effects on translocation are not typical. Uptake doubled under high-humidity conditions in poplar, and when spray droplets were rewetted after drying under low-humidity conditions, 2,4-D uptake increased (Sharma and Vandeborn 1970). Uptake was greater at humidity levels ranging from 70 to 74% compared with 34 to 48% and a difference was not measured between the drying times of herbicide spray droplets under the two humidity levels (Pallas 1960). Uptake of 2,4-D was greater when the humidity level was 100% as compared with 40%, and the rate of translocation to the meristematic region and rhizomes was much faster at 100% relative humidity in wolftail (*Carex cherokeensis* Schwein) (Burns et al. 1969). When the humidity

increased from 40 to 90%, 2,4-D uptake was 11% greater in grapes (*Vitis vinifera* cv. Lemberger), but translocation was not affected (Al-Khatib et al. 1992a).

Water Stress. Herbicide efficacy is influenced by drought stress in several herbicides because of reduced uptake and translocation. Plants under water or drought stress may display reduced herbicide uptake levels because of reduced stomatal openings and thicker or altered cuticle. Translocation may be reduced because of lower photo-assimilate production and transport as a result of reduced water availability and gas exchange. Short-term water stress typically has no effect on 2,4-D uptake but translocation levels are reduced. Uptake of 2,4-D acid was similar among turgidity levels ranging from 66 to 88% in common bean (*Phaseolus vulgaris* L.), but translocation from the treated leaf decreased when the soil moisture was at 13% (Basler et al. 1961). Similarly, 2,4-D trimethylamine salt uptake among various soil moisture contents did not differ, but translocation was twofold higher at 0.3-atm soil tension compared with 4 atm (Pallas and Williams 1962). In contrast to creating water stress through the soil, water stress in soybean [*Glycine max* (L.) Merr.] was created using a polyethylene glycol (PEG) solution (Kogan and Bayer 1996). When PEG was used, plant relative water content was reduced from 88 to 75% and leaf water potential was reduced from -0.55 to -1.21 MPa, resulting in 40% less 2,4-D foliar influx in soybean leaves under conditions that mimic water stress (Kogan and Bayer 1996). However, the short period of water stress induced by Kogan and Bayer using PEG and the lack of preconditioning that could alter the leaf anatomy may explain the contradictory results as compared with other moisture stress studies (Basler et al. 1961).

Plant Factors. Growth Stage and Environment. The age of the plant, leaf, and growing conditions can all affect herbicide uptake and translocation. Leaf age can influence herbicide uptake through the development and maturity of the cuticle, and the environment or growing conditions in which a plant develops can affect herbicide uptake and translocation. Since translocation of herbicides occurs in a source-to-sink fashion, translocation out of young, developing leaves may differ from

older, mature ones. The influence of leaf age and environment on 2,4-D uptake varies depending on species. Greater uptake was measured in immature leaves of common bean, pea (*Pisum sativum* L.), beet (*Beta vulgaris* L.), and sunflower (*Helianthus annuus* L.) than in mature leaves, but differences in uptake were not observed among leaf ages in corn (*Zea mays* L.) or cotton (*Gossypium hirsutum* L.) (Sargent and Blackman 1972). Developing leaves contain epicuticular wax that is less thick and has varying chemical composition compared with mature leaves, which can influence herbicide uptake (Baker and Hunt 1981). Less uptake was observed in 5-wk-old field bindweed (*Convolvulus arvensis* L.) seedlings compared with 6- and 16-wk-old mature plants, but greater translocation occurred in seedlings (Agbakoba and Goodin 1969). Grapes grown in the field had 26% less uptake compared with grapes grown in the greenhouse and was attributed to cuticle differences (Al-Khatib et al. 1992a).

Leaf and Cell Factors. Several features and characteristics of plant leaves can affect uptake and translocation of herbicides. Leaf angle, cuticle, pubescence, and other factors influence how much herbicide is absorbed by the plant, and the structure of plant cells within the leaf and stem can alter herbicide translocation patterns. Many studies have been conducted to determine the effects of cuticle thickness and chemical composition on 2,4-D uptake in various species. 2,4-D penetration was not correlated to cuticle thickness as measured in different species with varying tolerance levels (Norris 1974). A study of cuticle composition and chemistry found a wide variability among several plant species ranging in sensitivity, but a relationship with 2,4-D uptake was not found (Baker and Bukovac 1971). Even with varying leaf characteristics, differences in 2,4-D uptake were not found at 1, 3, or 7 d after application in alfalfa (*Medicago sativa*), grape, and pea (Al-Khatib et al. 1992a). It was initially theorized that 2,4-D uptake correlated with the amount of stomatal openings (Pallas 1960). However, this would require surface tensions that are below that of most herbicide formulations (Cobb and Reade 2010b) and the overall contribution of stomatal penetration to 2,4-D uptake is probably minor.

Translocation of 2,4-D follows the phloem-loading pathway and has been reviewed previously (Devine and Hall 1990). The general movement of

assimilates and phloem-mobile herbicides occurs from regions of carbohydrate synthesis (source) to regions of storage or utilization (Devine and Hall 1990). Transport is driven by a concentration gradient between the source and sink, resulting in the movement of water containing dissolved solutes and herbicides (Devine and Hall 1990). Herbicides and solutes can be transported either apoplastically (space between cells) or symplastically (directly between cells), resulting in different translocation rates and distribution (Devine and Hall 1990). 2,4-D displayed a strong symplastic movement in soybean and was found predominately in transit areas like the stem (Martin and Edgington 1981). 2,4-D is transported in the phloem via ion trapping (Riederer 2005). Weak acids, like 2,4-D, are trapped in the phloem because of the different pH levels between the phloem and xylem/apoplast (Riederer 2005). The phloem is more basic (pH = 8.5) relative to the apoplast (pH = 5.5), which causes the deprotonation of the weak acid to the negative ion, preventing it from crossing the cell membrane and trapping the ionic form in the phloem (Riederer 2005). Translocation of 2,4-D in this manner is similar to other systemic herbicides, but the presence of specific auxin herbicide carriers differentiates 2,4-D movement within the plant from most systemic herbicides. Inter- and intracellular indole-3-acetic acid (IAA) movement, and likely that of 2,4-D, is predominately dictated by auxin influx and efflux carriers (Enders and Strader 2015; Grones and Friml 2015). Within the cell, IAA concentration can be regulated by transporting IAA into the vacuole by the tonoplast-bound protein “walls are thin 1” (WAT1) (Grones and Friml 2015). Intercellular efflux carriers include members of the PIN-formed (PIN) and adenosine triphosphate (ATP)-binding cassette subfamily B (ABCB) families, and influx carriers include members of the auxin resistant 1/like aux1 (AUX1/LAX) family (Enders and Strader 2015). AUX1/LAX are plasma membrane bound only, whereas ABCB are plasma membrane and endomembrane localized and PIN carriers are found in the plasma membrane and endoplasmic reticulum (ER) (Enders and Strader 2015). The auxin influx carriers AUX1, LAX1, and LAX3 transport both IAA and 2,4-D, and the efflux carriers PIN2 and PIN7 can transport 2,4-D, whereas PIN1 cannot (Enders and Strader 2015). Fewer 2,4-D efflux

carriers relative to influx carriers allow the concentration of 2,4-D to increase within the cell. In tobacco cells (*Nicotiana tabacum*), the concentration of IAA was 118 nM compared with 1,106 nM 2,4-D concentration because of the lack of 2,4-D efflux from the cell (Delbarre et al. 1996). The 2,4-D concentration in isolated potato (*Solanum tuberosum* cv. Yukon) tissue was 15-fold that of the ambient solution, and efflux of 2,4-D out of the treated tissue was very slow (Martin and Edgington 1981). 2,4-D movement out of the cell was not detected and viewed as unlikely because of the low affinity of 2,4-D to the auxin efflux carrier and slow diffusion rates (Delbarre et al. 1996). Increasing the concentration of 2,4-D within the cell allows greater herbicidal activity.

Plant Species Effects. 2,4-D uptake and translocation can be affected by characteristics of each individual species. Many studies have documented the link between tolerance and sensitivity to 2,4-D uptake or translocation; in general, there is little correlation between sensitivity and uptake but a greater correlation with translocation (Pillmoor and Gaunt 1981).

The uptake of 2,4-D and sensitivity varies by species and results do not always correlate. The rate of uptake was slower in sensitive field bean than in wild oat (*Avena fatua* L.) (Holloway and Edgerton 1992). In contrast, tolerant oat (*Avena sativa* L.) had faster uptake than sensitive soybean but levels were equal by 24 h after application (Hall et al. 1982). Similar uptake levels were observed between tomato (*Solanum lycopersicum* Mill.) and eastern black nightshade (*Solanum ptycanthum*), even though eastern black nightshade showed greater tolerance (Hall and Swanton 1988). However, greater uptake of two different 2,4-D formulations was observed in sensitive pea than in tolerant eastern black nightshade (de Ruiter et al. 1993).

Though the link between uptake and tolerance is unclear, tolerant species tend to translocate less 2,4-D or distribute it differently as compared with sensitive species (Pillmoor and Gaunt 1981). After 24 h, only 5% of total radioactivity moved from the treated leaf in tolerant oats compared with 55% in sensitive soybean, and more C¹⁴-material was found in the roots (14.5%) and growing points (22.7%) in soybean compared with oats (3.1 and 1.6%, respectively) (Hall et al. 1982). Metabolism differences between tolerant and sensitive species (dis-

cussed in a later section) could be an explanation for the observed difference in translocation. Rapid conversion of 2,4-D to metabolites that can be sequestered in the cells of tolerant species prevents the translocation of the active herbicide or metabolites compared with sensitive species. The intermediate lipophilicity and weak acid nature of 2,4-D acid enable its movement in both the xylem and phloem. On the other hand, conjugation of 2,4-D with various plant compounds can alter these characteristics and limit movement (Cobb and Reade 2010b). Conjugation of 2,4-D is discussed later in this review.

Another means of tolerance to 2,4-D for some species is root exudation. Research on jimsonweed (*Datura stramonium*), honeyvine milkweed [*Ampe-lamus albidus* (Nutt.) Britt.], and Canada thistle (*Cirsium arvense*) have suggested that these species are more tolerant to 2,4-D by excreting the herbicide into the soil (Coble and Slife 1971; Fites et al. 1964; Turnbull and Stephenson 1985). Similarly, eastern black nightshade exuded 28% of applied 2,4-D into the soil compared with only 7% in tomato, and the extracted 2,4-D from the soil was unaltered (Hall and Swanton 1988).

Chemical and Application Factors. *Formulation.* The formulation of a herbicide can influence herbicide uptake and translocation. There are several formulations of 2,4-D that have been discussed in this review. Generally, uptake of 2,4-D ester is more rapid than amine formulations. In big leaf maple (*Acer macrophyllum* Pursh), uptake was greatest with the ethyl-hexyl ester formulation compared with the triethanolamine formulation (Norris and Freed 1966). Uptake of 2,4-D iso-octyl ester was 2.4 times and 1.3 times greater in eastern black nightshade and pea, respectively, than the trolamine salt (2,4-D plus 2-hydroxyethyl amine), but after 24 h, there was no difference in translocation among the formulations (de Ruiter et al. 1993). 2,4-D uptake was greater with long-chain amines, such as tetracyclamine and dodecylamine, compared with short-chain amines like dimethylamine salt in sunflower (Quehee and Sutherland 1973).

Effect of Adjuvants. Adjuvants improve herbicide uptake by increasing leaf wetting, reducing herbicide droplet surface tension, improving leaf surface and droplet contact, and many others. In relation to

2,4-D, adjuvants improve uptake but the observed effect on translocation is not as significant. Depending on the formulation of 2,4-D, adjuvants can either increase or decrease uptake. Adjuvants increased the uptake of several 2,4-D formulations (amine, sodium salt, and isopropyl ester) in soybean and corn (Hauser 1955). However, adding the surfactant Armoblen 600 increased uptake of the trolamine salt of 2,4-D but reduced uptake of the iso-octyl ester formulation (de Ruiter et al. 1993). Uptake of 2,4-D acid in honeyvine milkweed increased seven- to eightfold when 1.0% Tween 80 was added, and 2,4-D translocation also increased with the adjuvant but the change was not as significant as the increase in uptake (Coble et al. 1970). Similarly, addition of adjuvant increased uptake of 2,4-D trolamine salt by 4.8-fold in eastern black nightshade and 1.7-fold in pea (de Ruiter et al. 1993). Translocation was not affected when an adjuvant was included with 2,4-D trolamine salt or 2,4-D iso-octyl ester (de Ruiter et al. 1993).

Research has also been conducted on what adjuvant qualities influence 2,4-D uptake. Uptake was 19% greater with the use of a mixture of organosilicone and acetylinic diol ethoxylate surfactants compared with crop-oil concentrate in leafy spurge (*Euphorbia esula*) (Thompson et al. 1996). Ethylene oxide (EO) content ($-\text{CH}_2\text{CH}_2\text{O}-$) was inversely related to 2,4-D uptake (Thompson et al. 1996), and surfactants with EO of 5 had greater uptake (85%) in broad bean (*Vicia faba* L.) compared with EO 10 (61%) and EO 14 (50%) (Liu 2004). A lower EO value corresponds to a shorter EO chain and less polar surfactant molecule (Riechers et al. 1995). Uptake of 2,4-D increased when including a C13/C15 (i.e., 13- or 15-carbon chain per molecule, where more carbons make the surfactant more hydrophobic) alkanol surfactant compared with a C10 alkanol, and uptake was minimally increased using an octylphenol adjuvant (Liu 2004). However, 2,4-D uptake was not affected by surfactants with an EO value ranging from 6 to 18 in wild oat and bean in another study (Holloway and Edgerton 1992). The complexities of adjuvant properties, leaf properties, and interactions between them make it difficult to generalize about the effect of adjuvants on 2,4-D uptake.

Application Methods. The goal of a herbicide application is to deliver the active ingredient to

the target weed. This can be influenced by several factors including the size of the spray droplets and carrier volume. These factors can affect herbicide uptake by determining the spray coverage of the target, altering the concentration of the active ingredient in the droplet, and influencing droplet impact and retention on the leaf surface (Knoche 1994). Reducing the droplet size may increase 2,4-D efficacy in several weeds, which was also documented with other auxin herbicides (dicamba and MCPA), but droplet size did not affect glyphosate efficacy (Knoche 1994). Decreasing carrier volume did not affect 2,4-D performance, similar to many other systemic herbicides, but did improve glyphosate performance (Knoche 1994). An optimum droplet size, carrier volume, or ae concentration to maximize 2,4-D uptake in fava bean was not determined, and increasing the amount of 2,4-D applied reduced the efficiency of uptake (Stevens and Bukovac 1987). Similarly, 2,4-D dimethylamine uptake was not influenced by droplet size, but translocation decreased as droplet size increased in oriental mustard (*Sisymbrium orientale*) (Wolf et al. 1992). However, 2,4-D acid uptake was shown to be greatest with smaller droplets (0.5 μL compared with 10 μL) and with a larger total volume (100 μL compared with 10 μL) applied to the leaf of common bean (Knoche and Bukovac 1999). The concentration of 2,4-D in droplets did not affect uptake, but translocation was reduced by 10 to 14% when the concentration increased eightfold (Wolf et al. 1992). The true impact of droplet size and carrier volume on 2,4-D uptake and translocation may be difficult to determine because of differences between study methods used (i.e., nozzle types, spray pressure, application equipment, etc.) as well as differences between target plants used in the various studies.

pH. The pH of the spray solution can affect herbicide uptake and translocation. Altering the pH can dictate the ionic state of the herbicide molecule, and the movement of a charged molecule across the cuticle and membranes is more difficult than an uncharged molecule. The pH of the spray solution affects uptake to a greater degree than translocation since the ionic state of the molecule will be dictated by the interior of the plant. Greater 2,4-D uptake at pH 3 than pH 5 was measured in bean and sunflower cotyledon leaf surfaces, and the change in pH had a more dramatic effect on uptake in

sunflower (Szabo and Buchholtz 1961). Absorption of 2,4-D was greater at pH 3.5 compared with pH 8.5, and translocation (expressed as the percent absorbed by the plant) to the roots was greater at the lower pH in skeleton weed (*Chondrilla juncea* L.) (Greenham 1968).

Summary of Uptake and Translocation. Many factors can alter the uptake and translocation of 2,4-D but only a few have been shown to have a consistent effect. 2,4-D uptake is greater under conditions of higher temperatures and humidity. Water stress does not affect uptake, but translocation is reduced. Adjuvants, lower pH, and ester formulations of 2,4-D can increase uptake but translocation is generally not affected. 2,4-D uptake has little correlation to cuticle thickness or composition and plant sensitivity, but sensitive species translocate more 2,4-D than tolerant species. Future physiological research with 2,4-D in genetically engineered 2,4-D-resistant crops, in comparison with nontransformed isogenic lines differing in only the transgene, will determine if these factors influencing uptake and translocation of 2,4-D are consistent.

Plant Metabolism of 2,4-D

Research on the metabolism of 2,4-D has been extensively studied and reviewed since the 1950s (Loos 1969; Pillmoor and Gaunt 1981; Robertson and Kirkwood 1970; Sandermann et al. 1984). Recent reviews and information on 2,4-D and herbicide metabolism include Cobb and Reade (2010a) and Hatzios et al. (2005). Herbicide selectivity in many cases is dependent on plant metabolism. Plants usually metabolize herbicides via processes that convert the parent molecule to more polar products and insoluble residues (Hatzios et al. 2005). Interestingly, metabolic pathways of 2,4-D in sensitive and tolerant species share some commonalities. Although sensitive species in some cases may actually metabolize 2,4-D faster than tolerant species, the metabolites produced may be readily converted back to the parent acid. On the other hand, tolerant species usually produce metabolites of 2,4-D that are nonphytotoxic and irreversible. The metabolites formed during 2,4-D metabolism between sensitive dicots and tolerant monocots are similar, but vary in the amount of each metabolite formed, resulting in lower 2,4-D

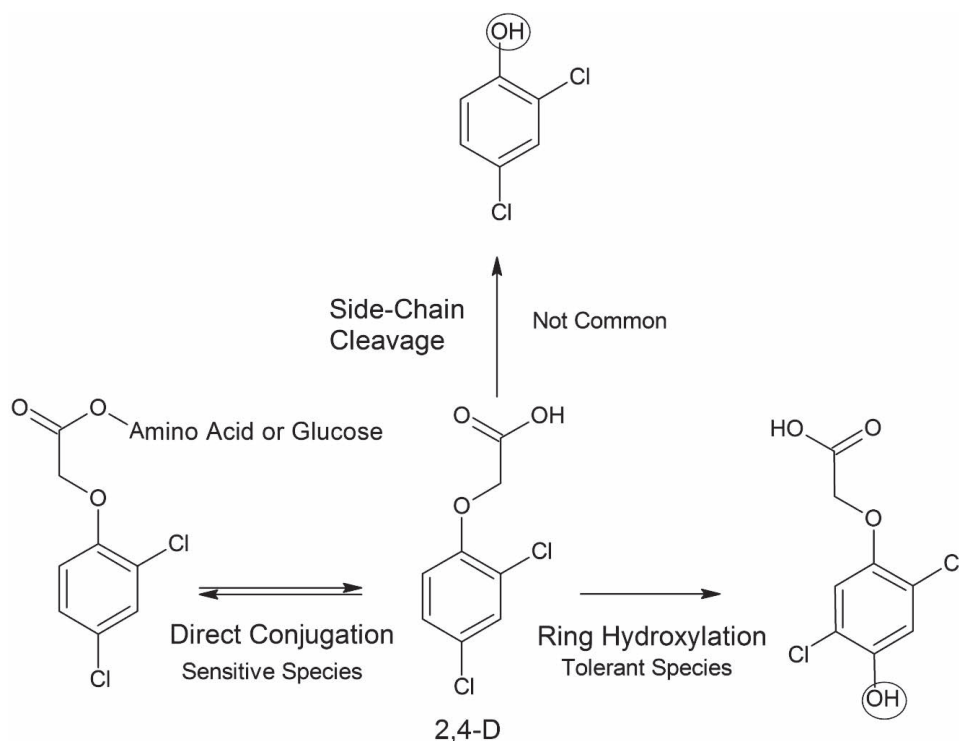


Figure 1. Pathways for metabolism of 2,4-D in higher plants.

concentrations in tolerant monocots compared with dicots (Pillmoor and Gaunt 1981). For example 2,4-D and IAA are both metabolized to temporary, reversible metabolites in dicots by modifying the carboxylic acid group, which can be converted back to active forms (Davidonis et al. 1980) by de-esterification. However, the selectivity of 2,4-D in monocots is primarily related to the formation and sequestration of nontoxic and permanent metabolites, typically by modifications of the phenyl or heterocyclic ring (Feung et al. 1975).

The objectives of this section are to summarize 2,4-D metabolism, highlighting the various pathways, enzymes involved, metabolites formed, and differences between species. Figure 1 depicts the three metabolic pathways, metabolites formed during 2,4-D metabolism, and hydroxyl groups (circled) capable of further metabolism through conjugation that will be discussed in this section. Metabolism of 2,4-D may be another mechanism for 2,4-D resistance in certain populations, but these populations and resistance mechanism will be discussed in a later section. The bioactivation of ester formulations of 2,4-D by carboxylesterase enzymes is another step in metabolism of 2,4-D ester that has been recently reviewed (Gershater et

al. 2006, 2007; Gershater and Edwards 2007), but only the metabolism of 2,4-D acid will be discussed in this section.

Side-Chain Cleavage. The cleavage of the side chain of 2,4-D has been observed in many plants, but only in a few species does it play a major role in metabolism, including red currant (*Ribes sativum* Syme), apple (*Malus domestica*), strawberry (*Fragaria × ananassa*), and garden lilac (*Syringa vulgaris*) (Loos 1969). Side-chain degradation occurs through a single oxidation to yield glycolic acid and 2,4-dichlorophenol (Pillmoor and Gaunt 1981). A result of side-chain degradation and a means of measuring 2,4-D metabolism by this pathway is a loss of carbon dioxide. A range of 7 to 33% was observed in the few species that predominantly utilize this pathway compared with < 1 to 2% in corn, soybean, cotton, and several other species (Loos 1969).

Direct Conjugation. The direct conjugation of 2,4-D with amino acids and glucose is a mechanism for metabolism that has been studied extensively (Feung et al. 1973, 1975, 1978; Hamilton et al. 1971; Montgomery et al. 1971). Amino acids, mainly glutamate and aspartate, and glucose can be

directly conjugated to the carboxylic acid group of 2,4-D to form amino acid conjugates or 2,4-D-glucose esters. The formation of amino acid conjugates dominates in soybean (Sandermann et al. 1984) and other sensitive dicots (Hatzios et al. 2005). Direct conjugation of IAA with amino acids occurs with the *GH3* gene, but this same enzyme cannot conjugate 2,4-D as a substrate (Staswick et al. 2005). The concentration of amino acid conjugates is much greater in comparison with other metabolites formed by dicots and the amount found in monocots. Amino acid conjugates are the first metabolite to form in dicots, with glutamate conjugation appearing initially, but over time the 2,4-D-glutamate conjugate is converted to other metabolites, including the 2,4-D-aspartate conjugate and sugar conjugates (Pillmoor and Gaunt 1981). Direct glucose conjugation of 2,4-D occurs with glucosyltransferase (GT) enzymes to form glucose esters (Hatzios et al. 2005), and only glucose is used as the form of sugar (Pillmoor and Gaunt 1981). Though glucose is the only form of sugar used in direct conjugation, other sugars are utilized to form larger macromolecules in ring hydroxylation discussed later in this section. Generally, the amino acid or glucose ester conjugates are more prevalent in sensitive dicots (Hatzios et al. 2005), induce auxin-related activity similar to 2,4-D (Feung et al. 1974), and are readily hydrolyzed back to 2,4-D acid (Pillmoor and Gaunt 1981). This pool of active 2,4-D and reversible 2,4-D conjugates allows the herbicide to exert its effects on these species. However, 2,4-D conjugates have been recovered from the vacuoles of dicots (Sandermann et al. 1984). This may potentially reduce herbicidal activity in species where this occurs.

Ring Hydroxylation. Tolerant monocots metabolize 2,4-D predominately through a ring hydroxylation reaction. A hydroxylation at the carbon-4 position on the aromatic ring of 2,4-D results in a migration or shift of the chlorine atom to the carbon-3 or carbon-5 position (Cobb and Reade 2010a; Loos 1969; Pillmoor and Gaunt 1981). Ring hydroxylation occurs through a reaction with cytochrome P450 (P450) enzymes (Hatzios et al. 2005), an enzyme family that is involved in the metabolism and detoxification of several herbicides. The main metabolites formed from ring hydroxylation of 2,4-D are 4-hydroxy-2,5-dichlorophenoxyacetic acid and 4-hydroxy-2,3-dichloro-

phenoxyacetic acid, and these metabolites are more readily observed in monocots than in dicots (Sandermann et al. 1984). Other hydroxylated metabolites have been observed, but 4-hydroxy-2,5-D is the most common (Feung et al. 1973, 1975, 1978; Hamilton et al. 1971; Montgomery et al. 1971). Formation of *O*-glucosides of the ring-hydroxylated metabolites by GT enzymes occurs rapidly after hydroxylation (Hatzios et al. 2005), as indicated in Figure 1. After glycosylation, metabolites can be further conjugated with other sugars, including malonic acid, to form larger structures. Malonylation occurs with *O*-malonyltransferase (*O*-MAT) enzymes and may help to stabilize conjugates against cellular digestion and signals for the removal of the conjugates into the vacuole or across the plasma membrane (Hatzios et al. 2005). Products from the ring hydroxylation metabolic pathway are more hydrophilic, non-phytotoxic, and polar compared with 2,4-D and cannot be hydrolyzed back to 2,4-D (Cobb and Reade 2010a). These nonphytotoxic, nonreversible ring hydroxylates are more readily sequestered than other types of metabolites in various locations, including the vacuole, or incorporated with structural polymers like lignin, pectin, and cellulose (Hatzios et al. 2005; Sandermann et al. 1984). Sensitive dicots form these same metabolites, but usually at much lower concentrations (Feung et al. 1978), indicating the greater utilization of other metabolic pathways.

Summary of Plant Metabolism of 2,4-D. Plant metabolism of 2,4-D occurs primarily through direct conjugation and ring hydroxylation, and to a smaller extent, side-chain cleavage (Figure 1). Herbicide selectivity is derived from what metabolic pathway is utilized by each plant species. The direct conjugation of 2,4-D with amino acids or glucose results in phytotoxic metabolites that can be hydrolyzed to 2,4-D and is more common in sensitive dicots. Ring hydroxylation of 2,4-D leads to a non- or partially phytotoxic metabolite that is permanent, can be further metabolized by GT and *O*-MAT enzymes, and is more common in tolerant monocots. In both dicots and monocots, metabolites and free 2,4-D can be found incorporated with structural polymers, but only metabolites are found in the vacuole. Future research to investigate the metabolism of 2,4-D in genetically engineered 2,4-D-resistant crops, compared with nontransformed

varieties, may reveal different rates of metabolism, enzymes utilized, and metabolites formed between these isogenic lines.

Mode of Action

Three Classes of Auxin Receptors. Since a previous review of auxin biology and signaling in relation to synthetic auxin herbicides (Mithila et al. 2011), a new category of auxin receptor protein has been identified, bringing the total number of known auxin receptors to three (Grones and Friml 2015; Salehin et al. 2015). These three auxin receptor or coreceptor systems have been characterized and their contributions to auxin-mediated signaling have been clarified in recent years (Grones and Friml 2015). The three proposed auxin receptors include: (1) auxin-binding protein 1 (ABP1) (Shi and Yang 2011; Tromas et al. 2010), localized predominantly at the ER and outer cell membrane/apoplast interface; (2) auxin-signaling F-box (TIR1/AFB) receptor protein homologs, localized in the nucleus (Dharmasiri et al. 2005; Guilfoyle 2007; Kepinski and Leyser 2005; Tan et al. 2007; Wang and Estelle 2014); and most recently (3) S-phase kinase-associated protein 2 (SKP2), also localized to the nucleus, which connects auxin signaling responses with cell division (Jurado et al. 2010). In addition to possessing different subcellular localizations, these three auxin receptors differ in their proposed functional roles in cell expansion, cell division, and regulating plant developmental processes (reviewed by Zazimalova et al. 2014, and chapters therein).

Until recently, the functions and roles of the plasmalemma- and ER-localized ABP1 protein in auxin perception and signaling responses had been well documented and established in *Arabidopsis* (Shi and Yang 2011; Tromas et al. 2010), but recently it was discovered that ABP1 is not an essential component for the auxin (IAA and 1-naphthaleneacetic acid [NAA])-signaling pathway, auxin-responsive gene expression, or regulation of *Arabidopsis* development under normal growth conditions (Gao et al. 2015). Additionally, a recent study reinvestigated the auxin-like phenotypic effects presumed to result from the mutant allele *abp1-5* in *Arabidopsis* (Enders et al. 2015). Upon sequencing the entire genome of the *abp1-5* line, researchers found additional unlinked site muta-

tions (i.e., not related to *abp1-5*) that may have contributed to these phenotypes (Enders et al. 2015), thereby casting more doubt on the actual role and function of ABP1 in auxin perception and signaling. These new results indicate that the roles of ABP1 in plant growth, development, and auxin-responsive gene expression (under normal and stressed conditions) require a re-examination and further research (Enders et al. 2015; Gao et al. 2015; Grones et al. 2015), particularly pertaining to the putative role of ABP1 in synthetic auxin herbicide responses in dicot weeds or as a possible mechanism of resistance to auxin herbicides (Mithila and Hall 2005; Mithila et al. 2011). However, since the role of ABP1 in IAA or 2,4-D signaling has not been established in dicots other than *Arabidopsis*, a brief discussion is presented below to summarize results that were reported before these recent contradictory papers (Enders et al. 2015; Gao et al. 2015; Grones et al. 2015).

Although primarily located on the ER, ABP1 is proposed to bind and perceive auxin in the slightly acidic apoplast surrounding the cell, where it may be involved with the rapid regulation of membrane potential and ion fluxes at the plasma membrane that govern auxin-induced cell expansion (Grones and Friml 2015). Recent findings have linked auxin perception on the cell surface via ABP1 with signaling in the cytosol via the identification of a transmembrane ABP1-interacting partner, a plasma membrane-localized transmembrane receptor-like kinase (reviewed by Grones and Friml 2015), to form a cell-surface, auxin-sensing complex that may regulate known auxin-dependent responses downstream of ABP1 binding (Chen et al. 2012; Grones and Friml 2015). Although ABP1 is mainly proposed as a key factor in regulating fast, nontranscriptional responses at the cell surface (Peer 2013), it may also be possible that ABP1 influences auxin-regulated transcriptional responses in the nucleus (Peer 2013; Shi and Yang 2011), as discussed below for the TIR1/AFB protein auxin receptor class.

The TIR1/AFB protein family of nuclear auxin receptors, in conjunction with binding auxin or 2,4-D as a “molecular glue,” are involved in a novel mechanism whereby transcription factors that repress auxin-responsive gene expression (Aux/IAAs) are rapidly degraded when auxin concentrations are high (Korasick et al. 2015; Salehin et al.

2015; Tan et al. 2007; Wang and Estelle 2014), leading to transcriptional activation of many auxin-responsive genes via “release from repression” (Pierre-Jerome et al. 2013). Recent progress has been made in establishing which of the *Arabidopsis* TIR1/AFB protein receptors are likely involved with synthetic auxin herbicide binding (Gleason et al. 2011 and reviewed by Enders and Strader 2015) and the structural basis for differential auxin binding (Lee et al. 2014). For example, AFB4 and AFB5 proteins are more distinct (from a primary sequence standpoint) in comparison with TIR1, and AFB5 has been implicated in preferentially binding the pyridine carboxylic acids (Lee et al. 2014; Walsh et al. 2006), quinclorac (Lee et al. 2014), and dicamba (Gleason et al. 2011), whereas TIR1, AFB1, AFB2, and AFB3 are thought to bind 2,4-D preferentially (Walsh et al. 2006). Interestingly, the auxin-transport mutant line *axr4-2* displayed resistance to 2,4-D but sensitivity to dicamba in *Arabidopsis* plants, and the AFB mutant lines *tir1-1* and *afb5* were resistant to dicamba, but only the *tir1-1* line was resistant to 2,4-D (Gleason et al. 2011). In addition, the double mutant line *tir1-1/afb5* exhibited an additive effect on dicamba resistance (Gleason et al. 2011). Thus, the differential binding affinities of synthetic auxin herbicides for auxin transporters or nuclear auxin receptors may lead to differences in the level of tolerance among plant species or broad or specific patterns of cross-resistance in dicot weeds, depending on which mutation(s) in which AFB(s) or auxin transport protein(s) are selected under field conditions (Mithila et al. 2011; Walsh et al. 2006).

The most recently discovered auxin receptor, SKP2, is involved in the degradation of nuclear transcription factors during the cell cycle process (Jurado et al. 2010). During the cell cycle, some transcription factors and other proteins need to be degraded for initiation of the next phase (Peer 2013). Binding of SKP2a protein with auxin enhances the interaction between SKP2a and cell division-related transcription factors, thus promoting their degradation and allowing cell division to proceed (Grones and Friml 2015). Overall, in addition to the TIR1/AFB-dependent auxin perception mechanism (Korasick et al. 2015), SKP2 might provide an alternative pathway that contributes to the final response to auxin in the nucleus (Grones and Friml 2015; Peer 2013).

Differences in Plant Responses to IAA and 2,4-D. In *Arabidopsis* seedlings, the inhibitory effects of IAA and its homolog 2,4-D occur at similar concentrations; however, the dose–response curves to IAA and 2,4-D are quite different (Enders and Strader 2015). For example, inhibition of root elongation in response to 2,4-D (at low nanomolar concentrations) initially occurs in a steeper dose–response curve than with IAA, suggesting that dicot responses to IAA are slightly attenuated in comparison with 2,4-D (Enders and Strader 2015). Three possible explanations may account for the differences in whole-plant responses to IAA and 2,4-D (Enders and Strader 2015), including (1) differences in auxin perception, (2) differences in cellular transport mechanisms, and (3) differences in auxin metabolism and homeostasis (or detoxification reactions in the case of 2,4-D). Since differences in cellular transport mechanisms and auxin perception do not appear to be large enough to account for the higher sensitivity of *Arabidopsis* roots to 2,4-D relative to IAA (i.e., 2,4-D is typically a poorer substrate for these processes), it appears most likely that qualitative and quantitative differences in auxin metabolism and mechanisms to maintain auxin homeostasis account for whole-plant differences in sensitivity between 2,4-D and IAA (Enders and Strader 2015; Kelley and Riechers 2007; McSteen 2010; Sterling and Hall 1997). For example, it has been postulated that detoxification of 2,4-D by phenyl-ring hydroxylation followed by glucose conjugation confers tolerance in grasses, whereas sensitive dicots can only perform reversible conjugation reactions with amino acids or sugars to modify the carboxylic acid group (Mithila et al. 2011; Staswick et al. 2005). Moreover, for each auxin receptor and cellular auxin transporter examined to date (Enders and Strader 2015), IAA is a better substrate than 2,4-D or has equal or greater binding affinity than 2,4-D (Lee et al. 2014; Tan et al. 2007).

Long-distance transport studies directly comparing the basipetal and acropetal transport of radiolabeled IAA and 2,4-D would greatly facilitate our understanding of the overall differences in individual auxin influx and efflux transporters (Grones and Friml 2015; Swarup et al. 2008; Yang and Murphy 2009), and how these differences may affect inter- and intracellular transport and dicot sensitivity to natural and synthetic auxins (Enders

and Strader 2015). As discussed earlier in this paper, within the cell, IAA concentration is regulated by transport to the vacuole by the tonoplast-bound protein WAT1 (Grones and Friml 2015). Intercellular auxin transport and auxin herbicide efficacy are affected by specific efflux carriers mentioned in the earlier discussion of 2,4-D translocation. Much less information has been reported for transporters within plant cells and tissues that are specific to other auxin herbicides such as dicamba, but as mentioned previously, the *Arabidopsis* auxin-transport mutant line *axr4-2* was resistant to 2,4-D but sensitive to dicamba (Gleason et al. 2011).

An interesting group of compounds called “phytotropins” inhibits the cellular efflux of IAA (facilitated by the PIN auxin-efflux carrier proteins) and synthetic auxins from the cell, and consequently their cell-to-cell polar transport away from plant meristems (Grossmann et al. 2002; Subramanian et al. 1997). This creates abnormally high auxin concentrations in growing tissues of meristematic shoot and root regions (Grossmann et al. 2002). Research reports in the published literature regarding herbicidal compounds with phytotropin activity, such as diflufenzopyr, have only reported synergistic activity with the synthetic auxin herbicides dicamba, quinclorac, and picloram (Grossmann et al. 2002), or the effect of the semicarbazone SCB-1 on cellular efflux of the synthetic auxin NAA (Subramanian et al. 1997). The insight provided by comparative whole-plant, long-distance translocation studies with IAA, NAA, dicamba, picloram, and 2,4-D would be enhanced greatly if specific PIN inhibitors could be identified that inhibit the basal efflux of phenoxyacetic acid herbicides in plant cells.

Physiological Activity. The derepression of auxin-response genes as described above initiates a cascade of physiological responses within the plant, ultimately leading to plant death in sensitive dicots. A review of auxin herbicide mode of action (Grossmann 2010) described in detail the physiological responses of sensitive dicots in three phases: stimulation, inhibition, and decay. The stimulation phase begins within minutes, characterized by a large increase in synthetic auxin concentration directly after the herbicide application, and plant symptoms become evident within a few hours. Ethylene production is stimulated and abscisic acid (ABA) hyperaccumulates during this phase, as well

as metabolic activation of ion channels and ATPases (Grossmann 2010). Visual symptoms of abnormal, unregulated growth, such as epinasty, tissue swelling, and stem twisting, begin to appear within hours. In the inhibition phase, excess production of reactive oxygen species, stomatal closure, and induction of physiological defense–stress responses reduce the overall production of amino acids, starches, nucleic acids, and other primary metabolites needed for growth and development. This unregulated cascade of events results in cessation of growth and ultimately onset of the decay phase, which occurs within 3 d after application, consisting of tissue/cell damage and plant death resulting from destruction of chloroplasts, membranes, and the plant vascular system (Grossmann 2010).

Recent molecular physiology research has identified several auxin-responsive genes (*SIIAA15* and *SIIAA29*) belonging to the AUX/IAA transcriptional repressor family of proteins in tomato in response to 2,4-D, ABA, and ethylene (Xu et al. 2015). These results may provide further insight into the mechanism of action of 2,4-D (Grossman 2010) and the interactions among auxins, ethylene, and ABA signaling pathways leading to phytotoxicity in sensitive dicots, as summarized above. Interestingly, overexpression of *SIIAA15*, but not *SIIAA29*, in transgenic tomato plants induced a phenotype similar to that following 2,4-D treatment, including epinasty and altered stomatal cell differentiation and leaf densities (Xu et al. 2015). In addition, transgenic lines either overexpressing (via 35S promoter) or underexpressing (via RNA interference) *SIIAA15* demonstrated altered sensitivities to exogenous applications of ABA, as measured by root elongation assays, but did not display altered phenotypes in response to ethylene. These recent findings indicate that 2,4-D-induced expression of *SIIAA15* may play an important role in mediating the downstream phytotoxic effects of 2,4-D or other synthetic auxin herbicides in tomato (Xu et al. 2015), in concert with the unregulated physiological effects triggered by several plant hormones (Grossman 2010).

Given these complex plant signaling mechanisms, phytohormone responses, and altered gene expression effects, applications of 2,4-D at sublethal doses or to relatively insensitive species such as grasses can still elicit a variety of physiological responses through up-regulation or down-regulation of vari-

ous enzymes. Some of these effects are observable when 2,4-D is used in conjunction with other herbicides or pesticides.

Antagonism of POST Grass Weed Control. Many cases of herbicide antagonism related to foliar applications of 2,4-D tank mixes have been reported with acetyl coenzyme A carboxylase (ACCase) inhibitors, in particular diclofop-methyl activity on wild oat, cultivated oats, and ryegrass (*Lolium rigidum*) (Fletcher and Drexler 1980; Kafiz et al. 1989; Olson and Nalewaja 1981; Shimabukuro and Hoffer 1991; Todd and Stobbe 1980). However, studies that investigated the combined effects of root-applied diclofop-methyl and 2,4-D on wheat, wild oat, or cultivated oat root growth did not report antagonism of diclofop-methyl activity (Jacobson et al. 1985; Todd and Stobbe 1980). The grass antagonism of 2,4-D against diclofop-methyl activity has been shown to be less with ester formulations of 2,4-D as compared with amines (Gillespie and Nalewaja 1989; O'Sullivan et al. 1977). Gillespie and Nalewaja (1989) attributed this difference in part to less diclofop uptake in combination with 2,4-D amine as compared with the ester. Subsequent work with haloxyfop (Mueller et al. 1990) demonstrated greater metabolism and less translocation of active haloxyfop in johnsongrass [*Sorghum halepense* (L.) Pers.] when mixed with 2,4-D. It is well documented that treatments of 2,4-D increase the expression of P450 genes in rice (*Oryza sativa* L.) (Hirose et al. 2007) and wheat (*Triticum aestivum* L.) (Pasquer et al. 2006), and up-regulate the metabolic activities of wheat P450 enzymes that hydroxylate lauric acid at various positions (lauric acid hydroxylases; LAH) as a natural substrate (Adele et al. 1981; Mougouin et al. 1991; Salaün et al. 1986; Zimmerlin et al. 1992) or ring-methyl-hydroxylate and *N*-demethylate the photosystem II-inhibitor chlorotoluron (Mougouin et al. 1991). Interestingly, one of the wheat LAH activities also metabolizes diclofop acid, leading to its rapid detoxification and tolerance in wheat (Forthoffer et al. 2001; Helvig et al. 1996; Zimmerlin and Durst 1992). Although a direct cause-effect relationship has not been established between 2,4-D induction of specific LAH activities in wheat and diclofop-acid hydroxylation activities in wheat, it is plausible that 2,4-D induces the expression and activity of the same P450 that hydroxylates both lauric acid and diclofop-acid in

wheat and possibly in other grasses as well (Barrett 1997), such as *Lolium* and *Alopecurus* spp., leading to antagonism.

Foliar applications of 2,4-D can also lead to more rapid metabolic detoxification of other herbicidal active ingredients, including ACCase inhibitors or acetolactate synthase (ALS) inhibitors (Han et al. 2013; Preston et al. 1996; Simpson et al. 1994). In the case of obtaining grass weed control with POST applications of ACCase inhibitors or ALS inhibitors, this type of interaction is antagonistic in that 2,4-D decreases the activity of the herbicide being used to control grasses by stimulating P450-based metabolism, thereby mimicking the metabolic mechanisms that frequently confer grass weed resistance or cereal crop tolerance (Han et al. 2013; Yu and Powles 2014). However, this stimulation of herbicide metabolism in grasses by 2,4-D appears to be temporary since the antagonistic effects are not evident if 2,4-D and POST graminicide applications are separated by 24 to 72 h (Mueller et al. 1990).

Physiological research has reported conflicting effects of 2,4-D on de-esterification of diclofop-methyl to the active diclofop-acid metabolite, but in general most laboratory studies indicate that 2,4-D increases the rate of detoxification of diclofop-acid to more polar, conjugated metabolites in conjunction with decreased translocation to meristematic tissues (Han et al. 2013; Hill et al. 1980; Shimabukuro and Hoffer 1991; Todd and Stobbe 1980). In addition to antagonism of grass control with ACCase inhibitors, 2,4-D also antagonized glyphosate activity on johnsongrass, a perennial grass, when applied in a tank mix with 2,4-D or dicamba (Flint and Barrett 1989a). Since glyphosate is a nonselective herbicide and undergoes limited metabolism in plants, the mechanism described for antagonism by 2,4-D in johnsongrass was related to decreased glyphosate uptake and translocation to roots (Flint and Barrett 1989a).

An area of research that warrants more attention is an investigation of the potential effects of 2,4-D applications on the activity of other pesticides applied POST, such as insecticides, fungicides, or nonauxinic plant growth regulators, or vice-versa (Taton et al. 1988). In theory, if 2,4-D increases the activity of P450s or other pesticide-degrading enzymes (analogous to herbicide safeners) (Riechers et al. 2010), then biokinetic analyses of foliar-

applied crop protection chemicals may require closer inspection, especially in soybean and cotton following commercialization of new 2,4-D-resistant crops (Wright et al. 2010).

Synergism of POST Broadleaf Weed Control. In contrast to antagonism of POST grass control, 2,4-D and dicamba have been shown to synergize the POST activity of glyphosate for control of perennial field bindweed (Flint and Barrett 1989b). As noted earlier, glyphosate is not metabolized significantly in plants but 2,4-D has POST activity on dicot weeds, so the mechanism for achieving a synergistic interaction may be more complex than in grass weeds. Research with field bindweed demonstrated that the combination of 2,4-D and glyphosate in a tank mix increased uptake of 2,4-D as well as increased the translocation of 2,4-D and glyphosate into the roots (Flint and Barrett 1989b). However, it was not stated in this study whether or not an adjuvant was included in these tank mixes. Field and greenhouse studies have shown that cationic, tallow amine surfactants optimize foliar uptake and activity of glyphosate (Riechers et al. 1995); as a result, it may be possible that these cationic surfactants also increase 2,4-D or dicamba uptake when applied in tank mixes, leading to the observed synergistic interactions in dicot weeds.

Crop Safening with 2,4-D Tank Mixes. As described above, 2,4-D applications can increase the activity of P450 enzymes in grasses, leading to more rapid metabolism of other active ingredients. In the case of a crop species this is a desirable herbicide interaction in that 2,4-D can protect from herbicide injury by increasing rates of herbicide detoxification, similar to the mechanism of action of herbicide safeners (Riechers et al. 2010). For example, organophosphate (OP) insecticides are often used in weed resistance studies to inhibit P450 enzymes that metabolize herbicides to assist in determining the nature of the resistance mechanism (Ma et al. 2013; Preston et al. 1996; Yu and Powles 2014). However, from the standpoint of crop safety the inhibition of P450 enzymes that metabolize herbicides, such as ALS inhibitors, results in herbicide synergism that increases crop injury and the potential for yield losses (Biediger et al. 1992; Kreuz and Fonné-Pfister 1992). By applying a tank-mix treatment containing 2,4-D and nicosulfuron to maize seedlings (previously treated with a soil

application of the OP insecticide terbufos), the injury caused by the terbufos–nicosulfuron interaction was prevented (Simpson et al. 1994). Thus, 2,4-D can act as a herbicide safener when P450 levels are not high enough in the crop to metabolize other herbicide active ingredients. As an example, grain sorghum [*Sorghum bicolor* (L.) Moench ssp. *bicolor*] injury from metsulfuron was decreased without a concurrent decrease in ivyleaf morning-glory (*Ipomoea hederacea* Jacq.) or velvetleaf (*Abutilon theophrasti* Medik.) control when tank mixed with 2,4-D or dicamba (Brown et al. 2004).

Weed Resistance to 2,4-D

Number of Dicot Weed Species and Recent Trends. There are 32 reported cases of weeds resistant to the synthetic auxin group of herbicides (Heap 2015), although some grass species are only resistant to quinclorac and will not be further discussed here. Among these 32 cases of synthetic auxin-resistant weeds are 28 distinct cases that describe resistance to 2,4-D: either 2,4-D alone, 2,4-D plus other synthetic auxin herbicides, or as part of a weed population displaying multiple herbicide resistance (Heap 2015). These 28 cases are comprised of 16 different dicot weed species, with some having multiple cases such as wild radish (*Raphanus raphanistrum*), wild carrot (*Daucus carota*), *Limnocaris flava*, *Papaver rhoeas*, and *Sphenoclea zeylanica*, and two species in the genus *Carduus*. Of particular interest are the most recent species to be added to this list since a previous review of auxinic-herbicide resistance (Mithila et al. 2011), including waterhemp (*Amaranthus tuberculatus*) (Bernards et al. 2012), several additional populations of wild radish (Jugulam et al. 2013; Walsh et al. 2007), a flixweed (*Descurainia sophia*) population that is resistant to the related phenoxy-acetic acid herbicide MCPA, and some populations of annual sowthistle (*Sonchus oleraceus*) (Heap 2015). Recent genetic studies determined that MCPA resistance in wild radish from Western Australia is governed by a single, incompletely dominant gene (Jugulam et al. 2013, 2014).

Potential for Target Site and Nontarget-Site Resistance (NTSR) Mechanisms to 2,4-D. To date, there are no published reports unequivocally describing an insensitive or less-sensitive auxin receptor or auxin-binding protein that confers weed

resistance to 2,4-D, although the role of ABP1 has been implicated in wild mustard (*Brassica kaber*) (Mithila and Hall 2005; Mithila et al. 2011). This resistant wild mustard population might have a different mechanism of resistance, however, since it was recently reported that ABP1 is not required for auxin signaling or regulation of *Arabidopsis* development (Gao et al. 2015). This indicates that the lack of 2,4-D binding to ABP1 may not be the true resistance mechanism in this wild mustard population, or that the roles of ABP1 differ between *Arabidopsis* and weedy *Brassica* spp. However, several papers have reported diverse NTSR mechanisms, including enhanced metabolism (Coupland 1994; Coupland et al. 1990) and decreased translocation (Jugulam et al. 2013). For example, a population of prickly lettuce (*Lactuca serriola* L.) resistant to 2,4-D displayed reduced uptake and translocation compared with a sensitive population, but rates of 2,4-D metabolism not different (Riar et al. 2011). It is important to note, however, that these two mechanisms may be physiologically linked; i.e., rapid production of polar metabolites often leads to measurements of decreased translocation since polar metabolites and herbicide conjugates are typically less phloem mobile than parent compounds (Han et al. 2013), most likely due to permanent sequestration in the vacuole via phase III transport and detoxification reactions (Devine and Hall 1990; Riechers et al. 2010).

Paradoxically, an interesting report involving MCPA-resistant wild radish demonstrated increased MCPA translocation to roots in the resistant population (in the absence of altered metabolism of MCPA between populations), which may have been related to extrusion of parent herbicide out of the roots into the soil as a novel exclusion mechanism (Jugulam et al. 2013). Additional reports of enhanced metabolism and resistance to phenoxyacetic acid herbicides have included common chickweed (*Stellaria media*) and horse-nettle (*Galeopsis tetrahit*) populations (Coupland 1994; Coupland and Jackson 1991; Coupland et al. 1990; Lutman and Heath 1990; Weinberg et al. 2006). Reports have yet to be published for other NTSR mechanisms conferring 2,4-D resistance, such as altered cellular uptake or sequestration (Mithila et al. 2011) or activity of 2,4-D–amino acid conjugation enzymes (Kelley and Riechers 2007; Staswick et al. 2005).

The notable absence of target-site-based resistance to 2,4-D may be related to several factors, including potential fitness penalties (Mithila et al. 2011; Powles and Yu 2010; Vila-Aiub et al. 2009; Walsh et al. 2006) and the recessive nature of some auxin herbicide resistance genes (Mithila et al. 2011; Sabba 2003; Van Eerd et al. 2004), functional redundancy of auxin receptors in binding natural auxins or synthetic auxin herbicides (Lee et al. 2014; Shimizu-Mitao and Kakimoto 2014; Walsh et al. 2006), and the complex nature of synthetic auxin herbicide mode of action (Enders and Strader, 2015; Mithila et al. 2011; Xu et al. 2015). However, it remains possible that a decrease in sensitivity to 2,4-D of an auxin receptor(s) belonging to any of the three auxin-binding protein families (Enders and Strader 2015; Groner and Friml 2015) could confer resistance to 2,4-D in a natural weed population, as proposed in detail below. An important factor to consider when assessing the risk of weed resistance to synthetic auxin herbicides is that it may be extremely difficult to “physiologically uncouple” the strong links between auxin receptors/binding proteins, auxin perception and signaling, auxin transport, metabolism, and homeostasis mechanisms, and subsequently plant responses to natural vs. synthetic auxin herbicides, as discussed previously in *Differences in Plant Responses to IAA and 2,4-D*. However, several theories for potential weed resistance mechanisms are discussed in more detail below.

Mutations in TIR1/AFB Nuclear Receptors Increase IAA Binding Affinity. Interestingly, a recent study showed that two distinct amino acid mutations in the leucine-rich repeat domain of the *Arabidopsis* TIR1 coreceptor increased sensitivity (i.e., increased binding affinity) to IAA, resulting in faster degradation of Aux/IAA transcriptional repressors and increased transcription of auxin-responsive genes (Pierre-Jerome et al. 2013). These mutations were also responsible for typical auxin hypersensitive-like symptoms (including epinasty) in mutant seedlings (Yu et al. 2013a). Further, the effects of each TIR1 mutation were additive, such that the double mutant TIR1 protein displayed an even higher binding affinity than either single mutation (Yu et al. 2013a). In addition to increased binding affinity for IAA, these two mutations increased the binding affinity for several other natural auxins and 2,4-D but not picloram, which is

consistent with previous research describing the preferred interaction of picloram with AFB4 and AFB5 (Lee et al. 2014; Walsh et al. 2006). These results indicate that it may be possible to modify the TIR1/AFB proteins from *Arabidopsis* or other dicot plant species to increase the flexibility of the auxin-inducible degron system (Yu et al. 2013a), including increasing or decreasing the binding affinity of these proteins for 2,4-D or other synthetic auxin herbicides, resulting in auxin hyper- or hyposensitivity (i.e., weed resistance) in plants (Gleason et al. 2011).

In addition to the potential for mutations in dicot TIR1/AFB proteins that may affect 2,4-D binding, it is also possible for mutations to occur in the Aux/IAA transcriptional repressor proteins that affect their ability to bind and interact with the TIR1/AFB family of auxin receptors as part of the overall auxin-signaling pathway (Pierre-Jerome et al. 2013). For example, mutations in conserved regions of several *Aux/IAA* transcriptional repressor genes have been identified in *Arabidopsis* (Mockaitis and Estelle 2008), as well as in three *Aux/IAA* genes in a nonflowering plant, the moss *Physcomitrella patens* (Prigge et al. 2010). The mutant lines in *P. patens* are termed NAA-resistant (*nar*) because of their auxin-resistant phenotypes (i.e., impaired responses to NAA and developmental transitions) (Prigge et al. 2010). In wild-type *P. patens*, NAA stimulates transcription of all three *Aux/IAA* genes within 1 h, indicating that auxin causes rapid changes in gene expression in both flowering and nonflowering plants and that the basic auxin-perception mechanisms are conserved (Prigge et al. 2010). In dicot plants, however, such mutations in TIR1/AFB or Aux/IAA proteins and resulting auxin-resistant phenotypes may confer significant fitness costs in the absence of synthetic auxin herbicide treatment, thereby limiting their frequency in natural weed populations (Mithila et al. 2011).

Cross-Resistance Patterns to Other Auxin Herbicides and Other Sites-of-Action Inhibitors. In many cases of reported resistance to 2,4-D, cross-resistance to other synthetic auxin herbicides is not described or mentioned (Heap 2015). However, some reported cases of resistance to 2,4-D demonstrated resistance to closely related phenoxyacetic acids (such as MCPA or mecoprop) but not to other chemical classes of auxin herbicides such as the benzoates (e.g., dicamba) or picolinates (e.g.,

picloram) (Heap 2015). These diverse patterns may be due to either a lack of testing with various synthetic auxin classes or a true lack of genetic cross-resistance. The recently reported population of waterhemp resistant to 2,4-D also was less sensitive to dicamba, though the difference between the resistant and sensitive biotypes was not as great as for 2,4-D (Bernards et al. 2012). More recent work (Jugulam and Godar 2014) reported reduced sensitivity to dicamba in the 2,4-D-resistant waterhemp population as well as a population of 2,4-D-resistant wild radish from Australia. The same study also indicated reduced sensitivity to 2,4-D in a dicamba-resistant kochia [*Kochia scoparia* (L.) Schrad.] population. Given the complex binding patterns of auxin herbicides discussed earlier and the involvement of various auxin transport proteins in the ultimate herbicidal effect, it is not surprising that cross-resistance patterns between 2,4-D and other auxin herbicides is complex.

Another issue to consider is the possibility that P450s or glutathione *S*-transferases (GSTs) could detoxify herbicides from more than one target site/family, leading to cross-resistance or multiple resistance (Preston 2004; Yu and Powles 2014). If NTSR mechanisms for 2,4-D are governed by single dominant or incompletely dominant genes, then the spread of weed resistance to 2,4-D (and possible pleiotropic effects on resistance to other herbicides) would be predicted to be quite rapid (Yu and Powles 2014), assuming widespread use and the absence of resistance management practices. This scenario could be particularly troublesome in the case of selecting for increased activity of P450s or GSTs that can metabolize herbicides that have yet to be commercialized or discovered (Ma et al. 2013; Preston 2004). Currently, increased metabolism of 2,4-D in 2,4-D-resistant wild radish has been reported (Goggin and Powles 2014), but a similar investigation of MCPA resistance in this species did not indicate differential metabolism between resistant and susceptible populations (Jugulam et al. 2013).

Low-Dose Herbicide Selection Pressure: Implications for 2,4-D Resistance. In addition to the frequency in which herbicides are applied and crop/herbicide site-of-action rotations (Powles and Yu 2010), the rate at which a herbicide is applied can affect the selection for herbicide-resistant weed populations (Manalil et al. 2011; Yu et al.

2013b). In the case of ACCase- and ALS-resistant rigid ryegrass populations from Australia, recurrent selection of diclofop-methyl-sensitive populations with reduced rates (i.e., less than a labeled rate) of diclofop-methyl resulted in NTSR to diclofop-methyl within only three generations (Manalil et al. 2011). Resistant plants metabolized diclofop-acid into polar metabolites about two- to threefold faster than sensitive plants and accumulated about twofold less diclofop-acid in foliage (Yu et al. 2013b), presumably as a result of enhanced P450-catalyzed detoxification or glucose conjugation reactions. These results suggest that metabolic-based resistance to 2,4-D and other synthetic auxin herbicides in dicot weeds could also rapidly evolve if full herbicide rates are not utilized. However, it has been shown that use of herbicide tank mixes can delay the selection for herbicide-resistant biotypes (Beckie and Reboud 2009; Diggle et al. 2003; Mithila et al. 2011), which underscores the importance of using glyphosate, glufosinate, or other tank-mix partners when applying 2,4-D to corn, soybean, or cotton upon commercialization of 2,4-D-resistant crop varieties (Wright et al. 2010). Additionally, soil-applied herbicides with residual activity on dicot weeds, as well as preventative, cultural, and mechanical methods, should be incorporated into integrated weed management systems for effective, sustainable dicot weed management in agronomic crops (Mithila et al. 2011).

Weed Control and Crop Tolerance

Overview of Use. 2,4-D has been used worldwide to control a wide spectrum of broadleaf weeds and woody plants in numerous small grain, fruit, nut, and vegetable crops, pastures and rangeland, residential lawns and turf grasses, rights-of-way, and aquatic and forestry sites. Few, if any, other herbicides have as many registered uses as 2,4-D. Currently, there are over fifty 2,4-D products labeled in the United States and a similar number of products that contain 2,4-D in combination with other herbicides (CDMS 2015). Many estimates of the total use of 2,4-D have been developed since its commercialization. The sources of these estimates often do not denote whether they represent acid equivalent, active ingredient, or actual product. For purposes of this review the authors assume the reported quantities to be of acid equivalent.

Between 1945 and 1950 production of 2,4-D increased from 416,000 kg to over 6,350,000 kg (Peterson 1967). As new and more selective herbicides were developed, use of 2,4-D on major field crops in the United States declined from about 18.1 million kg and 34% of total herbicide use by farmers in 1966 to about 15.4 million kg and 15% of total use in 1971 (Andrilenas 1974). However, from 1960 through 1971, 2,4-D accounted for most of all pesticide use in wheat (Lin et al. 1995) and even into the early 1990s 2,4-D was still used on 40 to 60% of spring and durum wheat hectares, 15 to 20% of winter wheat hectares, 10% of corn hectares (Delvo and Lin 1994), and more than 9% of grain sorghum hectares (Morrison et al. 1994). A comprehensive review of phenoxyacetic acid herbicides reported 2,4-D registration for use on over 65 crops and numerous noncropland uses with more than 21 million kg of 2,4-D acid equivalent used in the United States in 1992 (Burnside et al. 1996). As recent as 2012, 2,4-D was used on 13% of winter wheat hectares at an average use rate of 600 g ha⁻¹, totaling nearly 1.1 million kg of active ingredient (NASSHighlights 2013). In comparison, 2,4-D usage on spring wheat and durum in North Dakota, the top producing state of those crops, had dropped below 9% of planted hectares, and to about 5% of barley and 14% of oat hectares in favor of other more selective herbicides (Zollinger et al. 2012). The U.S. Environmental Protection Agency (USEPA) Office of Pesticide Programs estimated that approximately 65% of annual 2,4-D usage in the United States from 1992 through 2000 (21 million kg) was for agriculture; the remainder was for nonagricultural uses (Borges et al. 2004). About one-fourth of total usage and slightly more than one-third of the agricultural usage was for pasture and rangeland and about one-fourth of agricultural usage was for spring and winter wheat. Another one-fourth of total usage and nearly three-fourths of nonagricultural 2,4-D usage was on residential lawns, either alone or mixed with fertilizer. Total use in the United States has increased only slightly since that time (SM McMaster, 2,4-D Task Force, personal communication). Table 2 provides a summary of the various crop and noncrop uses as estimated by EPA (Borges et al. 2004). These data may underreport the use of 2,4-D somewhat since use in premix products may not have been counted. Other estimates, such as provided by the 2,4-D

Table 2. Uses of 2,4-D and labeled application timings and rates (USEPA 2005).

Crop or site	Treated area	% Treated	Stage(s) of application	Maximum rate
	ha			kg ae ha ⁻¹
Pasture/rangeland	6,080,213	3	POST	2.24
Wheat, spring	3,919,115	51	POST/preharvest	1.40/0.56
Wheat, winter	2,656,451	15	POST/preharvest	1.40/0.56
Corn, field	2,595,341	9	Preplant/PRE/POST/preharvest	1.12/1.12/0.56/1.68
Soybean	1,480,797	5	Preplant	0.56 to 1.12
Fallow, summer	626,880	7	Between crops	2.24
Barley	865,249	36	POST/preharvest	1.40/0.56
Sorghum	429,387	12	POST	1.12
Rice	174,426	13	Preplant/POST	1.12/1.68
Pome fruits	40,875	19	POST	2.24
Stone fruits	< 40,000		POST	2.24
Nut orchards	< 40,000		POST	2.24
Forestry	< 40,000		Not designated	4.48
Aquatic ditchbanks	—	—	POST	2.24
Aquatic surface	—	—	POST	4.48
Noncrop	—	—	POST	2.24 to 4.48

Task Force, indicate higher use as indicated in Figure 2 (Industry Task Force II on 2,4-D 2009a). The introduction of new 2,4-D-based technologies discussed later in this review may result in increased use of 2,4-D on corn, soybean, and cotton. Some authors have suggested as much as a 30-fold increase in 2,4-D use associated with these technologies (Benbrook 2012). However, the assumptions and methods underlying these estimates have been called into question (Brookes et al. 2012), and given greater competition between various herbicide technologies in the coming years, it is unlikely that any given product will reach the same level of adoption as glyphosate-resistant crops.

In the broadest sense, 2,4-D is effective for the control of broadleaf weeds with limited effect on grasses. Though 2,4-D can have limited soil residual activity and early investigation found some effectiveness as a PRE herbicide (Peterson 1967), its major use has been POST. Application rates vary widely by use pattern but usually fall within the range of 280 to 1,120 g ae ha⁻¹ for most crop uses (Anonymous 2005). In the United States, rates higher than 1,120 g ae ha⁻¹ (in some cases as high as 4,480 g ae ha⁻¹) are labeled for use against perennial broadleaf weeds for some nongrain crop uses such as turf, fallow, hay, and pasture. Rates will also vary by formulation, with higher rates allowed for salt formulations than for esters when applied POST because of crop tolerance issues. However, labeled use rates can vary among commercial products of

the same concentration and formulation. Often the variance between products of the same concentration is in the maximum labeled rate of application. Also, some 2,4-D products are not labeled for all the same uses as other 2,4-D products. For example, some products do not include preharvest treatment on oats or orchard and horticultural uses. Therefore, end users should only apply products that list the intended use on the product label.

Some broad generalizations can be made about the sensitivity of broadleaf weeds to 2,4-D. Annual weeds in the *Asteraceae*, *Leguminosae*, *Cruciferae*, and *Convolvulaceae* tend to be among the most sensitive to 2,4-D, whereas those in the *Polygonaceae*, *Labiatae*, and *Solanaceae* are usually more tolerant. However, in some families it is not possible to generalize. Within the *Chenopodiaceae* common lambsquarters (*Chenopodium album*) is quite sensitive to 2,4-D, whereas kochia is tolerant. Weeds in the *Amaranthus* are usually well controlled at higher rates of 2,4-D (840 to 1,120 g ae ha⁻¹). Ester formulations of 2,4-D typically generate a more rapid plant response, especially under conditions of environmental stress (Monaco et al. 2002; Nice et al. 2004). However, although decreased crop tolerance has been documented, an advantage in overall control for ester formulations is difficult to find in the literature and is more often noted for perennial weeds than with annuals when comparisons are found. Early work comparing amine and ester formulations in a greenhouse experiment did

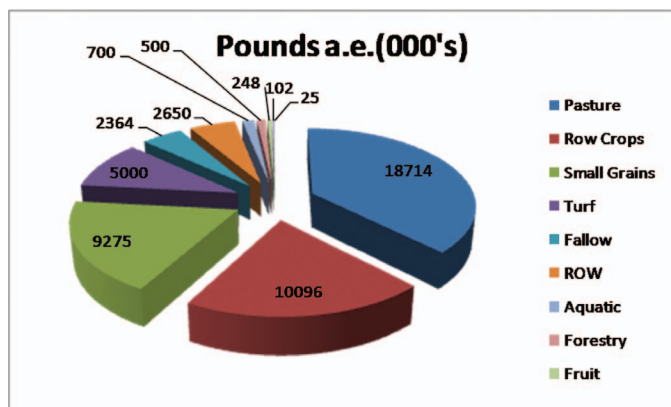


Figure 2. Uses of 2,4-D as reported by the 2,4-D Task Force circa 2005 and available at <http://www.24D.org>.

not indicate any inherent difference between the two (Gertsch 1953). Since both amine and ester forms exist as 2,4-D acid inside the plant, the main advantage of ester formulations is related to faster uptake due to better penetration of waxy leaf cuticles. This may cause greater crop injury since faster uptake may overrun the crop's ability to metabolize 2,4-D to nontoxic molecules. It may not always translate into greater weed control across all weed species or under all conditions.

Usually 2,4-D is applied in combination with other herbicides, especially when used in cereals or turf. This broadens the spectrum of activity, allows lower application rates to reduce chances of crop injury, and in some cases provides overlapping control that helps avoid evolution of resistant weeds. As discussed in previous sections, 2,4-D can interact with other herbicides in either an antagonistic or synergistic manner. Labels for some ACCase-inhibiting herbicides, such as sethoxydim or tralkoxydim, recommend against tank mixing with 2,4-D amine or require higher rates to overcome the reduced grass activity. In some cases 2,4-D ester is allowed but amine formulations are not (Anonymous 2009, 2012).

Preplant Uses. The expansion of no-till has expanded the use of 2,4-D before row crop planting. Most of the use attributed to crops such as soybean, cotton, and other broadleaf crops (Table 2) occurs in the spring before planting to remove winter annual, early spring-emerging weeds, or help control perennials. In 1992, 2,4-D preplant application on no-till soybean was 1% of planted hectares, grew to 7% the next year (Delvo and

Lin 1994), and to 11% in 2012 (NASS Highlights 2012). Often 2,4-D is applied in combination with another broad-spectrum product such as glyphosate or paraquat in these situations to control grasses and improve control of weeds such as henbit (*Lamium amplexicaule* L.), clover (*Trifolium* spp.), prickly lettuce, and glyphosate-resistant weeds such as marehail or horseweed [*Conyza canadensis* (L.) Cronq.] (Loux et al. 2015). Though 2,4-D has a relatively short soil half-life of approximately 6 d (Wilson et al. 1997), there are some label restrictions regarding the interval between burn-down applications and planting of some crops (Anonymous 2005). These plant-back intervals can vary by formulation, with 2,4-D ester generally having a shorter interval than 2,4-D amine (Anonymous 2005). This is not likely due to differences in soil half-life since the ester is rapidly converted to the acid form in most soils (Wilson 1975) and comparisons have found no difference in the rate of soil degradation in the field (Wilson and Cheng 1976). The general thought is that the difference in label recommendations is due to the lower water solubility of the ester formulation and associated lower movement through soil to the roots of sensitive crops (Hager 2012). Aldrich and Willard (1952) found that corn stand reduction and reduced growth resulting from PRE-applied 2,4-D occurred only if the 2,4-D was leached into the seed zone and a butyl ester formulation of 2,4-D ester did not move as freely in percolating water as did a triethanolamine salt formulation. Field studies in 2007 (Thompson et al.) did not find a difference in soybean tolerance to ester vs. amine formulations of 2,4-D applied preplant. Similar work with cotton tolerance to preplant applications did not find a difference related to formulation (Miller et al. 2003).

Use in Cereal Grains. Numerous spring and winter annual broadleaf weeds and several biennial and perennial broadleaf weeds are common in both spring- and fall-seeded cereal grains [wheat, barley (*Hordeum vulgare* L.), oats, rice, and rye (*Secale cereale* L.)]. Weed spectrum varies by production region, when the crop is planted (fall- or spring-sown), and cropping rotation. Moderate to dense infestations of broadleaf weeds can cause significant yield reductions in wheat, ranging from approximately 10 to 50% depending on species and length

of competition (Conley and Bradley 2005; Peterson 1997; Swan 1971).

Fall-sown crops generally have fewer weeds than spring-sown crops unless conditions favor weed emergence simultaneously or soon after winter crop emergence. For that reason, 25 to 40% of winter wheat receives herbicide application for weed control compared with > 90% of spring wheat and durum in the northern Great Plains receiving herbicide treatment (Delvo and Lin 1994). Even after 70 yr of use, 2,4-D is still one of the most commonly applied herbicides in wheat and barley (NASS Highlights 2013). 2,4-D is most often applied for control of the mustards, lambsquarters, and pigweed. It is often mixed with ALS-inhibiting herbicides such as thifensulfuron, tribenuron, or metsulfuron, other auxins such as fluroxypyr or clopyralid, or with contact herbicides such as bromoxynil to improve control of broadleaf weeds such as kochia, henbit, and wild buckwheat (*Polygonum convolvulus* L.).

Cereal Tolerance. Extensive research in the late 1940s and early 1950s helped determine how best to use 2,4-D and served as the basis of present-day use recommendations (Anonymous 1953; Derscheid et al. 1951; Klingman 1953; Olson et al. 1951; Price and Klingman 1958). Though tolerance can vary somewhat by crops within the cereals, the optimum growth stages to apply 2,4-D is usually when plants have at least three to four tillers but before stem elongation. In wheat this may correspond to Feekes 3 through Feekes 5 growth stages. Cereals are especially prone to injury when they are in the boot stage, which in wheat is Feekes growth stage 10. Varieties within crops have been reported to differ in susceptibility to 2,4-D (Derscheid et al. 1951; Nalewaja and Arnold 1970; Price and Klingman 1958; Shaw et al. 1955). Preplant application of 2,4-D too close to seeding may interfere with stand establishment. Generally, it is safe to seed wheat 2 wk after receiving at least 15 mm of rainfall or irrigation after 2,4-D application (Phillip Stahlman, personal communication). POST 2,4-D application to cereal grains before tillering can reduce stands, cause twisting and curling of leaves characterized by Olson et al. (1951) as onion-like leaves, and reduce tillering and grain yields. 2,4-D application during early stages of stem elongation (jointing) can cause prostrate growth or leaning of spike-bearing tillers and can

impair spike emergence from the boot. Spikes that emerge from the boot may be deformed such as shown in Olson et al. (1951). Application near anthesis can cause sterility. Freyman and Hamman (1979) observed that treatment with 2,4-D can adversely affect the cold tolerance of winter wheat. Application on winter cereals in late fall during periods of inactive growth is not advised as significant injury and yield reduction can result (Loux 2010). Olson et al. (1951) discussed the two general timings of small grain sensitivity to 2,4-D in the context of when reproductive cells were in a primordial stage. This was later shown by Loubser and Cairns (1989) via scanning electron micrographs of barley meristems after treatment at various stages with 2,4-D. Johanson and Muzik (1961) observed that applications of 2,4-D to either wheat foliage or directly to roots themselves increased root initiation but inhibited elongation in lateral roots.

Low-volatile ester formulations may be more efficacious than amine, salt, or acid formulations under dry conditions because they more readily penetrate through the waxy cuticle of leaves, but under conditions conducive to rapid crop growth ester formulations pose greater risk of crop injury, often expressed as prostrate tiller growth and spike abnormalities (Anonymous 1948; Derscheid et al. 1951; Friesen and Walker 1956). Recommended use rates for wheat, barley, and rye range from 280 to 560 g ae ha⁻¹ depending on target weed susceptibility and growth stage and environmental conditions, with rates of 840 g ae ha⁻¹ permitted if an increased risk of crop injury is acceptable (Moechnig et al. 2011). Currently, preharvest application of 2,4-D at 0.6 kg ha⁻¹ (previously up to 1.2 kg ha⁻¹) can be made when wheat is in the mid- to hard-dough stage and nodes are no longer green to prevent perennial weeds in the bud to bloom stage, such as field bindweed, from producing seed (necessary in fields grown for certified seed) or when annual weeds threaten to seriously interfere with harvesting. The preharvest interval for 2,4-D harvest-aid applications is 14 d (Anonymous 2005), so growers desire to spray as early as possible. Applying 2,4-D too early, when stem nodes are still green, can cause stem breakage, usually at the top node during the waiting period and may reduce crop seed germination (Klein 2013; Moechnig and Deneke 2009). Weed control with preharvest applications is often not satisfactory because of the

large size of the weeds at application. Also, stems of treated broadleaf weeds often become brittle and break into small pieces during the harvesting process, resulting in greater foreign material in the harvested grain. The preharvest use of 2,4-D has declined since glyphosate was registered for this use.

Use in Rice. Weeds reduce rice yield by direct competition and can reduce grain quality and grade (Scott et al. 2013). Smith (1988) cited estimated yield losses due to weeds in U.S. rice-producing states ranging from 12 to 35%, with average loss of 17%. Some common broadleaf rice weeds controlled by 2,4-D include ducksalad [*Heteranthera limosa*, (Sw.) Willd.], dayflower (*Commelina diffusa* Burm. f.), eclipta (*Eclipta prostrata* L.), hemp sesbania [*Sesbania herbacea* (P. Mill.) McVaugh], morningglory species (*Ipomoea* spp.), and water hyssop (*Bacopa* spp.). In 1990, 2,4-D was applied on nearly 17% of U.S. rice hectares at an average rate of 1 kg ha⁻¹ (NASS 1991), but it was used on only 7% of planted rice hectares in 2013 (NASS 2015).

Rice Tolerance. Kaufman (1953) published an extensive article on the tolerance of rice to 2,4-D and other phenoxy herbicides. Similar to small grains, highest injury was noted before tillering and after the boot stage, with little damage noted with applications occurring between these two times. Injury symptoms (stem and leaf twisting, head malformations, and floret sterility) observed in rice were similar to those in wheat or barley. Root malformation and inhibition can also occur (Kaufman 1953). In rice-producing states other than California, amine, salt, and acid formulations of 2,4-D at rates up to 1.1 kg ha⁻¹ ae may be applied 2 to 4 wk before planting or at rates up to 1.33 kg ha⁻¹ when rice is in the late tillering stage of development, usually about 6 to 9 wk after emergence. Preplant interval and maximum POST use rates may vary with 2,4-D product. However, 2,4-D herbicides should not be applied when the internode length of rice stems exceeds 1.3 cm or after panicle initiation as severe crop injury and yield loss may result.

Wild rice (*Zizania palustris*) is grown in the Great Lakes area of North America. 2,4-D amine at 280 g ha⁻¹ is the only herbicide available for use on wild rice to control common water plantain in Minnesota. However, crop tolerance is marginal and 2,4-

D should not be applied before the early- to mid-tillering stage of crop development (Nelson et al. 2003).

Use in Corn, Sorghum, and Millets. Up until the 1970s, 2,4-D was widely used on corn and sorghum crops despite risks of crop injury if application was not timely. Development of a variety of alternative herbicides during the 1980s and 1990s, as well as the advent of herbicide-resistant corn, is believed largely responsible for a drop in 2,4-D usage on corn from 13% of total herbicide active ingredient in 1968 to only 1% in 2008 (Fernandez-Cornejo et al. 2014).

Corn yield loss from weed competition is highly variable but typically ranges from 15 to 40% without herbicide treatment and up to 15% with herbicide treatment (Bridges 1992). Typical yield losses in sorghum from weed competition range from 30 to 50%, but complete crop failure can result in extreme cases (Stahlman et al. 2000). Perennial weeds such as field bindweed, common milkweed (*Asclepias syriaca* L.), hemp dogbane, and bur ragweed (*Ambrosia acanthicarpa* Hook) also infest sorghum fields and generally are more competitive in grain sorghum than in corn. Key annual broadleaf weeds common in corn and sorghum such as common lambsquarters, pigweed species, morningglory species, ragweed species, waterhemp, and velvetleaf can be well controlled by POST applications of 2,4-D. Most common broadleaf weeds can be economically controlled with 2,4-D in pearl millet and proso millet (Lee et al. 2012; Lyon et al. 2008); however, not all 2,4-D products are labeled for use on millet. Application rates range from 280 to 560 g ha⁻¹ though the lower rates are often used to avoid crop injury. The use of lower rates does limit the effectiveness of 2,4-D, however, and its use in corn and sorghum has decreased ever since the introduction of *s*-triazines and other effective preplant and PRE herbicides that control weedy grasses as well as broadleaf weeds (CAST 1975). In some instances, such as control of deep-rooted perennials or occasionally in tank mixes with other herbicides, 2,4-D is still sometimes used in corn. Research has shown that 2,4-D amine improves broadleaf weed control and reduces injury to grain sorghum when tank mixed with metsulfuron (Brown et al. 2004) and fluthiacet-methyl (Reddy et al. 2014).

Tolerance in Corn, Sorghum, and Millets. Optimum growth stages for broadcast application of 2,4-D are when corn plants are less than 20 cm tall, sorghum plants are 10 to 25 cm tall, and after pearl millet plants are well tillered and 20 cm tall (Lee et al. 2012). Applications to proso millet should occur after two to five leaves are present but before the early boot stage and only using the amine formulation of 2,4-D. Symptoms of 2,4-D effects on corn, sorghum, and millets include stalk leaning and brittleness; retarded, malformed, and fused brace roots; leaf rolling, commonly called “onion leafing” or “buggy whipping;” occasional lodging after heading; and yield reductions (Phillips 1958; Rodgers 1952). Over a 4-yr period root injury was most severe when 2,4-D was applied at the five- to seven-leaf growth stage of Midland grain sorghum and there was no difference in grain yields between alkanolamine salt and isopropyl ester formulations (Phillips 1958). Low-volatile ester formulations of 2,4-D generally provide greater broadleaf weed control but are more likely to injure grain sorghum than amine formulations (Stahlman et al. 2000). With all formulations crop injury risks are dosage dependent and greater when crops are growing rapidly under conditions of high temperature and humidity and high-soil moisture content (Stahlman et al. 2000). Application on sorghum plants less than 10 cm tall may cause severe lodging or plant death and application to all these crops during floral development (preboot and boot stages) may cause partial floret sterility and reduced yields (Stahlman et al. 2000). Application when plants are more than 20 to 25 cm tall should be made with drop nozzles to reduce foliar interception of the spray as much as possible and reduce potential for crop injury (Stahlman et al. 2000). Corn and sorghum hybrids are known to vary in tolerance to 2,4-D and the herbicide should only be applied on hybrids known to be tolerant (Burnside and Wicks 1972; Marshall and Nel 1981). Even with lower application rates applied at recommended timing, injury to corn can still sometimes occur.

Use in Pasture and Rangeland. Weed problems in pastures, rangeland, and perennial grasslands not in agricultural production (such as the U.S. Conservation Reserve Program) vary from poisonous native plants that cause frequent and sometimes major economic losses from livestock death and illness to nonnative invasive weeds that negatively affect

forage quantity and quality, reduce species diversity, and alter the way ecosystems function. On the basis of U.S. state and federal survey data, it was estimated that 51 million ha of rangeland, pastureland, national parks, natural preserves, and other wildlands were infested with 16 selected invasive plants: downy brome (*Bromus tectorum* L.), musk thistle (*Carduus nutans* L.), Russian knapweed [*Acroptilon repens* (L.) DC.], diffuse knapweed (*Centaurea diffusa* Lam.), spotted knapweed (*Centaurea stoebe* L.), yellow starthistle (*Centaurea solstitialis* L.), Canada thistle, leafy spurge, hawkweeds (*Hieracium* spp.), perennial pepperweed (*Lepidium latifolium* L.), Sericea lespedeza [*Lespedeza cuneata* (Dumont) G. Don], dalmatian toadflax [*Linaria dalmatica* (L.) P. Mill.], purple loosestrife (*Lythrum salicaria* L.), tropical soda apple (*Solanum viarum* Dunal), medusahead [*Taeniatherum caput-medusae* (L.) Nevski], and saltcedar or tamarisk (*Tamarix ramosissima* Ledeb.) (Duncan and Clark 2005). The large majority of infestations of most species was in the 17 western states from North Dakota to Texas and west to the Pacific Coast.

Most of the 2,4-D used on grazing lands is applied to managed pastures, which often receive treatment annually as opposed to infrequent treatment of smaller portions of rangeland. Treatment with a phenoxy herbicide in 1971 amounted to 2 million ha or approximately 5% of the 40 million ha of pasture in the United States and only 0.85 million ha (0.4%) of more than 214 million ha of rangeland (CAST 1975). From 1992 through 2000, 2,4-D was applied on only about 3% of all pastureland and rangeland but accounted for 36% of agricultural usage and 24% of total usage (Borges et al. 2004).

Usually 2,4-D is used in mixtures with other growth-regulator herbicides, most often aminopyralid, dicamba, fluroxypyr, picloram, or triclopyr, for broadcast and spot treatment applications. Typical dosages are up to 1.1 kg ha⁻¹ for control of susceptible annual biennial weeds and from 1.1 to 2.2 kg ha⁻¹ for biennial and perennial weeds and woody plants (Anonymous 2005). A second application may be needed no sooner than 30 d after the first application for hard-to-control weeds and woody plants. The preharvest interval for forage cut for hay is 7 d (Anonymous 2005).

Use in Turfgrasses. Managed turfgrasses including residential and nonresidential lawns, parks, athletic and recreational facilities, and golf courses are estimated to cover more than 16 million ha in the United States (Milesi et al. 2005). Sod and turfgrass rank among top agricultural commodities in some eastern and southern states. A recent analysis of the turfgrass industry placed its value at \$57.9 billion (Haydu et al. 2009). Maintaining turf weed free accounts for a major portion of management costs. Primary broadleaf weeds found in turf include dandelion (*Taraxacum officinale* G.H. Weber ex Wiggers), broadleaf plantain (*Plantago major* L.), buckhorn plantain (*Plantago lanceolata* L.), clovers, curly dock (*Rumex crispus* L.), speedwells (*Veronica* spp.), and creeping wood sorrel (*Oxalis corniculata* L.). POST applications of 2,4-D can provide control of these weeds, superior to many other turf herbicides (Elmore 1996b). Most turfgrass species are tolerant but a few, such as bentgrass (*Agrostis* spp.) and St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze], can exhibit some injury (McCarty 1994).

The most readily available herbicides for selective, POST control of broadleaf weeds include 2,4-D, 2,4-DP-p, MCPP-p, and dicamba. Commercial products for homeowners and urban markets are available as single active ingredients or as combinations of two or more active ingredients available in liquid formulations (sprayable), and often in granular formulation along with a fertilizer that are applied with a drop or broadcast spreader (CAST 1975). Combined fertilizer and herbicide products are popularly known as “weed-n-feed” products; they should be used with caution near ornamentals that might be susceptible to the herbicide active ingredient in the product. The USEPA estimates that more than 5.2 million kg of 2,4-D active ingredient are applied on lawns annually (Borges et al. 2004).

Use rates of commercial lawn and garden products vary because of different percentages or concentrations of herbicide active ingredient in granular and liquid products. However, use on ornamental turf is limited to two broadcast applications per year per treatment site at maximum application rate of 1.7 kg ha⁻¹ (Anonymous 2005). The maximum application rate for grass grown for seed or sod is 2.2 kg ha⁻¹ (Anonymous 2005).

People or pets should not enter treated areas until spray on the foliage has dried (Anonymous 2005).

Use in Forestry, Rights-of-Way, and Roadways.

The forest products industry reports using herbicides for site preparation for new plantings, herbaceous weed control, and to release trees from weedy competition (Shepard et al. 2004). The goal is not to provide complete weed control but to provide a short-term growth advantage for trees over competitive vegetation. Estimates of herbicide use in commercial forestry are imprecise as there is no national system that tracks forestry herbicide use in the United States (Shepard et al. 2004). However, the U.S. Forest Service tracks pesticide use on federal lands and applies herbicides primarily to control noxious weeds and indicated that in 2004 2,4-D was the second most commonly used herbicide, after picloram, with applications on over 20,000 ha of federal forest and rangeland (Cota 2004). Currently, other herbicides are more widely used than 2,4-D, though it is still used for site preparation and conifer release.

Control of weeds and brush along roads, railways, ditch banks, utility rights-of-way, pipelines, and industrial sites is often discussed as industrial vegetation management. Usually the main objective is to prevent interference in travel or other operations by herbaceous weeds or brush in these settings. Other auxin herbicides, such as triclopyr, picloram, and aminopyralid, are often applied in combination with 2,4-D to improve activity on woody species and perennial weeds. More 2,4-D active ingredient is used on roadways than the combined use on electric utilities, industrial sites and pipelines, and railroads. All of these uses account for about 5% of total 2,4-D usage annually (Borges et al. 2004). Herbicides are used in industrial forestry for site preparation for tree establishment, herbaceous weed control, and to release crop trees from woody plant competition (Shepard et al. 2004).

Use in Tree Fruits and Nuts and Vineyards.

Orchards can benefit from a well-managed vegetative orchard floor cover that is limited to the area between the tree rows. This vegetative cover, either via resident vegetation or planted cover crops, provides a stable surface for machinery, reduces compaction, and improves soil structure and water infiltration. Although resident vegetation makes a

good cover, it may contain weeds that invade the tree row and become difficult to control. On the other hand, properly managed cover crops can prevent invasion of the orchard by weeds. It is desirable to keep the area within the tree row relatively weed free to reduce competition for water and nutrients as well as reduce infestations of insects, rodents, and diseases (Smith 2015). Tillage is one weed-control method for orchards but it has significant drawbacks in terms of labor and fuel requirements as well as damage to tree roots and increased erosion. Mowing is a more practical mechanical weed-control method but also has most of the same labor, fuel, and equipment constraints as tillage. Herbicides, often mixtures of glyphosate with a soil-residual product such as dichlobenil, diruon, or oryzalin, are an effective and efficient means of maintaining orchard floors. Applications of 2,4-D are usually made in orchards for perennial broadleaf weeds occurring in the tree row or in the vegetative cover between the rows (Elmore 1996a). Amine-, salt-, and acid-formulated 2,4-D are used in orchards and vineyards at rates up to 1.6 kg ae ha⁻¹ primarily to control small, actively growing annual and perennial broadleaf weeds on orchard floors and for grass suppression (chemical mowing) between rows of trees (Peachey 2014). Other uses include green sucker control in hazelnuts (*Corylus avellana* L.) and preharvest application of the isopropyl ester of 2,4-D to delay fruit abscission (fruit drop) and to increase fruit size of oranges (*Citrus × sinensis*) and grapefruit (*Citrus × paradisi*). The preharvest interval for pome fruits is 14 d; for stone fruits, 40 d; and for nut orchards, 60 d (Anonymous 2005). No other phenoxy herbicides are registered for use in these crops. In 1993, nearly 0.62 million kg of 2,4-D were used on pome and stone fruits at an average rate of 0.9 kg ha⁻¹ (NASS 1994). Estimates for two important nut crops, almonds and filberts, indicate 35,000 kg of 2,4-D applied across approximately 22,000 ha (Borges et al. 2004). Though one of the smaller markets in terms of 2,4-D usage, the herbicide is important for these crops.

2,4-D can also be used in grapes in California after shatter following bloom and before shoots reach the ground, or during the dormant season with a preharvest interval of 100 d (Anonymous 2014b). However, grapes are very sensitive to 2,4-D

and it should not be applied to foliage, shoots, or stems (Lange et al. 1968).

Use in Aquatic Habitats. Invasive aquatic vegetation threatens ecosystem diversity, degrades water quality, interferes with commercial and recreational activities, and impedes navigation and water movement in streams and irrigation canals. Many aquatic species are more than nuisance vegetation because they produce toxins that endanger human and animal health and alter critical habitat for threatened and endangered species (CAST 2014). Decaying aquatic plants and algae produce unpleasant taste and odors and freshwater toxins produced by cyanobacteria (commonly referred to as blue-green algae) are harmful to vertebrates, including humans.

Several emergent and submersed aquatic weeds are controlled by 2,4-D, but this herbicide is primarily used for selective control of water hyacinth [*Eichhornia crassipes* (Mart.) Solms] and Eurasian water milfoil (*Myriophyllum spicatum* L.) (Haller 2014). A liquid amine formulation is used to control emergent and submersed plants and a granular BEE formulation is used for submersed weed control. In addition, a granular amine formulation has been recently registered. Typical use rates are 0.5 to 4 ppm for submersed dicot weeds and 2.2 to 4.5 kg ha⁻¹ for foliar application. Some native emergent plants—including water lilies (*Nymphaea* spp.), spatterdock [*Nuphar lutea* ssp. *advena* (Ait.) Kartesz & Gandhi], and bulrush (*Scirpus* spp.)—are susceptible to 2,4-D, so care should be taken to avoid injury to these plants.

Aquatic herbicides are sprayed directly onto floating or emergent aquatic plants or are applied to the water in either liquid, granular, or pellet forms. The granular formulation contains the low-volatile BEE ester formulation of 2,4-D and the liquid formulations contain the dimethylamine salt of 2,4-D. The granular form is effective in controlling submerged weeds and liquid formulations are most effective in spring when weeds emerge. Use of aquatic herbicides is regulated (restricted use) in most states. The BEE ester formulation of 2,4-D cannot be used in waters with threatened and endangered salmon in the Pacific Northwest (Anonymous 2015b). Swimming is restricted for 24 h after application of 2,4-D BEE products (no swimming restrictions for amine formulations) applied to aquatic sites and there

are specific restrictions related to applications near drinking water intakes (Anonymous 2005).

Minor Uses. Many small-area crops depend on 2,4-D for weed control. These include asparagus, blueberries, cranberries, strawberries, and hops (Anonymous 2005). Sugarcane is a relatively small crop in area in the United States but much more important in tropical areas of the world. Amines, salts, or acid formulations of 2,4-D at $2.2 \text{ kg ae ha}^{-1}$ can be broadcast for control of emerged broadleaf weeds before canes appear or applied POST after cane emergence through canopy closure (Anonymous 2005).

Lesser-known but interesting uses of 2,4-D include application to certain varieties of potatoes (*Solanum tuberosum* L.) to enhance tuber color (Fulfs and Payne 1955) and improve fruit retention and size in citrus (Stewart and Hield 1950).

The above uses are supported by residue tolerances established by regulatory agencies around the world. Tolerances have also been established for indirect or inadvertent exposures to crops without labeled uses, including a range of fruit and vegetable crops (USGPO 2012)

Volatility and Drift

Vapor movement of herbicides occurs when molecules of the active ingredient convert from a liquid to a gas and are transported off the intended application site by wind or air currents. Vaporization is a function of both the inherent physical properties of the herbicide and the environmental conditions that are present during and after the application (Behrens and Lueschen 1979, Burgoyne and Hites 1993).

The acid form of 2,4-D has a vapor pressure of 1.4×10^{-7} mm of Hg at 25 C, which is considered to have a relatively low potential for vapor drift (Shaner 2014). Vapor pressure measurements for salts of 2,4-D are not meaningful since the value is generally reflective of the acid form after dissociation from the counterion. Actual vapor loss and movement of 2,4-D from salts depends on the strength of association between 2,4-D anion and the associated cation as well as that cation's stability. Salts that readily dissociate will have vaporization that generally approximates that of the acid, especially if the cation is susceptible to loss. This is the case with dimethylamine salts of 2,4-D where

the dimethylamine ion is subject to vapor loss. Those salts with a lower dissociation and more stable counterions, such as the choline salt, will have significantly less vapor loss and movement (Hillger et al. 2012).

Ester formulations can be considered low volatile or high volatile depending on the length of the carbon chain in the alcohol used to make the ester. Esters made from alcohols with an alkyl chain of four carbons or fewer are considered highly volatile (Gile 1983; Moore 2008). This group includes the methyl, isopropyl, and butyl esters, which were not reregistered in the United States in 2005 (USEPA 2005b). On the other hand, esters of 2,4-D made from alcohols with an alkyl chain of more than four carbons are classified as low-volatile esters (Moore 2008). These include BEE and 2-EHE. Although these low-volatile esters are much less likely to result in vapor drift than early ester formulations, they are still an order of magnitude more volatile than salt formulations (Gervais et al. 2008).

Several environmental factors can influence the volatilization of herbicides in the field. A primary environmental influence on volatility is temperature (Quehee and Sutherland 1974). Another factor is atmospheric mixing related to wind currents.

Physical drift is the movement of small spray particles by wind currents. Given that this is not unique to 2,4-D, the topic is better covered elsewhere.

Nontarget Plant Sensitivity

Low doses of auxin herbicides such as 2,4-D on sensitive plants can often cause visual symptoms ranging from minor leaf malformations to severe stem twisting depending on the species, active ingredient, dose, and stage of growth at time of exposure (Gunsolus and Curran 1991). Off-target injury can be associated with spray particle drift, volatilization, or contamination of sprayers. Downwind deposition from a typical ground application can range from 0.1 to 9% at 0 to 2 m outside the spray swath, dropping to 0.02 to 4% at 3 m and exponentially beyond that distance (Carlsen et al. 2006). If one assumes 2,4-D application rates that often range from 560 to $1,120 \text{ g ae ha}^{-1}$, testing plant sensitivity at rates of 5 to 10 g ae ha^{-1} to approximate drift injury at distances of 0 to 10 m and rates of 0.5 to 1.0 g ae ha^{-1} at longer distances

would appear to be reasonable. Some authors have assumed 1/1000th of the applied rate to represent exposure to vapor drift (Egan et al. 2014)

Although the various sources of these low-rate exposures can cause similar symptoms in sensitive crops, determining a rate response for some sources experimentally can be difficult. Plant response to varying vapor concentrations is especially challenging since consistent generation of known doses is extremely difficult. Researchers often attempt to simulate plant response to herbicide drift on sensitive plant species by applying rates representing fractional amounts of the standard application rate applied to a target crop. These “simulated drift rates” are usually applied in spray volumes of 100 to 200 L ha⁻¹ to ensure uniform coverage and accurate dosages in the experiments. Most of the available data regarding plant sensitivity have been generated using this technique. However, these conditions may not accurately reflect a true drift situation where the actual volume of spray solution impinging on a plant is many orders of magnitude lower. Often phytotoxic effects are positively correlated with spray volume and plant coverage (Bode 1988); these simulated drift studies may overestimate the amount of damage caused by a given exposure from drift. In a few cases, leaf penetration by a herbicide may be concentration dependent, in which case the more concentrated solutions found in drifted spray droplets may cause greater injury. Herbicide effects from volatilization exposure would be more highly overestimated in these simulated drift experiments since spray exposure is chronic as opposed to an acute and transient vapor exposure (due to air mixing and lack of particle deposition). On the other hand, some herbicides can show an increased response when applied at lower spray volumes and therefore higher concentrations (Banks and Schroeder 2002; Roeder et al. 2008). However, these studies can be instructive for correlating visual symptoms with other plant parameters such as growth and grain yield.

Another complicating factor in some studies of plant sensitivity to 2,4-D is the addition of surfactants in several cases (Bhatti et al. 1996; Marple et al. 2008; Ogg et al. 1991). These adjuvants may have increased coverage and penetration of 2,4-D over that found in actual practice since 2,4-D adjuvants are not typically recommended on 2,4-D product labels. However, since 2,4-D

is often applied in combination with other herbicides that may require the addition of surfactants, it may be that these studies could be considered as “worst case” scenarios.

For many crop species, plants can effectively recover from low-level auxin herbicide injury without significant yield loss. In some plants very low doses of auxin herbicides can actually stimulate growth and reproduction (Appleby 1998; Cedergreen 2008). Most species have a biphasic response to low-dose and high-dose exposure to auxin herbicides. Low-dose ranges are characterized by limited dose response and temporary injury, whereas high-dose ranges have a more defined dose response that results in long-lasting injury. As mentioned above, species vary in their inherent sensitivity to auxin herbicides; in fact, they differ among various active ingredients of the same mode of action.

Soybean represent an auxin-sensitive species that is often grown adjacent to areas treated with auxin herbicides such as 2,4-D. Andersen et al. (2004) simulated drift by foliar applications of 2,4-D at 11.2, 56, or 112 g ae ha⁻¹ applied to soybean at the third vegetative (V3) growth stage, which resulted in maximum visual injury of 5, 23, and 33%, respectively. The 11.2 g ae ha⁻¹ rate of 2,4-D did not cause significant reduction in plant biomass and only the highest rate, 112 g ae ha⁻¹ (representing 10 to 20% of a full application rate of 2,4-D), resulted in reduced grain yield. This would not be a realistic dose for drift exposure. Treatment with 11.2 or 56 g ae ha⁻¹ of 2,4-D resulted in yields statistically the same as the untreated check. More recent research (Robinson et al. 2013) conducted a nonlinear regression analysis of soybean injury data generated from foliar applications applied at V2, V5, and second reproductive stage (R2). This analysis determined the rate of 2,4-D needed to cause visual injury of 20% 14 d after application (DAA) of 77, 29, and 109 g ae ha⁻¹ for the V2, V5, and R2 growth stages, respectively. Regression analysis correlating visual injury and yield determined that visual injury of 35% at 14 DAA was required to reduce seed yield by 10%. Other studies required rates of simulated drift exposure to 2,4-D exceeding 140 g ae ha⁻¹ to achieve statistically significant yield reductions (Kelley et al. 2005; Wax et al. 1969). A 2014 meta-analysis of soybean injury and yield reduction over nine studies conducted across 49 yr

indicated that “soybean has surprisingly high tolerance to 2,4-D” (Egan et al. 2014). The analysis predicted that soybean would have no significant yield loss to exposures of up to 5.6 g ae ha⁻¹ at either the vegetative or reproductive stages, and that yield loss at 56 g ae ha⁻¹ would be slight (1.5 to 3.0%). These studies show that although visual injury may sometimes raise concerns with affected growers, the risk of soybean yield loss from 2,4-D exposure is relatively low.

Cotton is another species that is sensitive to auxin herbicides and is one of the most sensitive plants to 2,4-D exposure. A 2008 study (Marple et al. 2008) evaluated cotton response to 2,4-D at rates ranging from 0.48 to 2.8 g ae ha⁻¹. One set of experiments examined cotton response at different growth stages and another experiment evaluated the effect of multiple applications. Cotton injury from single applications of 2,4-D at 1.4 g ae ha⁻¹ ranged from 8 to 75% 28 DAA depending on location-year and growth stage, with higher injury occurring when applications were made at the earlier growth stages. However, there was no significant yield loss from this rate in spite of the apparent foliar injury. Multiple applications of 1.4 g ae ha⁻¹ of 2,4-D caused significant visual injury and yield loss but lower rates had only moderate to no yield loss, depending on year, despite high levels of visual injury. Everitt and Keeling (2009) conducted similar studies exposing cotton to rates from 0.28 to 280 g ae ha⁻¹ of 2,4-D at various growth stages from cotyledon to full bloom. Despite auxin herbicide injury being observed, visual injury ratings overestimated eventual yield losses, especially at early application timings where injury ranging from 12 to 50% did not significantly affect yield up to 2.8 g ae ha⁻¹. A more recent paper (Sciumbato et al. 2014) found that an exposure of 0.53 g ae ha⁻¹ at the four- to six-leaf stage resulted in visual injury ranging from 2 to 16%, depending on year. Significant yield reductions in those studies generally occurred at rates higher than 5.3 g ae ha⁻¹, though cotton yield at that rate tended to be numerically lower. The 2014 meta-analysis (Egan et al.) of 2,4-D effects on cotton predicted a 19% yield reduction from an exposure of 0.56 g ae ha⁻¹ at the vegetative stage and 9% yield reduction from the same rate at reproductive stages. However, the data were highly variable, with regression coefficients (r^2) of 0.28 at the vegetative stage and 0.38 to 0.48 at

the reproductive stages. Similarly, the analysis found a low correlation between visual injury from 2,4-D and cotton yield ($r^2 = 0.32$). A possible explanation for this degree of variability may be related to cotton response to environmental conditions after exposure. Under favorable growing conditions the plants may be able to recover and compensate for earlier injury.

Grape is another crop species of concern for off-target injury by auxin herbicides. Like cotton, visual symptoms of herbicide exposure can be seen at very low doses; however, low-rate simulated drift applications (even multiple exposures) have been shown to yield amounts equivalent to untreated checks (Ogg et al. 1991). These studies showed that grapes treated with simulated drift rates between 1 and 10 g ae ha⁻¹, applied one to four times during the growing season, could recover as long as visual injury did not exceed 2 on their rating scale (2 = symptoms clearly visible, up to 20% reduction in leaf blade size, growth restriction of interveinal tissue, cupping of leaf margins, roughness to leaf surface). This level of injury was generally not observed with single applications at the rates tested but were exceeded by multiple applications of 10 g ae ha⁻¹. The authors did indicate some 2,4-D symptoms on the untreated control that may have been the result of ambient 2,4-D exposure from the surrounding area.

Tomato sensitivity to auxin herbicides has been the subject of several published studies (Bennet 1989; Breeze and West 1987; Fagliari et al. 2005; Hemphill and Montgomery 1981; Jordan and Romanowski 1974; Smith and Geronimo 1984; Van Rensburg and Breeze 1990). Visual symptoms can be observed at 1 to 2 g ae ha⁻¹ of 2,4-D and gross yield reductions have been reported in the range of approximately 3 to 20 g ae ha⁻¹ for plants in the flowering stage. Much less yield reduction occurs at later growth stages (Fagliari et al. 2005; Jordan and Romanowski 1974). Though overall yield may be reduced at these rates, several authors indicated observing malformed fruits and delayed maturity at around 2 g ae ha⁻¹ (Jordan and Romanowski 1974). Smith and Geronimo (1984) reported a tomato yield reduction ranging from 2 to 84% as the foliar application rate of 2,4-D amine increased from 1.1 to 560 g ae ha⁻¹. Similar to tomato, pepper is most sensitive to yield reductions from 2,4-D when exposure occurs near flowering

and is less affected postbloom (Gilreath et al. 2001). This may be a result of disruption of reproductive tissues at this time.

Other vegetable crops show a range of sensitivity to low rates of 2,4-D. Hemphill and Montgomery (1981) reported no significant yield effects on broccoli (*Brassica oleracea* var. *italica* L.), cabbage (*Brassica oleracea* var. *capitata* L.), carrot [*Daucus carota* subsp. *sativus* (Hoffm.) Schübl. & G. Martens], cauliflower (*Brassica oleracea* var. *botrytis* L.), cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L.), onion (*Allium cepa* L.), radish (*Raphanus sativus* L.), rutabaga (*Brassica napus* var. *napobrassica* L.), or turnip (*Brassica rapa* subsp. *rapa*) at 2,4-D rates up to 20.8 g ae ha⁻¹. However, none of the root crops produced marketable roots at this rate. More recent work (Mohseni-Moghadam and Doo-han 2015) indicated that injury to broccoli and bell pepper (*Capsicum annuum*) after applications of 2,4-D at doses ranging from 2.1 to 16.8 g ae ha⁻¹ varied significantly over years. Overall, broccoli was less sensitive to 2,4-D than bell pepper, with broccoli injury of less than 10% in most instances where rates below 16.8 g ae ha⁻¹ were applied. However, bell pepper injury was as high as 33% 7 DAA of 4.2 g ae ha⁻¹. In both cases visual injury declined by 28 DAA. Yield of broccoli was only affected by the highest rate in 1 of the 2 yr of the experiments. Although overall yield of bell pepper was generally not affected by 2,4-D, yield at the earliest harvest date was reduced by the 16.8 g ae ha⁻¹ rate. In contrast to the root crop injury reported by Hemphill and Montgomery, potatoes appear to have tolerance to low levels of 2,4-D and certain varieties are treated with 2,4-D to enhance color (Bussan et al. 2014).

Other Field Crops. Peanuts (*Arachis hypogaea* L.) have demonstrated considerable tolerance to low rates of 2,4-D (Johnson et al. 2012; Leon et al. 2014), with rates of 70 g ae ha⁻¹ or greater required to cause significant yield loss. Wall (1996) investigated injury to buckwheat [*Fagopyrum tataricum* (L.) Gaertn.], canola (*Brassica napus* L.), field pea, lentil (*Lens culinaris* Medik.), and sunflower across 3 yr from rates of 2,4-D ranging from 9.5 to 151.2 g ae ha⁻¹. Sunflower was the most sensitive of these crops, with 9.5 g ae ha⁻¹ resulting in 9 to 83% visual injury, depending on year. Canola was relatively insensitive, with 0 to 5% injury at rates of 75.6 g ae ha⁻¹ or less.

The references above indicate that there are indeed visible injury symptoms from unintended exposure of sensitive dicot crops to auxin herbicides, sometimes at very low rates. Yield reductions do occur in several of these studies when higher doses (e.g., > 0.1× of use rates) are applied. However, normal drift (or possible vapor exposure) often results in recoverable injury symptoms with little or no yield penalty in many cases, especially under favorable growing conditions. Effects on quality of horticultural produce may be more complicated, justifying further research.

Ornamentals. Injury to trees and ornamental plants from off-target movement of 2,4-D has been reported since the 1950s and 1960s (Linn et al. 1959; Phipps 1963) and is often a source of complaints to state regulatory authorities and extension offices. Sometimes these incidents are a result of drift from agricultural applications made to fields adjacent to homes and gardens. In other cases the exposure may occur during application of 2,4-D to lawns bordered by susceptible plants. In either instance visual symptoms can sometimes be confused with those caused by biotic agents such as mites and diseases (Al-Khatib et al. 1992b). Studies have been conducted to quantify these effects at rates ranging from 1 to 375 g ae ha⁻¹ (Al-Khatib et al. 1992b,c; Hatterman-Valenti et al. 1995; Hatterman-Valenti and Mayland 2005; Samtani et al. 2008). Injury to rose (*Rosa dilecta*) was 7 to 10% 30 DAA and 0 to 5% 60 DAA of 11.2 g ae ha⁻¹ (Al-Khatib et al. 1992b). Seedling white oak (*Quercus alba* L.) exhibited moderate levels of damage (2 to 4 on a rating scale of 1 to 10) when exposed to 15 g ae ha⁻¹ at the leaf-unfolding and fully expanded leaf stages, but injury was not always significantly different from the control and varied significantly by year (Samtani et al. 2008). An evaluation of 2,4-D injury to nine different annual bedding flowers (Hatterman-Valenti et al. 1995) reported values of approximately 10 to 15% visual injury from rates ranging from 1 to 64 g ae ha⁻¹. The exceptions included petunia (*Petunia x hybrida* Hort. Vilm.), which exhibited 28% visual injury at 4 g ae ha⁻¹.

The above examples have been largely generated through the direct spray application methods already discussed. Plant response to exposure to herbicide vapors presents a unique situation and associated challenges regarding experimental design (Breeze 1993). Generation of consistent, measure-

able vapor concentrations requires complex apparatus and is difficult to replicate (Breeze 1993; Breeze and West 1987). In the field, atmospheric mixing, duration of exposure, and environmental factors affecting plant sensitivity will greatly affect observed responses of sensitive dicots to 2,4-D in the vapor phase. Although some field studies have been able to demonstrate qualitative differences between herbicides and formulations (Eytcheson et al. 2012; Sosnoskie et al. 2015), quantitative correlations between measured air concentrations of 2,4-D and broadleaf crop injury have been inconsistent (Farwell et al. 1976; Sandmann et al. 1991). Other attempts at quantification relied on correlation of visual plant response under vapor conditions with dose–response curves generated via direct spray applications (Sciumbato et al. 2004).

Toxicology

Numerous controversies regarding the impact of 2,4-D on human health have been raised over decades of use. Since its introduction in 1946, the toxicology and human health effects of 2,4-D have been studied intensively, making it one of the most extensively evaluated chemical compounds. This information has been previously reviewed in detail (Bus and Leber 2001; Garabrant and Philbert 2002; Gingell et al. 2001; Kennepohl et al. 2001; Munro et al. 1992). A brief overview is presented here as an update on the status of the potential human health risks associated with the use of 2,4-D.

Acute Toxicity. The most likely human or animal exposure to 2,4-D would be short term or acute. A review by the USEPA of numerous acute toxicological studies has concluded, “2,4-D generally has low acute toxicity via the oral, dermal and inhalation routes of exposure (Toxicity Category III or IV). 2,4-D is neither a skin irritant nor a skin sensitizer. Although ester forms are not eye irritants, the acid and salt forms are considered to be eye irritants.” (USEPA 2005a). The observed dermal absorption of < 6% (Kennepohl et al. 2001; Maibach and Feldmann 1974; USEPA 2004) is considered to be low.

Subchronic and Chronic Toxicity (Including Carcinogenicity). Once absorbed, 2,4-D is rapidly and completely excreted in urine (Timchalk 2004; Van Ravenzwaay et al. 2003). Therefore, it does not

accumulate in animal tissues. The toxicological kinetics of 2,4-D are well understood via numerous animal studies (Charles et al. 1996a,b) and, when coupled to human exposure information, significantly improves confidence in human risk extrapolation (Saghir et al. 2013). These animal studies have found only minimal effects at the highest doses (300 mg kg⁻¹ d⁻¹) tested (Charles et al. 1996a,b). Often the higher doses exceeded the maximum tolerated dose, the highest dose of a treatment that will produce an effect without unacceptable toxicity to the test animal, and were therefore not representative of true subchronic exposure.

Although highly publicized National Cancer Institute studies in the 1980s pointed to a link between 2,4-D and non-Hodgkin’s lymphoma (Hoar et al. 1986), further analysis of the data as well as additional studies concluded that there was no such correlation (Cantor et al. 1992; De Roos et al. 2003; Zahm et al. 1990). Other studies claimed a link of residential use of 2,4-D to malignant lymphomas in pet dogs with access to treated areas (Hayes et al. 1991). However, a re-evaluation of the raw data from this study did not confirm either a dose–response relationship or actual association between 2,4-D use and occurrence of canine malignant lymphoma (Kaneene and Miller 1999).

Recent published reviews and analyses support the position that epidemiology data are inadequate to establish a causal association between 2,4-D exposure and cancer (Burns and Swaen 2012; Goodman et al. 2015; von Stackelberg 2013). Many reviewers point out insufficient quality with respect to exposure and a lack of consistency across multiple studies.

This large body of work regarding carcinogenicity of 2,4-D has been reviewed by numerous scientific and regulatory groups including the USEPA (2005b, 2012), Health Canada Pest Management Regulatory Agency (PMRA) (2005, 2007, 2008), the World Health Organization (WHO) (1996), New Zealand Environmental Risk Management Authority (2003), and the European Commission (2001). All of these regulatory reviews have found no evidence of carcinogenicity.

In 2015, the International Agency for Research on Cancer (IARC) assessed the cancer hazard of 2,4-D and assigned a classification of “2B – possibly carcinogenic to humans” (Loomis et al. 2015). This conclusion is at odds with comprehensive cancer

reviews completed by health and safety regulators worldwide. This classification does not mean that 2,4-D causes or is even likely to cause cancer in people. IARC has assigned its 2B grouping to many other common products including aloe vera, coffee, and pickled vegetables (WHO 2015).

Genotoxicity, Teratogenicity, Reproductive Toxicity, and Neurotoxicity. The potential for 2,4-D to produce birth defects, alter reproductive processes, or affect endocrine or neurological function has been thoroughly assessed (Bus and Leber 2001; Charles et al. 1996a,b, 2001; Gingell et al. 2001; Gollapudi et al. 1999). None of these studies has identified a link between 2,4-D and substantive effects in this area. The 2005 USEPA 2,4-D reregistration eligibility decision concluded that it is not “biologically plausible” that 2,4-D and related compounds are associated with adverse effects on development or reproduction in humans (USEPA 2005a). A more recent extended one-generation study examining systemic toxicity, developmental neurotoxicity, developmental immunotoxicity, reproductive toxicity, endocrine modulation, and thyroid effects was published in 2013 (Marty et al. 2013). Critical evaluation of the results of this study by USEPA confirmed that there is no indication of reproductive toxicity, developmental neurotoxicity, or endocrine disruption by 2,4-D (USEPA 2013). Other studies in fish and frogs further support the no-effect determination with regard to 2,4-D and endocrine disruption (Coady et al. 2013, 2014).

A key fundamental in USEPA’s risk assessment of 2,4-D toxicity was that dose results above renal saturation would not be considered (USEPA 2005b). The mechanisms responsible for renal clearance of 2,4-D have been investigated in several species. 2,4-D is actively secreted by the proximal tubules. This mechanism of renal clearance is consistent with results seen with other phenoxy acids. Observed dose-dependent, nonlinear pharmacokinetics of 2,4-D are primarily due to the saturation of this renal secretory transport system. Developmental toxicity, neurotoxicity, and reproductive toxicity were only observed after exposure to 2,4-D and its amine salts and esters at dose levels that were at or above the threshold of saturation of renal clearance. USEPA’s evaluation only considered dose level results below renal saturation for toxicity of 2,4-D.

As to effects on the immune system, studies have found 2,4-D to have no impact on antibody formation or cells associated with the immune response (Coady et al. 2014).

Relevance to Human Health. Potential human risks to pesticide exposures can be estimated by comparing the ratio of no-observed adverse-effect level values obtained from animal toxicity studies to estimated human exposures. This is known as the margin of exposure (MOE). Since 2,4-D is completely and rapidly excreted in urine in humans, collection of total 24-h urine samples provides reasonable estimates of immediate 2,4-D exposures. For professional workers employed as commercial yard sprayers, total 2,4-D exposure has been estimated as $0.003 \text{ mg kg}^{-1} \text{ d}^{-1}$, resulting in a calculated MOE of 1,700 (Yearly 1986). For nonprofessional home and garden 2,4-D users, exposure is estimated at $0.0001 \text{ mg kg}^{-1}$, resulting in a MOE of 50,000 (Solomon et al. 1993). In both cases the large MOE between a dose causing no toxic effects in animals and actual estimates of human exposures under real-world use conditions suggests a high margin of safety for approved uses of 2,4-D.

Hays et al. (2012) recently compared external dose-based risk assessments to biomonitoring-based assessments (Aylward et al. 2010) using the concept of bioequivalents. The findings of this assessment indicate that the external dose-based assessments result in estimates of exposure and resulting hazard quotients that are consistently several-fold higher than those based on biomonitoring data. Both approaches provide consistent findings of substantial margins of safety in the U.S. population for exposures to 2,4-D under current product stewardship practices for both acute and chronic exposure scenarios. This highlights that assessments based on both external and internal dose approaches support the conclusions that current use practices for 2,4-D are safe for human health.

Effects on Wildlife

Pesticides may have effects on wildlife through either direct exposure, indirect exposure, or impact on habitat. Exposures of wildlife to 2,4-D, whether from direct spraying or consumption of treated vegetation, is of low toxicological significance. Current studies show that 2,4-D is practically

nontoxic to fish and amphibians (frogs), only slightly toxic to aquatic invertebrates, and practically nontoxic to honeybees and earthworms (Industry Task Force II on 2,4-D 2009b).

All 2,4-D formulations are considered to be only slightly toxic to practically nontoxic to birds, as represented by the mallard duck and bobwhite quail in 8-d dietary studies (USEPA 2005b). Evaluation of an avian reproduction study (quail) found that the no-effect concentration was greater than 1,000 ppm, practically nontoxic for a wide range of measurements such as egg laying and shell thickness (USEPA 2005b). Terrestrial invertebrates, including bees and earthworms, have a low sensitivity to 2,4-D (USEPA 2005b; WHO 1997). In a similar fashion, most formulations of 2,4-D have been shown to be practically nontoxic to aquatic invertebrates (USEPA 2005b; WHO 1997).

Indeed, the greatest effect of 2,4-D on wildlife is likely to be the presentation of an enhanced habitat after spraying, which allows the infiltration of lower-growing fruit-bearing plants. In a study covering 2 decades, many common game species were shown to prefer habitat created by a sprayed utility right-of-way (Bramble and Burns 1974).

Environmental Fate

2,4-D has a relatively short half-life and has limited mobility in the soil. In 35 recent field dissipation studies across the United States, less than 5% of applied 2,4-D moved downward more than 6 inches. The average lowest depth detected ranged from 6 to 12 inches in soils of the southern United States and 16 to 24 inches in low organic soils where greater movement would be expected (Wilson et al. 1997).

Soils were sampled to a depth of 48 inches and analyzed for 2,4-D plus its soil metabolites until two analyses provided a result of “nondetectable” at each sampled depth. Even though laboratory solubility studies indicated that 2,4-D is potentially mobile, rapid degradation in the soil and removal from soil by plant uptake minimizes leaching under application conditions within normal limits.

Field dissipation studies found that 2,4-D had an apparent soil half-life of 6.2 d with a range of 1.7 to 13.1 d. The moisture content of the soil appears to have a major effect on the half-life, since the main route of degradation is by microorganisms. The

commonly used 2,4-D amine salts and 2,4-D esters are not persistent under most environmental conditions. Dissociation of 2,4-D amine salt is expected to be instantaneous (< 3 min) under most environmental conditions. Ester forms of 2,4-D biotransform and hydrolyze rapidly to the acid in natural soil and water conditions, typically in less than 2 d. Under these conditions, the environmental exposure from 2,4-D esters and 2,4-D amines is expected to be minimal in both terrestrial and aquatic environments (Wilson et al. 1997).

Bioaccumulation–Bioconcentration. Environmental fate and animal data show that 2,4-D is relatively short lived. Animal metabolism studies demonstrate that the herbicide is rapidly eliminated and has a low potential for bioaccumulation or bioconcentration. A Canadian study spanning several decades showed no accumulative effect in soil (Smith 1989).

2,4-D-Resistance Traits

For some time, researchers have been attempting to incorporate resistance to 2,4-D into desirable plants that are normally sensitive either through selective breeding (Devine et al. 1975; Munoz et al. 2015; Taylor et al. 1989) or biotechnology (Bayley et al. 1992; Charles et al. 2007; Wright et al. 2010). The objectives were generally to allow use of 2,4-D on the crop of interest or incorporate greater tolerance to low rates occurring from off-target movement.

It has long been understood that 2,4-D is degraded in soil by microorganisms (Audus 1950; Steenson and Walker 1957). By the late 1980s specific enzymes responsible for 2,4-D mineralization by soil bacteria had been identified and later were inserted into crop plants to confer limited tolerance to applications of 2,4-D (Bayley et al. 1992; Perkins et al. 1987; Streber et al. 1987). However, these early efforts often did not produce crops that could withstand application rates required for effective weed control (Charles et al. 2007). The discovery and development of bacterial genes that code for aryloxyalkanoate dioxygenase enzymes (AADs) has provided a path to new transgenic crops that are robustly resistant to application rates of 2,4-D, thus enabling new use patterns for the molecule and providing a means of adding additional modes of action to weed control

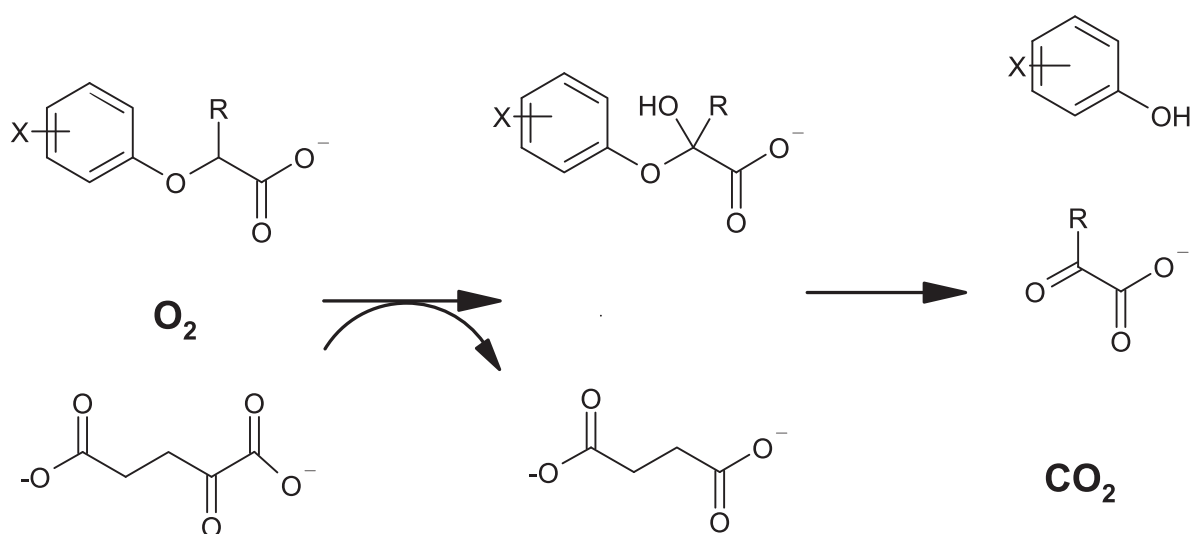


Figure 3. General reaction catalyzed by aryloxyalkanoate dioxygenase enzyme (adapted from Wright et al. 2010).

programs in corn, cotton, and soybean (Wright et al. 2010). Originally isolated from the soil bacteria *Sphingobium herbicidivorans* and *Delftia acidovorans*, these enzymes catalyze a two-step dioxygenase reaction that degrades 2,4-D acid into the non-herbicidal metabolite dichlorophenol (Figure 3). Genes that encode these enzymes have been successfully introduced into several plants, including *Arabidopsis*, maize, soybean, cotton, rice, canola, and tobacco (Peterson et al. 2011). Currently, maize, soybean, and cotton are being commercialized for use in several countries across the Americas. The tolerance enabled by these traits has been shown to be highly robust at 2,4-D rates up to 4,480 g ae ha⁻¹ applied POST to these crops at a range of growth stages (Peterson et al. 2011; Robinson et al. 2015; Wright et al. 2010).

POST applications of 2,4-D at rates from 800 to 1,100 g ae ha⁻¹ in combination with glyphosate provide broad-spectrum control of grass and broadleaf weeds, including broadleaf weeds that have developed resistance to glyphosate (Peterson et al. 2011; Robinson et al. 2012). This is a higher rate range than has typically been applied in most crops POST. A premix of 2,4-D choline plus glyphosate (195 g ae L⁻¹ + 205 g ae L⁻¹) has been developed by Dow AgroSciences for use in conjunction with AAD-enabled crops (Li et al. 2010, 2013). The currently labeled rates for this product in the United States range from 1,640 to 2,185 g ae ha⁻¹, thus providing 800 to 1,065 g ae ha⁻¹ of 2,4-D choline and 840 to 1,120 g ae ha⁻¹ of glyphosate

(Anonymous 2014a). Other work has been conducted to examine the performance of 2,4-D combined with glufosinate as an alternative to combinations with glyphosate (Craigmyle et al. 2013).

Optimum weed control and best management practices for resistance are best served by applying these combinations as a part of a weed management program that utilizes a combination of diverse weed control tools. Applications of 2,4-D choline + glyphosate after burn-down or PRE applications of soil-residual herbicides have provided consistent control of several glyphosate-resistant species (Ellis et al. 2014; Ruen et al. 2014; Simpson et al. 2014).

Summary

Despite having been discovered more than 70 yr ago, 2,4-D continues to provide significant benefits to farmers, ranchers, homeowners, land managers, and many others who work to control weeds. The body of research generated by hundreds of scientists over this time frame is truly amazing and forms the basis for much of our knowledge of herbicide action and physiology. Studies conducted as early as the 1940s and 1950s must be admired for their contributions to weed science, especially considering the tools of the day. More recent investigations of 2,4-D mechanisms of action and general activity in plants provide unique insights into the function of plant auxins. Innovations around this molecule are expanding its utility and helping to address

issues associated with its use, extending its useful life well into the future.

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