Introduction

The composition of woody weed species varies throughout the southeast. Each different species mix requires a unique herbicide prescription. There is no standard herbicide mix that can provide sufficient efficacy in all circumstances. At extremely high rates, all woody weeds can be controlled, but the objective of most herbicide applications is to provide maximum control while minimizing cost and using a minimum amount of chemical. Each herbicide application results in a response that changes according to the species mix, the chosen herbicide, and the formulation used. The decision to use herbicides for forestry applications needs to be based on site specific knowledge. Forestry and right-of-way vegetation specialists need conclusive research that can provide the necessary species efficacy information for different mixtures of herbicides.

Pesticide development, testing and EPA registration can take up to 10 years and can cost $50 million for a single new product (NACA, 1993). Due to the difficulty and expense of new herbicide registration, herbicide mixtures have become a resourceful method to improve vegetation management practices. The combination of already registered herbicides gives a vegetation specialist the opportunity to utilize individual product strengths and expand the spectrum of weed control. Herbicide mixtures can also allow the use of lower rates, which can increase crop tolerance in release operations, reduce the amount of herbicide left in the soil, and possibly reduce the cost of the spray operation (Barret and Witt, 1987).

It is also possible that the combination of the two products may produce a response that is greater than the sum of the separate product responses. This phenomenon is known as a synergistic response. While synergism can provide a beneficial increase in efficacy, a vegetation specialist needs to be aware of the potential for a negative interaction between the combined herbicides. This is referred to as an antagonistic response. Antagonism between herbicides can reduce overall efficacy of the spray operation, and may require higher rates of the combined herbicides to be used.

Chemical Synergism and antagonism are phenomena that have received attention from scientists in numerous fields. In the last few years, the medical community has spent a great deal of resources to combat antagonistic drug interactions. Database alert systems are being developed to warn pharmacists of dangerous combinations among a patient prescriptions. Agricultural researchers have found the same type of interaction problems can occur when certain agrichemicals are mixed together and applied (Hatzios and Penner, 1985). Currently, much pesticide and fertilizer screening is conducted to indicate the occurrence of synergism and antagonism.

The existence of synergistic and antagonistic herbicide combinations in forestry and right-of-way applications has not been fully explored. Synergism and antagonism has been hypothesized but not conclusively proven for certain combinations of woody plant herbicides (Ezell et al., 1995; Lawrie and Clay, 1993). By utilizing the methods used in industrial, pharmaceutical and agricultural research, tests can be made to determine the interaction that may be occurring among commonly used forestry and right-of-way herbicides. Also, these methods can be used as tools to predict the efficacy of herbicide combinations on different weed ecosystems. The determination of interactions in herbicide mixtures provides a method to explain and predict dose-response relationships given a wide spectrum of different woody weed species.

This study focuses on two herbicide mixtures, imazapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-(1H-imidazol-2-y1]-3-pyridinecarboxylic acid) plus glyphosate [N-(phosphonomethyl) glycine] and imazapyr plus triclopyr butoxyethyl ester (3,5,6-trichloro-2-pyridinyloxyacetic acid). Both mixtures are commonly used in forestry and right-of-way vegetation management applications. There has been research conducted on both of these herbicide mixtures, but few of these studies have produced data that are adequate for the determination of synergism or antagonism. This research utilizes rapid primary screening trials of herbicide mixtures on woody trees and shrubs to determine if synergism or antagonism is occurring. The methods conducted in the research can be used to update

ChESS (Chemical Expert System for Silviculture) (Zedaker, 1993), which currently does not make prescriptions based on variable mixture information.

Objectives

This study will attempt to:

1) Determine if the joint action of triclopyr ester plus imazapyr tank mixtures results in synergistic, antagonistic or additive effects at a species level.

2) Determine if the joint action of glyphosate plus imazapyr tank mixtures results in synergistic, antagonistic or additive effects at a species level.

3) Develop response surface models that explain the efficacy of variable tank mixtures of glyphosate plus imazapyr and triclopyr ester plus imazapyr on woody weed species.

Literature Review

Synergistic and Antagonistic Response

When mixing two active herbicides, there are a number of different ways that these compounds may effect each other. The herbicide mixture can alter the effects of each individual herbicide biochemically, competitively, physiologically, or chemically (Green et al., 1997). These interactions can drastically effect the efficacy of an herbicide on a target weed species as well as the effect on the crop species. Two important interactive effects of mixing herbicides are synergism and antagonism.

Synergism is defined as the cooperative action of different chemicals in a mixture such that the total effect is greater than the sum of the independent effects (Green et al., 1997). In relation to herbicides, the total response induced by an herbicide mixture is greater than the sum of the responses by each herbicide alone (Anderson, 1996). A synergistic response in an herbicide mixture can be very desirable. A study by Bovey and Whisenant (1992) found that a tank mix of clopyralid (3,6-dichloro-2-pyridinecarboxylic acid monoethanolamine salt) and triclopyr butoxyethyl ester applied post-emergent to honey mesquite (*Prosopis glandulosa*) exhibited 87% control. When applied alone at the same dosage, neither herbicide controlled more than 27% of the treated plants. When mixed together, clopyralid and triclopyr exhibited cooperative action that was much larger than the expected response, indicating a synergistic interaction.

Antagonism in a chemical mixture can be defined as having cooperative action such that the total effect is less than the sum of the independent effects. The total response induced by an herbicide mixture is less than the expected sum of the responses induced by each herbicide alone (Anderson, 1996). An antagonistic response can severely increase the cost of an herbicide application. A study by Grichar (1991) found that mixing 2,4-DB (4-(2,4-Dichlorophenoxy) butyric acid, dimethylamine salt) with sethoxydim (2-[1-(ethoxyimino)butyl-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) reduced the annual grass control efficacy of sethoxydim by 65%. The antagonistic response required an additional 50% more sethoxydim to be applied to obtain the desired annual grass control (Anderson, 1996). When mixed, sethoxydim and 2,4-DB can lead to an expensive antagonistic interaction.

Antagonistic responses can also be beneficial. Antagonism between herbicides has been used to reduce crop injury while maintaining weed efficacy. When MCPA (2-methyl-4-chlorophenoxyacetic acid dimethylamine salt) is added to fenoxaprop-ethyl ((±)-ethyl2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoate), antagonism reduces crop injury to wheat (*Triticum aestivum*) and barley (*Horduem vulgare*), while efficacy on wild oat (*Avena fatua*) weed control remains the same (Deschamps et al., 1990). The recognition of antagonism can allow the use of mixture to protect the crop against damage.

An additive response is what would be expected if no interactive effects occurs between the herbicides in a mixture. The total response induced by the herbicide mixture is equal to the sum of the responses by each herbicide applied alone (Anderson, 1996). At equivalent biological rates, the two herbicides could replace each other in the mixture without a significant change in the response (Green and Streibig, 1993).

Useful methods used to study synergism and antagonism include Additive Dose Models (ADM) and Multiplicative Survival Models (MSM). Additive Dose Models determine the mixture response based on the additive effect of the doses (Green et al., 1997). A deviation from the expected additivity of the herbicide mixture response will indicate a synergistic or antagonistic effect. ADM methods are recommended if the herbicides in the mixture exhibit similar response on the treated weeds (Morse, 1978; Hatzios and Penner, 1985; Green et al., 1997). As long as the herbicides in a mixture exhibit similar responses, such as plant dieback and mortality, ADM methods are the best measure for synergism and antagonism determination (Hatzios and Penner, 1985). The herbicides must exhibit similar response if an assumption of additive effects is going to be made. If two herbicides do not exhibit similar responses, there is no basis for assuming that they will have an additive effect when they are combined.

Multiplicative Survival Models determine the mixture response based on the multiplicative effect of combining doses (Green et al., 1997). A reference model determines an expected response at a mixture level, and the observed response at that dose will indicate synergism or antagonism. MSM methods should be used if the herbicides exhibit independent responses (Morse, 1978; Green et al., 1997; Hatzios and Penner, 1985). If two herbicides exhibit independent responses, such that both do not effect the response being measured, then determination of synergism or antagonism based on an assumed multiplicative effect is a suitable method. The herbicides may produce their effects in different ways, thereby having no expected influence on the effectiveness of each other (Morse, 1978). With two herbicides that have independent responses, only the active herbicide will have an assumed effect on the weed species, so any increased or decreased activity from adding the inactive herbicide will indicate synergism or antagonism. A good example of this relationship is shown by the antagonistic effect 2,4-DB has on grass control when added to sethoxydim (Grichar, 1991). 2,4-DB is not active on grass species, so any decreased grass control caused by adding 2,4-DB is best shown through an MSM method.

The determination of synergism and antagonism in herbicide mixtures has been explored by a number of agricultural researchers. A popular MSM method is known as the Colby method (Green and Bailey, 1987). Colby (1967) developed a method of recognizing the extent of synergism and antagonism in herbicide mixtures by calculating the expected response of the mixture, and then comparing the expected response with the actual response found for the mixture. The expected response is calculated as follows:

$$E(Y) = A_p + B_q - (A_p * B_q)/100$$

where: $E(Y)$ = expected percent control

A_p = Herbicide A at rate p kg/ha applied alone

B_q = Herbicide B at rate q kg/ha applied alone

If the actual mixture percent control response is greater the expected response, then the mixture exhibits synergism. If the actual mixture percent control response is less than the expected response, then the mixture exhibits antagonism.

The Colby method is attractive due to the ease of application and simple mathematical computations (Akobundu et al., 1975). The Colby method will indicate synergism and antagonism within each mixture combination individually, making it easier to indicate the range of the interaction. When the mixture results in a deviation from this value of no expected influence (expected percent control), then synergism or antagonism is determined. There is criticism that the Colby method undervalues synergism and overvalues antagonism (Rummens, 1975). The expected responses given by the Colby method tend to shift away from a sigmoidal response, causing the expected responses to ignore synergistic interactions and overestimate antagonistic interactions (Rummens, 1975). The Colby method may also give false confidence to the "expected response" (Akobundu et al., 1975).

An ADM method was developed by Tammes (1964) from a popular agricultural and pharmaceutical research method. The Tammes isobologram method uses a graphical display of the response to the herbicide mixture(Figure 1). A selected response, such as the ED50, is graphed for the herbicide mixture, and the shape of the resulting curve will indicate if synergism or antagonism had occurred. The ED50 (Effective Dose 50) is the amount of herbicide that will result in 50% control of the plant species. A synergistic joint action will result in an concave isobole, showing that the mixture requires smaller rates to obtain the same control. An antagonistic response will result in an convex isobole, showing that the mixture requires larger rates to obtain the same amount of control. A linear isobole shows the additive effect that the herbicides have on control (Tammes, 1964; Green and Streibig, 1993).

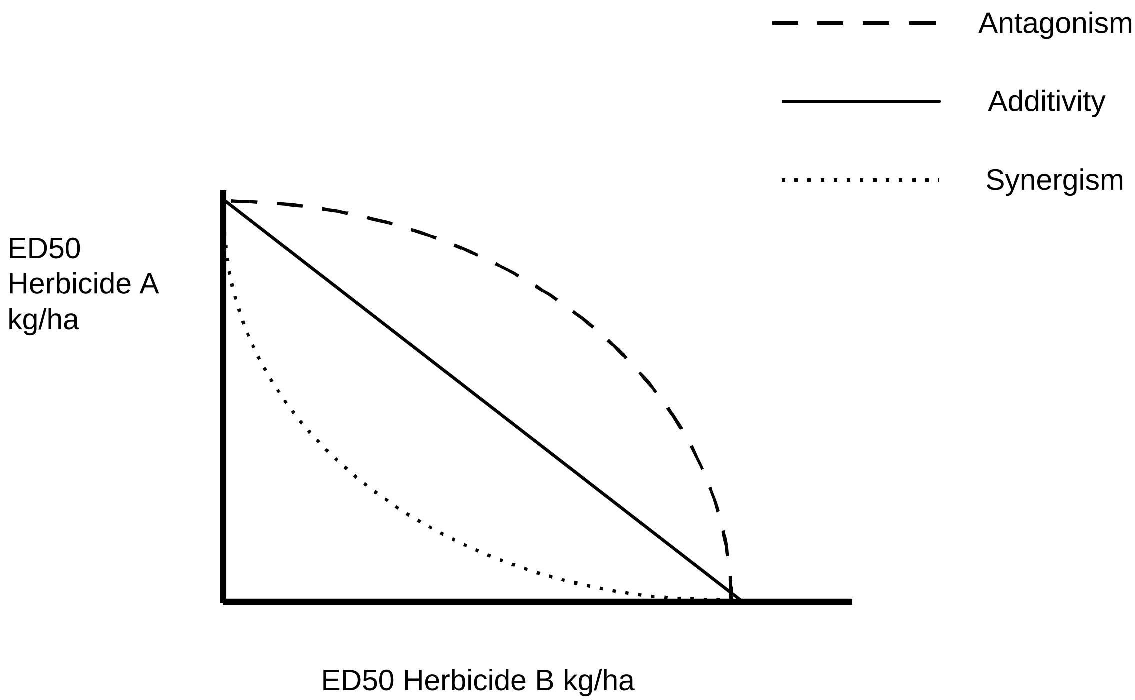


Figure 1: The isobologram method using ED50 values to display synergism or antagonism.

The Tammes isobologram method has been used to determine the effects of pharmacological drug mixtures (Tammes, 1964), as well as agricultural chemical mixtures (Poch et al., 1990). The isobologram method, like other ADM methods, is considered a reasonable reference model when the herbicides exhibit similar responses (Green et al., 1997; Morse, 1978). If the two herbicides in a mixture effect a plant in similar ways, there can be an assumed additive effect. In other words, one of the herbicides in a mixture can be replaced with a biologically equivalent amount of the other herbicide with no change in response (Morse, 1978).

Due to the complex computations involved with the calculation of ED50 values and other standard control values, the isobologram method has failed to gain popular acceptance (Akobundu et al., 1975). The isobologram method can be utilized in situations where probit values are readily available (Akobundu et al., 1975), or where ED50 values can be calculated using regression techniques, such as response surface analysis.

Using regression analysis procedures, Myers and Montgomery (1995) constructed a similar ADM method of determining synergism and antagonism in industrial mixtures. The method is referred to as *synergism or antagonism due to nonlinear blending*. Beginning with the linear model:

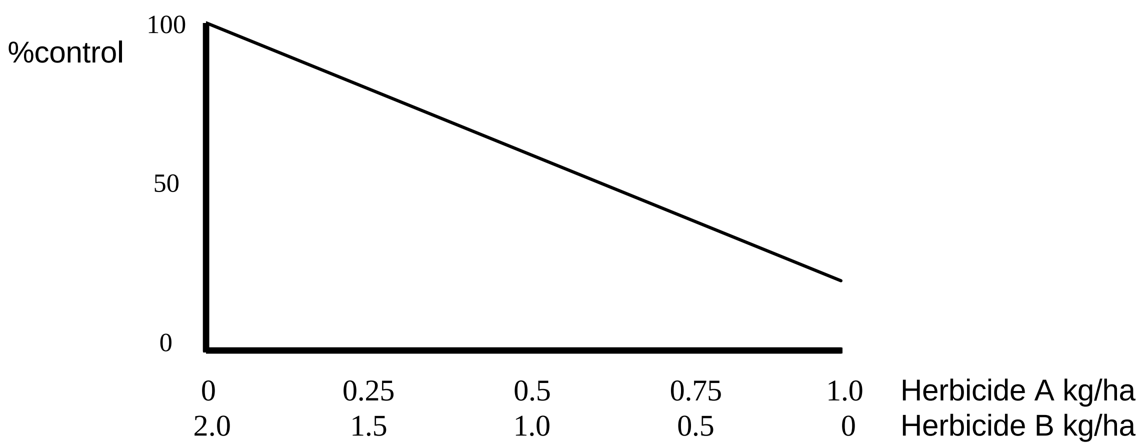
$$E(Y) = ß_1X_1+ß_2X_2$$

where: $E(Y)$ = expected percent control

X_1 = Herbicide rate kg/ha for herbicide 1

X_2 = Herbicide rate kg/ha for herbicide 2

the fitted line for two components of the mixture show the linear trend of complete additivity (Figure 2a).



$$E(Y) = B_1X_1 + B_2X_2$$

where: $E(Y)$ = expected percent control

X_1 = imazapyr kg/ha

X_2 = triclopyr kg/ha

Figure 2a: Fitted line for an herbicide mixture showing complete additivity.

The fitted line is a replacement series isobole. Any additional response from the mixture, either positive or negative, can be represented by the addition of the interaction term $ß_{12}X_1X_2$ shown below in the quadratic nonlinear model:

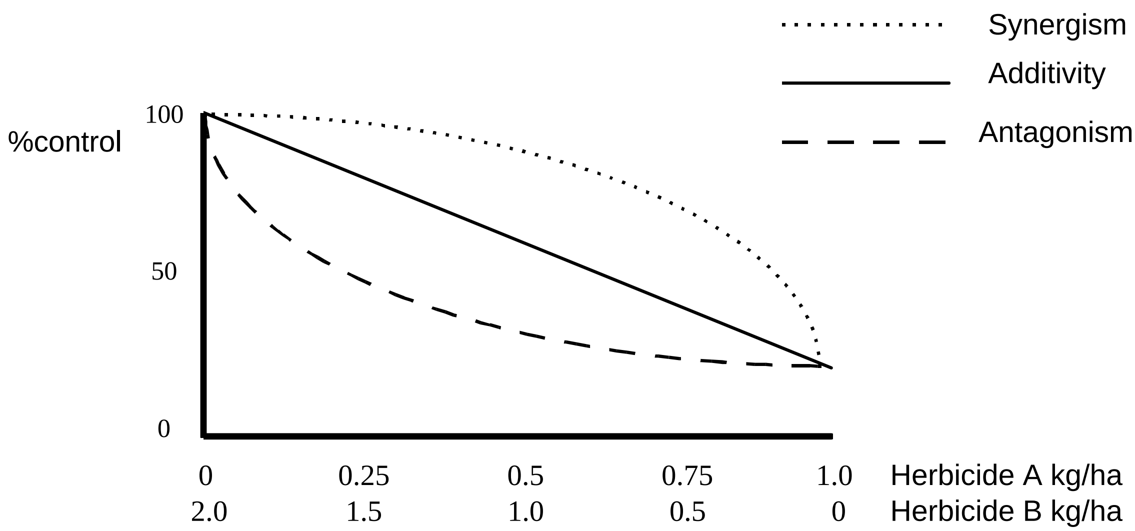
$$E(Y) = ß_1X_1 + ß_2X_2 + ß_{12}X_1X_2$$

where: $E(Y)$ = expected percent control

X_1 = Herbicide rate kg/ha for herbicide 1

X_2 = Herbicide rate kg/ha for herbicide 2

$ß_{12}X_1X_2$ represents the excess response from the quadratic model (Figure 2b). A positive value for $ß_{12}$ will indicate synergism, and a negative value will indicate antagonism.



$$E(Y) = B_1X_1 + B_2X_2 + B_{12}X_1X_2$$

where: $E(Y)$ = expected percent control
X_1 = imazapyr kg/ha
X_2 = triclopyr kg/ha

Figure 2b: Nonlinear blending method used to determine synergism or antagonism.

The nonlinear blending method utilizes regression techniques, and appears to be a sound approach to synergism and antagonism determination. The herbicide mixture can be shown to conform or deviate from the expected additive response. Like other ADM methods, The nonlinear blending method should only be used with combinations of herbicides that exhibit similar action. The nonlinear blending method is based on the assumption that the two herbicides in the mixture are additive.

Empirical response surface analysis can provide a prediction surface that takes into account the different interactive effects of an herbicide combination. Since there is no theoretical response function for imazapyr plus triclopyr ester and imazapyr plus glyphosate mixtures, empirical response surface modeling offers a method to represent the interaction between these herbicides. The main features of the mixture response surfaces can be approximated by fitting a quadratic empirical response surface. The quadratic response surface can be fitted using:

$$E(Y)=\beta_0+\beta_1X_1+\beta_2X_2+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{12}X_1X_2$$

where: Y = percent control

X_1 = rate of Herbicide A

X_2 = rate of Herbicide B

Quadratic response surfaces can approximate a variety of possible surface shapes, including maximums, stationary ridges, rising ridges and saddles (Box and Draper, 1987).

Shiver et al.(1991) used a quadratic response surface model to fit the control response from picloram (4-amino-3,5,6-trichloro-2-pyridine-carboxylic acid) plus triclopyr ester mixtures on a selection of woody species. The response surface was fit using:

$$E(Y)=\beta 0 +\beta_1 P+\beta_2 P^2+\beta_3 T+\beta_4 T^2+\beta_5 PT$$

where: Y = expected control

P = picloram kg/ha

T = triclopyr kg/ha

The Shiver et al.(1991) response surface will be adequate for an herbicide mixture that may exhibit a quadratic response. The least squares method can be used to fit the empirical response surface.

Knowe et al.(1995) mapped quadratic empirical response surfaces for imazapyr plus glyphosate and imazapyr plus triclopyr ester mixtures to determine optimal rates and timing for red alder(*Alnus rubra*) and vine maple (*Acer circinatum*) control with a field study. Knowe et al.(1995) suggest that response surface modeling should include at least three or more rates of each herbicide to develop an adequate surface.

The sources of variation include the main and interactive effects of the herbicides, represented as linear or quadratic components of the response surface. The interaction between increasing levels of the herbicides will result in a variety of shapes in the response surfaces. Response surface analysis creates a model that describes the trends that may indicate a synergistic or antagonistic reaction. Synergism or antagonism will be described by the interactive component of the surface model. The interactive component ($\beta_{12}X_1X_2$) will show a trend of increasing or decreasing activity with the addition of each herbicide.

Herbicide Mixture Research

Imazapyr is an herbicide in the imidazolinone family used for woody plant control. The primary mode of action of imazapyr is the inhibition of acetolactate synthase(ALS). Inhibition of the ALS enzyme deprives a plant of amino acids needed for DNA synthesis and cell growth (Anderson, 1996). The forestry product formulation of imazapyr at 478 grams ae/liter is Arsenal Applicators Concentrate®. For site preparation applications, Arsenal AC has a suggested rate of 1.75 to 2.9 liter/ha (24-40 oz/ac) (American Cyanamid product label, 1994).

Glyphosate is considered a nonfamily herbicide. Glyphosate inhibits the shykimic acid pathway as well as blocking porphyrin ring synthesis (Anderson, 1996). Glyphosate is similar to imazapyr in that they both inhibit the synthesis of amino acids needed for plant metabolic processes. The forestry product formulation of glyphosate at 478 grams ae/liter is Accord®. For site preparation applications, Accord has a suggested rate of 4.65 to 23.24 liters/ha (2 to 10 qts/ac) (Monsanto product label, 1993).

Tricolpyr is a growth regulator in the pyridine herbicide family. The primary mode of action for triclopyr is not known (Anderson, 1996). The auxin-like properties of triclopyr interfere with nucleic acid metabolism and disrupt cell transport systems (Anderson, 1996). The forestry product formulation of triclopyr ester at 478 grams ae/liter is Garlon 4®. For site preparation applications, Garlon 4 has a suggested rate of 9.3 to 18.6 liters/ha (4 to 8 qts/ac) (DowElanco product label, 1995).

Mixtures that include imidazolinones have been found to result in synergism and antagonism. Liu et al.(1994) found that imazamethabenz ((±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imadazol-2-yl]-4(and 5)-methylbenzoic acid(3:2)) plus fenoxaprop mixtures resulted in antagonism on wild oat weed populations. A study by Riley and Shaw(1988) found that the addition of imazapyr to various rates of imazethapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid) or imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid) created a synergistic increase in both johnsongrass (*Sorghum halepense*) and pitted morningglory (*Ipomoea lacunosa*) control. Mixtures of imazaquin or imazethapyr plus AC263,222(imazameth)(2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid) were found to result in either synergistic or additive responses in johnsongrass, pitted morningglory and entireleaf morningglory (*Ipomoea hederacea*)(Shaw and Wixson, 1991). Given the interactive effects of other imidazolinone mixtures, Imazapyr may also exhibit an interactive effect from combination with glyphosate or triclopyr ester.

Triclopyr butoxyethyl ester (Garlon 4) has been suggested to exhibit interactive effects when mixed with other herbicides. Mixtures of triclopyr ester with clopyralid have seemed to result in a synergistic response on honey mesquite (Bovey and Whisenant, 1992), yet a complete spectrum of rates was not tested and no specific test for synergism was done. Picloram, another pyridine family herbicide, was found to be antagonistic to glyphosate when applied to Canada thistle(*Cirsium arvense*) (O'Sullivan and Kossatz, 1982).

Imazapyr plus triclopyr ester (Arsenal AC plus Garlon 4 and Chopper EC® plus Garlon 4) applications are also commonly used in site preparation applications. In a study to determine the effect of foliarly applied imazapyr mixtures, Ezell et al.(1995) applied a number of different imazapyr plus triclopyr ester mixtures to sweetgum (*Liquidambar styraciflua*). Ezell et al.(1995) reported that there is no significant effect of adding triclopyr ester to imazapyr. The percent mortality results showed no significant improvement from adding 32-128 oz./acre of triclopyr ester to 16 and 24 oz./acre of imazapyr. It should be noted that this study measured the response of a single species (sweetgum). These results may not be indicative of the effect of imazapyr plus triclopyr ester mixtures on a full range of southern woody species. In the same study, Ezell et al.(1995) found that triclopyr amine (Garlon 3A®) seemed to be antagonistic to imazapyr.

Lawrie and Clay (1993) found that mixing imazapyr and triclopyr ester seemed to depress the activity of each component, yet imazapyr plus triclopyr ester mixtures appeared to have an increased effect at some doses. In the same experiment, Lawrie and Clay (1993) found that sequential applications of imazapyr and triclopyr ester (imazapyr treatment, followed by a triclopyr ester treatment 2 days later, and a triclopyr ester treatment followed by an imazapyr treatment) on *R. ponticum* appeared to have an antagonistic response.

Knowe et al.(1995) discovered that imazapyr plus tricopyr ester mixtures generally exhibited an additive effect on red alder and vine maple. However, one of the data sets in their study showed that imazapyr plus triclopyr ester exhibited a negative interactive effect at higher rates of the two herbicides (Knowe et al., 1995). Knowe et al.(1995) explained that the negative interactive effect was likely due to phloem damage caused by high triclopyr ester rates in the mixture, causing a decrease in the response from the imazapyr.

A study of imazapyr plus triclopyr ester (Garlon 4 plus Chopper) mixtures used in basal applications found the addition of triclopyr ester to imazapyr significantly improved control of imazapyr resistant species such as hackberry (*Celtis occidentalis*), loblolly pine (*Pinus taeda*), winged elm (*Ulmus alata*), black locust (*Robinia pseudoacacia*) (Ezell et al., 1996). A similar study testing basal applications of imazapyr plus triclopyr ester found significant improvements in efficacy from adding triclopyr ester to imazapyr in reduced volume bark applications to sweetgum, southern red oak(*Quercus falcata*), and hickory(*Carya spp*.), especially at lower imazapyr rates (Williams et al., 1996). The increased spectrum from adding triclopyr ester to imazapyr may prove to be effective.

Imazapyr plus glyphosate (Arsenal AC plus Accord) is a commonly used herbicide mixture for forestry site preparation and release activities. Knowe et al.(1995) found that imazapyr plus glyphosate mixtures exhibited an additive effect on crown reduction of red alder and vine maple. A positive interactive effect between imazapyr and glyphosate was found, but they did not feel that this indicated an overall pattern of synergism. Lawrie and Clay (1993) studied the effects of herbicide mixtures on container-grown *Rhododendron ponticum*. Mixtures of imazapyr and glyphosate did not interact synergistically, and glyphosate did not consistently enhance the activity of imazapyr.

Foliar applications of imazapyr plus glyphosate have been found to provide a high level of efficacy on a variety of hardwood species (Burkhalter, 1992; Ramsey et al., 1992). Several researchers have concluded that tank mixes of Arsenal plus Accord seem to provide better hardwood control than applications of Arsenal or Accord alone.

This phenomenon could be the synergistic effect of the mixture on a community basis. Each herbicide is highly effective on a selection of woody trees and shrubs, and ineffective on other woody trees and shrubs. But when they are combined, the range of effective species control is increased, whereby creating a synergistic community response. Ramsey et al.(1992) found that adding glyphosate while lowering the rate of imazapyr was effective at controlling species that are tolerant to imazapyr alone, but individual species synergism was not reported. Imazapyr plus glyphosate mixtures may be best for sites with tolerant species such as black cherry (*Prunus serotina*), dogwood (*Cornus florida*) and red oak (*Quercus spp.*) (Ramsey et al., 1992). Some studies have found that the combination of imazapyr and glyphosate increases the individual species efficacies, creating an overall increase in control. Imazapyr and glyphosate have comparable modes of action and interior plant movement, yet they have different spectrums of control, making the combination of the two herbicides very effective (Burkhalter et al., 1990).

For a mixture to be truly synergistic, the combination of two herbicides would result in a higher efficacy on individual species. The combination of imazapyr and glyphosate may result in a synergistic community efficacy, but it may not be synergistic at a species level. Specific imazapyr plus glyphosate mixtures necessary to optimize efficacy on sites with variable species composition cannot presently be determined.

Many of the past studies of imazapyr plus triclopyr ester and imazapyr plus glyphosate mixtures suggest that there could be interactive effects of applying herbicides together. However, none of the studies used analytical methods that are designed to determine synergism or antagonism in mixtures and apply these methods to a wide range of woody species.

Rapid Primary Screening

Rapid Primary Herbicide Screening (RPHS) is an herbicide research method that investigates the effects of herbicidal compounds on woody plants in an accelerated manner. Herbicide efficacy information can be obtained in a short amount of time by controlling temperature and light levels after an herbicide application to accelerate the physiological age of the treated seedlings.

RPHS for woody plants can provide herbicide efficacy information similar to field trial data in less time and with fewer resources (Zedaker and Seiler, 1988). Unlike herbaceous primary screening, woody species field trials require enough time for the seedlings to produce secondary woody tissue. From the beginning to the final assessment, woody species field trails may take up to 26 months to complete (Zedaker and Seiler, 1988). The time required for testing can be reduced into a single year or less through a RPHS process. The amount of herbicide needed for the trials is also greatly reduced (Zedaker and Seiler, 1988).

During a RPHS process, seedlings are grown from seed in greenhouse conditions. The seedlings are physiologically manipulated though a series of controlled environments. The seedlings are rotated between greenhouses, shade houses and cold rooms so that the seedlings develop the woody tissue similar to that of field grown plants (Zedaker and Seiler, 1988).

When compared to field trials, the efficacy rates found in RPHS tests tend to require lower rates for the same degree of control, yet the efficacy trends between a species and an herbicide appear to be consistent (Zedaker and Seiler, 1988). In a comparison between commercial field applications and RPHS applications, glyphosate rates at 0.8 ai/ac in RPHS trials had similar efficacies to 1.5 ai/ac rates in commerical field applications (Zedaker and Seiler, 1988). A study of fluroxypyr found that a field application of 2 lbs ai/ac had similar efficacies to RPHS at 0.5 lbs ai/ac (Zedaker and Seiler, 1988) Although field grown trees generally require higher rates than greenhouse grown trees, the RPHS process reveals important information about how a species may respond to an herbicide in the field. The efficacy and selectivity of herbicides on seedlings in the greenhouse have been shown to parallel field grown seedlings (Bunn et al., 1996).

A number of studies have shown similar herbicide responses between greenhouse grown and field grown plants. A study analyzing compiled data from the University of Oklahoma PHYTOTOX data base showed that taxonomic differences between plants had a much greater influence on response to herbicides than whether the plants were grown in the greenhouse or the field (Fletcher et al., 1990). Deschamps et al.(1990) found that MCPA antagonized fenoxaprop when applied to wheat (*Triticum aestivum*) and barley (*Horeum vulgare*) in the greenhouse and the field.

There are also many studies that show different herbicide responses between greenhouse grown and field grown plants. Bovey and Whisenant (1992) found that applications of triclopyr plus clopyralid on honey mesquite resulted in synergism in the field, but found no increased activity in greenhouse grown plants. Moore and Banks (1991) found antagonism between mixtures of paraquat with naptalam and bentazon when applied to greenhouse grown plants, but in the field, the excellent efficacy from paraquat masked any antagonistic effects. Greenhouse and field grown plant can react differently to the same herbicide due to environmental conditions and stresses.

Because the size and physiological condition of a tree are important determinants of herbicide efficacy, the RPHS process cannot always predict the exact relationship between an herbicide and a field grown tree. Also, most past studies only included herbaceous species, so the comparison of woody plants grown in the greenhouse and field needs to be explored further.

Methods and Materials

The rapid primary screening procedure was used to test for synergism and antagonism between imazapyr plus triclopyr and imazapyr plus glyphosate mixtures with a selection of woody weed species. The rapid primary screening procedure was conducted in a greenhouse, shade house and cold room located at the Reynolds Homestead Forestry Resources Research Center in Critz, VA.

Species that are abundant southeastern woody weeds and that have shown some tolerance to either imazapyr, glyphosate, triclopyr ester were chosen for the rapid primary screening. Red maple (*Acer rubrum*), loblolly pine, black locust, black cherry, winged elm, water oak (*Quercus nigra*) (Table 1) and cabbage palmetto (*Sabel palmetto*) were chosen because they are hard to control (published efficacy values for cabbage palmetto were not found). Sweetgum was chosen for its abundance in the southeast.

The seeds were purchased from commercial seed sources (Louisiana Forest Seed Co., Lecompte, LA USA; F.M. Schumaker, Sandwich, MA USA), and stratified according to the individual species requirements beginning in the Summer of 1996. The seeds were germinated in the Fall of 1996. The water oak, loblolly pine, sweetgum, black locust and winged elm were germinated in conetainers (164 cm^3), and thinned to one seedling per conetainer. The black cherry, red maple and cabbage palmetto were germinated in soil trays on propagation mats and transferred to conetainers after sprouting. The growing medium used consisted of a 50-50 mix of Promix Bx® potting soil and sand.

Table 1: Herbicide efficacy for controlling the tested species at suggested field rates.

	Imazapyr***	Glyphosate***	Triclopyr ester***
Red Maple	Good**	Marginal*	Good**
Sweetgum	Good**	Good**	Good**
Loblolly Pine	Tolerant*	Marginal*	Good**
Black Locust	Tolerant*	Good**	Good**
Black Cherry	Marginal*	Marginal*	Marginal*
Winged Elm	Tolerant*	Good**	Marginal**
Water Oak	Good**	Marginal**	Marginal*

* according to Miller and Mitchell (1990).

** according to ChESS efficacy matrices, (Zedaker, 1993).

*** commericial formulations of Arsenal AC®, Accord®, and Garlon 4® respectively.

Control of less than 40% = Tolerant

Control of between 40% and 80% = Marginal

Control of more than 80% = Good

The seedlings were exposed to a 5 month growing season in the greenhouse. The greenhouse had daytime temperatures between 25 °C and 35 °C and nighttime temperatures between 18 °C and 24 °C. The seedlings received a combination of ambient sunlight and electric sodium arc light to maintain a 16-hour photoperiod. The seedlings were kept well watered to avoid drought stress. Banrot® fungicide was applied to the soil monthly at 2.0 grams per 3.78 liters of water to prevent seedling mortality. The seedlings were also fertilized monthly with Miracle Gro® at 5 milliliters per 3.78 liters of water.

The imazapyr plus triclopyr ester and imazapyr plus glyphosate mixtures were tested at six different levels for each herbicide in each mixture combination. The herbicide treatment levels were selected to include an entire response curve; from the no effect level to the 100% mortality level. The experiment included 66 herbicide combinations (Table 2), and was replicated three times. Each treatment contained five seedlings of each of the following for all three replications: black cherry, winged elm, sweetgum, red maple, black locust, and loblolly. The first replication also contained five water oak seedlings for each treatment. Five water oak were included in the selected treatments for the second and third replications. The cabbage palmetto seedlings were included in selected treatments for all three replications.

Table 2: Treatment design and numbers of rapid primary screening experiment.

		Triclopyr kg/ha						Glyphosate kg/ha				
		0	0.05	0.5	1.0	1.5	2	0.05	0.5	1.0	1.5	2
Imazapyr	0	1	7	13	19	25	31*	37	43	49	55	61*
kg/ha	0.025	2	8	14	20	26	32	38	44	50	56	62
	0.25	3	9	15	21	27*	33	39	45	51	57*	63
	0.5	4	10	16	22*	28	34	40	46	52*	58	64
	0.75	5	11	17*	23	29	35	41	47*	53	59	65
	1	6*	12	18	24	30	36	42	48	54	60	66

*treatments used for nonlinear blending method, see page 33.

The herbicide treatments were applied under controlled conditions using a spray booth located at the Critz, VA facility. The spray tests were conducted on March 18, 1997. The day was overcast, with periodic rainshowers and high humidity. The weather required hanging plastic tarps to allow enough dry area to stage the spray test.

The 66 treatments were applied in a randomized block design. All species within a treatment replication were sprayed in a single application with the spray booth. The seedlings in each replication were mounted in seedling racks, with adequate spacing between seedlings to allow for full coverage by the spray swath. The species groups were arranged randomly on the racks to avoid spray location bias. The seedlings were individually lifted on the seedling racks so that a uniform height is attained by all seedlings within a treatment. This prevented the seedlings from receiving variable amounts of spray solution due to height differences.

The spray booth was fitted with 8001EVS spray nozzle and the seedling rack was placed to accommodate a 24 inch spray swath. The spray booth was calibrated to deliver 150 liters of carrier per hectare. The spray booth was calibrated two to three times per block to insure a constant delivery rate and speed.

A 200 ml volume of each herbicide mixture was prepared in plastic 16 oz bottles. The 200 ml of herbicide mixture was a sufficient spray volume for all 3 replications for each mixture. The herbicide mixtures were formulated using commercial formulations of imazapyr (Arsenal AC), glyphosate (Accord) and triclopyr ester (Garlon 4). 4.0 liters at the 2 kg/ha rate was mixed for the glyphosate and triclopyr, and 8.0 liters at 1 kg/ha was mixed for the imazapyr. For the 2 kg/ha mix of glyphosate, 222 ml of Accord was added to 4.0 liters of tank mix (Accord contains 478 grams ae per liter). For the 2 kg/ha mix of triclopyr, 222 ml of Garlon 4 was added to 4.0 liters of tank mix (Garlon 4 contains 478 grams ae per liter). For the 1 kg/ha mix of imazapyr, 222 ml of Arsenal AC was added to 8.0 liters of tank mix (Arsenal AC contains 478 grams a.e. per liter). All of the treatments were produced by diluting the 2 kg/ha mix of glyphosate and triclopyr and the 1 kg/ha mix of imazapyr. For example, the mixture of 0.5 kg/ha of glyphosate plus 0.25 kg/ha of imazapyr, 50 ml of the 2 kg/ha glyphosate mix and 50 ml of the 1 kg/ha imazapyr mix was diluted into an additional 100 ml of distilled water.

The spray nozzle was flushed after each application with a 50% acetone 50% distilled water solution followed by distilled water to avoid contamination between replications. The interior of the spray booth was rinsed with tap water between applications.

After the seedlings were treated, they were watered from below for the entire acclimation and dormancy period so that the herbicide would not be washed off the leaves of the seedlings. Four days after the seedlings were sprayed, they began an 8 week acclimation in a cooler that was outfitted with electric sodium arc lights, where the temperature was gradually dropped to between 5° and 10° C and the photoperiod was gradually shortened to stimulate the seedlings to harden off. The first week had a 12 hour dark period, the second week had a 14 hour dark period, and the third through eighth week had 16 hour dark periods. The electric sodium arc lights would raise the temperature of the cooler if left on for the entire photoperiod, so the lights would switch off twice for one hour long cooling periods during the photoperiod.

By mid May 1997, after the 8 week acclimation period, the seedlings were cooled for an additional 1000 hour period (approximately 40 days) to stimulate seedling dormancy. The cooler temperatures were kept between 5 °C and 10 °C, with a 8 hour photoperiod (with two short cooling periods) and 16 hour dark period.

On June 17, 1997, the seedlings were returned to the greenhouse environment to allow them to break dormancy. The seedlings were grown with a 16 hour photoperiod until the control seedlings had fully developed new foliage.

The post-treatment efficacy measurements began on August 4, 1997. The above ground height of the individual seedlings was measured to the nearest 0.5 cm. The seedlings were harvested and grouped according to species within each treatment. The treatment samples were placed in a drying oven for 7 days at approximately 65° C until a constant weight was obtained. Following a drying process, the treatment samples were weighed to obtain the above ground dry weight to the nearest 0.01 gram. Along with the height and biomass measurements, a live/dead count was obtained. Percent mortality values were calculated.

Analysis

Of the original eight species, sweetgum, red maple and black locust suffered unacceptible mortality due to unknown factors that occured during the rapid screening process. Within the sweetgum, red maple and black locust seedlings, the control treatments as well as the lowest rate treatments experienced high levels of mortality. The control treatments in sweetgum, red maple and black locust experienced between 40% and 100% mortality These three species were excluded from further analysis.

The hypotheses for these rapid primary screening tests are as follows:

1. H_0: Imazapyr plus triclopyr mixtures result in an additive response on the selected woody weed species.

   H_1: Imazapyr plus triclopyr mixtures result in a synergistic or antagonistic response on the selected woody weed species.

2. H_0: Imazapyr plus glyphosate mixtures result in an additive response on the selected woody weed species.

   H_1: Imazapyr plus glyphosate mixtures result in a synergistic or antagonistic response on the selected woody weed species.

The hypotheses were tested using response surface analysis, the Colby method, the nonlinear blending method, and the isobologram method. The efficacy data for the black cherry and winged elm were analyzed using response surface analysis, the nonlinear blending method and the isobologram method. The Colby method was not performed on black cherry and winged elm because imazapyr, triclopyr ester and glyphosate all exhibited similar responses on these species. Multiplicative Survival Models such as the Colby method should only be utilized when the herbicides in the mixture exhibit dissimilar response (Morse, 1978). Loblolly pine was analyzed using response surface analysis and the Colby method. Loblolly pine was not included in the nonlinear blending method or the isobologram method because imazapyr exhibited a dissimilar response than triclopyr ester and glyphosate on loblolly. Additive Dose Model methods should only be utilized when the herbicides in a mixture exhibit similar response (Morse, 1978). The water oak and cabbage palmetto seedlings were analyzed using the nonlinear blending method.

Percent mortality was calculated according to Zedaker and Miller (1991):

$$\text{Percent Mortality} = \frac{(\text{no. of treated stems judged dead})}{(\text{total no. of treated stems})} * 100$$

The percent mortality values were used in the percent control calculations for the Colby, nonlinear blending and the isobologram methods. The percent control values for the response surface analysis was obtained using the height measurements, biomass measurements, or percent mortality values as the response. Percent control was calculated according to Zedaker and Miller (1991):

$$\text{Percent Control} = 1 - \frac{(\text{treated rep response})}{(\text{control rep response})} * 100$$

The percent control calculations utilized the height, biomass, or percent mortality measurements, depending on which of these response variables appears to be the best predictor of the response surface.

Percent control response surfaces were fitted for black cherry, winged elm and loblolly using Response Surface Regression (RSREG), Regression (REG) and 3 Dimensional Graph (3Dgraph) procedures on SAS (PC version). Percent mortality, height and dry weight values were transformed into percent control values. The fitted surface values were given a minimum of 0% and a maximum of 100%. Any fitted value less than 0% was set to 0% and any value greater than 100% was set to 100%.

The response surfaces explain the effect of a herbicide mixture on a species in relation to increasing rates of each herbicide as well as the interactive effect of the increasing herbicide rates. The hypotheses for the response surfaces can be determined by observing the significance values for the surface, and then by observing the graphical display of the surface for trends that imply a synergistic or antagonistic response. R-Square and Lack-of-Fit values were used to determine which response variable is the most explanatory for each response surface model.

The hypothesis for the Colby test is:

H_0: $Y = E(Y)$ (additive)

H_1: $Y > E(Y)$ (synergism)

$Y < E(Y)$ (antagonism)

where E(Y) equals the Colby value for each combination of imazapyr plus triclopyr ester and imazapyr plus glyphosate. The Colby method was descriptively analyzed by comparing the relative size of the difference between the observed and expected values. Large differences between the observed and expected values, and trends within the treatments were analyzed.

The nonlinear blending method was tested by fitting the quadratic model:

$$E(Y)= ß_1X_1+ß_2X_2+ß_{12}X_1X_2$$

The hypothesis for the nonlinear blending method is:

H_0: $ß_{12} = 0$ (additive)

H_1: $ß_{12} > 0$ (synergism)

$ß_{12} < 0$ (antagonism)

The hypothesis was determined by testing the significance of $ß_{12}$ with a least squares regression (REG) procedures using SAS (PC version). The model was fitted with the no intercept (NOINT) option. A significance of $p < 0.05$ will be used as a criteria for a synergistic or antagonistic interaction. Only the replacement series treatments were included in the analysis for the nonlinear blending method (see Table 2).

The isobologram method results in a graphical display of the mixture response at biologically equivalent rates. The isobolograms were constructed using the ED50 values (Effective Dose for 50% control).

The hypothesis for the isobologram method is:

H_0: the isobole is linear (additive)

H_1: the isobole has a concave trend (synergism)

the isobole has a convex trend (antagonism)

The ED50 was predicted using the response surface models that were developed for each herbicide mixture. A variable selection was conducted on the response surface models to remove insignificant variables from the models. Each variable with a significance of $p < 0.15$ was included in the final model for ED50 determination. After the siginificant variables were selected for each species, the percent mortality data was fitted into the final model. ED50 predictions were made using the fitted models. Synergism or antagonism were graphically determined by observing relatively large deviations from the expected additive line.

Results

In the week following the spray booth application, most of the treatments exhibited epinasty in the terminal buds and slight leaf malformation. Epinasty and leaf malformation is typical growth regulator damage. One explanation for this damage is that there was a small amount of triclopyr ester volatilization following the spray booth application when the seedlings were grouped in an open shed to dry. Another explanation is that the Timberland 90 surfactant may have caused the epinasty and leaf malformation. The damage was not sufficient to cause excessive mortality among the eight treated species.

Response Surface Analysis

Imazapyr plus Triclopyr ester

A) Black Cherry

The response surface models were developed for percent control using mortality, height and dry weight measurements for black cherry, winged elm and loblolly pine. The quadratic response surface for imazapyr plus triclopyr mixtures on black cherry that appears to be the best fit was developed with the percent mortality data, yet all three response surfaces show significant lack of fit ($p < 0.05$)(Table 3). With a significant lack of fit, there is strong evidence that the quadratic response surface model is not descriptive enough to account for the trends in the data. Even with a significant lack-of-fit, the two herbicide rates account for 68% of the variation in the percent mortality of black cherry.

Table 3: Fit of the response surface models for imazapyr plus triclopyr ester mixtures on black cherry using percent control derived from mortality, height and dry weight measurements.

	Mortality	Height (cm)	Dry weight (g)

	Mortality		Height (cm)		Dry weight (g)	
parameter	estimate	Prob>|T|	estimate	Prob>|T|	estimate	Prob>|T|
β_0	20.707	0.0000	45.426	0.0000	55.675	0.0000
β_1	73.033	0.0001	68.695	0.0001	62.843	0.0000
β_2	96.157	0.0000	65.691	0.0000	52.470	0.0000
β_3	-23.614	0.1862	-28.395	0.0767	-28.443	0.0437
β_4	-33.758	0.0000	-27.031	0.0000	-23.484	0.0000
β_5	-28.557	0.0000	-19.370	0.0000	-15.269	0.0000
R^2	0.6822		0.5625		0.5229	
Lack-of-fit	0.0106		0.0008		0.0000	

$E(Y) = \beta_0 + \beta_1 I + \beta_2 T + \beta_3 I^2 + \beta_4 IT + \beta_5 T^2$, where Y = percent control of black cherry, I = imazapyr kg/ha, T = triclopyr ester kg/ha.

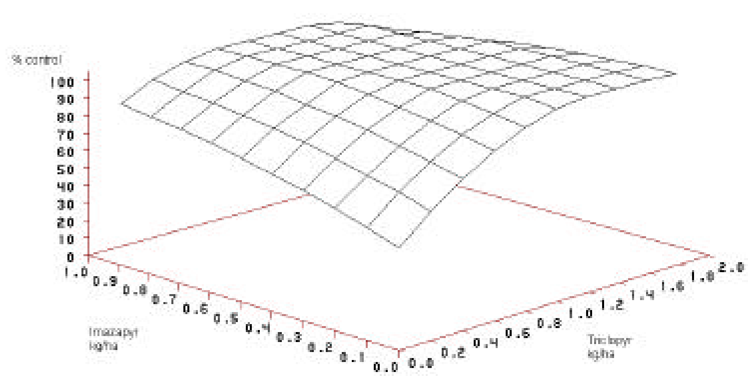


Figure 3: Fitted response surface for imazapyr plus triclopyr ester mixtures on black cherry using percent mortality data. The fitted model: $E(Y) = 20.7 + 73.0I + 96.2T - 23.6I^2 - 33.8IT - 28.6T^2$ where Y = percent control of black cherry, I = imazapyr kg/ha, T = triclopyr kg/ha

The linear effects for both imazapyr(β_1) and triclopyr ester(β_2) were significant for black cherry percent mortality(Table 3), indicating that both herbicides positively effect percent mortality (Figure 3). There is also a significant negative interactive effect (β_4) ($p < 0.0001$) from imazapyr plus triclopyr ester mixtures using the percent mortality data. This can be explained by the decrease in slope as imazapyr and triclopyr are added. At higher rates, the imazapyr plus triclopyr ester mixtures show a decrease in percent control (Figure 3), indicating that antagonism may be occuring when the herbicides are combined at high rates. At the lower rates the imazapyr plus triclopyr ester mixtures appear to have an additive effect (Figure 3).

B) Winged Elm

The percent mortality data appear to provide the best fit for the imazapyr plus triclopyr ester mixtures on winged elm (Table 4). In the fitted quadratic response surface for imazapyr plus triclopyr ester mixtures on winged elm, the herbicide rates account for 72% of the variation in the percent mortality data (Table 4), and the lack-of-fit statistic was not significant (Table 4). There was a significant negative interactive effect (β_4) ($p < 0.0009$) (Table 4) with the percent mortality data, which can be explained by a decrease in slope with the addition of both imazapyr and triclopyr. As the percent control increased from a single herbicide, the effect of adding the other herbicide decreased (Figure 4). There appears to be a general additive effect when imazapyr plus triclopyr ester is applied to winged elm (Figure 4)

Table 4: Fitted response surface models for imazapyr plus triclopyr ester mixtures for winged elm using percent control derived from mortality, height and dry weight measurements.

	Mortality		Height(cm)		Dry weight(g)	
parameter	estimate	Prob>|T|	estimate	Prob>|T|	estimate	Prob>|T|
β_0	7.485	0.1238	34.615	0.0000	15.395	0.0055
β_1	68.196	0.0005	60.067	0.0041	50.064	0.0210
β_2	89.562	0.0000	68.994	0.0000	95.905	0.0000
β_3	-23.756	0.1967	-24.674	0.2130	-6.678	0.7461
β_4	-23.359	0.0009	-20.589	0.0060	-24.907	0.0016
β_5	-23.055	0.0000	-19.004	0.0002	-27.432	0.0000
R^2	0.7262		0.5349		0.6558	
Lack-of-fit	0.4248		0.3843		0.2539	

$E(Y) = \beta_0 + \beta_1 I + \beta_2 T + \beta_3 I^2 + \beta_4 IT + \beta_5 T^2$, where Y = percent control of winged elm, I = imazapyr kg/ha, T = triclopyr ester kg/ha.

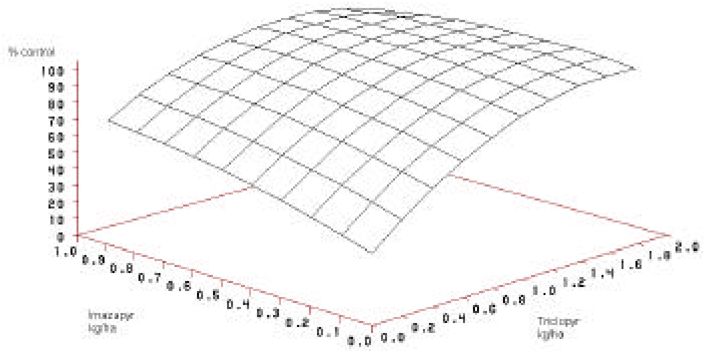


Figure 4: Fitted response surface for imazapyr plus triclopyr ester mixtures on winged elm using percent mortality data. The fitted model: $E(Y) = 7.5 + 68.2I + 89.6T - 23.8I^2 - 23.4IT - 23.0T^2$ where Y = percent control of winged elm, I = imazapyr kg/ha, T = triclopyr ester kg/ha.

C. Loblolly Pine

The dry weight data appear to provide the best fit for the imazapyr plus triclopyr ester mixtures on loblolly pine (Table 5). For imazapyr plus triclopyr ester mixtures on loblolly pine, the herbicide rates explain 70% of the variation in the dry weight data (Table 5), with no significant lack-of-fit (Table 5). For the dry weight data, the linear(β_1) and quadratic(β_3) effects of imazapyr were not significant) (Table 5), indicating that the imazapyr rate does not have a significant effect on the dry weight of loblolly pine. There was also a negatively significant interactive effect(β_4) for the dry weight data (Table 5), which can be explained by a decreased change in percent control as the rate of imazapyr and triclopyr is increased (Figure 5). Figure 5 appears to exhibit additivity, with no distinct deviations to indicate synergism or antagonism.

Table 5: Fitted response surface models for imazapyr plus triclopyr ester mixtures for loblolly for percent control derived from mortality, height and dry weight measurements.

	Mortality		Height(cm)		Dry weight(g)	
parameter	estimate	Prob>|T|	estimate	Prob>|T|	estimate	Prob>|T|
β_0	0.195	0.9530	41.064	0.0000	1.380	0.7020
β_1	-22.090	0.0921	3.949	0.9022	6.0483	0.6701
β_2	5.774	0.3761	-13.581	0.3987	28.595	0.0001
β_3	27.713	0.0290	6.419	0.8357	10.684	0.4350
β_4	-14.926	0.0018	-17.939	0.1219	-14.455	0.0054
β_5	9.726	0.0024	12.460	0.1095	3.140	0.3591
R^2	0.5795		0.0538		0.7042	

Lack-of-fit	0.5261	0.2897	0.4332

$E(Y) = \beta_0 + \beta_1 I + \beta_2 T + \beta_3 I^2 + \beta_4 IT + \beta_5 T^2$, where Y = percent control of loblolly, I = imazapyr kg/ha, T = triclopyr ester kg/ha.

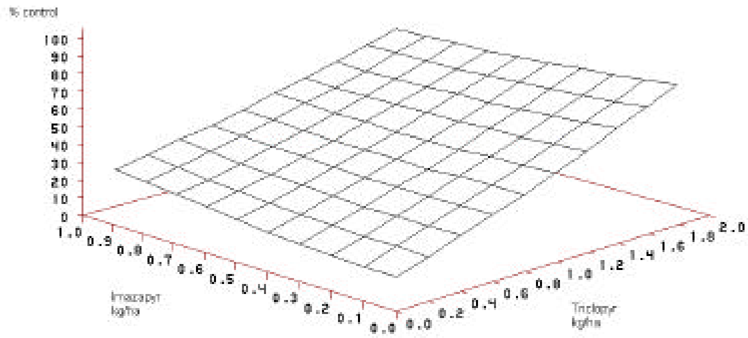


Figure 5: Fitted response surface for imazapyr plus triclopyr ester mixtures on loblolly using dry weight data. The fitted model: $E(Y) = 1.4 + 6.0I + 28.6T + 10.7I^2 - 14.4IT + 3.1T^2$ where Y = percent control of loblolly, I = imazapyr kg/ha, T = triclopyr ester kg/ha.

Table 6: Fitted response surface models for imazapyr plus glyphosate mixtures for black cherry for percent control derived from mortality, height and dry weight measurements.

	Mortality		Height(cm)		Dry weight(g)	
parameter	estimate	Prob>|T|	estimate	Prob>|T|	estimate	Prob>|T|
β_0	5.832	0.2565	21.009	0.0000	38.623	0.0000
β_1	88.363	0.0000	123.371	0.0000	103.188	0.0000
β_2	48.890	0.0000	49.578	0.0000	40.053	0.0000
β_3	-22.328	0.2517	-58.831	0.0005	-50.650	0.0005

β_4	-30.612	0.0000	-34.555	0.0000	-29.397	0.0000
β_5	-2.704	0.5778	-6.544	0.1137	-5.653	0.1094
R^2	0.6784		0.6839		0.6474	
Lack-of-fit	0.3143		0.3927		0.0004	

$E(Y) = \beta_0 + \beta_1 I + \beta_2 G + \beta_3 I^2 + \beta_4 IG + \beta_5 G^2$, where Y = percent control of black cherry, I = imazapyr kg/ha, G = glyphosate kg/ha.

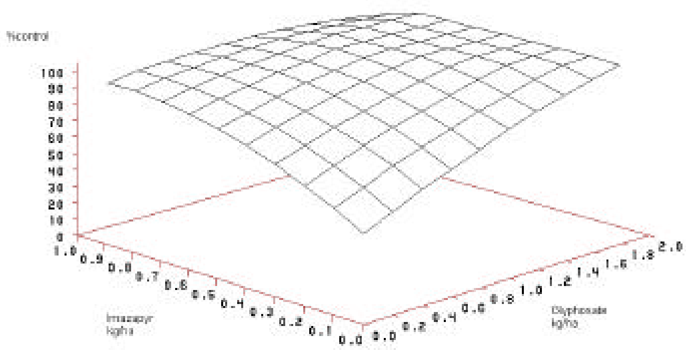


Figure 6: Fitted response surface for imazapyr plus glyphosate mixtures on black cherry using height data. The fitted model: $E(Y) = 21.0 + 123.4I + 49.6G - 58.8I^2 - 34.6IG - 6.5G^2$ where Y = percent control of black cherry, I = imazapyr kg/ha, G = glyphosate kg/ha.

Imazapyr plus Glyphosate

A. Black Cherry

The height data appear to be the best fit for the imazapyr plus glyphosate mixtures on black cherry, where the herbicide rates explain 68% of the variation in the height data (Table 6), with no significant lack-of-fit (Table 6). The linear effects of imazapyr(β_1) and glyphosate(β_2) are both significant and positive for height data (Table 6), indicating that both herbicides have a positive effect on the control of height of black cherry (Figure 6). The quadratic effects of imazapyr(β_3) and glyphosate(β_5) are both significant and negative for height data (Table 6), which can be explained by a leveling off of percent control as it approaches 100% (Figure 6). The significant negative interactive effect (β_4) (Table 6) is shown by the decrease in slope as the rates of imazapyr and glyphosate increase (Figure 6). The response surface for imazapyr plus glyphosate on black cherry appears to be additive (Figure 6).

B) Winged Elm

For the imazapyr plus glyphosate mixture on winged elm, the percent mortality data provides the best fit, with the herbicide rates explaining 69% of the variation in percent mortality (Table 7), and the lack-of-fit statistic is significant to the 0.10 level, but not the 0.05 level. The linear(β_2) and quadratic(β_5) effects of glyphosate are significant (Table 7), indicating that glyphosate effects winged elm mortality (Figure 7). The linear(β_1) and

Table 7: Fitted response surface models for imazapyr plus glyphosate mixtures for winged elm for percent control derived from mortality, height and dry weight measurements.

	Mortality		Height(cm)		Dry weight(g)	
parameter	estimate	Prob>|T|	estimate	Prob>|T|	estimate	Prob>|T|
β_0	4.467	0.4288	10.730	0.0269	15.811	0.0035
β_1	33.233	0.1363	38.851	0.0414	21.448	0.3058
β_2	89.011	0.0000	98.867	0.0000	95.908	0.0000
β_3	-0.326	0.9878	7.593	0.676	17.550	0.3839
β_4	-20.308	0.0120	-28.613	0.0000	-23.882	0.0019

β_5	-20.558	0.0002	-26.446	0.0000	-26.342	0.0000
R^2	0.6917		0.7385		0.6812	
Lack-of-fit	0.0552		0.0045		0.0118	

$E(Y) = \beta_0 + \beta_1 I + \beta_2 G + \beta_3 I^2 + \beta_4 IG + \beta_5 G^2$, where Y = percent control of winged elm, I = imazapyr kg/ha, G = glyphosate kg/ha.

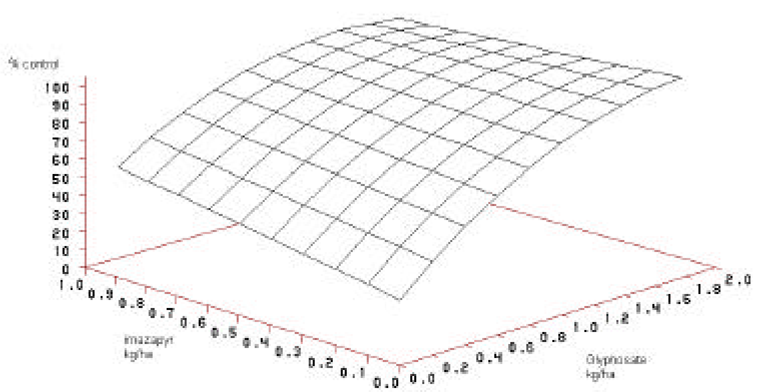


Figure 7: Fitted response surface for imazapyr plus glyphosate mixtures on winged elm using percent mortality data. The fitted model: $E(Y) = 4.5 + 33.2I + 89.0G - 0.3I^2 - 20.3IG - 20.6G^2$ where Y = percent control of winged elm, I = imazapyr kg/ha, G = glyphosate kg/ha

quadratic(β_3) effects of imazapyr are not significant (Table 7), indicating that imazapyr does not have a large effect on winged elm mortality(Figure 7). The interactive effect(β_5) of imazapyr plus glyphosate mixtures on winged elm is significant as well (Table 7), showing the decrease in slope as imazapyr and glyphosate rates are increased (Figure 7). The imazapyr plus glyphosate mixture appears to have an additive effect on winged elm (Figure 7).

C. Loblolly Pine

For imazapyr plus glyphosate mixtures on loblolly pine, the percent mortality data appear to provide the best fit, even though there is a significant lack-of-fit in all three response variables (Table 8). The quadratic response surface is not sufficicient to explain the trends in the data, but the herbicide rates still explain 91% of the variation in the percent mortality data. The linear(β_2) and quadratic(β_5) effects of glyphosate are both significant (Table 8), indicating that glyphosate causes mortality in loblolly pine (Figure 8). The linear(β_1) and quadratic(β_3) effects of imazapyr are not significant (Table 8), indicating that imazapyr does not effect loblolly pine mortality (Figure 8). The interactive effect (β_4) for imazapyr and glyphosate on loblolly pine is not significant as well (Table 8). The imazapyr plus glyphosate mixtures on loblolly appear to have an additive effect on loblolly pine mortality(Figure 8).

Table 8: Fitted response surface models for imazapyr plus glyphosate mixtures for loblolly for percent control derived from mortality, height and dry weight measurements.

	Mortality		Height(cm)		Dry weight(g)	
parameter	estimate	Prob>|T|	estimate	Prob>|T|	estimate	Prob>|T|
β_0	0.079	0.9815	8.237	0.0154	6.773	0.0517
β_1	0.430	0.9745	10.148	0.4423	12.042	0.3759
β_2	142.103	0.0000	132.251	0.0000	138.773	0.0000
β_3	-3.162	0.8069	-11.741	0.3564	-10.192	0.4363

β_4	-1.444	0.7645	-1.984	0.6753	-3.448	0.4796
β_5	-46.675	0.0000	-43.987	0.0000	-47.112	0.0000
R^2	0.9150		0.9030		0.9003	
Lack-of-fit	0.0216		0.0136		0.0000	

$E(Y) = \beta_0 + \beta_1 I + \beta_2 G + \beta_3 I^2 + \beta_4 IG + \beta_5 G^2$, where Y = percent control of loblolly, I = imazapyr kg/ha, G = glyphosate kg/ha.

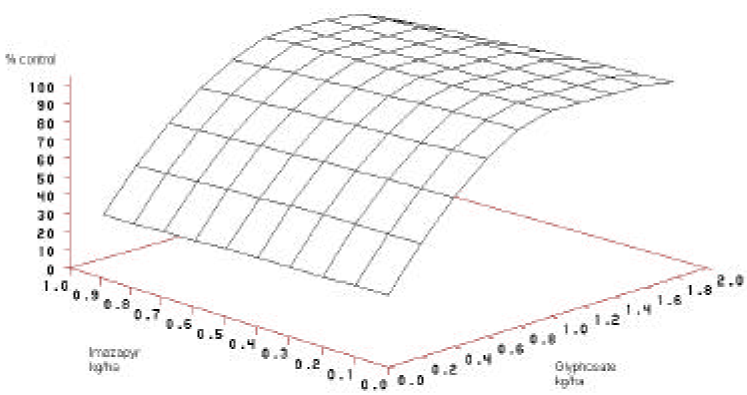


Figure 8: Fitted response surface for imazapyr plus glyphosate mixtures on loblolly pine using percent mortality data. The fitted model: $E(Y) = 0.1 + 0.4I + 142.1G - 3.2I^2 - 1.4IG - 46.7G^2$ where Y = percent control of loblolly pine, I = imazapyr kg/ha, G = glyphosate kg/ha.

Nonlinear Blending Method

Imazapyr plus Triclopyr ester

A.) Black Cherry

The nonlinear blending method was performed using percent control calculations from the mortality data for black cherry, winged elm, water oak and cabbage palmetto. The nonlinear blending method for imazapyr plus triclopyr ester mixtures show a significant interactive term (β_{12})($p<0.05$) on black cherry, indicating a synergistic interaction (Figure 9).

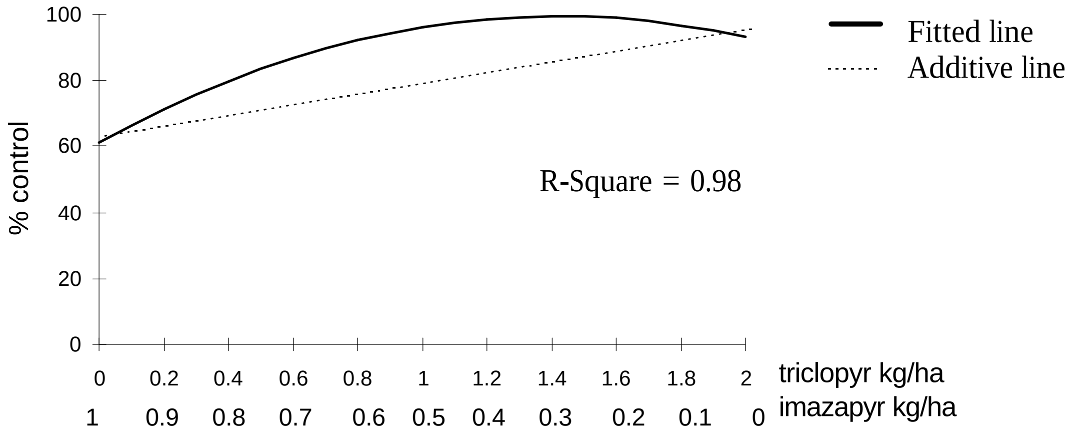


Figure 9: Imazapyr plus triclopyr nonlinear blending on black cherry using percent mortality data. Fitted model: E(Y) = 61.1I*** + 46.6T*** + 38.1IT*, where Y = percent control of black cherry, I = imazapyr kg/ha, T = triclopyr ester kg/ha. * significant to p <0.05. *** significant to p < 0.0005.

B.) Winged Elm

The nonlinear blending method indicates that imazapyr plus triclopyr ester mixtures resulted in an additive response on winged elm (Figure 10). The imazapyr*triclopyr term is not siginificant, and the imazapyr plus triclopyr ester mixture appears to have a linear response (Figure 10), which implies that the mixture had an additive effect on winged elm.

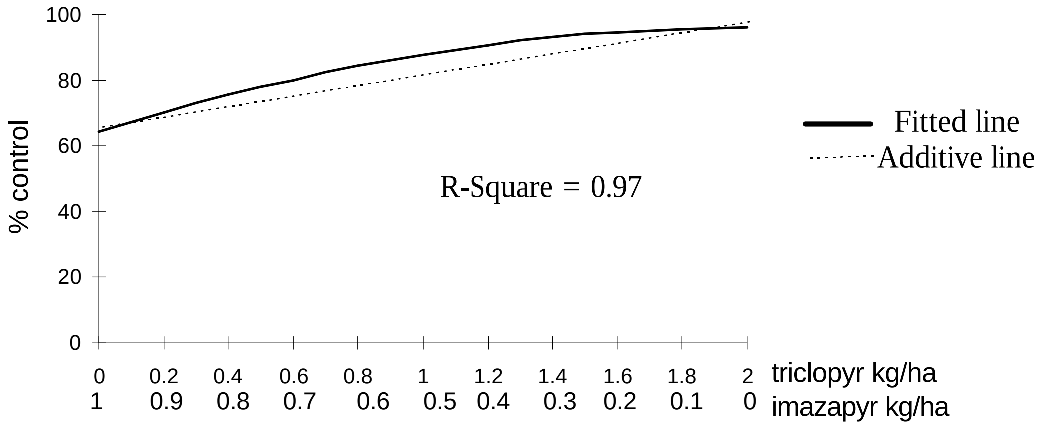


Figure 10: Imazapyr plus triclopyr nonlinear blending on winged elm using percent mortality data. Fitted model: E(Y) = 64.2I*** + 48.1T*** + 15.2IT, where Y = percent control of winged elm, I = imazapyr kg/ha, T = triclopyr ester kg/ha. *** significant to p < 0.0005.

C.) Water Oak

The results of the nonlinear blending method for water oak response to imazapyr plus triclopyr mixtures is additive. The nonlinear blending method indicates that imazapyr plus triclopyr has no significant synergistic or antagonistic effect on water oak (Figure 11).

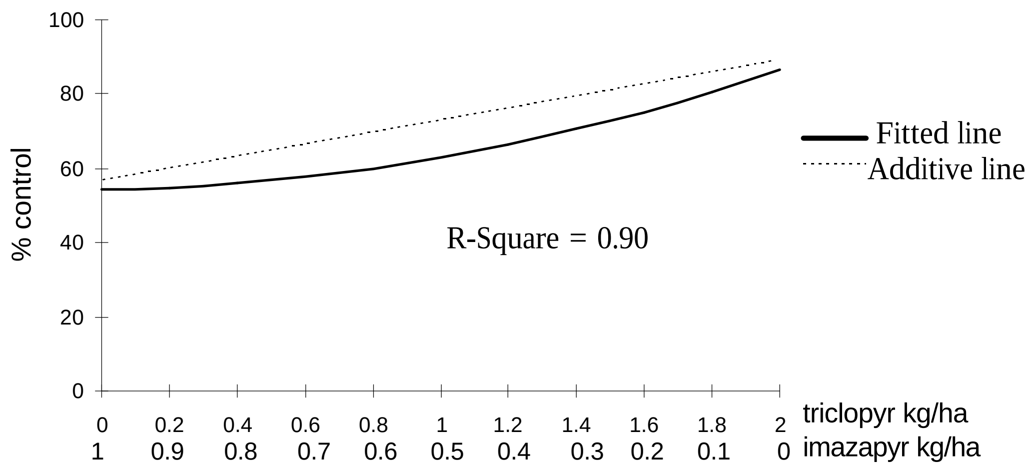


Figure 11: Imazapyr plus triclopyr nonlinear blending on water oak using percent mortality data. Fitted model: $E(Y) = 54.5I^{**} + 43.2T^{***} - 15.2IT$, where Y = percent control of water oak, I = imazapyr kg/ha, T = triclopyr ester kg/ha. ** significant to $p < 0.005$, *** significant to $p < 0.0005$.

D.) Cabbage Palmetto

The cabbage palmetto response to imazapyr plus triclopyr ester mixtures is additive. The nonlinear blending method indicates that imazapyr plus triclopyr has no significant synergistic or antagonistic effect on cabbage palmetto (Figure 12).

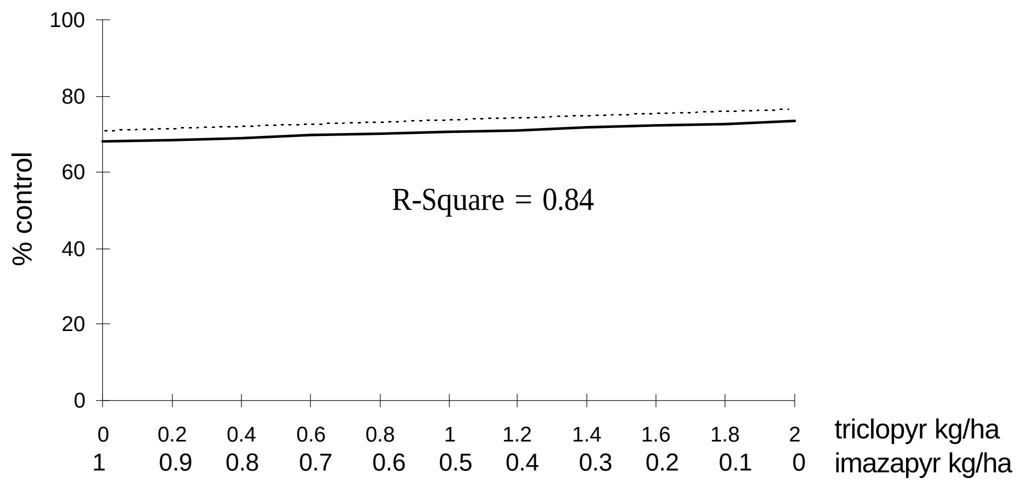


Figure 12: Imazapyr plus triclopyr nonlinear blending on cabbage palmetto using percent mortality data. Fitted model: E(Y) = 68.0I** + 36.7T** + 0.0IT, where Y = percent control of cabbage palmetto, I = imazapyr kg/ha, T = triclopyr ester kg/ha. ** significant to $p < 0.005$.

Imazapyr plus Glyphosate

A.) Black Cherry

The nonlinear blending method indicates that imazapyr plus glyphosate has an additive effect on black cherry. The imazapyr*glyphosate term is not significant (Figure 13), which indicates that imazapyr and glyphosate may not interact when applied to black cherry. Figure 13 does not deviate much from a linear response, indicating that imazapyr plus glyphosate does not have a significant interactive effect on black cherry.

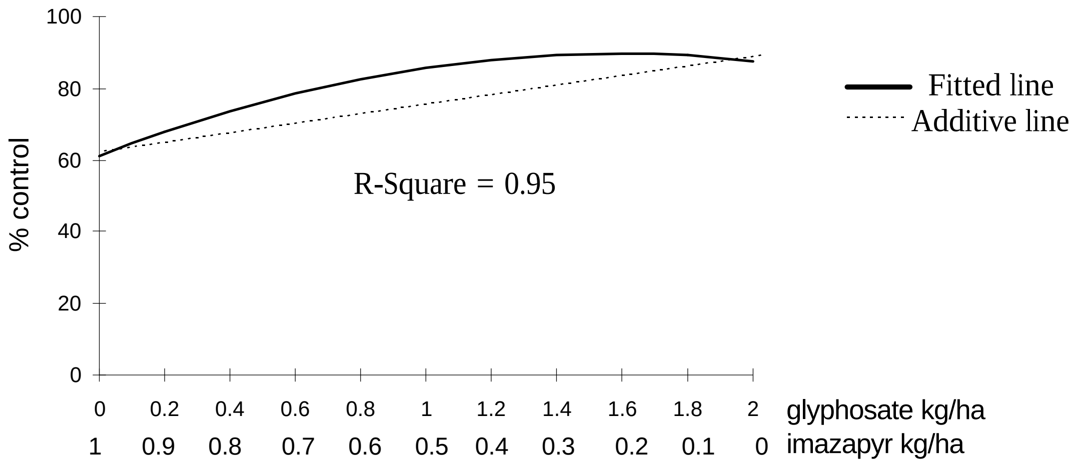


Figure 13: Imazapyr plus glyphosate nonlinear blending on black cherry using percent mortality data. Fitted model: E(Y) = 60.9I*** + 43.8G*** + 22.9IG, where Y = percent control of black cherry, I = imazapyr kg/ha, G = glyphosate kg/ha. *** significant to $p < 0.0005$.

B.) Winged Elm

The nonlinear blending method indicates that imazapyr plus glyphosate has as additive effect on winged elm. The imazapyr*glyphosate term is not significant (Figure 14), which shows that there is a linear trend in winged elm response to imazapyr plus glyphosate.

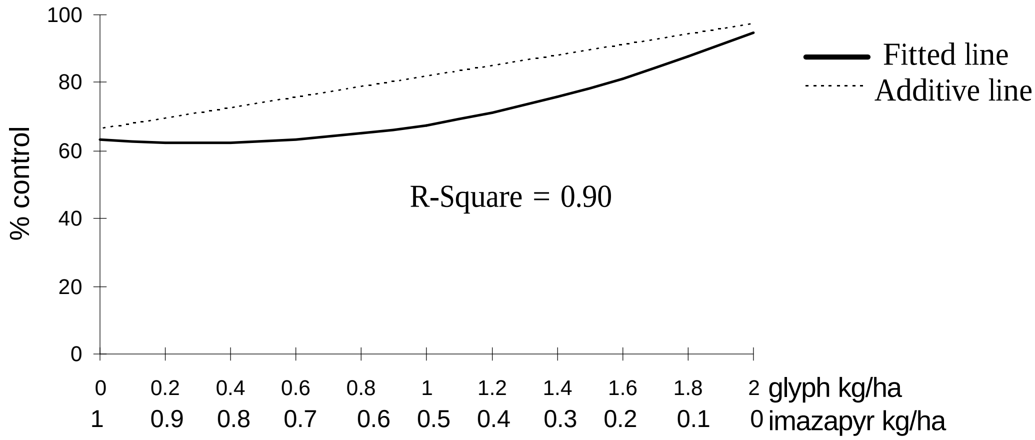


Figure 14: Imazapyr plus glyphosate nonlinear blending on winged elm using percent mortality data. Fitted model: E(Y) = 63.0I** + 47.5G*** - 22.9IG, where Y = percent control of winged elm, I = imazapyr kg/ha, G = glyphosate kg/ha. ** significant to p < 0.005, *** significant to p < 0.0005.

C.) Water Oak

According to the nonlinear blending method, imazapyr plus glyphosate mixtures have an additive effect on water oak. The imazapyr*triclopyr term is not significant (Figure 15), which indicates that water oak percent mortality does not deviate much from a linear trend. Figure 15 appears to exhibit antagonism, but the imazapyr*triclopyr term is not significant.

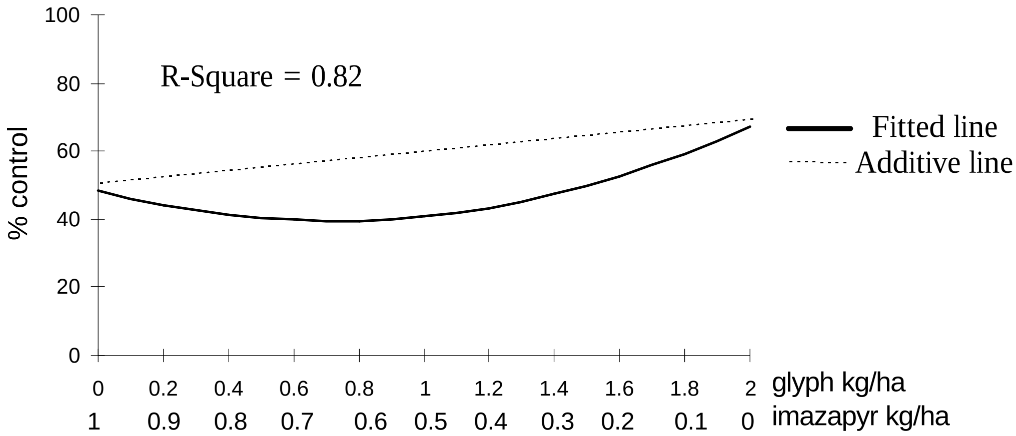


Figure 15: Imazapyr plus glyphosate nonlinear blending on water oak using percent mortality data. Fitted model: E(Y) = 48.6I** + 33.6G*** - 34.3IG, where Y = percent control of water oak, I = imazapyr kg/ha, G = glyphosate kg/ha. ** significant to $p < 0.005$, *** significant to $p < 0.0005$.

D.) Cabbage Palmetto

Cabbage palmetto results in an additive response to imazapyr plus glyphosate as well. The imazapyr*glyphosate term is significant to p = 0.11 (Figure 16), which is not a large enough deviation from a linear trend to meet a synergistic interaction, even though the fitted line deviates from the additive line.

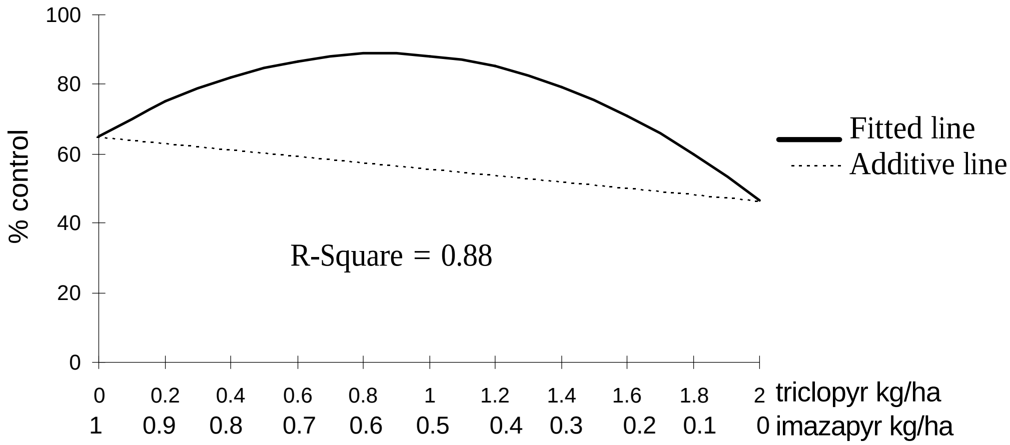


Figure 16: Imazapyr plus glyphosate nonlinear blending on cabbage palmeto using percent mortality data. Fitted model: E(Y) = 65.1I** + 23.2G* + 64.8IG, where Y = percent control of cabbage palmetto, I = imazapyr kg/ha, G = glyphosate kg/ha. * significant to $p < 0.05$, ** significant to $p < 0.005$.

Isobologram Method

<u>Imazapyr plus Triclopyr ester</u>

A.) Black Cherry

The final model for determining ED50 values of imazapyr plus tricopyr ester mixtures using black cherry mortality data was:

$$E(Y) = 22.86 + 50.57I + 96.16T - 33.76IT - 28.56T^2$$

where:

Y = percent control of black cherry

I = imazapyr rate (kg/ha)

T = tricolyr ester rate (kg/ha)

The fitted model explains 68% of the variation in black cherry mortality with the imazapyr plus triclopyr ester mixtures (Figure 17). The isobologram method indicates that additivity is occuring with black cherry. The ED50 isobologram (Figure 17) shows a linear trend at biologically equivalent rates of triclopyr ester and imazapyr.

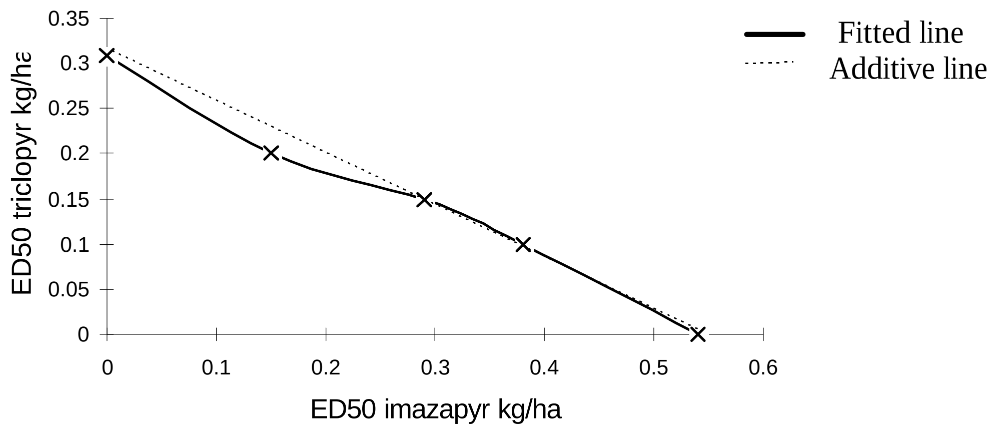


Figure 17: Fitted ED50 isobologram for imazapyr plus triclopyr ester on black cherry using percent mortality data. Fitted model: $E(Y) = 22.86 + 50.57I + 96.16T - 33.76IT - 28.56T^2$, where Y = percent mortality of black cherry, I = imazapyr rate kg/ha, T = tricolyr kg/ha. R-Square = 0.6767

B.) Winged Elm

The final model for determining the ED50 rates for the isobologram method for imazapyr plus triclopyr ester mixtures using winged elm mortality is:

$E(Y) = 9.57 + 45.6I + 89.56T - 23.36IT - 23.06T^2$

where:

Y = percent control of winged elm

I = imazapyr rate (kg/ha)

T = triclopyr ester rate (kg/ha)

The fitted model explains 72% of the variation in winged elm mortality from imazapyr plus triclopyr ester mixtures (Figure 18). The isobologram method shows an additive effect of imazapyr plus triclopyr ester on winged elm (Figure 18). At biologically equivalent rates, imazapyr and triclopyr ester remain additive.

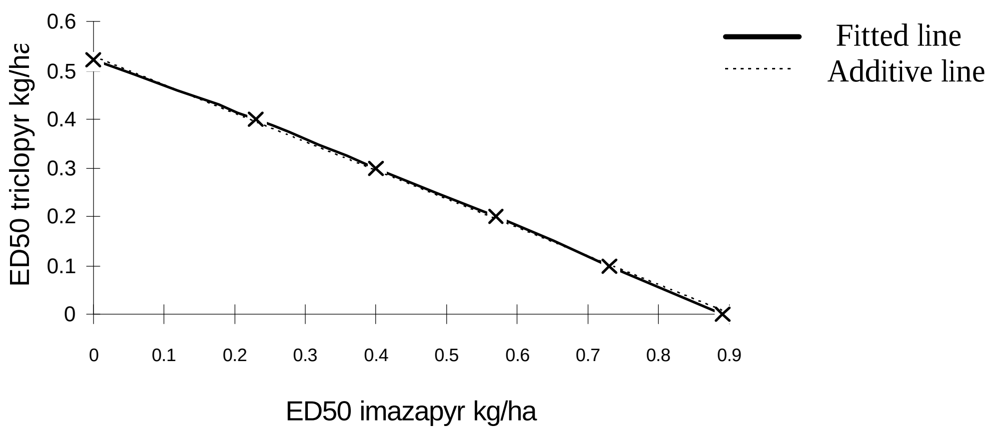


Figure 18: Fitted ED50 isobologram for imazapyr plus triclopyr ester on winged elm using percent mortality data. Fitted model: $Y = 9.57 + 45.6I + 89.56T - 23.36IT - 23.06T^2$, where Y = percent control of winged elm, I = imazapyr rate kg/ha, T = triclopyr rate kg/ha. R-Square = 0.7216

<u>Imazapyr plus Glyphosate</u>

A.) Black Cherry

The final model used to determine ED50 values for imazapyr plus glyphosate mixtures using black cherry mortality is:

E(Y) = 8.74 + 67.13I + 43.74G - 30.61IG

where:

Y = percent control of black cherry

I = imazapyr rate (kg/ha)

G = glyphosate rate (kg/ha)

The fitted model explains 67% of the variation in black cherry mortality from imazapyr plus glyphosate mixtures (Figure 19). The isobologram method shows a slight antagonistic trend with biologically equivalent rates of imazapyr and glyphosate (Figure 19), but the deviation does not appear large enough to imply antagonism. The isobologram method indicates additivity

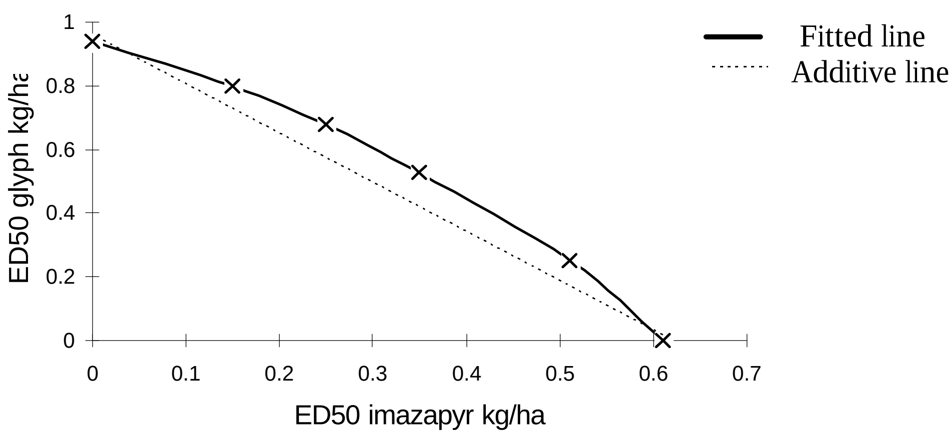


Figure 19: Fitted ED50 isobologram for imazapyr plus glyphosate on black cherry using percent mortality data. Fitted model: Y = 8.74 + 67.13I + 43.74G - 30.61IG, where Y =

percent control of black cherry, I = imazapyr kg/ha, G = glyphosate kg/ha. R-Square = 0.6733

B.) Winged Elm

The final model for determining ED50 values for imazapyr plus glyphosate mixtures using winged elm mortality is:

$$E(Y) = 4.38 + 33I + 90.24G - 20.28IG - 21.18G^2$$

where:

Y = percent control of winged elm

I = imazapyr rate (kg/ha)

G = glyphosate rate (kg/ha)

The fitted model explains 69% of the variation in winged elm mortality from imazapyr plus glyphosate mixtures (Figure 20). The isobologram method shows an additive effect of imazapyr plus glyphosate on winged elm (Figure 20). At biologically equivalent rates, there does not appear to be a significant deviation from an additive isobole when imazapyr plus glyphosate was applied to winged elm.

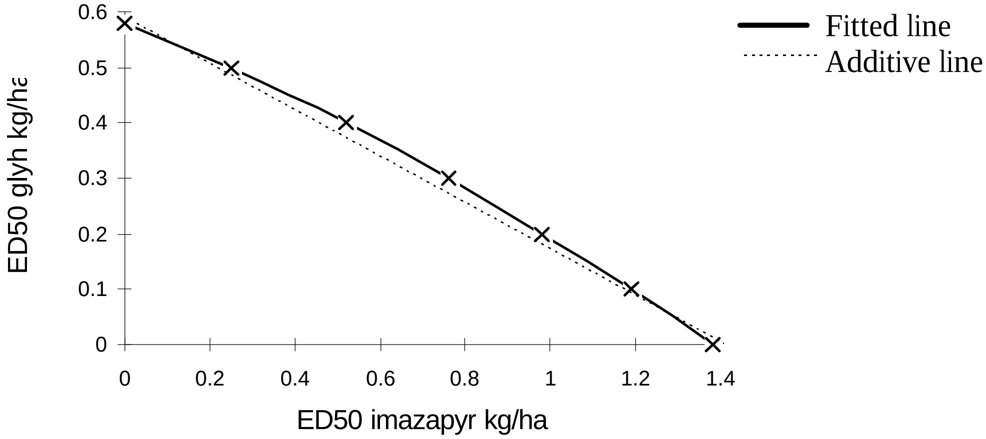


Figure 20: Fitted ED50 isobologram for imazapyr plus glyphosate on winged elm using percent mortality data. Fitted model: E(Y) = 4.38 + 33I + 90.24G - 20.28IG - 21.18G^2 where Y = percent control of winged elm, I = imazapyr kg/ha, G = glyphosate kg/ha.

The Colby Method

<u>Imazapyr plus Triclopyr ester</u>

The Colby method shows an antagonistic loblolly pine response in imazapyr plus triclopyr ester. At higher rates of triclopyr ester, adding 0.25-1 kg/ha of imazapyr resulted in an antagonistic response to loblolly pine mortality (Table 9). For example, at a mixture of 1.5 kg/ha of triclopyr ester and 0.025 kg/ha of imazapyr, 33% less control was observed than we would expect at these rates (Table 9). The observed mortality was lower than the expected mortality, which supports a possible antagonistic response.

Table 9: Colby values for imazapyr plus triclopyr ester on loblolly pine.

		Triclopyr rates (kg/ha)				
		0.05	0.5	1	1.5	2
Imazapyr	0.025	0	0	6	-33	7
rates	0.25	0	0	0	-43	-26
(kg/ha)	0.5	0	0	0	-46	-33
	0.75	0	0	0	-40	-13
	1	0	0	0	-33	-33

<u>Imazapyr plus Glyphosate</u>

Colby method indicates an antagonistic interaction when imazapyr plus glyphosate was appied to loblolly pine (Table 10). In the middle rates of glyphosate, the Colby values indicate that there is an antagonistic interaction when imazapyr is added to glyphosate.

Table 10: Colby values for imazapyr plus glyphosate on loblolly pine.

		Glyphosate rate (kg/ha)				
		0.05	0.5	1	1.5	2
Imazapyr	0.025	0	-34	0	0	0
rate	0.25	0	-27	0	-7	0
(kg/ha)	0.5	0	-14	-7	0	0
	0.75	0	-7	-20	0	0
	1	0	-27	-13	-7	0

Summary

According to the methods used, the rapid screening data resulted in one possible antagonistic response with response surface analysis, one possible synergistic response using the nonlinear blending method, and two possible antagonistic responses using the Colby method (Table 11). The remaining responses were additive. Imazapyr plus triclopyr ester appeared to exhibit synergism when applied to black cherry using the nonlinear blending method (Table 11), with antagonism at very high rates of both herbicides, as was indicated by the response surface method (Table 11). Imazapyr plus triclopyr ester and imazapyr plus glyphosate both appeared to exhibit antagonism when applied to loblolly pine using the Colby method (Table 11).

Table 11: Summary of interactions for imazapyr plus triclopyr ester and imazapyr plus glyphosate mixtures using four different methods.

Species	Response surface	Nonlinear Blending	Colby	Isobologram
<u>Imaz + Tric</u>				
Black Cherry	antagonism	synergism	---	additive
Winged Elm	additive	additive	---	additive
Loblolly Pine	additive	---	antagonism	---
Water Oak	---	additive	---	---
Cabbage Palmetto	---	additive	---	---
<u>Imaz + Glyph</u>				
Black Cherry	additive	additive	---	additive
Winged Elm	additive	additive	---	additive
Loblolly	additive	---	antagonism	---
Water Oak	---	additive	---	---
Cabbage Palmetto	---	additive	---	---

Discussion

Imazapyr plus Triclopyr ester

Knowe et al. (1995) suggest that imazapyr plus triclopyr ester mixtures are generally additive, with a slightly negative effect at higher rates of both herbicides. Our findings agree with the Knowe et al. (1995) findings. The black cherry response surface showed slight depressed activity at high rates of imazapyr and triclopyr ester, indicating antagonism. The Colby method suggests that antagonism occurs when imazapyr is added to high rates of triclopyr ester and applied to loblolly pine, but the response surface analysis of imazapyr plus triclopyr ester shows additivity on loblolly pine. Because there is little supportive evidence of the imazapyr plus triclopyr ester antagonism on loblolly pine, the Colby method appears to be creating an antagonistic interaction where one may not exist, as Rummens (1975) suggested may happen.

Lawrie and Clay (1993) found that mixing imazapyr plus triclopyr ester has an antagonistic effect on *Rhododendron ponticum*, but it appeared to have a synergistic effect at some doses. The imazapyr plus triclopyr ester mixtures appear to exhibit general synergism on black cherry, but response surface analysis found antagonism occuring at high rates of imazapyr plus triclopyr ester. This indicates that imazapyr plus triclopyr ester mixtures can provide good control of imazapyr tolerant woody weeds, but the highest rates of triclopyr ester with the highest rates of imazapyr should not be used.

Triclopyr ester had a large effect when added to imazapyr, especially on species that are relatively tolerant to imazapyr applications. These findings do not agree with the Ezell et al. (1995) who found that there is no significant effect of adding triclopyr ester to imazapyr. The difference in findings may be due to the fact that the Ezell et al. (1995) study was based on field data. The effect of environmental conditions and the effectiveness of foliar imazapyr applications in the field study may have masked the effect of adding triclopyr ester; as happened in the Moore and Banks (1991) paraquat study, where effective field applications of paraquat masked any antagonism with naptalam and bentazon.

Imazapyr plus Glyphosate

Knowe et al. (1995) and Lawrie and Clay (1993) found that imazapyr plus glyphosate mixtures exhibited an additive effect on a number of different woody species. These findings generally agree with the results found here. Imazapyr plus glyphosate exhibited an additive effect with black cherry, winged elm, water oak and cabbage palmetto using response surface analysis and nonlinear blending. The Colby method found antagonism at certain rates when imazapyr plus glyphosate was applied to loblolly pine, but the response surface analysis did not find a significant antagonistic interaction when imazapyr plus glyphosate was applied to loblolly pine. Again, there is no supportive evidence that the Colby method is showing a true antagonistic interaction. The Colby method may be creating or overvaluing antagonism as suggested by Rummens (1975).

Ramsey et al (1992) reported that adding glyphosate while lowering the imazapyr rate was effective at controlling imazapyr tolerant species such as black cherry. The imazapyr plus glyphosate nonlinear blending and response surface analysis show this to be true. Because glyphosate is more effective on black cherry, efficacy is increased with higher rates of glyphosate. Synergism is not occurring, but the additive effect of increased amounts of glyphosate provides better control than imazapyr alone.

Imazapyr plus triclopyr ester and imazapyr plus glyphosate mixtures did not show a consistent trend of synergism or antagonism. When applied to southeastern woody weeds that are hard to control, only black cherry exhibited a trend of synergism with imazapyr plus triclopyr ester mixtures with antagonism at high rates of both herbicides. Imazapyr plus triclopyr ester and imazapyr plus glyphosate both exhibited antagonism on loblolly pine, but the evidence is not conclusive. The remaining species and mixtures that were tested did not conclusively show synergism or antagonism.

Synergism and Antagonism Determination Methods

The response surface analysis method seems useful at determining synergism and antagonism. A quadratic response surface is not capable of showing small trends in the data, so only the large trends are recognized. This is useful when the purpose of the experiment is to document a distinct trend in a region of interest, such as the depression in activity on black cherry at high rates of imazapyr and triclopyr ester. Response surface analysis is more expensive in that it should include five or more rates from each herbicide, which limits the use of response surface development to greenhouse studies or extensive field studies where 25 or more treatments can be replicated and analyzed.

Nonlinear blending was a very useful method at determining a general trend of synergism or antagonism. With as few as five replicated treatments, an herbicide mixture can be shown to deviate from an assumed additive line. The nonlinear blending method has not been used in past agricultural research to determine synergism and antagonism. Regression analysis methods have been described (Green, Streibig, and Jensen, 1997; Hatzios and Penner, 1985), but the use of a specific and simplistic regression technique such as the nonlinear blending method needs to be explored by more researchers who suspect synergism or antagonism among agricultural and forest herbicide mixtures. The nonlinear blending method could be utilized in field and greenhouse studies.

The isobologram method was useful as a descriptive device, even though it is limited as a statistical determination of synergism or antagonism. If statistical significance can be determined using regression techniques, then the isobologram method offers support in showing a trend of synergism or antagonism. The isobologram method did not support the imazapyr plus triclopyr ester general synergism found with nonlinear bending and antagonism at high rates found with response surface analysis. Using the isobologram method, imazapyr plus triclopyr ester was found to be additive at biologically equivalent rates. The nonlinear blending method used the actual percent control values to document changes in the rate of each herbicide in the mixture, where the isobologram method used predicted ED50 values to document changes in the rate of each herbicide in the mixture.

The isobologram method could have been more useful if it was built with more relevant biologically equivalent rates, such as ED80 or ED90 values. If the isolologram were built with ED80 or ED90 values, the antagonism at high rates of imazapyr plus triclopyr ester on black cherry may have been indicated by the isobologram as well as the response surface. When nonlinear blending was used, the percent control values appear to be a much stronger measure of interaction response in the herbicide mixture, and statistical significance can be tested as well. The isobologram method is descriptive, but the interaction response was not recognized from the predicted ED50 values.

The Colby method appeared to exhibit antagonism in loblolly where the response surface analysis did not support the finding. Past research has found that the Colby method overvalues antagonism and undervalues synergism (Rummens, 1975). The imazapyr plus triclopyr and imazpayr plus glyphosate mixtures show a deviation from the expected response using the Colby method given the efficacy of triclopyr and glyphosate, but the deviation was not large enough to show significant depressed activity in the response surface analysis. Our findings support Rummens (1975) idea of overvalued antagonism, but more importantly, our findings indicate the statistical weakness of the Colby method. Much of the synergism and antagonism registered by the Colby method could be random variation in the data, so it is difficult to decide how large a difference between the expected and actual value indicates a significant interaction. The Colby method does not stand alone as a satisfactory method of determining herbicide interaction. The Colby method, as with any other reference model, needs to be used only as a descriptive device (Morse, 1978).

Applications

Research to determine synergism or antagonism with woody plant herbicides can be used in a number of useful ways. Herbicide manufacturers must continually develop better herbicide formulations to improve the effectiveness of chemical silviculture prescriptions.

Herbicide formulations can be changed to utilize the knowledge of synergistic or antagonistic interactions. By isolating the cause of an interaction, an herbicide can be formulated to either enhance or remove the synergistic or antagonistic interactions that occur with common mixing partners.

When enough knowledge about the interactions that occur when woody plant herbicides are mixed is available, guidelines can be established about how forestry herbicides should be combined. If an herbicide mixture is found to exhibit antagonism at higher rates of both herbicides (which was found in imazapyr plus triclopyr ester mixtures on black cherry and loblolly), a mixture ratio should be established to avoid the rates that can create ineffective herbicide applications.

Herbicide prescriptions can be improved when sufficient information is available on the interactive effect of herbicide mixtures. Because woody weed communities vary through out the southeastern U.S., an herbicide prescription should be written to maximize the efficacy of the application on the most common woody weeds on a particular site. Decision support systems, such as ChESS (Zedaker, 1993), can be improved to account for the effect of variable mixture rates on the woody weed community. Decision support systems can be programmed to select the best herbicides to use for a specific site, based on the individual responses surfaces of each woody weed to the available herbicide mixtures. Factors such as percent control, cost, as well as the amount of herbicide used, can be used as criteria to select the best possible combination of woody plant herbicides.

Rapid primary screening has provided useful information on synergism and antagonism from variable herbicide mixtures. By fitting response surfaces using the rapid primary screening data, descriptive information about the trends that occur in forestry herbicide mixtures was obtained. Response surface analysis can also be used as a predictive tool for forestry herbicide applications in the field. The next step to developing useful predictive response surfaces is to conduct field screenings to determine the actual rates needed to control different woody species. Field screenings can also validate the response surface trends from the rapid primary screening. Once adequate information is known about the relationship between rapid primary screening studies and field studies, larger assumptions about community control can be made from the rapid primary screening data.

Literature Cited

Akobundu, I.O., Sweet, R.D., Duke, W.B., 1975, A Method of Evaluating Herbicide Combinations and Determining Herbicide Synergism, *Weed Science*, v. 23(1): 20-25.

Anderson, W.P, 1996, Chapter 8 in *Weed Science, Principles and Applications*, 3rd edition, West Publishing Company, St. Paul, MN

Barret, M., Witt, W.W., 1987, Maximizing Pesticide Use Efficiency, from *Energy In World Agriculture*, edited by Helsel, Z.R., Elsevier publishing, v. 2: 250.

Bovey, R.W., Whisenant, S.G., 1992, Honey Mesquite(*Prosopis glandulosa*) Control by Synergistic Action of Clopyralid: Triclopyr Mixtures, *Weed Science*, v. 40(4): 563-567.

Box, G.E.P. and Draper, N.R., 1987, *Empirical Model Building and Response Surface Analysis*, Wiley Publishing, New York, N.Y.

Bunn, B.H., Zedaker, S.M., Kreh, R.E., Seiler, J.R., 1996, Evaluation of Herbicide Rapid Screening at Four Developmental Stages in Woody Plants, Abstract from 1996 *Proceedings, 49th Southern Weed Science Society* SWSPBE 49: 84.

Burkhalter, A.P., Moore, J.W., Voth, R.D., 1990, Accord and Accord plus Arsenal for Site Preparation, Abstract from *Proceedings, 43rd Southern Weed Science Society* SWSPBE 43: 287.

Burkhalter, A.P., 1992, Update On Glyphosate Tank Mixes for Forestry Site Preparation, Abstract from *1992 Proceedings, 45th Southern Weed Science Society* SWSPBE 45: 259.

Colby, S.R., 1967, Calculating Synergistic and Antagonistic Responses of Herbicide Combinations, *Weeds*, v.15: 20-22.

Deschamps, R.J.A., Hsiao, A.I., Quick, W.A., 1990, Antagonistic Effect of MCPA on Fenoxaprop Activity, *Weed Science*, v. 38: 62-66.

Ezell, A.W., Vollmer, J., Minogue, P.J., Zutter, B., 1995, A Comparison Of Herbicide Tank Mixtures for Site Preparation Evaluation Of Treatment Efficiency and Possible Antagonism, Paper from *Proceedings, 48th Southern Weed Science Society* SWSPBE 48: 142-148.

Ezell, A.W., Nelson, L.R., Vollmer, J., Minogue, P.J., Catchot, A.L., 1996 A Comparison of Basal Applications of Imazapyr and Triclopyr Tank Mixtures For Hardwood Control, Paper from *Proceedings, 49th Southern Weed Science Society* SWSPBE 49: 90-96.

Fletcher, J.S., Johnson, F.L., McFarlane, J.C., 1990, Influence of Greenhouse Versus Field Testing and Taxonomic Differences on Plant Sensitivity to Chemical Treatment, *Environmental Toxicology and Chemistry*, v. 9: 769-776.

Green, J.M., Bailey, S.P., 1987, Herbicide Interactions with Herbicides and Other Agricultural Chemicals, a chapter from *Methods of Applying Herbicides*, edited by

C.G. McWhorter and M.R. Gebhardt, Weed Science Society of America Monograph 4: 37-61.

Green, J.M., Jensen, J.E., Streibig, J.C., 1997, *Defining and Characterizing Synergism and Antagonism for Xenobiotic Mixtures*, from *Regulation of Enzymatic Systems Detoxifying Xenobiotics in Plants*, edited by K.K. Hatzios, Kluwer Academic Publishers, Dordrecht, Netherlands.

Green, J.M., Streibig, J.C., 1993, Herbicide Mixtures, Chapter 7 in *Herbicide Bioassay*, edited by J.C. Streibig and P. Kudsk, P., CRC Press, Inc.: 117-135.

Grichar, W.J., 1991, Sethoxydim and Broadleaf herbicide interactions effects on annual grass control in peanuts (*Arachis hypogyaea*), *Weed Technology* v. 5: 321-324.

Hatzios, K.K., Penner, D., 1985, Interactions of Herbicides with Other Agrochemicals in Higher Plants, *Reviews of Weed Science*, WSSA v. 1: 1-63.

Knowe, S.A., Cole, E.C., Newton, M., 1995, Response Surface Analysis of Control of Red Alder and Vine Maple with Glyphosate-Imazapyr and Triclopyr-Imazapyr, *Western Journal of Applied Forestry*, v. 10(4): 127-131.

Liu, H.S., Hsiao, A.I., Quick, W.A., 1994, Interaction between imazamethabenz and fenoxaprop in wild oat control and crop tolerance, *Crop Protection*, vol. 13(7): 525-530.

Lawrie, J., Clay, V., 1993, Effects of Herbicide Mixtures and Additives on *Rhododendron ponticum*, *Weed Research*, v. 33: 25-34.

Miller, J.H., Mitchell, R.J., 1990, *A Manual on Ground Applications of Forestry Herbicides*, USDA Management Bulletin R8-MB 21, Atlanta, GA

Moore, J.D., Banks, P.A., 1991, Interactions of Foliarly Applied Herbicides on Three Weed Species in Peanut (*Arachis hypogoea*), *Weed Science*, v. 39(4): 614-621.

Morse, P.M., 1978, Some Comments on the Assessment of Joint Action in Herbicide Mixtures, *Weed Science*, v. 26(1): 58-71.

Myers, R.H., Montgomery, D.C., 1995, *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*, John Wiley and Sons Inc., New York, N.Y.: 542-544.

N.A.C.A., 1993, *from Lab to Label: The Research, Testing and Registration of Agricultural Chemicals*, Washington, DC: 2.

O'Sullivan, P.A., Kossatz, V.C., 1982, Influence of picloram on *Cirsium arvense* (L.) Scop. control with glyphosate, *Weed Research* v. 22: 251-256.

Poch, G., Reiffenstein, R.J., Unkelbach, H. D., 1990, Application of the isobologram technique for the analysis of combined effects with respect to additivity as well as independence, *Canadian Journal of Physiology and Pharmacology*, v. 68: 682-688.

Ramsey, C.L., Glover, G.R., Lauer, D.K., 1992, Screening of Glyphosate and Imazapyr Tank Mix Rates for Loblolly Pine Release, Abstract from *Proceedings, 45th Southern Weed Science Society* SWSPBE 45: 231.

Riley, D.G., Shaw, D.R., 1988, Influence of Imazapyr on the Control of Pitted Morningglory(*Ipomoea Lacunosa*) and Johnsongrass(*Sorghum halepense*) with Chlorimuron, Imazaquin, and Imazethapyr, *Weed Science*, v. 36(5): 663-666.

Rummens, F.H.A., 1975, An Improved Definition of Synergistic and Antagonistic Effects, *Weed Science*, v. 23(1): 4-6.

Shaw, D.R., Wixson, M.B., 1991, Postemergence Combinations of Imazaquin or Imazethapyr with AC 263,222 for Weed Control in Soybean, *Weed Science*, v. 39(4): 644-649.

Shiver, B.D., Knowe, S.A., Edwards, M.B., Kline, W.N., 1991, Comparison of Herbicide Treatments for Controlling Common Coastal Plain Flatwoods Species, *Southern Journal of Applied Forestry*, v. 15: 187-193.

Streibig, J.C., Rudemo, M., Jensen, J.E., 1993, Dose-response Curves and Statistical Models, Chapter in *Herbicide Bioassay*, edited by Streibig, J.C. and Kudsk, P., CRC Press, Inc.: 29-55.

Tammes, P.M.L., 1964, Isoboles, a graphic representation of synergism in pesticides, *Netherlands Journal of Plant Pathology*, v. 70: 73-80.

Williams, R.A., Yeiser, J.L., Earl, J.A., 1996, Efficacy of Reduced Rates of Basal Applied Herbicides for Woody Plant Control of Selected Hardwoods, Paper from *Proceedings,49th Southern Weed Science Society* SWSPBE 49: 97-100.

Zedaker, S.M., 1993, *Chemical Expert System for Silviculture Users Manual version 2.0*, Virginia Tech Intellectual Properties.

Zedaker, S. M., Miller, J.H., 1991, Measurement of Target and Crop Species, Chapter 4 in *Statistical Methods for Forest Herbicide Research*, edited by J.H. Miller and G. Glover, Southern Weed Science Society: 22-40.

Zedaker, S.M., Seiler, J.R., 1988. Rapid Primary Screenings For Forestry Herbicides, Paper from *5th biennial Southern Silvicultural Research Conference*: 349-352.

Vita

Mathew C. Nespeca graduated high school in Potomac, Maryland in 1989. After high school, Mathew spent four years at Auburn University and received a Bachelor's of Science in Forest Resource Management in 1993. Mathew worked for two years in the lumber industry as a quality supervisor for the Southern Pine Inspection Bureau. He worked in numerous lumber mills and secondary manufacturing facilities all over the Eastern U.S. In 1995, Mathew decided to return to school to pursue a graduate education at Virginia Tech. Following two years of study and research, Mathew received a Master's of Science in Forestry, with a concentrated interest in forest herbicide use and chemical silviculture. Mathew is currently working for American Cyanamid as a Sales Associate in Parsippany, New Jersey.