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Herbicides for Natural Area Weed Management

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<http://dx.doi.org/10.5772/56183>

1. Introduction

Natural areas represent a significant resource for many countries. In the U.S. natural areas can be defined as conservation lands set aside for preservation or restoration, such as city or county park, private woods, state or national park, Bureau of Land Management (BLM) lands, or other areas [1,2]. In many cases these areas are utilized for recreation, ecosystem services or other non-agricultural purposes [3,4]. Given this broad definition, natural areas encompass a huge portion of the land mass of the United States and represent incredible biological diversity. According to the U.S. National Vegetation Classification in 2012 there are 8 major classifications in the U.S. with 430 groupings and over 6100 associations [5]. Some of the more common ecological communities include deciduous temperate forests, temperate coniferous forests, grasslands, and wetlands such as swamps, tidal marshes, and riparian zones.

Many natural areas are managed to some degree for a variety of uses, but due to the complexity of many natural area systems, the management techniques developed for, and utilized in these areas is diverse. Some areas are managed exclusively for recreation and include water attractions, hiking and biking trails, horse trails, or camping. In these cases, user satisfaction, human health and safety are the primary goals, with ecological community diversity being a secondary, but often equally important, goal [6]. Other areas that are managed for conservation (including hunting), preservation or restoration may not require as intense or frequent management [7].

Vegetation management in natural areas is performed for a variety of purposes but falls broadly into two primary categories: 1) maintaining the existing vegetation at desirable levels and species composition or 2) restoring the ecosystem to a desirable state. With the latter category, restoration can include reintroduction of naturally occurring species,

reintroduction of a natural ecological process such as fire or water fluctuations, and/or providing an environment that allows for natural reintroduction/colonization of native species [8].

Within the past 2 decades, vegetation management in natural areas has intensified due to issues with invasive species. Invasive weedy species represent one of the biggest threats to the diversity and utility of many natural areas [9]. Moreover, invasive species are considered to be a major threat to endangered species, second only to habitat loss [10]. Currently there are over 400 invasive non-native plants impacting approximately 133 million acres in the U.S. alone and it is estimated that invasive species are spreading at the rate of 1.7 million acres annually [11]. In 1999, a mandated executive order specifically addressed invasive species and their impacts, leading to the formation of the National Invasive Species Council (NISC) and the Invasive Species Advisory Committee (ISAC) [12]. These organizations and many more at the regional, state and local level dedicated to invasive species management has greatly influenced natural area vegetation management.

This chapter will provide an overview of the types of management practices used in a range of natural area systems and detail those herbicides used in natural areas. Weed management in aquatic systems will not be discussed in this chapter.

2. Herbicide registration and regulation for use in natural areas

Herbicides are labeled for use on a specific crop or site as defined by the U.S. Environmental Protection Agency [13]. Many herbicides can be used in natural areas, but labeling may be restricted to only specific uses within the broader context of a 'natural area'. In addition, many states, agencies, and/or local governments may prohibit or restrict usage of a particular product or compound. It is not the intent of this chapter to list those specific sites where a particular herbicide could be used, but rather provide details of how the herbicide is applied, its mode of action, its spectrum of activity and environmental considerations associated with use.

3. Overview of natural area herbicides and their mechanisms/modes-of-action

This section will provide background of those herbicides used in natural areas and will include information on chemistry, formulations, mode-of-action and selectivity. Specific details to each herbicide are listed in Table 1.

Herbicide (common name)	Mechanism of Action ²	Rate range kg/ha ³	Application Methods ⁴	Environmental Dissipation ⁵	*Common Application Methods and General Spectrum of Control ⁶
2,4-D	O (4)	0.56-3.8	F, B, I, CS	Microbial (7-10)	POST – annual, perennial broadleaves
Diclorprop	O (4)	4.1-10.4	F, S, I, CS	(10)	PRE, POST – annual, perennial BL's, brush
Dicamba	O (4)	0.28-2.2	F, S, B, CS	Microbial (4-14)	PRE, POST – annual, perennial BL's, brush
Picloram	O (4)	0.14-1.12	F, S, B, I, CS	Microbial (90-300)	PRE, POST – perennial BL's, brush, trees
Triclopyr	O (4)	0.56-9.0	F, B, I, CS	Microbial (30)	POST – perennial BL's, brush, trees
Fluroxypyr	O (4)	0.14-0.56	F	Microbial (38)	POST – annual, perennial BL's, brush
Clopyralid	O (4)	0.14-0.56	F, S	Microbial (40)	PRE, POST – annual, perennial BL's, brush
Aminopyralid	O (4)	0.09-0.25	F, S, I	Microbial (35)	PRE, POST – annual, perennial BL's, brush
Aminocyclopyrachlor	O (4)	0.06-0.28	F, S, B, I	Microbial (60)	PRE, POST – annual, perennial BL's, brush
Simazine	C ₁ (5)	2.2-8.9	S	Microbial (70-90)	PRE – annuals, perennials
Diuron	C ₂ (7)	4.5-18	S	Microbial (90)	PRE – annuals, perennials
Tebuthiuron	C ₂ (7)	0.84-4.48	S	Microbial (400)	PRE – perennial herbs, brush, trees
Hexazinone	C ₁ (5)	2.5-7.5	S	Microbial (90)	PRE – perennial grass, brush, trees
Bromacil	C ₁ (5)	1.8-13.4	S	Microbial (60)	PRE – annual, perennial, brush
Prometon	C ₁ (5)	8.9-36	S	Microbial (450)	PRE – perennial grass, brush, trees
Glyphosate	G (9)	1.1-5.6	F	Irreversibly bound	POST – annuals, perennials, brush
Fosamine	Z (27)	2.24-26.9	F	Microbial (8)	POST – woody brush, trees
Glufosinate	H (10)	0.32-1.56	F	Irreversibly bound	POST – annuals, limited perennials
Paraquat	D (22)	0.71-1.14	F	Irreversibly bound	POST – annual species, no soil activity
Sethoxydim	A(1)	0.31 - 0.53	F	Microbial (4-11)	POST - annual grasses only
Clethodim	A(1)	0.11 – 0.28	F	Microbial (3)	POST - annual and perennial grasses only
Fluazifop-p-butyl	A(1)	0.13 – 0.42	F	(7-21)	POST - annual and perennial grasses only
Imazapyr	B (2)	0.56 – 1.70	F, S, B, I, CS	Microbial (25-140)	PRE, POST – perennial grass, brush, trees
Imazapic	B (2)	0.05 – 0.21	F, S	Microbial (60-120)	PRE, POST – annuals, perennial grasses
Imazamox	B (2)	0.14 – 0.56	F	Microbial (20-30)	POST – annuals, brush, trees
Chlorsulfuron	B (2)	0.018-0.15	F, S	Hydrolysis (40)	PRE, POST - rangeland annual/perennials
Metsulfuron-methyl	B (2)	0.012-0.17	F, S	Hydrolysis (30)	PRE, POST – annuals, perennials, brush
Sulfometuron-methyl	B (2)	0.065-0.4	F, S	Hydrolysis (20-28)	PRE, POST – annual, perennials, brush
Flumioxazin	E (14)	0.28-0.42	S	Microbial (12-18)	PRE – annual species
Oxyfluorfen	E (14)	0.56-2.24	S	Photolysis (35)	PRE- annual species
Isoxaben	L (21)	0.56-1.12	S	Microbial (50-120)	PRE – seedling annual species
Pendimethalin	K ₁ (3)	0.84-3.36	S	Photolysis (44)	PRE – seedling annual species

Herbicide (common name)	Mechanism of Action ²	Rate range kg/ha ³	Application Methods ⁴	Environmental Dissipation ⁵	*Common Application Methods and General Spectrum of Control ⁶
Oryzalin	K ₁ (3)	2.24-6.72	S	Photolysis (20-90)	PRE – seedling annual species
Diclobenil	L (20)	4.5-22.4	S	Microbial (60)	PRE – seedling annual species, nutsedge
S-Metolachlor	K ₃ (15)	1.4-2.8	S	Microbial (67)	PRE – seedling annual species, nutsedge

¹ Information presented derived from sources including, but not limited to: 2007 Herbicide Handbook, Weed Science Society of America, Lawrence, KS. 458p; ExToxNet - The EXtension TOXicology NETwork, <http://extoxnet.orst.edu/>; Crop Data Management Systems, Inc., <http://www.cdms.net/>.

² Mode of action classification based on Herbicide Resistance Action Committee (HRAC) – [letters and subscript numbers] and the Weed Science Society of America (parentheses). HRAC <http://www.hracglobal.com/> WSSA <http://www.wssa.net/Weeds/Resistance/WSSA-Mechanism-of-Action.pdf>

³ Rate range based on current label guidelines for control in natural areas or non-cropland sites. Rate expressed in kilograms of active ingredient per hectare.

⁴ Application methods include: F - foliar, S – soil, B – basal bark, I – stem injection, CS – cut stump

⁵ Environmental dissipation includes the major means of breakdown and half-life range in days in soil. In some cases, the mechanism of breakdown is not available.

⁶ abbreviations: POST – postemergence activity/application; PRE – preemergence soil activity; BL's – broadleaf species.

*General application information only – refer to product label and local/state recommendations for specifics on use rates, application methods and timing, species controlled and restrictions for use.

Table 1. Properties and application methods of commonly used herbicides used in natural areas¹.

3.1. Synthetic auxins or growth regulators

The growth regulator herbicides represent the oldest and possibly the most widely used of the herbicides used in natural areas. These materials are mechanistically classified as synthetic auxins [14] and include herbicides in the phenoxy-carboxylic acids, benzoic acid and pyridine carboxylic acid (picolinic acid) chemical groups.

2,4-D is the principle herbicide in the chemical group phenoxy-carboxylic acids and has been used for broadleaf weed control since the late 1940's. This compound was first noted to have growth regulator properties in 1942, and registered as an herbicide after World War II [15]. There have been 28 different chemical formulations registered for 2,4-D, including the parent acid, amine salts and esters [14]. Salt formulations are characterized by fairly high water solubility and low volatility, while esters are more prone to volatility and more soluble in liquid fertilizers [16]. Ester formulations show greater phytotoxicity per acid equivalent basis due to greater cuticle penetration and foliar uptake. Short chain esters are highly prone to volatilization, and no longer registered for use. As of 2005, there were 9 formulations of 2,4-D supported for reregistration by the United States Environmental Protection Agency [17]. These include the parent acid, the sodium, diethanolamine, dimethylamine, isopropylamine, and triisopropanolamine salts, and the 2-butoxyethyl, 2-ethylhexyl, and isopropyl esters. In general salts are formulated as wettable powders, granules or soluble concentrates, while the water-insoluble esters are formulated as emulsifiable concentrates or mixed with oils or liquid fertilizers.

In addition to 2,4-D, there have been several other phenoxy-carboxylic acid herbicides developed that are used in natural areas. These include MCPA, diclorprop, and mecoprop. Once again several formulations of each have been developed, including salt and ester forms. Of the three, diclorprop is used most extensively in natural areas [18], while MCPA and mecoprop are mainly used in grass crop and turf situations for annual and perennial broadleaf weed control [19]. As of 2007, the parent acid and the dimethylamine salt and ethylhexyl ester formulations of dichlorprop are registered for use by the US EPA. This herbicide has better activity on woody brush and trees, compared to 2,4-D. The ester formulation is often used alone or in oil-based carriers for spot specific plant treatments such as fencerows and rights of ways.

For many years, the phenoxy herbicide 2,4,5-T was the standard treatment for woody brush and tree control in pastures and rangeland [20]. This herbicide was highly active on several species and possessed considerable soil persistence, which contributed to its effectiveness. 2,4,5-T was cancelled for use by the U.S. EPA in the early 1980's due to concerns from the contaminant dioxin during certain manufacturing processes. Dioxin has been demonstrated to be a known carcinogen and was present in considerable quantities of 2,4,5-T used during the Vietnam war [21]. The herbicide known as 'Agent Orange' was actually a combination of 2,4,5-T and 2,4-D used for widespread aerial-applied jungle defoliation [22]. However, the levels of dioxin in commercially produced 2,4,5-T after the war were very low, but continuing concerns and public outcry lead to the cancellation of this herbicide [23].

The benzoic acid herbicide chemical family contains only one currently available herbicide for use in natural areas, dicamba. Dicamba is formulated only as a salt, with the following salts registered for use by the US EPA: dimethylamine (DMA) salt, sodium (NA) salt, isopropylamine (IPA) salt, diglycolamine (DGA) salt, and potassium (K) salt [14]. Interestingly, this herbicide can volatilize and move off target, despite being formulated as a salt. Dicamba is highly effective on many weeds in crops and is widely used in pasture/rangeland situations for perennial weed management [24]. It is considered to have superior perennial broadleaf weed control compared to many of the phenoxy herbicides, while still providing selectivity towards crops (primarily corn and sorghum). Dicamba also possesses greater soil persistence than phenoxy, which also contributes to its control [25].

The pyridine or picolinic acid herbicide chemical family comprises several herbicides that are widely used for natural area weed control. In general these herbicides are more potent compared to equivalent rates of phenoxy herbicides, and many possess considerable soil residual activity. The first picolinic acid herbicide developed was picloram in 1963 by Dow Chemical [14]. Similar to 2,4-D, picloram is formulated as salts (triisopropanolamine and potassium) and ester (ethylhexyl/isooctyl). Picloram is used in a wide range of natural areas, particularly open rangeland, for woody brush control [18]. Several formulations are also used in permanent pasture situations for perennial broadleaf weed control. The use of picloram is limited in certain areas over potential groundwater contamination concerns due to high water solubility and relatively long soil half-life. Moreover, many crops are highly sensitive to picloram at very low rates (<ppb), which also limits use in tolerant crops due to rotational concerns [26].

Triclopyr is probably the most widely used picolinic acid herbicide in natural areas, especially for woody brush species [27]. This herbicide is formulated as the triethylamine salt and the butoxyethyl ester, both of which are used across a wide range of natural, forest and pasture/rangeland situations. It possesses good activity on many annual and perennial broadleaf weeds and brush, but at rates slightly higher when compared to picloram [20]. However, unlike picloram, triclopyr has limited soil activity and is generally considered to be non-soil active [14].

The picolinic acid herbicide fluroxypyr also has limited soil activity, and is used primarily for broadleaf weed control in cereals, fallow cropland and pastures. It is formulated as a meptyl and butometyl ester and is often combined with other growth regulator herbicides to broaden weed control spectrum [28]. The use of fluroxypyr in natural areas is limited, primarily rights of ways, mainly due to superior weed control spectrum from other picolinic acid herbicides and labeling restrictions.

Clopyralid is another picolinic herbicide with moderate utility in natural areas. This herbicide was discovered in 1961 by Dow Chemical Company but was not registered for herbicidal use in the U.S. until 1987 [14]. It is mainly formulated as the monoethanolamine salt, but ester formations are also available. Clopyralid has moderate soil persistence and may cause problems with sensitive crops planted after clopyralid use in the previous crop [28]. This herbicide has broadleaf weed activity, similar to the picolinic acid herbicides as a whole, but has greater specificity and therefore selectivity towards many legume, solanaceous and composite type weeds [29,30,31].

Aminopyralid is a relatively new picolinic acid herbicide registered for use in pastures/rangeland, forestry and natural areas [14]. Aminopyralid is only formulated as the potassium salt. It has moderate soil persistence, and like clopyralid, has specificity towards legume, composite and solanaceous weeds [32]. In fact, one of the primary registrations for this herbicide is for the control of tropical soda apple (*Solanum viarum*) in southeastern U.S. pastures [33]. In other areas of the U.S. the primary target species is composites such as thistles (*Cirsium spp.*) and species of knapweeds (*Centaurea spp.*) [34]. It is formulated as a salt and often combined with other herbicides to increase weed spectrum.

Aminocyclopyrachlor is the most recent herbicide to be registered for use in natural areas [35]. This herbicide possesses the typical growth regulator mode of action, but does not fit within the chemical classifications listed above. The uses of this compound are still being developed, but like aminopyralid and clopyralid, it has remarkable specificity at low use rates [36]. Aminocyclopyrachlor is primarily formulated as a salt, but ester formulations have been tested for basal bark applications in oil carriers. This herbicide is very active on a range of broadleaf species, but also possesses considerable activity on certain grasses, including many perennial grasses [37].

The mode of action of the synthetic auxin herbicides is not well understood, but appears to disrupt the normal cellular and tissue response to auxin. Auxin is present in plants at very small concentrations (nanomolar) and acts as a signaling molecular for a wide range of cellular functions and responses [38]. Auxin levels must be precisely controlled within the plant for

normal regulation of plant responses to growth, development and environmental stimuli [39]. Auxin is regulated through two processes; metabolism via biosynthesis, conjugation, de-conjugation and degradation or transport and distribution within and between cells. The distribution of auxin, including directional flow, is regulated by the presence and activity of auxin transporters in the plasma membrane. Because auxins are weak acids, they are dissociated in the presence of neutral cellular pH (7.0) and trapped as anions within the cell. Thus transport out of the cell can be mediated through plasma membrane located facilitators specific for auxin.

Herbicides within this classification are considered auxin mimics, and are thought to act like auxin within plant tissues. Earlier research suggested that these herbicides acted to acidify the cell wall by activating a membrane bound ATPase proton pump and this acidification induced cell elongation [40]. Other work showed an increase in RNA polymerase, leading to increases in cell division and uncontrolled growth. Ethylene generation has also been reported, likely to counteract the stimulatory effect of auxin [41]. However, recent work has shown 2,4-D to be transported by influx carriers into the cell [42] and also through efflux carriers [43]. Due to limited metabolism, the auxin-effect of these herbicides presumably causes rapid cell division in some cells and a complete cessation of growth in other cells. This unregulated growth results in stem twisting, leaf strapping, puckering, and a plethora of other symptoms associated with growth regulator herbicides.

Synthetic auxin herbicides are chemically weak acids, and although some possess soil activity, these herbicides are applied to the foliage of plants. Once applied these herbicides are rapidly absorbed by leaf tissue and remobilized, similar to carbohydrate movement, to areas of meristematic growth via the phloem [14]. They possess the similar anion trapping mechanism as natural auxins, and this likely contributes to their effectiveness in herbicidal activity. Soil uptake of these herbicides occurs through the xylem where upward movement to shoots and leaves takes place. However, once diffusing from the xylem into leaf tissues, the herbicide is transported, in a similar manner to carbohydrates, to regions of meristematic growth.

The ability to metabolize is the primary selectivity mechanism for tolerant plant species. In most cases, grasses are moderately to highly tolerant to growth regulator herbicides through the ability to conjugate these herbicides with amino acids or sugars [25]. Most of these herbicides are slowly degraded regardless of plant species, but grasses appear to have the ability to shunt the herbicide conjugate to the vacuole, where it is either sequestered from sites of action, and/or slowly degraded. Many picolinic acid herbicides such as picloram, aminopyralid and clopyralid are sequestered in the vacuole of tolerant plants, but the compound remains intact and thus herbicidally active [44]. This has led to many issues with off-target damage due to removal of the herbicide sequestering plant tissue and subsequent release of the herbicide in the environment.

This phenomenon was first observed with picloram, and later with clopyralid and aminopyralid. In the case of picloram, animals grazing on treated forage grasses were observed to have the ability to transfer the herbicide through urination or defecation. Concentrating of the herbicide, coupled with soil persistence lead to problems with sensitive crops planted in fields after grazing. Dried hay, either degraded as plant biomass or via manure, transferred from

treated fields to other areas has also been shown to cause problems [45]. Manure from animals fed on treated forage that is used for compost and fertilizer is another source of contamination. More recently, grass clippings from treated turf, primarily clopyralid, can also be a problem [46]. The sequestration rather than degradation, coupled with high sensitivity at very low rates (parts per billion) for many broadleaf crop species is the reason for this major problem. This issue has led to the cancellation of this herbicide in many areas, due to contamination in municipal compost for use by the general public [47]. Product labels containing these herbicides explicitly restrict the movement of treated plant biomass, and manure from livestock fed with treated forage in an effort to minimize off-target injury.

Recently genes for the metabolism of dicamba and 2,4-D have been inserted from bacteria into soybeans, cotton and corn, affording the ability to utilize these herbicides for weed control [48,49]. However, there are many concerns over the use of this technology, including the accelerated development of resistance by weeds as observed with the widespread use of glyphosate in glyphosate tolerant crops. Several weeds have developed resistance to growth regulator herbicides including kochia (*Kochia scoparia*) and lambsquarters (*Chenopodium album*) resistance to dicamba, yellow starthistle (*Centaurea solstitialis*) resistance to clopyralid and picloram and 2,4-D resistance in common chickweed (*Stellaria media*) and most recently common waterhemp (*Amaranthus tuberculatus*) [50,51]. The mechanism of resistance in most of these cases is not known.

3.2. Acetolactate (ALS) inhibitors

Herbicides within this classification are broadly represented by two major chemical families; the sulfonylureas and the imidazolinones. These herbicides are used in a wide range of cropping systems but many are also used in natural areas [14]. Both chemistries are highlighted by low use rates, low mammalian toxicity, and extreme specificity [52,53]. Interestingly, both classes of herbicide target the same plant enzyme, and were simultaneous discoveries by 2 separate agrochemical companies in the 1980's, DuPont for the sulfonylureas and American Cyanamid for the imidazolinones [54].

The first herbicide registered for use from this class was chlorsulfuron by DuPont in 1982 [52]. Chlorsulfuron is predominantly used in the western United States for broadleaf weed control in cereal grains and pasture/rangelands, but more recently for invasive species control by the Bureau of Land Management [55]. Other sulfonylurea herbicides developed by DuPont include sulfometuron and metsulfuron, which were initially labeled for use in forestry and industrial sites, but later labeling included uses for metsulfuron in pastures and natural areas and uses for sulfometuron for invasive species management [55,56].

Like the synthetic auxin herbicides, sulfonylurea herbicides have activity on a wide range of natural area broadleaf weeds but their activity also includes some grasses [57]. In general, and at rates labeled for use, chlorsulfuron is used for annual and short-lived perennial weed control in open rangeland and natural areas, while sulfometuron and metsulfuron have more control of woody brush and trees [58]. Both of these latter herbicides are used for hardwood control in commercial conifer forests and also for broad spectrum weed control in industrial sites such as railroads, rail yards, highway rights-of-way and electrical substations. However, all three

of herbicides also contain labeling specific to natural areas. Metsulfuron has a special local needs (SLN) label for the control old world climbing fern (*Lygodium microphyllum*) in south Florida natural areas [59].

Extremely low use rates and remarkable specificity set these herbicides apart from the traditional phenoxy herbicides [60]. It is difficult to make broad generalizations regarding the activity of the sulfonylureas because some species are controlled while other species, even within the same genus, are not. Therefore, uses for these products are regional or even local, depending on the species to be controlled and not controlled. These herbicides also have considerable soil activity, and this contributes to their long-lasting control in perennial systems [61]. However, this high level of activity can also cause problems with rotational crops, but this is not a common situation in areas where sulfometuron and metsulfuron are applied [60].

The imidazolinone herbicides used in natural areas include imazapyr, imazamox and imazapic. Imazapyr was first registered in 1985 for use in forestry and industrial sites such as railroads, rail yards, and powerline and highway rights-of-way [53]. At typical use rates, this herbicide has very broad spectrum activity that includes annual and perennial broadleaves, and several brush, vine and hardwood tree species. This herbicide also has tremendous activity on perennial grasses, both rhizomatous and bunch type grasses [62,63]. While initially developed for the industrial market, imazapyr is widely used in many natural areas for invasive species management. Imazapyr does have a registration for use in imidazolinone resistant crops, but its usage as such is limited [64].

Imazapic is registered for use in peanuts and certain forages, but is widely utilized for grass and broadleaf weed management in native perennial grass prairies [65]. Many perennial grasses such as eastern gamma grass, big bluestem grass (*Andropogon gerardii*), indiagrass (*Sorghastrum spp.*), switchgrass (*Panicum virgatum*) and buffalograss (*Bouteloua dactyloides*) have good tolerance to imazapic, although some injury is observed at seedling stages or during spring regrowth. Imazapic is also labeled for wildflower planting and for seedhead suppression of bahiagrass in turf settings. Imazapic is also used for the control of several invasive species in natural areas. These include Dalmatian toadflax (*Linaria vulgaris*), yellow starthistle (*Centaurea solstitialis*), leafy spurge (*Euphorbia esula*), Russian knapweed (*Acroptilon repens*), and tall fescue (*Schedonorus phoenix*) [66,67,68,69].

Imazamox is the most recent registration from the imidazolinone herbicide group in natural areas for the control of submersed and emergent vegetation [70]. It is particularly effective on Chinese tallow tree (*Triadica sebifera*), which is a major invasive species throughout much of the southeastern United States. Imazamox is also effective for several emergent and ditchbank species, and preliminary research indicates good control of cattail (*Typha spp.*). This herbicide has limited grass activity, and is most effective on broadleaf species.

The sulfonylurea herbicides chlorsulfuron, sulfometuron and metsulfuron are formulated as dry flowable granules that readily mix with water. Sulfonylureas are weak acid compounds with very high water solubility [14]. These herbicides are readily absorbed by roots from soil applications and transported via the xylem to shoots and leaves of plants. Once in the leaves, these herbicides are often remobilized in the phloem to growing regions - tracking a similar

pattern of flow as carbohydrates. Sulfonylureas are also absorbed from applications to plant foliage, entering the leaves and stems, and translocated to areas of high meristematic activity in manner similar to root uptake [60].

The imidazolinone herbicides are also highly water soluble but formulated as salts. They are generally marketed as aqueous solutions, but some older formulations were dry flowable granules. Imidazolinone herbicides are variable in soil activity but if present can be readily absorbed by plant roots [71]. They are transported to leaves and stem tissues via the xylem and can be remobilized to meristematic tissues. This pattern of reallocation occurs in the phloem, similar to carbohydrate movement. Imidazolinones are also absorbed from applications to plant foliage, entering the leaves and stems, and translocated to areas of high meristematic activity in manner similar to root uptake [72].

Mechanistically, the imidazolinones and sulfonyl-ureas act in the same manner by inhibiting the activity of the enzyme acetolactate synthase (ALS), which is also referred to as acetohydroxy acid synthase (AHAS, EC 2.2.1.6) [73]. This enzyme catalyzes the conversion of 2-ketobutyrate to 2-acetohydroxybutyrate through the addition of a 2 carbon unit using hydroxymethyl thiamine pyrophosphate (TPP). This is the initial step in the formation of the amino acid isoleucine. The ALS enzyme also catalyzes the conversion of pyruvate to form 2-acetolactate, once again utilizing TPP to add a 2-carbon unit [74]. This reaction is the initial step in the formation of valine and leucine. Thus by inhibiting acetolactate synthase, the formation of three essential branched chain amino acids cannot occur and inhibition occurs through a binding of the herbicide across the channel leading to the active site [75]. Herbicides in both groups bind at entrance of this channel, effectively blocking entrance to substrates and co-factors needed for the reaction to occur.

The inability of the plant to produce these essential amino acids leads to a cessation of protein/enzyme synthesis and plant growth. Since these compounds accumulate in areas of new growth, meristematic activity is stopped. The plant cannot continue to make new cells and eventually dies [60]. Symptoms from these herbicides are generally manifested as discoloration in the growing regions, especially newly emerging leaves and shoot tips. Internode length is markedly decreased, and leaves may be malformed or misshaped [76]. Generalized chlorosis is a common symptom, although imidazolinones may show purple discoloration, especially in effected grasses. In annual species, a characteristic symptom of sulfonylurea injury is a reddening of the abaxial leaf veins.

Selectivity of these herbicides in plants is primarily metabolism based, and is often mediated through mixed-function oxidases (MFO's) [77]. These compounds catalyze several reactions in plants, including the breakdown of harmful xenobiotics such as herbicides. Tolerant plants generally are able to metabolize sulfonyl-ureas and/or imidazolinones through this mechanism, thus imparting selectivity [60]. In cropping systems, crop selectivity is compromised if certain insecticides, such organo-phosphates, are used that disrupt MFO activity, allowing the herbicide to affect the target enzyme [78].

Interestingly, resistance development by weedy species occurs through amino acid substitutions of the target enzyme at the binding site [79]. In most cases, only a single amino acid change

will confer resistance, and several substitutions (single amino acid changes) will cause resistance in sulfonyl-ureas. Conversely, very few impart resistance in imidazolinones and only one confers resistance across both herbicide families. The substitutions that confer resistance also appear to have little to no effect on enzyme efficiency, and thus growth of herbicide resistant biotypes varies little from non-resistant biotypes [80].

3.3. Photosynthetic inhibitors

Those herbicides that directly inhibit photosynthesis have been used for several years and were developed in the 1950's and 1960's [81]. While several chemical families are represented within this broad mode of action classification, the substituted ureas and triazines are those used most widely for natural area weed control. These products were originally developed for use in pasture/rangelands and forestry situations, but like several other herbicides, have been adopted for use in natural areas.

The triazine herbicides used in natural areas include hexazinone, simazine, and prometon. Simazine was originally developed for broadleaf and grass weed control in corn and sorghum, but later uses included grass and broadleaf control in established fruit and nut crops, albeit much higher rates of application per acre [82,83]. It was also used in aquatic situations for algae control, sold under the trade name "Aquazine", but this was cancelled in the 1990's [83]. Its use in natural areas currently is limited, primarily because simazine lacks broadspectrum control of perennial plants, particularly brush, vines and trees.

Prometon has been used for many years in industrial settings for broad-spectrum annual and perennial grass and broadleaf weed control [85]. This herbicide has considerable activity on many hardwood tree species, and is often marketed as a soil sterilant. This tremendous activity limits its use in many situations that require selectivity, and that includes forestry and most natural areas. Therefore, labeling as such is confined to areas where little to no vegetation is desired such as powerline substations, under asphalt paving, sidewalks, railyards and similar industrial sites [86]. Consequently prometon use in natural areas is very limited.

Hexazinone is an asymmetrical triazine that was originally developed for use in the conifer forest industry for hardwood control, and often used in a manner called pine release [87]. This situation occurs 2-4 years after pine seedling establishment, where hexazinone is broadcast applied to provide control of regenerating hardwood species, allowing the pines to be 'released' from the competing hardwood saplings. Hexazinone also has a label for use in bahiagrass (*Paspalum notatum*) and bermudagrass (*Cynodon dactylon*) pastures for the control of broadleaf species, but most often targeting smutgrass (*Sporobolus indicus*) [88]. It can be used in many natural area settings where hardwood tree, brush/shrubs or possibly vines are the target, but many native forbs and some native grasses may also be injured. Hexazinone works wells in areas where pines are the primary species, possibly where undesirable species are dominant under pines, and understory selectivity is not paramount. Once these species have been removed, revegetation can then be accomplished.

Diuron and tebuthiuron comprise those herbicides in the substituted urea chemical family that are used in natural areas. Diuron is similar to simazine in that it was first developed for use in

crops – corn and cotton, with later registrations including broadleaf and grassy weeds in established fruit and nut crops [89,90]. Diuron has good activity on a number of annual species, but lacks control of perennial plants. It is often a component in combination herbicides for broad-spectrum weed control in industrial sites such as railroads, railyards, powerline rights of way and substations [86]. The goal of these applications is to provide a vegetation free zone for extended periods of time. The use of diuron in natural areas is limited due to spectrum of activity; too much injury on desirable annual grasses and forbs and limited control of larger, more woody shrubs, vines and trees.

Tebuthiuron however, has tremendous activity on a wide range of woody species, particularly hardwood trees such as oaks (*Quercus spp.*), maple (*Acer spp.*), poplar (*Populus spp.*), and sweet gum (*Liquidambar styraciflua*). [91]. This species is also very effective on shrubs, vines and herbaceous perennials [92]. It is often used in non-crop land and industrial settings for broadleaf vegetation control, including vines and hardwoods. Tebuthiuron is utilized in powerline corridors and around utility poles to promote healthy grass stands to maintain cover for grazing for livestock and wildlife and also erosion control [93]. This herbicide also is labeled for use in certain forestry situations, primarily for non-desirable vegetation control in conifers [94].

Bromacil is another photosynthetic inhibitor that belongs to the uracil chemical family that has limited uses in natural areas. It has similar use patterns as diuron and simazine, including vegetation management in industrial sites such as powerline substations, railroads, railyards, and rights-of-way [86]. Bromacil can also be used in certain fruit crops such as citrus for broadleaf and grass weed control [14,95]. While this herbicide has tremendous activity on annual species, it has less than adequate control of perennial vines, trees and shrubs compared to other herbicides; therefore wide spread utility in natural areas is limited [96].

As a group, photosynthetic inhibitors have low water solubility and limited foliar uptake [97]. Most are formulated dry as wettable powders or pellets, or liquid as clay-suspended flowables. Hexazinone is the only exception with a liquid formulation. These herbicides are soil applied; even applications over the top of existing foliage are active only when reaching the soil [14]. Photosynthetic inhibitors are readily taken up by plant roots and translocated to leaves and shoots through the water stream facilitated by xylem tissue [98]. Once reaching leaves, these herbicides partition into individual cells. As the plant continues to transpire, more herbicide is moved to the leaves, with older leaves and leaf tips transpiring the most water. These areas tend to demonstrate chlorosis first and most strongly simply because these tissues have transpired more water, and thus taken up more herbicide, compared to newer tissues. This causes the characteristic pattern of chlorosis often observed with these herbicides. Subtle differences in water solubility between herbicides and subsequent partitioning into leaf tissue of various species produce variations in chlorotic patterns, such as veinal chlorosis and/or interveinal chlorosis [99].

Differences in water solubility and to a lesser extent degradation, dictate the uses and selectivity of these products. Diuron, simazine, prometon, and bromacil are very non-water soluble and tend to remain in the upper soil profile [14]. This maintains the herbicides in the zone of germinating annual weeds, thus providing extended weed control. Perennial fruit and nut

crops avoid herbicide injury primarily through limited uptake, since the roots of most trees are below the concentrated herbicide zone [100]. Conversely hexazinone and tebuthiuron are more water soluble and move deeper into the soil profile, which limits their utility for long-term vegetation management because annual weeds begin to infest the zone above the herbicide [101]. However, this places these herbicides into the root zone of many perennial forbs, vines, shrubs and trees where it is absorbed and translocated, causing injury and often mortality. Even large trees, especially oaks, can be killed if sufficient herbicide is placed in the root zone. Typically the leaves become chlorotic, necrotic and abscise. New leaves emerge, and follow the same chronological pattern, but generally do not expand to more than half normal size. After 2 to 3 cycles of leaf emergence and abscission, the trees succumb to death due to the lack of carbohydrate reserves needed for growth [102]. Depending on species, rate applied, and geographic location, death can take 1-2 years. Unfortunately, these herbicides are sometimes used in malicious attacks to destroy trees or shrubs; and in some cases trees of historic value, such as the Toomer Oaks on the campus of Auburn University, Auburn, Alabama (tebuthiuron) in 2010 or the Treaty Oak of Austin, Texas (hexazinone) in 1989 [103].

Photosynthetic inhibitors, regardless of chemical family, work in the same manner to interrupt the light reactions of photosynthesis. These reactions serve to capture the light energy from sunlight through excitation of chlorophyll molecules and the subsequent removal of an electron from a molecule of water; producing free oxygen and hydrogen [104]. Electrochemical energy is passed through a series of reactions (mainly photosystem II, cytochrome B, plastocyanin and photosystem I) to form NADPH+H. During this transfer, a proton gradient is formed across the chloroplast membrane, sufficient to generate ATP. These herbicides bind to a protein (specifically the D1 protein) within the photosystem II complex that does not allow electron transfer to occur [81]. This blockage of electron flow inhibits the formation of NADPH +H, and indirectly inhibits ATP formation as well. Energy continues to be absorbed by the chlorophyll molecules and transferred to the reaction centers associated with photosystem II, but cannot be dissipated [105]. This excess, or non-transferable, energy is then passed on to free oxygen, creating radical oxygen. Oxygen is a highly toxic radical that quickly reacts within the chloroplast to form hydroxyl radicals, peroxide, and/or lipoxides. Ultimately chloroplast and other cellular membranes become damaged and leaky, chlorophyll molecules are destroyed, and the tissue degrades.

While many photosynthetic inhibitors can be considered total vegetation control herbicides, certain species have the ability to tolerate these herbicides through metabolism. Metabolism is achieved primarily by glutathione and/or carbohydrate conjugation, whereby the herbicide molecules are bound with these compounds and shuttled to the vacuole for further breakdown [106]. However, in natural area systems - especially at rates typically used, placement and differential uptake is the primary mechanism of selectivity. Many conifers, pines (*Pinus spp.*) in particular, have the ability to tolerate hexazinone presumably through metabolism, but the mechanism is not known.

3.4. Glyphosate

Glyphosate is one of the most widely used herbicides in the world, and has been extensively used in natural areas for nearly 4 decades [107]. It is non-selective and provides control of a wide range of species, including annual and perennial grasses, annual forbs, short lived perennials, vines and many tree species [108,109,110]. It has limited activity on conifers, but time of year dictates use during periods of no or slow growth. This is generally the fall months prior to winter, termed hardening-off [111]. While active on many species of larger perennials, it is often mixed with other herbicides for greater control.

Glyphosate is chemically a weak acid, and is readily translocated in phloem tissues to areas of new growth. It is absorbed through foliar tissues such as leaves, shoot tips and green stems, but uptake is limited by woody tissues. Root uptake is possible, but rarely occurs due to irreversible binding of glyphosate to soil particles once the herbicide comes in contact with the soil. As in the case of other weak acid herbicides, glyphosate accumulates in meristematic regions, following a similar movement to that of carbohydrates [112]. Glyphosate affects the ability of plants to produce essential aromatic amino acids by blocking an initial step in the shikimic acid pathway. More specifically, this herbicide inhibits the activity of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSP synthase) which catalyzes the conversion of EPSP from shikimate-3-phosphate and phosphoenolpyruvate [14]. This enzyme is a key enzyme in the shikimate acid pathway, which produces the aromatic amino acids tryptophan, phenylalanine, and tyrosine, along with a multitude of other secondary compounds including phenolics, flavonoids and coumarins [113]. Glyphosate also greatly influences carbon allocation and flow within the cell, as uncontrolled shikimate accumulation occurs as a result of this inhibition.

The typical symptoms of glyphosate injury include an initial cessation of growth followed by chlorosis in the meristematic regions of growth [14]. Chlorosis is often lighter in color compared to the photosynthetic inhibitors, and in some species may almost appear white or cream colored. Necrosis occurs several days after initial symptoms and complete plant death results in 21 to 35 days depending on species and maturity/size of treated plants. Glyphosate is extremely difficult to metabolize by plants and is readily translocated to areas of new growth [114]. This stability within plant tissues is the reason it has excellent activity on many perennial plants, allowing glyphosate to be 'stored' in overwintering tissues such rhizomes and root-stocks [115]. When plants begin to reallocate carbohydrates for spring regrowth, glyphosate is remobilized to these areas. Another unique symptom of glyphosate, particularly in regrowing perennial species, is the phenomenon of bud fasciation [116]. Bud fasciation is where several buds/shoot tips arise from a single meristematic region, forming a cluster of tightly packed shoots and leaves. The exact mechanism is not well understood, but appears to be related to a loss of apical dominance and deregulation of auxin activity.

Resistance to glyphosate has increased in annual cropping systems (Roundup-Ready technology) but resistance has not been documented in natural areas systems [117]. Several plants have the ability to tolerate and outgrow applications of glyphosate, especially trees, shrubs and woody vines. In these cases, limited uptake and/or dilution within non-metabolically active tissues is the likely reason for poor activity.

3.5. Fosamine

Fosamine has been used in industrial right of way situations for many years and more recently used for invasive species control in natural areas such as natural savannahs and prairies. Brush control is the target for this herbicide, but it can be used for the control of herbaceous weeds such as leafy spurge (*Euphorbia esula*). Fosamine is tolerated by certain species of conifers, but hardwoods and other deciduous trees are often damaged. Fosamine is applied to the foliage of target plants where it is slowly absorbed by leaf tissues [118]. This herbicide has little to no soil activity and is rapidly degraded by soil microbes, limiting its environmental persistence [119]. This herbicide is recommended for late summer/autumn applications – typically one to two months prior to leaf drop. Fosamine appears to have limited translocation out of treated foliage and does not exhibit symptoms on treated tissue [120,121]. The effect of fosamine is not apparent until the following spring where leaves often fail to emerge or if emerged will be small and spindly in appearance. The mechanism of fosamine is not clear, but some evidence suggests an inhibition of mitosis or the inability of new developing cells to effectively transport calcium [14]. The limited translocation within plant tissues allows the use of this herbicide as a ‘side-trim’ treatment, where a portion of tree can be controlled without affecting the entire tree. This type of application is used in powerline and railroad situations to chemically trim a tree to remove unwanted limbs and foliage [86].

3.6. Inhibitors of Acetyl CoA Carboxylase (ACCase inhibitors)

Herbicides within this group fall into two broad chemical families – the cyclohexanediones or the aryl-oxy-phenoxy propionates [14]. There are several herbicides within these families labeled for use in non-crop/natural areas, but the most widely utilized include sethoxydim, clethodim and fluazifop-butyl [86]. These herbicides are characterized by their selectivity towards annual and perennial grasses, with minimal to no activity on other monocots or dicot species [122]. They are primarily applied to the foliage due to a lack of appreciable soil activity through binding to soil particles and rapid microbial degradation.

ACCase inhibiting herbicides are applied to the foliage of grasses, where they are readily absorbed. Similar to other weak acid herbicides, they are translocated to areas of meristematic growth following the pattern of carbohydrate flow [112]. Cyclohexanediones and aryl-oxy-phenoxy propionate herbicides inhibit the activity of acetyl CoA carboxylase [123]. This enzyme is the initial step in the formation of fatty acids, which are the primary building blocks of cell membranes and other cellular components necessary for normal growth. New growth is stopped and grasses often become chlorotic or purple in color. Another characteristic symptom is the water soaked browning of stems when pulled from the whorl.

The utility of these herbicides is limited to annual and perennial grass control. Clethodim and fluazifop have superior activity on perennial grasses, and are often used for the control/suppression of reed canarygrass (*Phalaris arundinacea*), cogongrass (*Imperata cylindrica*), Japanese stiltgrass (*Microstegium vimineum*) to name a few [124,125,126]. However, complete control of well established grass stands is often not achieved with a single application and multiple treatments are usually required.

3.7. Glufosinate and paraquat

Glufosinate and paraquat are contact type herbicides that can be used in a wide range of non-cropland, industrial, rights-of-way areas and natural areas [86]. Both of these herbicides are contact in activity, requiring complete coverage of the target foliage to attain good control [14]. In addition, both paraquat and glufosinate do not possess soil activity due to immediate and irreversible binding to soil particles [127]. These herbicides are very effective on annual broadleaf and grassy weeds, but only marginally effective on well established perennial plants. Since these herbicides do not translocate out of treated foliage, perennial plants can usually regrow following treatment [112].

Glufosinate is rapidly absorbed by leaf tissue and is active in the chloroplast of cells. Specifically, glufosinate inhibits the enzyme glutamine synthase, which catalyzes the incorporation of free ammonia into the amino acid glutamate to form glutamine [128]. This reaction is the primary mechanism by which plants incorporate nitrogen for use in cellular products such as amino acids, nucleotides, enzymes and storage proteins. The lack of nitrogen incorporation, however, is not the primary means by which the plant dies. Free ammonia levels increase in the chloroplast where this molecule begins to uncouple membranes. Uncoupling is the action where membranes can no longer maintain a gradient that drives energy formation in photosynthesis [129]. Damage becomes visible generally after 4 to 5 days and appears as chlorotic lesions followed by rapid necrosis of treated leaves.

Paraquat herbicide was developed in the early 1960's for broad spectrum weed control in non-crop land and other vegetation free sites. Paraquat is rapidly absorbed by leaf tissues and is active primarily in the chloroplast, although it may also impede mitochondrial function [112]. Paraquat affects the light reactions of photosynthesis in the photosystem I complex, more specifically at the site of electron transfer from ferredoxin to NADPH+H reductase [130,131]. Paraquat does not bind or disrupt enzyme activity, but rather steals/diverts the electron to become a reduced paraquat molecule. Paraquat in this reduced form quickly passes the electron energy to oxygen, creating oxidized paraquat and radical oxygen ($O^{\cdot -}$). Paraquat becomes reduced again by another electron, oxidized through transfer to oxygen and the cycle continues. Subsequently, the ability of the plant to make NADPH+H is compromised, but more importantly radical oxygen reacts with water and lipids to produce hydrogen peroxide, hydroxyl radicals and lipoxides. These radicals interact with the lipid fraction of membranes, destroying the chloroplast and eventually the plasma membrane [132]. Symptoms from paraquat can be evident within 12 to 24 hours after application. Leaves first appear water soaked, followed quickly by necrotic lesions that coalesce to encompass the entire leaf. High light levels promote faster necrosis, and complete damage is generally achieved within 2-4 days.

Glufosinate and paraquat require good coverage and therefore must be applied in higher carrier volumes compared to systemic herbicides. There appears to be some movement with glufosinate out of treated tissues, but translocation to perennial structures such as rhizomes or tubers does not occur to an appreciable extent [133]. The utility of paraquat and glufosinate for natural area plant management is limited for several reasons. First most weeds in natural areas are perennials, so applications of these herbicides will only provide temporary control.

Secondly, these herbicides are non-selective - causing damage to any plant that is contacted, desirable and undesirable vegetation [14]. Thirdly, these herbicides lack soil activity so long term control cannot be realized.

3.8. Protox inhibitors

There are several herbicides and herbicide families that encompass this mode of action category [14]. Protox inhibitors are primarily used in annual cropping systems for broadleaf, grasses and nutsedge (*Cyperus spp.*) control [134,135,136]. These herbicides possess good soil activity with moderate to long soil persistence and many also have tremendous foliar activity [137].

Protox inhibitors are readily taken up by plant roots and translocated to leaves and shoots through the water stream facilitated by xylem tissue [138]. Once reaching leaves, these herbicides partition into individual cells. As plant continues to transpire, more herbicide is moved to the leaves, with older leaves and leaf tips transpiring the most water. These areas tend to demonstrate damage initially and most strongly simply because these tissues have transpired more water, and thus taken up more herbicide, compared to newer tissues. Damage appears as bronzing or necrotic lesions in leaf tissue. These lesions generally lack pattern, but eventually coalesce into more wide-spread damage and eventual leaf drop. Stem tissues may also exhibit similar necrotic injury. In grasses and sedges, a browning of leaf tissue along the midvein is often observed.

Foliar activity shows a similar pattern, with necrotic lesions developing in random areas on leaf tissues, with complete necrosis occurring in 3-5 days [139]. Even tolerant plants will show some damage from foliar applications on treated tissue, but to a much lesser extent and quickly outgrow the injury. There is no translocation from foliar applications of protox inhibiting herbicides, only those areas contacted will be damaged [139]. However, subsequent damage may occur from root uptake, if an appreciable amount of herbicide reaches the soil and remains active. This is highly dependent on whether the herbicide has soil activity, application rate, and foliar coverage at the time of application.

Protox inhibiting herbicides have a very unique mode of action that was not clearly understood for many years [140]. Mechanistically, these herbicides inhibit the enzyme protoporphyrinogen oxidase which catalyzes the conversion of protoporphyrinogen IX to protoporphyrin IX in the chloroplast [141]. This step is an intermediate process in the production of chlorophyll molecules. Excess protoporphyrinogen IX leaks out of the chloroplast envelope into the cytoplasm where it is converted by a cytoplasmic (insensitive) version of protoporphyrinogen oxidase to protoporphyrin IX [142]. This molecule has the ability to absorb light energy, but can only dissipate this energy to oxygen. This forms singlet oxygen, a highly reactive form of oxygen that quickly interacts to form other highly toxic radicals that destroy cell membranes. Cells become leaky, rupture, die and eventual tissue degradation follows.

Utility of the protox inhibiting herbicides in natural areas is limited. Foliar activity is contact only, therefore perennial plants quickly regrow. In addition, at the rates needed to garner control, selectivity is lost or severely compromised. Appreciable control can be achieved from

soil uptake and activity, but those rates of herbicide application necessary may also reduce selectivity, and in some cases may not be within label guidelines. Flumioxazin and oxyfluorfen are protox inhibiting herbicides that may be used in non-crop areas, but applicability to natural areas has not been widely studied. These herbicides may have some use in restoration situations, providing control of undesirable vegetation prior to or immediately after an augmented restoration planting. However, there has been limited research to determine which herbicide product is most effective as a function of selectivity and desirable persistence.

3.9. Growth inhibitors

Herbicides that are categorized as growth inhibitors fall into three major mechanisms of action, but produce the common effect of inhibiting seedling emergence. The three mechanisms include: 1) interruption of mitosis through a blockage of spindle fiber formation, 2) interruption of cell wall formation through an inhibition of cellulose biosynthesis, and 3) interruption of cell membrane formation through a blockage of very long chain fatty acid synthesis. In nearly all situations, these herbicides are applied to the soil where they are absorbed by germinating seedlings, preventing seedling growth [14].

These compounds are characterized by extremely low water solubility, maintaining the herbicides in the upper soil profile [127]. As seeds germinate, the roots and emerging shoots come in contact with the herbicide, where it is rapidly absorbed, inhibiting growth and killing seedlings before they emerge from the soil. These herbicides do not translocate within plant tissues, so the growing regions of the plant must come in contact to be effective. Foliar applications are ineffective because the herbicides remain in the cuticle or epidermal cells, and cannot come in contact with meristematic tissues which are generally shielded within the bud structure. Selectivity is achieved through placement, whereby the shoots of tolerant germinating seedlings can emerge with minimal herbicide uptake in meristematic regions *and* the roots can grow below the treated layer. In cropping systems, this is most often achieved with broadleaf crops possessing hypogeal germination patterns. Perennial crops also exhibit good tolerance because the roots are well below the treated soil layer and foliar uptake is minimal. Examples of growth inhibiting herbicides used in non-crop areas include diclobenil, pendimethalin and metolachlor, but applicability to natural areas has not been widely studied.

4. Herbicide application methods in natural area weed management

This section will detail the various methods used for applying herbicides for management of weedy species in natural areas. The complexity of natural areas dictates a unique and often non-conventional approach to herbicide application to 1) maintain selectivity, 2) provide control of large specimens, and 3) minimize off-target damage to the natural environment. Selectivity is much more difficult to achieve and maintain in natural areas. Herbicides are generally developed for weed control in cropping systems, and then secondarily labeled for use in non-cropland areas. In crops only selectivity towards the crop plant is desired, and damage to all other plants is beneficial, advantageous or inconsequential. However, in natural



Figure 1. Postemergence application of herbicide to woody brush. Photo Courtesy James Miller, U.S. Department of Agriculture, Forest Service, <http://www.forestryimages.org>

area weed management, only one species is the target and damage to other species is not desirable – especially injury to rare or endangered plants.

4.1. Post-emergence foliar applications

This is the most common method of application, whereby the herbicide in diluted solution is applied as a spray over the top of targeted species (Figure 1). For larger areas, treatments are made to both target and non-target species utilizing an aerial (propeller plane or helicopter), tractor or all-terrain vehicle (ATV) mounted broadcast spray boom. Herbicides are applied as the amount of active ingredient per unit land area, and calibrated to deliver this amount based on carrier volume output. Smaller, more isolated or higher selectivity required sites will utilize a backpack sprayer with a hand-held spray wand or boom. Backpack applications cannot be calibrated in the same manner; herbicides are applied as a percentage of undiluted herbicide in a variable carrier output [18,143].

Aerial applications are highly restricted and only certain herbicides can be applied aerially, and in some cases only during certain times of the year to minimize off-target injury. For

example, the state of Florida restricts the use of organo-auxin from aerial applications from January 1 until May 1 of each year [144]. Aerial treatments often utilize very low gallon spray volumes (3-10 gallons per acre) to maximize efficiency with weight and spray volume [145]. This restricts aerial applications to systemic herbicides that are not dependent on high carrier volume for effectiveness.

Tractor or ATV boom-mounted sprayer applications can utilize a range of carrier volumes and thus not restricted to systemic herbicides only. These types of application equipment generally utilize a rear mounted boom with flat fan nozzles. The size or width of the boom varies, but ATV mounted booms are generally less than 15 feet while tractor booms may reach 30 feet or greater. Regardless, boom width is restricted compared to traditional agricultural applications due to unevenness of terrain to be covered, obstacles such as trees, shrubs, etc. and limitations on pump and tank capacity on smaller tractors and ATVs. Boom applications, especially those utilizing boom widths greater than 15-20 feet, require relatively flat ground, uniform height and high density of target species. As such, many land managers cannot utilize this type of equipment in many natural area systems.

Boom-less nozzles are often used in industrial applications and have some merit for use in natural area weed control. These nozzles are specifically designed to produce a multi-stream pattern across a 12-15 foot-wide spray swath. When mounted on an ATV or truck, these nozzles can produce a sizable sprayed area, without the issues associated with a fix boom to avoid obstacles and uneven terrain. However, coverage with these types of nozzles is not uniform and generally high volume output is required to maintain proper spray pattern. In addition, the actual nozzle is very expensive compared to a standard fan fan system. Due to difficulties with application uniformity and issues with achieving selectivity, most natural area weed managers will rely heavily on small backpack sprayers. This type of sprayer consists of a 5 gallon/20 liter (on average) tank, a hand-held, single nozzle spray wand, and a small diaphragm pump with an attached lever. The operator uses the pump to pressurize the tank, forcing the liquid spray mixture through the spray wand. Pressurization is under the control of the operator, and is generally maintained to provide a proper pattern from the adjustable orifice on the spray wand. As the name suggests, the apparatus is worn on the back of the applicator using shoulder straps and often a waist strap to stabilize weight distribution. In most cases, the user operates the wand with one hand and pressurizes the tank with the other.

Backpack applications utilize diluted herbicide solution and mixed as a percent solution; in most cases between 0.5 and 3% solution. Applications are made to target species on a visual 'spray to wetness' observation. To achieve some degree of uniformity among applicators, the basis for adequate spray delivery is when spray droplets begin to drip from the leaf surfaces. This 'spray to runoff' technique is common regardless of target species or herbicide. While it is difficult to accurately measure volume output on a per acre basis, most researchers estimate these types of applications to range from 30 to 50 gallons per acre. In many cases, post-emergence foliar applications contain herbicides with soil residual activity, either from an herbicide that possesses both foliar and soil activity or soil active herbicides that are tank-mixed to provide extended control. Regardless, the application technique is the same for most boom-mounted sprayers. For soil applications using a backpack sprayer, the applicator self-calibrates

by placing a known amount of liquid in the sprayer and sprays a defined area. Once the area has been completely sprayed, the amount of liquid used by the applicator is calculated to determine individual spray output per area (in most cases ft²).

4.2. Soil basal applications

Soil basal applications are used for 2 primary purposes - 1) provide control of an existing plant or group of plants, or 2) provide preventative control of potential plant problems around stationary objects such as power poles. In either scenario, the herbicide is placed in often high concentrations around the base of the treated plant or object. The herbicide may be applied in liquid or granular form, and in a variety of placement patterns to achieve maximum root uptake of the intended target(s). Some herbicides, especially soil active photosynthetic herbicides, are formulated as pellets, which are essentially larger, more concentrated granules. Dry formulated granules or pellets are often easier and more accurate to apply as basal soil treatments. In these situations a certain number of pellets or dry volume of granule is placed as a function of targeted plant circumference. The pattern of placement varies considerably among applicators and may include circular, piles of pellets, or even gridlines in the case of larger infestations [146]. Soil basal herbicides include many of the photosynthetic inhibitors and several of the ALS and growth regulating herbicides. While the growth inhibiting and protox herbicides possess good soil activity, their effectiveness on established and larger plants is limited due to lack of root uptake and translocation or short-term control. Uses are generally restricted to those situations where preventative control is the primary objective.

4.3. Basal-bark applications

Basal-bark applications are utilized to provide control of larger specimens, where over-the-top foliar applications are not feasible for logistical or selectivity reasons. As the name suggests, basal-bark treatments are made near the ground to the trunks of small trees or shrubs [143]. Treatments are applied using a hand-held spray bottle or backpack sprayer to provide a tight stream of liquid onto the bark (Figure 2). Techniques for basal-bark applications vary widely among practitioners and weed specialists, but most agree that complete coverage around the trunk base is necessary for control. The width of the spray band around the tree varies as a function of species, size and herbicide being used, but most common is a 12 inch (30 cm) width band. Applications are generally made to the point of visual dripping or running of the liquid down the bark surface.

Basal-bark treatments utilize an oil carrier (often referred to as basal oil) in which the herbicide is diluted at a high concentration or undiluted [147]. Diesel fuel or kerosene was used as carriers for many years, but environmental and economic restrictions limit current usages in many areas. In some cases, depending on herbicide formulation, the herbicide may be applied in undiluted form. Regardless of carrier, the herbicide must be in an oil soluble/lipophilic form to allow for penetration into the bark tissues. The objective is to maximize herbicide penetration through the outer epidermal layers (periderm) and reach the secondary phloem and cambium [143]. Once reaching these layers, the herbicide may be remobilized in the phloem, penetrate and affect the dividing cambium cells, or possibly enter the water stream via the xylem



Figure 2. Basal bark application to small tree. Photo Courtesy BASF.

sapwood. There is little research as to the actual mechanism of mortality but is surmised that the herbicide is translocated slowly throughout the plant, accumulating in regions of active growth and killing meristematic tissues. The resiliency of many large woody trees and shrubs requires that the herbicide remain available within the plant, and presumably in translocatable form, for a period of time that allows the specimen to exhaust food reserves and/or meristems to provide complete control.

Basal bark herbicides are limited to ester formulations of triclopyr, picloram, 2,4-D, 2,4-DP. Dicamba and oil-soluble formulation of imazapyr have also been used, often in combination with other herbicides [148]. To be effective as a basal treatment, the herbicide must be able to solubilize in oil, which is needed to penetrate the bark layers. The herbicide must also be systemic to allow translocation once reaching the vascular tissues. For these reasons, basal bark treatments are exclusively weak acid herbicides, but only those chemistries that can be formulated to be oil soluble such as esters. Several weak acid herbicides, including the sulfonylureas, are not effective as basal treatments because of low oil solubility.

4.4. Stem injection applications

Stem injection applications are generally made to trees or shrubs with larger than 4 inch (20 cm) diameter trunk bases, which is the upper limit for effective basal treatments. In this type of application – also called hack and squirt, the herbicide is placed into a cut or frill made into the bark of the specimen (Figure 3). A hatchet, axe, machete, or other hand-held cutting device is used to make a downward cut/incision that penetrates the bark to the cambium layer, creating a cavity to contain a small amount of herbicide solution [147]. Although highly dependent on herbicide and species, incisions are made evenly around the trunk, or in the case of larger trees a complete girdle might be necessary. One rule of thumb is one incision per inch of trunk diameter [149]; another is no incisions more than 3 inches (10 cm) apart [150]. Herbicide activity on a given species is generally what dictates the number of cuts that is required. Additionally, it is useful to place these cuts near the base of the stem. Making the application higher on the stem will often increase the likelihood of stem-sprouting below the application site.



Figure 3. Hack and squirt application to larger diameter tree. Photo courtesy James Miller, U.S. Department of Agriculture, Forest Service, <http://www.forestryimages.org>

Unlike basal bark applications, this type of application can utilize water and oil soluble formulations, providing greater flexibility in herbicide options. In addition to those herbicides mentioned for basal bark, glyphosate, triclopyr amine salt, and hexazinone can be effectively used. Typical concentrations for injections range from 33 to 50% solution in water. In some cases, undiluted herbicide is used. Only a small amount of liquid is placed per cut (< 5 ml) and applied using a single nozzle backpack sprayer, or a hand-held spray bottle. A marker dye is often used to help applicators visualize and keep track of treatment applications. There have been several pieces of equipment developed to ‘inject’ herbicide into woody plant tissues, combining the mechanical cutting operation with liquid dispensing operation [151]. The ‘hypo-hatchet’ delivers a pre-measured amount of liquid through a pore in the hatchet blade when inserted into the trunk tissue [152]. Injector bars (Figure 4) contain the herbicide mixture within the bar which is



Figure 4. Stem injection of herbicide into trunk of target tree. Phot credit James Miller, U.S. Department of Agriculture, Forest Service, <http://www.forestryimages.org>

jabbed into a tree, and a lever is pulled allowing a pre-measured amount of liquid to flow through the end of the bar [147]. Some bar type devices will insert a granular pellet during each injection. Other injection tools include a hand-held gun, with a large diameter needle that can be inserted into softer perennial tissues, once again with a premeasured amount that is injected.

4.5. Cut-stump applications

Cut-stump applications occur, as the name implies, to the cut portion of a felled tree or shrub. The purpose of the application is to prevent regrowth of the plant from shoots arising from the cambium layer of the cut stump. Herbicide is applied to the cut surface, making sure to cover the entire outer cambium layer [86,147]. Placement of the herbicide across the entire stump is not necessary, since the majority of the inner tissues consist of non-living heartwood (Figure 5). Applications should occur within 30 minutes of cutting to avoid the layer becoming scabbed over, reducing herbicide uptake and penetration.

Triclopyr amine or ester, picloram, 2,4-D, dicamba, imazapyr or glyphosate can be used for cut stump applications. Ester formulations can be applied as 25% solution in basal oil, while amine/salt formulations are applied as 50% solution in water. Sometimes undiluted herbicide can be used, but care must be taken to avoid 'flashback'. Flashback is a phenomenon where the herbicide is absorbed by the trunk and roots of the felled specimen, translocated through the root system, and passed through root grafting to the roots of neighboring plants [149]. Neighboring plant roots can also absorb the herbicide from soil around the treated stump, where herbicide is washed



Figure 5. Herbicide application with marker dye made to cut stump, targeting only outer cambium region. Photo credit James Miller, U.S. Department of Agriculture, Forest Service, <http://www.forestryimages.org>

off the stump or root crown. Regardless, applicators are encouraged to use only the amount necessary to provide control of resprouting, and limit excessive herbicide use.

4.6. Ballistic herbicide application

This unique approach to applying herbicides has been developed by Dr. James Leary with the University of Hawaii [153]. In this system, herbicides are encapsulated in paint ball pellets and distributed to the target species via a commercially available paint ball gun. Each 'ball' contains a known amount of herbicide and rate is calculated by the number of balls fired at each specimen. The applicator targets the apical regions of the plant, or the larger stems to increase the 'splatter' effect that helps distribute the herbicide within the plant architecture.

Dr. Leary has performed nearly all initial testing with imazapyr and triclopyr, which readily translocates within plant tissues. Imazapyr also possesses good soil residual activity, which aids in effectiveness. This technology is still in the evaluation phase, but holds good promise for treating invasive species in remote and inaccessible areas. Most of the treatment evaluations have been performed on the slopes of tropical mountains in Hawaii, where the only means of treatment has previously been a single nozzle suspended from a helicopter. The nozzle is embedded in a heavy ball that helps reduce swaying and the pilot attempts to direct the nozzle over the crown of the targeted specimen. This approach is time consuming, precarious and expensive. With the ballistic approach, the applicator fires a number of balls into the crown to deliver the herbicide. This allows for more specimens to be treated per helicopter flying time, eliminates the need for unwieldy spray equipment and provides for more precise herbicide

application [154]. As mentioned, this technology is still under intense evaluation, and commercialization of the process has not been undertaken.

5. Integrated approaches to natural area weed management

Regardless of herbicide or application method used, chemical weed control must be used in an integrated approach for controlling weeds. Other methods of weed control such as prevention, biological, cultural and mechanical techniques are often utilized to complement chemical control programs. For example, mechanical felling of large trees, followed by chemical treatment of regrowth, is a common operational strategy for forestry. Chemical control to provide initial kill of vegetation, followed by the introduction of a biocontrol agent is very effective for management of *Melaleuca quinquenervia* in south Florida. A critical aspect of management in many systems is the use of fire to reduce ground litter, promote seed germination and flowering, and provide control of undesirable species. Fire can also be used to reduce biomass and promote regrowth, which often results in more efficacious herbicide treatments. Conversely, intense fires by excessive fuel generated from invasive species can cause severe damage, especially to desirable over-story trees.

Restoration is another very important component of natural area management. This aspect involves: 1) promoting the existing desirable vegetation through regrowth or regeneration from a seed bank, or 2) intentional planting of desirable species through physical transplanting or sowing of seed. Previous control methods can have a profound effect on restoration. Mechanical tillage can disrupt the seedbank through exposure seed on the surface or bury beyond the point of emergence. Residual activity from herbicides used to control invasive plants can also be deleterious to recolonizing desirable species. Studies to determine herbicide longevity and sensitivity of species is important when developing both control strategies and subsequent restoration plans as components of an overall management plan.

6. Conclusions

Herbicides are a critical component to managing undesirable species in natural areas. However, several considerations must be addressed for effective and environmentally safe usage. Proper herbicide selection, timing of application, type of application methodology and application rate must be adhered according to the product label. Actual site of usage must also fall within product label guidelines. Herbicides should never be used as a stand-alone approach but rather as a component of an integrated long-term management strategy for invasive species control and natural area restoration.

Acknowledgements

This publication is a contribution of the University of Florida Institute for Food and Agricultural Sciences and the Florida Agricultural Experiment Station. The authors also wish to thank

the Center for Aquatic and Invasive Plants at the University of Florida for support in publication of this document.

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