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Inheritance and Physiological Basis for 2,4-D Resistance in Prickly Lettuce (*Lactuca serriola* L.)

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ABSTRACT: Experiments were conducted to determine the inheritance and physiological basis for resistance to the synthetic auxinic herbicide (2,4-dichlorophenoxy)acetic acid (2,4-D) in a prickly lettuce biotype. Inheritance of 2,4-D resistance in prickly lettuce is governed by a single codominant gene. Absorption and translocation were conducted using ^{14}C-2,4-D applied to 2, 4-D-resistant and -susceptible biotypes. At 96 h after treatment (HAT), the resistant biotype absorbed less applied 2,4-D and retained more 2,4-D in the treated portion of the leaf compared to the susceptible biotype. The resistant biotype translocated less applied 2,4-D to leaves above the treated leaf and crown at 96 HAT compared to the susceptible biotype. No difference in the rate of metabolism of 2,4-D was observed between the two biotypes. Resistance to 2,4-D appears to originate from a reduced growth deregulatory and overstimulation response compared to the susceptible biotype, resulting in lower translocation of 2,4-D in the resistant prickly lettuce biotype.

KEYWORDS: (2,4-dichlorophenoxy)acetic acid, 2,4-D, absorption, auxinic herbicides, herbicide resistance, inheritance, *Lactuca serriola* L., metabolism, translocation

INTRODUCTION

Prickly lettuce is a well-adapted facultative winter or spring annual weed of the Pacific Northwest (PNW) region of the United States. It is a common and troublesome weed in cereal crops, conservation reserve program land, and range and noncropland areas.[1] Auxinic herbicides (phenoxycarboxylic acid-, benzoic acid-, pyridinecarboxylic acid-, and quinolinecarboxylic acid-type herbicides) have historically been effective and widely used for control of prickly lettuce in cereal crops. In range and noncropland areas, 2,4-D has been commonly used due to its affordability.[1] Recently, a 2,4-D-resistant prickly lettuce biotype was identified in the inland PNW.[1]

Prickly lettuce has a history of herbicide resistance and was the first weed species discovered resistant to acetolactate synthase (ALS)-inhibiting herbicides.[2,3] Generally, auxinic herbicide resistant biotypes are thought to require more generations for selection relative to other herbicide modes of action, particularly ALS and acetyl-coenzyme A carboxylase (ACCase) inhibitor herbicides.[4,5] Only 28 auxinic herbicide resistant weed biotypes have been reported worldwide. Of those, 15 are resistant to 2,4-D. In comparison, other modes of action exhibit herbicide resistance more frequently. There are currently 107 ALS inhibitor and 68 photosystem II inhibitor herbicide resistant weed species.[6] Auxinic herbicides have been in use longer than most other herbicide modes of action, supporting the hypothesis that auxinic herbicides are more resilient to selection pressure. Treated species require a greater number of generations to develop resistance to this mode of action.

Affinity for the auxin receptor and F-box protein *TIR1* and subsequent signal transduction processes is thought to be the primary site of herbicide action and, as a consequence, to cause auxin overdose.[7] Indeed, in *Arabidopsis*, increasing doses of 2,4-D induced up-regulation or down-regulation of different genes in the ethylene and abscisic acid pathways, indicating that there may be several receptor sites depending on 2,4-D dose and plant species.[8] It is therefore not surprising to find inconsistencies in identifying probable causes of 2,4-D resistance when comparing various 2, 4-D-resistant species. A common inheritance model for auxin herbicide resistance has yet to be determined. Inheritance studies in auxinic resistant wild mustard have reported a single dominant gene conferring resistance to picloram and 2,4-D[9] and dicamba.[10] Dicamba resistance in kochia was also reported to be inherited by a single dominant nuclear gene.[11] A single recessive gene imparts quinclorac resistance in false cleavers (*Galium spurium* L.)[12] and clopyralid and picloram resistance in yellow starthistle.[13] Conversely, Weinberg et al.[14] reported 4-chloro-2-ethylpheonxyacetic acid (MCPA) resistance in hemp-nettle (*Galeopsis tetrahit* L.) as a quantitative trait and indicated that the resistance is governed by two or more nuclear genes with additive effects. One possible reason for such irregularity in resistance mechanisms is that in each of these instances, one or more signal receptor sites or downstream genes in the signaling pathway(s) contained mutations. Different inheritance patterns would exist depending on the gene mutation.

When target-site resistance has been implicated as the cause for auxinic herbicide resistance, both resistant and susceptible biotypes exhibit similar physiological behaviors. Several of the previously cited examples of auxinic herbicide resistance exhibit similar patterns of absorption and translocation in both resistant and susceptible biotypes. Differential absorption, translocation, or metabolism was not the basis for picloram-resistant yellow starthistle,[15] dicamba- or picloram-resistant wild mustard,[16] or quinclorac-resistant false cleavers.[17] Only in hemp-nettle were differences observed in the physiological behavior of MCPA. Resistant hemp-nettle biotypes had no difference in absorption of MCPA but, rather, a reduced rate of translocation and increased

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rate of metabolism compared to susceptible biotypes.[14] Although these studies addressed the physiological basis for auxinic herbicide resistance, each instance of resistance appears to have unique characteristics that are likely related to the complex interactions between the signal receptor sites and the herbicides.[7]

Therefore, studies were conducted to determine the inheritance and physiological basis for 2,4-D resistance in prickly lettuce. The objectives of the study were to (1) determine the inheritance of 2,4-D resistance in prickly lettuce and (2) determine if differential absorption, translocation, or metabolism of 2,4-D is occurring between the susceptible and resistant biotypes.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Resistant and susceptible prickly lettuce biotypes identified by Burke et al.[1] were used in these studies. The doses required to reduce growth 50% (GR_{50}) for resistant and susceptible field-collected prickly lettuce are 150 and 6 g ae ha^{-1} 2, 4-D, respectively, resulting in a resistant biotype that is 25 times more resistant to 2,4-D than the susceptible biotype.[1] Seeds of resistant and susceptible biotypes were used for uptake, translocation, metabolism, and inheritance studies. Plants for all experiments were grown under controlled environment glasshouse conditions of 32/25 ± 3 °C day/night temperature and 14 h photoperiod consisting of natural light supplemented with light from sodium vapor lamps. For all experiments, 10 seeds were planted in 0.5 L volume plastic pots filled to capacity with commercial potting media, LC1Mix (Sun Gro Horticultural Distribution Inc., Bellevue, WA). Following emergence, plants were thinned to one plant per pot. Pots were subirrigated as needed.

For the inheritance study, plants were grown in three sets with intervals of 2 weeks between planting of sets to synchronize flowering of resistant and susceptible biotypes to ensure successful crossing. Susceptible and resistant plants were kept in different rooms of a glasshouse before and after crossing. Plants were transplanted to 4 L pots when they reached the 5−8-leaf stage of growth.

Inheritance of 2,4-D Resistance in Prickly Lettuce. Four resistant (R13, R18, R21, and R23) and two susceptible (S11 and S33) prickly lettuce plants, all from the third generation of self-pollination, were crossed in all combinations for a total of 100 crosses. Crosses using susceptible biotypes as female (S × R; designated F_1 crosses) and resistant biotype as female [R × S; designated reciprocal F_1 (RF_1) crosses] were made for each pairing of resistant/susceptible biotypes. Crosses were made using the clip-and-wash method of emasculation.[18] Following crossing, female parent plants were covered with transparent cloth bags and individual flowers were tagged with the respective cross information. Seeds produced from F_1 and RF_1 crosses were collected and stored until needed for further studies.

Prickly lettuce is a nearly obligate self-fertilizing plant.[19] Therefore, it is crucial to determine the success of the crossing procedure. As there is no morphological marker for 2,4-D resistance in prickly lettuce, F_1 and RF_1 plants were challenged with 2,4-D to evaluate cross success. Susceptible plants were used as controls. Plants were grown in a growth chamber set to provide 16/8 h light/dark and 22/15 °C day/night temperature. All plants were sprayed with 430 g ae ha^{-1} of 2,4-D at the 5−8-leaf stage to determine 2,4-D susceptibility. The herbicide dose was based on GR_{50} values calculated by Burke et al.[1] for resistant and susceptible prickly lettuce biotypes. Plants were sprayed using an indoor spray chamber using an 8002E flat-fan nozzle and calibrated to deliver 186 L ha^{-1} at 190 kPa.

F_1 plants from the S11 × R23 cross were self-pollinated to produce F_2 seed. Ninety-six F_2 plants were grown in a growth chamber under the same conditions as F_1 plants and sprayed at the same rate of 2,4-D when they had reached the 5−8-leaf stage. Eight plants each of resistant and susceptible parents were also sprayed under the same condition as a control. The experiment was conducted twice. Visual injury, based on leaf discoloration and apparent biomass reduction, were estimated on a scale from 0 (no injury) to 100 (complete control or complete death). The visual ratings were then sorted into six categories based on the visual ratings. The frequency of F_2 plants within each class was tabulated for overall injury.

Chi square analysis was performed to find the best fit for segregation of 2,4-D resistance in prickly lettuce. Goodness-of-fit for the null hypothesis of 1:3, 1:2:1, 1:15, 9:3:3:1, and 1:64 phenotype ratios was determined by chi square (χ^2) test at $P \leq 0.05$.[20] The Yates correction factor was used if there was only one degree of freedom.

Absorption and Translocation of [^{14}C] 2,4-D: 4 Day Experiment. Nonformulated 2,4-D with ^{14}C-labeled benzene ring (specific activity of 9.25 MBq; Sigma-Aldrich Co., St. Louis, MO) was dissolved in a water/7 M dimethylamine (13:1, v/v) solution to make a stock solution with specific activity of 0.33 kBq μL^{-1}. When prickly lettuce plants reached the 5−6-leaf stage, a 2 cm portion of the second fully expanded leaf was marked halfway between the stem and leaf tip. The marked area of each plant was covered with a 2 cm wide plastic tab to intercept spray. Plants were then oversprayed with nonradiolabeled 2,4-D amine at a rate of 514 g ae ha^{-1}. Ten 0.5 μL spots of radioactive herbicide solution containing in total 3.33 kBq of [^{14}C] 2,4-D were applied evenly on the adaxial side of the middle marked portion of leaf. A 25 μL syringe (Microliter, Hamilton Co., Reno, NV) equipped with a repeating dispenser delivered 0.5 μL droplets and was used to apply the [^{14}C]-2,4-D solution. Plants were harvested 1, 4, 8, 24, and 96 HAT. Each plant was cut at the soil surface and dissected into six plant parts: the marked portion treated with radioactive 2,4-D (treated portion), the nontreated portion of the treated leaf (above and below the treated portion), the portion of the plant above the treated leaf (upper leaves), the portion of the plant below the treated leaf (lower leaves), the crown, and the roots.

The treated portion of the treated leaf was rinsed for 15 s with 1 mL of a methanol/water (1:1 v/v) solution to remove nonabsorbed 2,4-D. Rinse solution was collected in a 25 mL scintillation vial, mixed with 20 mL of scintillation fluid, and radioassayed by Tri-Carb 2900TR liquid scintillation analyzer (LSA; PerkinElmer Life and Analytical Sciences, Shelton, CT) to determine the amount of nonabsorbed ^{14}C. All plant parts were dried for 48 h at 40 °C, weighed, and oxidized using a biological sample oxidizer, OX500 (R. J. Harvey Instrument Corp., Tappan, NY). The evolved CO_2 was trapped in 15 mL of scintillation fluid and radioassayed by LSA. Treatments were replicated four times. The experiment was conducted twice.

Absorption and Translocation of [^{14}C] 2,4-D: 21 Day Experiment. As injury and regrowth are observed in the resistant biotype, absorption and translocation of 2,4-D were studied over the recovery period following 2,4-D treatment, approximately 3 weeks. The materials and methods were identical to those used for the short-term 2,4-D absorption and translocation experiment, with the exception that plant harvests were taken at longer intervals. Harvest intervals were 1, 4, 7, 14, or 21 days after treatment (DAT). Additionally, relative growth rates were calculated using whole plant dry weights according to the method o Hoffmann and Poorter.[21]

Metabolism of [^{14}C]-2,4-D. All procedures for growing, treating, and harvesting plants were similar to those of the absorption and translocation study except a greater quantity of radioactive 2,4-D (7.9 kBq) was applied and one extra harvest interval (168 HAT) was included. The treated leaf was not dissected into two parts as in the absorption and translocation study. Instead, the entire treated leaf was processed for analysis. After dissection, all plant parts were wrapped in aluminum foil and stored in −20 °C until extraction.

All plant portions were ground individually with a Polytron tissue grinder (Brinkmann Instruments, Westbury, NY) using 10 mL of methanol. The homogenate was centrifuged (Beckman Instruments Inc.) at 2100*g* for 10 min in a 15 mL VWR centrifuge tube (VWR International Inc., West Chester, PA), and the supernatant was decanted

into a new 50 mL tube (Evergreen Scientific Inc., Los Angeles, CA). The remaining pellet was rinsed and centrifuged (2100*g*) twice with 5 mL of methanol. The supernatant was decanted into the same 50 mL tube after each centrifugation step. Following the third centrifugation, the pellet was air-dried, wrapped in aluminum foil, and oxidized in a biological oxidizer as previously described to determine the efficiency of the extraction. The extraction efficiency for the individual plant portions averaged 98%. The supernatant from each plant portion was air-dried and re-eluted with 500 μL of methanol. To evaluate potential postextraction degradation, 5 μL of radiolabeled herbicide solution was added to extracts from nontreated plants and used as a control.

Normal-phase thin-layer chromatography (TLC) was used to separate [^{14}C]-2,4-D from ^{14}C-labeled metabolites. Silica gel plates (20 × 20 cm; Whatman) were used as the stationary phase, and a mixture consisting of methylene chloride, methanol, acetone, and glacial acetic acid (8:1:0.5:1, v/v/v/v) was used as the mobile phase. Plates were divided into nine 1 cm wide lanes, and a 250 μL aliquot of each sample was spotted 3 cm above the bottom edge of the plate. The center lane was loaded with standard 0.5 μL stock solution (1.69 kBq of radioactivity) containing [^{14}C]-2,4-D dissolved in a mixture of water/7 M dimethylamine (13:1, v/v). Additionally, a nonradiolabeled 2,4-D standard was also spotted to the extreme left lane to allow visual verification of [^{14}C]-2,4-D. The air-dried plates loaded with plant samples and standards were placed in developing tank containing 5 mm deep mobile phase solution. All of the plates were developed until the mobile phase had traveled 185 mm above origin. The radioactive positions, proportions, and corresponding relative mobility (R_f) of [^{14}C]-2,4-D and ^{14}C-labeled metabolites were determined by scanning TLC plates with a radiochromatogram scanner, AR-2000 (Bioscan Inc., Washington, DC). Radioactive trace peaks were integrated with Win-Scan software. Peaks below 1% of total radioactivity were rejected. The radioactive parent herbicide in each extract was identified by comparing R_f values from the corresponding radiolabeled standard and verified with the nonradiolabeled standard.

Analysis of Absorption, Translocation, and Metabolism Experiments. The absorption and translocation experiments were organized as a randomized complete block designs with split−split plot treatment arrangement. Prickly lettuce biotype was the main plot, harvest interval was the subplot, and plant part was the subsubplot. The experimental units were individual plants. Treatments were replicated four times, and both the short-term harvest and long-term harvest experiment were conducted twice. The absorption and translocation were expressed as percent of applied [^{14}C]-2,4-D. Data were tested for normality using the PROC Univariate procedure in SAS, version 9.1 (SAS Institute Inc., Cary, NC). Data were subjected to arcsine square root transformation prior to ANOVA to improve the homogeneity of variance. The sums of squares were partitioned to reflect a split−split plot treatment arrangement and trial effects using the MIXED procedure in SAS. ANOVA indicated that trial effects for absorption, total translocation out of the treated leaf, and translocation to the root were not significant for any plant species or harvest interval ($P > 0.05$); therefore, data were pooled over trials. Absorption and accumulation in the treated leaf were fit to a hyperbolic two-term model as the equation

$$y = a \times x/(b + x) \quad (1)$$

where y is the percent absorption expressed as the percent of the applied dose, a is the upper asymptote or theoretical absorption maximum, x is time (expressed as hours or days after treatment), and b is the time to reach half of the maximum absorption.

A three-term sigmoidal equation was used to relate translocation over time. The sigmoidal equation was

$$y = a/(1 + \exp(-(x - x_0)/b)) \quad (2)$$

where y is the percent translocation expressed as the percent of the applied dose, a is the asymptote or the maximum translocation expressed as the percent applied, x is time (expressed as hours after treatment), x_0 is time to 50% of a, and b is the slope of the curve at x_0.

Coefficients of determination (R^2) were calculated for all regressions. For the Gompertz and exponential maximum equations fitted to the data, an approximate R^2 value was obtained by subtracting the ratio of residual sums of squares to corrected total sums of squares from 1. The R^2 and residual mean squares were used to determine goodness of fit to nonlinear models.[20]

The metabolism experiment organization was similar to the absorption and translocation study. Treatments were also replicated four times, and the experiment was conducted twice. Statistical procedures were similar to the absorption and translocation study. Additionally, sums of squares were partitioned to test trial replication and linear, quadratic, or higher order polynomial effects of metabolism of ^{14}C-2,4-D in resistant and susceptible plants over time.[22] Data consisted of area normalized percentage of 2,4-D DMA, 2,4-D acid, or polar metabolites. Statistical procedures were similar to the absorption and translocation study, and percentage metabolism was modeled using the equation

$$C = y_0 + C_0\, e^{-kt} \quad (3)$$

where y_0 is the point where the decay rate equals 0, C_0 is the percent in the treated leaf at time zero minus y_0, k is the first-order rate constant (h^{-1}), and t is time (h). 2,4-D half-life ($t_{1/2}$) was calculated from eq 4

$$t_{1/2} = \ln 2/k \quad (4)$$

where k is the first-order rate constant calculated in eq 3.

RESULTS AND DISCUSSION

Inheritance of 2,4-D Resistance. All F_1s and RF_1s expressed 2,4-D resistance equivalent to the resistant parent based on visual injury (data not presented), and the phenotypic screening of F_1s and RF_1s confirmed the homozygosity of the parental genotypes. These results suggest that the resistant trait is dominant and nuclear-encoded, as the RF_1s (where the pollen donor is the resistant biotype) exhibited a level of resistance similar to that of the resistant biotype.

The inheritance of the 2,4-D resistance trait was also evaluated in the F_2 progeny by evaluating plants from S11 × R23 derived cross in the F_2 generation. All plants were sprayed with 430 g ae ha^{-1} of 2,4-D at the 5−8-leaf stage to determine susceptibility, a dose that discriminated the parental lines.[1] Of 192 F_2 plants, 51 (25%) plants exhibited visual injury between 0 and 30% (highly resistant), 100 (55%) plants had visual injury symptoms between 31 and 70% (intermediate resistance), and 41 (21%) plants had an injury rating above 70% (highly susceptible). The presence of a high number of resistant plants (25%) in the F_2 population does not support a polygenic inheritance of 2,4-D resistance in prickly lettuce like that observed in hemp-nettle.[14] On the basis of chi-square analyses, data fit a Mendelian segregation ratio of 1:2:1. Segregation of plants in the F_2 generation supports a monogenic, codominant, inheritance model for 2,4-D resistance.

Previous studies have described both polygenic and monogenic inheritance of auxinic herbicide resistance. Resistance to auxinic herbicides in hemp-nettle was reported to be under polygenic control.[14] Conversely, previous inheritance studies in wild mustard for 2,4-D and picloram resistance[9] and dicamba resistance[10] indicated that auxinic herbicide resistance can also be governed by a single dominant gene. Single nuclear encoded genes also control auxinic resistance to clopyralid and picloram in yellow starthistle[13] and dicamba resistance in kochia.[11]

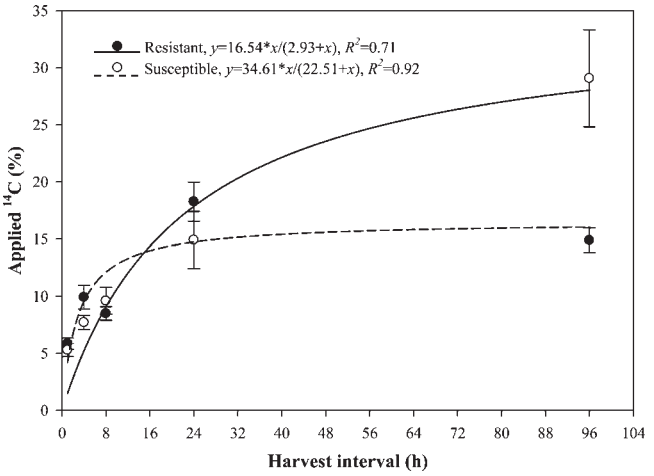

Figure 1. Absorption of foliar applied ^{14}C-2,4-D into susceptible and resistant prickly lettuce biotypes over 4 days, expressed as percent of applied. Error bars represent the standard error of the mean, where $n = 8$.

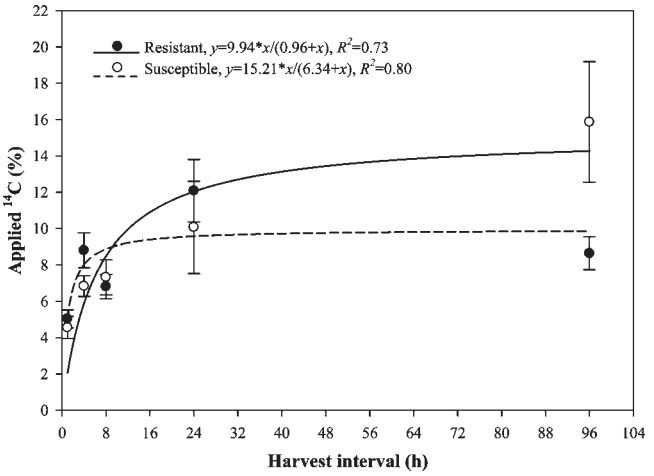

Figure 2. Accumulation of foliar applied ^{14}C-2,4-D in the treated leaf of susceptible and resistant prickly lettuce biotypes over 4 days, expressed as percent of applied. Error bars represent the standard error of the mean, where $n = 8$.

Absorption and Translocation of [^{14}C]-2,4-D: 4 Day Experiment. Recovery of ^{14}C material from both resistant and susceptible prickly lettuce biotypes averaged 90%. Absorption, expressed as percent of applied [^{14}C]-2,4-D, increased from 5.8 and 5.3% at 1 HAT to 14.9 and 29.1% at 96 HAT in resistant and susceptible prickly lettuce biotypes, respectively (Figure 1). Absorption was biphasic, with the rate of absorption greatest in early harvest intervals in each biotype.

Total radioactivity left in the treated leaf was similar in both biotypes through 24 HAT (Figure 2). Thereafter, radioactivity in the treated leaf of the resistant biotype decreased, whereas radioactivity in the susceptible biotype increased. The resistant biotype translocated less radioactivity out of the treated leaf to the leaves above the treated leaf, crown, and roots compared to the susceptible biotype (Figure 2). The susceptible biotype translocated 15.9% of the applied radioactivity at 96 HAT harvest out of the treated leaves. The resistant biotype translocated less radioactivity, 3.1% of the applied, than the susceptible biotype

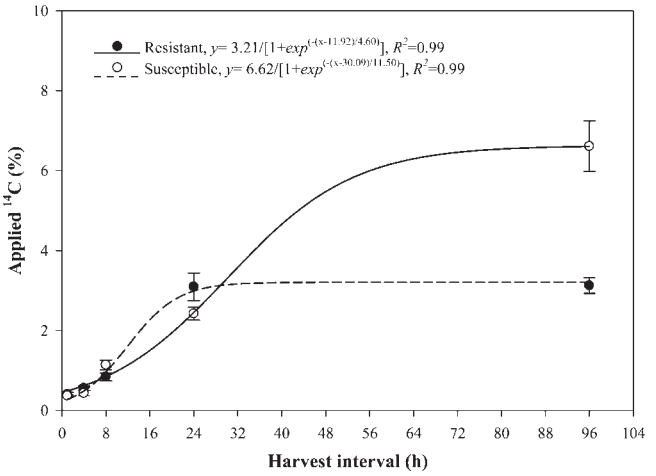

Figure 3. Total translocation of foliar applied ^{14}C-2,4-D out of the treated leaf of susceptible and resistant prickly lettuce biotypes over 4 days, expressed as percent of applied. Error bars represent the standard error of the mean, where $n = 8$.

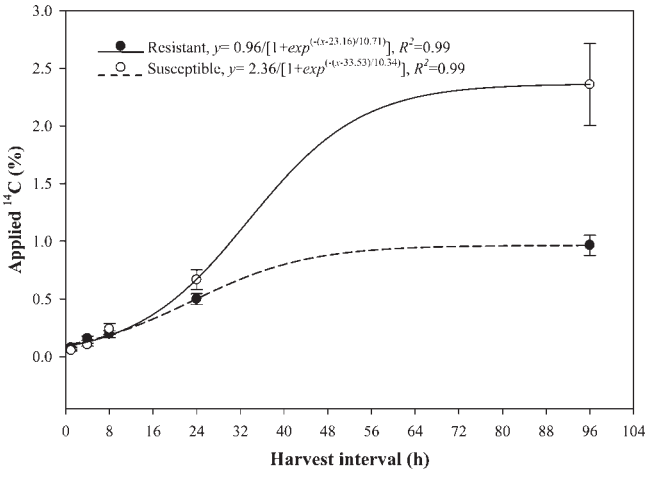

Figure 4. Accumulation of foliar applied ^{14}C-2,4-D in the crown of susceptible and resistant prickly lettuce biotypes over 4 days, expressed as percent of applied. Error bars represent the standard error of the mean, where $n = 8$.

(6.6% of the applied) at 96 HAT (Figure 3). The majority of translocated radioactivity moved to the crown and leaves above the treated leaf in each biotype. Accumulation of radioactivity in the leaves above the treated leaf was similar in each biotype. However, the crown of the resistant biotype accumulated less radioactivity at 96 HAT than the susceptible biotype (0.9 and 2.4% of the applied, respectively) (Figure 4). Both biotypes accumulated similar amounts of radioactivity in older leaves below the treated leaf (data not presented). A very small portion of radioactivity was recovered from the roots.

Absorption and Translocation of [^{14}C] 2,4-D: 21 Day Experiment. Recovery of ^{14}C material from both resistant and susceptible prickly lettuce biotypes decreased over time ($y = -2.0x + 85.5$, $r = 0.94$). Absorption, expressed as percent of applied [^{14}C]-2,4-D, increased from 15.4 and 15.9% at 1 DAT to 26.4 and 21.7% at 96 HAT in resistant and susceptible prickly lettuce biotypes, respectively (Figure 5). Absorption was similar

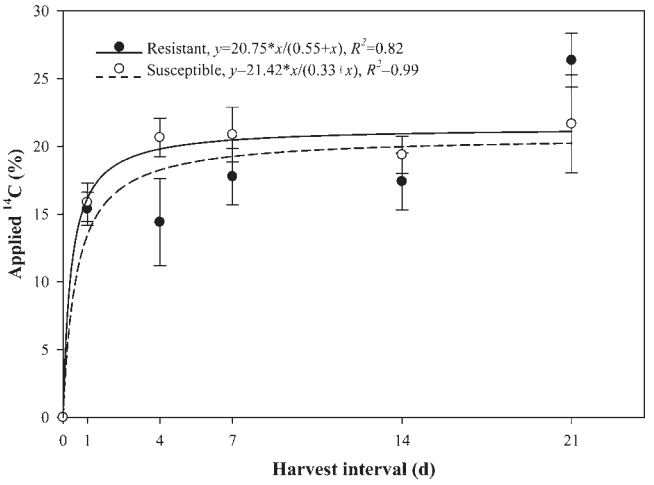

Figure 5. Absorption of foliar applied ^{14}C-2,4-D into susceptible and resistant prickly lettuce biotypes over 21 days, expressed as percent of applied. Error bars represent the standard error of the mean, where $n = 8$.

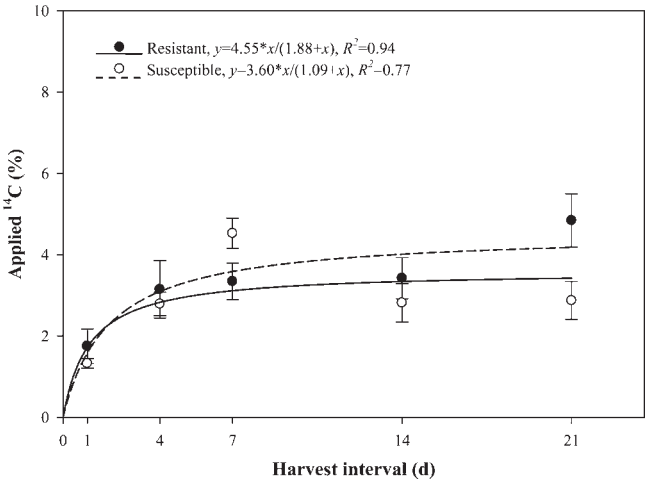

Figure 6. Total translocation of foliar applied ^{14}C-2,4-D out of the treated leaf of susceptible and resistant prickly lettuce biotypes over 21 days, expressed as percent of applied. Error bars represent the standard error of the mean, where $n = 8$.

in both biotypes at each harvest interval. Interestingly, over the interval from 14 to 21 DAT, the absorption of applied 2,4-D increased in the resistant biotype from 17.4 to 26.4%. Over the same interval, absorption of applied 2,4-D remained similar in the susceptible biotype (19.4% at 14 DAT, 21.7% at 21 DAT). By 21 DAT, the resistant biotype begins to regrow from the 2,4-D injury, whereas the susceptible biotype does not. Regrowth was observed in the 21 day experiment and may be responsible for increased absorption at 21 DAT in the resistant biotype.

Total translocation of the applied radioactivity in the long-term experiment followed a pattern similar to that of absorption (Figure 6). At 7 DAT, translocation in the susceptible biotype was greater at 4.5% of the applied than the resistant biotype at 3.2% of the applied. As with absorption, over the interval from 14 to 21 DAT, the translocation of applied radioactivity increased in the resistant biotype from 3.4 to 4.8%. Over the same interval, translocation of applied 2,4-D remained similar in the susceptible

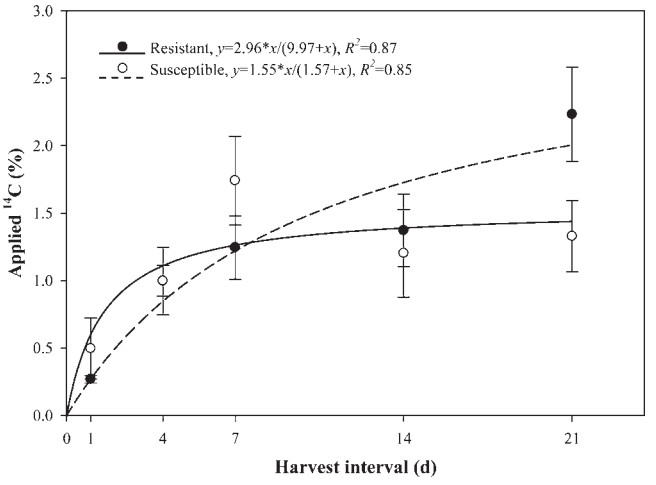

Figure 7. Accumulation of foliar applied ^{14}C-2,4-D in the crown of susceptible and resistant prickly lettuce biotypes over 21 days, expressed as percent of applied. Error bars represent the standard error of the mean, where $n = 8$.

biotype (2.8% at 14 DAT, 2.9% at 21 DAT). Accumulation of radioactivity followed a similar trend (Figure 7). By 21 DAT, nearly double the radioactivity had accumulated in the crown of the resistant biotype (2.3% of the applied) than had accumulated in the crown of the susceptible biotype (1.3% of the applied).

Environmental and species effects on absorption and translocation of 2,4-D are well documented.[24−26] Values for absorption of 2,4-D have varied from 18 to 70% depending upon plant species, study duration, and environmental conditions.[24−28] For more recent example comparisons, the biphasic absorption of 2,4-D in Drummond's goldenweed [*Isocoma drummondii* (Torr. & A. Gray) Greene][28] and perennial *Glycine* species [29] were similar to the absorption pattern observed in both biotypes of prickly lettuce. Studies of auxin resistance in hemp-nettle revealed 50% less acropetal movement of ^{14}C in MCPA-resistant compared to MCPA-susceptible biotype.[14] Absorption and translocation of 2,4-D specifically and auxinic herbicides in general appear to be species dependent and likely related to the sensitivity of the target site. Greater sensitivity and more rapid deregulation of source-sink relationships may reduce absorption and translocation of auxinic herbicides. Relative comparisons may not be appropriate between species, as each species likely perceives the synthetic auxin signal in a unique manner.

After herbicide treatment, the crown of the resistant prickly lettuce biotype is less injured when compared to the susceptible biotype.[1] Growth after treatment with 2,4-D in resistant prickly lettuce occurs from lateral or apical meristems arising from the crown.[1] Increased translocation of radioactivity out of the treated leaf in the susceptible biotype compared to the resistant biotype may have reduced the concentration gradient in the treated portion of the treated leaf and associated cuticle, contributing to greater absorption by the susceptible biotype at the 96 HAT harvest. Greater ^{14}C translocation from the treated leaves of the susceptible compared to the resistant biotype was also reported in hemp-nettle,[14] and differential absorption was also believed to be a mechanism of resistance in 2,4-D resistant ground ivy.[23] Alternately, if the resistance is due to altered or reduced sensitivity at the target site, an overstimulation of growth in the susceptible biotype may have caused the observed differences in absorption and translocation at the end of the 4 day experiment and at 4 DAT in the 21 day experiment.

Table 1. Relative Growth Rates (and Standard Errors, where $n = 8$) for Susceptible (S) and Resistant (R) Biotypes Treated with [^{14}C]-2,4-D

harvest interval (days)	biotype	relative growth rate (mg mg^{-1} day^{-1})
1−4	R	18.1 (32.1)
	S	95.7 (29.1)
4−7	R	45.8 (41.9)
	S	−6.0 (24.1)
7−14	R	22.0 (42.3)
	S	14.6 (15.7)
14−21	R	33.2 (43.1)
	S	4.5 (26.6)

Relative Growth Rates. There are many effects of auxinic herbicides on plants; most notable are a rapid overstimulation and deregulation of growth after application, often occurring within hours after application.[7] Relative growth rates of susceptible biotypes between 1 and 4 DAT were greater than the relative growth rate for the resistant biotype over the same interval (Table 1). The relative growth rate of the susceptible biotype for the duration of the experiment was lower than that of resistant biotype and indicated that growth of susceptible biotype had virtually ceased. The resistant biotype continued to grow for the duration of the experiment. The magnitude of overstimulation of growth observed in the susceptible biotype was not observed in the resistant biotype.

The susceptible biotype appears to have absorbed and translocated more 2,4-D than the resistant biotype due to the effects of the auxinic herbicide, including overstimulation and deregulation of growth. Lower absorption and translocation should perhaps be expected from what appears to be an altered signal receptor site: the 2,4-D signal is not received by the resistant biotype, and the auxinic overdose response, including overstimulation of growth, is mitigated. The result is more absorption and translocation of 2,4-D by the susceptible biotype over a narrow interval between application and 96 HAT.

Metabolism. The R_f value of 2,4-D was 0.65. Any peak identified with an R_f value of <0.65 was considered to be more polar than 2,4-D and designated a polar metabolite. A single peak with an R_f value of >0.65 was identified as the dimethylamine salt of 2,4-D. The relative proportion of polar metabolites, dimethylamine salt of 2,4-D, and parent 2,4-D was similar in the two biotypes at each harvest interval. Thus, data were pooled over biotype at each harvest interval (Figure 8). The proportion of parent 2,4-D and dimethylamine salt of 2,4-D in the treated leaf decreased from 76 and 22% at 1 HAT to 42 and 9% at 168 HAT, respectively. The calculated half-life for 2,4-D in prickly lettuce was 34.7 h. In contrast, the proportion of polar metabolites increased from 3% at 1 HAT to 49% at 168 HAT. A variety of metabolic degradation pathways for 2,4-D are known and include side-chain degradation, side-chain lengthening, ring hydroxylation, conjugation, and ring cleavage.[24] The resistant and susceptible biotypes metabolized the parent compound at a similar rate, and there were no differences in the composition of the polar metabolites by TLC. However, further investigation of the chemical makeup of the metabolites might identify differences in the composition of the polar metabolites produced by the two biotypes using a different analytical technique.

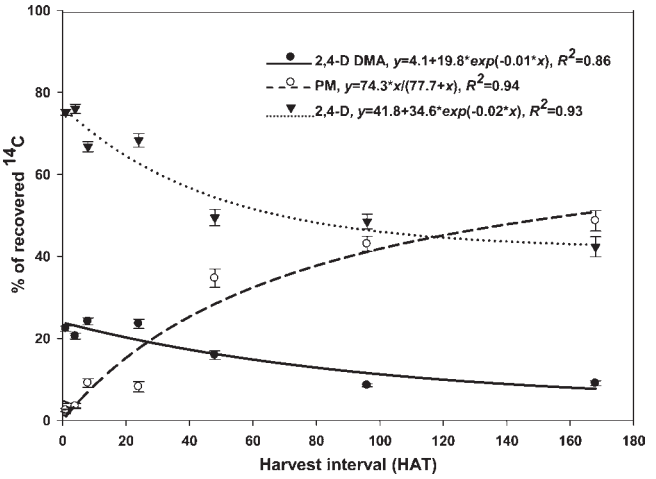

Figure 8. Percent radioactivity from thin layer chromatographic separations of ^{14}C-2,4-D and metabolites from treated leaves of prickly lettuce ($n = 16$), averaged over trials and biotypes. Abbreviations: 2,4-D DMA, dimethylamine salt of 2,4-D; HAT, hours after treatment; PM, sum percentage of metabolites more polar than 2,4-D.

The crown portion of susceptible and resistant biotypes at 96 HAT was also evaluated to determine if the radioactivity present was the parent compound or a metabolized derivative. The percentages of metabolites and parent 2,4-D were similar in the crowns of susceptible and resistant biotypes, but ≥54% of the radioactivity present in crown was 2,4-D, and no dimethylamine salt of 2,4-D was recovered. The metabolism experiment confirmed that the major proportion of radioactivity reaching the crown was 2,4-D acid, and the balance was an unidentified metabolite. Similar results for susceptible and resistant biotypes of other resistant species have been observed. Differential metabolism was not the basis for dicamba-resistant kochia,[30] nor was metabolism of quinclorac different in quinclorac-resistant goosegrass (*Eleusine indica* L.) and quinclorac sensitive large crabgrass (*Digitaria sanguinalis* L.).[31]

The F_1 and reciprocal F_1 plants were resistant to 2,4-D, indicating a dominant gene action for 2,4-D resistance in prickly lettuce. However, the codominant segregation (1:2:1) of F_2 plants, coupled with a decreased magnitude of resistance in the intermediate group, suggests that there may be one or more additional genes modifying the effect of a single major gene. Physiological studies indicating differences in absorption and translocation between resistant and susceptible biotypes appear be related to a signal receptor site modification, perhaps at *TIR1*. Differences in absorption and translocation may be indicative of a reduced auxinic response by the resistant biotype. Studies are in progress to identify the molecular basis for the 2,4-D-resistant trait and to assess other physiological aspects of 2,4-D resistance.

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ABBREVIATIONS USED

2,4-D, 2,4-dichlorophenoxyacetate; DAT, days after treatment; HAT, hours after treatment; LSA, liquid scintillation analyzer.

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