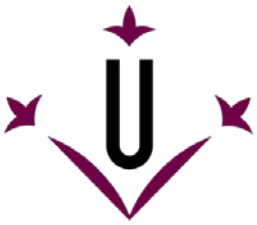

Unravelling the resistance mechanisms to 2,4-D (2,4-dichlorophenoxyacetic acid) in corn poppy (*Papaver rhoeas*)

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Abstract

In southern Europe, the intensive use of 2,4-D (2,4-dichlorophenoxyacetic acid) and tribenuron-methyl in cereal crop systems has resulted in the evolution of resistant (R) corn poppy (*Papaver rhoeas* L.) biotypes. Experiments were conducted to elucidate (1) the resistance response to these two herbicides, (2) the cross-resistant pattern to other synthetic auxins and (3) the physiological basis of the auxin resistance in two R (F-R213 and D-R703) populations. R plants were resistant to both 2,4-D and tribenuron-methyl (F-R213) or just to 2,4-D (D-R703) and both R populations were also resistant to dicamba and aminopyralid. Results from absorption and translocation experiment revealed that R plants translocated less [^{14}C]-2,4-D than S plants at all evaluation times. There was between four and eight-fold greater ethylene production in S plants treated with 2,4-D, than in R plants. Overall, these results suggest that reduced 2,4-D translocation is the resistance mechanism in synthetic auxins R corn poppy populations and this likely leads to less ethylene production and greater survival in R plants.

Keywords: Auxinic herbicide, cross resistance, ethylene production, herbicide resistance, radioactivity, translocation.

1. Introduction

Agricultural weeds cause major crop losses by competing for nutrients, water or light. Even though non-chemical methods have been used for controlling weeds, herbicides are considered the most effective means of weed control [1]. 2,4-D (2,4-dichlorophenoxyacetic acid), an auxinic herbicide, was commercially released in 1946 becoming the first successful selective herbicide to specifically target dicotyledonous weeds. 2,4-D still remains as one of the most commonly used herbicides in the world as a consequence of its low cost, selectivity, efficacy and wide spectrum of weed control [2]. The auxinic herbicide family (group O according to the Herbicide Resistance Action Committee, HRAC; and group 4 according to the Weed Science Society of America, WSSA) contains four chemical groups, including pyridine-carboxylic acids (i.e. aminopyralid), quinolinecarboxylic acids (i.e. quinclorac), benzoic acids (i.e. dicamba), and phenoxy-carboxylic acids (i.e. 2,4-D).

After 60 years of widespread and repeated usage, few examples of resistance to this mode of action have been reported. Generally, the selection of synthetic auxin resistant biotypes requires more generations than for other modes of action herbicides, particularly acetolactate synthase (ALS) and acetyl-coenzyme A carboxylase (ACCase) inhibitors [3]. Several reasons have been proposed to explain this phenomenon, including low mutation rates, fitness penalties and redundancy in auxin receptors within the plant [2,4]. Nowadays, there are 32 auxinic herbicide resistance species, 15 of those being resistant to 2,4-D [5]. The precise mode of action for these herbicides, and consequently, the resistance mechanisms in weeds are, however, still poorly understood [2,6]. Nonetheless, new discoveries including nuclear auxin receptors (F-box proteins), influx and efflux carriers and plasma membrane bound receptors have provided basic clues as to the molecular mode of action of these herbicides [6–10].

The characterization of resistance mechanisms has been investigated in few auxinic herbicide-resistant weeds. Differential absorption, translocation, or metabolism were not the basis for resistance in the majority of the assessed species [11–15]. Only in a few weeds these non-target-site mechanisms (NTSM) have been related with the resistance response [3,16,17]. Additionally, it has been reported that the application of auxinic herbicides stimulates ethylene biosynthesis in sensitive, but not in resistant plants [13,15,18]. This unregulated auxin response and the resulting hyperaccumulation of ethylene, abscisic acid (ABA) and reactive oxygen species (ROS) in auxinic herbicide sensitive plants may be involved in the induction of tissue damage and cell death after synthetic auxins application [19].

Corn poppy (*Papaver rhoeas* L.) is a major weed of cereal crops in Southern Europe [20]. Its extended germination period, high seed production, and seed bank persistence makes it especially difficult to manage. It has been estimated that corn poppy can decrease wheat yields up to 32% [21]. Moreover, the increase in both monoculture farming and overuse of 2,4-D (since the 60s) followed by tribenuron-methyl application (early 80s) have selected ALS and/or 2,4-D herbicide-resistant biotypes. The International Survey of Herbicide Resistant Weeds records ALS inhibiting herbicide-resistant biotypes of corn poppy in ten different European countries. Furthermore, 2,4-D resistant biotypes have been detected in Italy [5]. While it is well known that resistance to ALS inhibitors in corn poppy is caused by a single point mutation in the ALS gene (target-site mechanisms, TSM) [20,22–24], no studies have attempted to understand the resistance mechanisms to synthetic auxins in this species. A better understanding of the 2,4-D resistant mechanisms in corn poppy may also improve resistance management by better defining herbicide use patterns to delay or avoid resistance to this mode of action [4].

This study was thus conducted in order to (1) determine the herbicide rate causing 50% mortality (GR_{50}) and the resistance index (RI) of resistant (R) and a susceptible (S) populations to 2,4-D and tribenuron-methyl, (2) characterize the cross-resistance response of R and S plants to other synthetic auxins chemical groups used in cereals systems, (3) compare the physical (contact angle) and physiological features (absorption and translocation of [^{14}C]-2,4-D) between R and S plants and (4) to examine the ability of 2,4-D to induce ethylene biosynthesis in R and S corn poppy plants.

2. Material and Methods

2.1. Plant material

Before winter cereal harvest, mature capsules form at least twenty different corn poppy plants were collected in two fields where failure of corn poppy control with ALS inhibitors and/or 2,4-D had been reported. F-R213 population, suspected to be multiple resistant, was collected from a field located in Baldomar, north of Spain (41º54'39.0"N and 1º00'21.2"W) in 2013. D-R703 population, with suspected resistance to 2,4-D, was collected from a field located in Almacelles (41°43'39.6"N and 0°27'29.5"E) in 2003. Two susceptible populations (H-S013 and S-S012) were included in this study. H-S013 was obtained from a seed dealer (Herbiseed, Twyford, UK) in 2008, and S-S012 was collected in 2012 from a cereal field in Almenar (41º47'30.5"N and 0°27'29.5"E) where no resistance problems had been reported. Corn poppy seeds were sterilized in a 30% hypochlorite solution. Sterilized seeds were sown in Petri dishes with 1.4% agar supplemented with 0.2% KNO_3 and 0.02% gibberellin GA_3. Seeds were placed in a growth chamber at 20/10 °C day/night, 16 h photoperiod under 350 µmol photosynthetic photon-flux density m^{-2} s^{-1}. After 14 days, seedlings were transplanted in 7 x 7 x 7 cm plastic pots filled with the following soil mixture: silty loam soil 40%

(w/v), sand 30% (w/v), peat 30% (w/v). Pots were placed in a greenhouse in Lleida, north-eastern Spain (41° 37'N, 0° 38'W) and were watered regularly to field capacity.

2.2. Dose-response experiments

Five seedlings were sown per pot and after establishing, were thinned to three per pot. At the six leaf stage (5-6 cm), all populations were tested with tribenuron-methyl and 2,4-D. Tribenuron-methyl (Granstar 50 SX, DuPont, 50%) was applied at 0, 4.6, 9.3, 18.7 (field dose), 37.5, 75, 150, 600 and 1200 g a.i.·ha^{-1} to R plants and at 0, 0.25, 0.5, 1.1, 2.3, 4.6, 9.3, and 18.7 g a.i.·ha^{-1} to S plants. 2,4-D (Esteron 60, Dow AgroSciences, 60%) was applied at 0, 75, 150, 300, 600 (field dose), 1200 and 4800 g a.i.·ha^{-1} to R populations and at 0, 9.3, 18.75, 37.5, 75, 150, 300 and 600 g a.i.·ha^{-1} to S plants. Non-treated plants were used as controls. A total of four replicates (three plants per pot) were included at each dose. Herbicides were applied using a precision bench sprayer delivering 200 L·ha^{-1}, at a pressure of 215 kPa. Four weeks after treatment, plants were harvested (above ground) and the dry weight (65 °C for 48 h) was measured.

2.3. Cross-resistance patterns of synthetic auxins

Both R populations (D-R703 and F-R213) and H-S013 plants were sprayed with dicamba (Benzoic acid) and aminopyralid (Pyridine-carboxylic acid) in order to study the effects of other synthetic auxins. Dicamba (Banvel D, Syngenta, 48%) and aminopyralid (Dow AgroSciences, 3.9%) were sprayed at their field rates (144 and 9.9 g a.i.·ha^{-1}, respectively) as well as two times their field rates. Five replicates (three plants per pot) and five control pots (non-treated plants) were included at each dose. Applications and evaluations were done as described above.

2.4. [^{14}C]-2,4-D uptake and translocation experiments

Ring labeled [^{14}C]-2,4-D with specific activity of 1576 MBq·mmol^{-1} was provided by Dow AgroSciences (Dow AgroSciences, Indianapolis, USA). Seedlings from H-S013 and both R populations at six true leaves of development (5-6 cm), were treated with four droplets of 0.5 µL (2 µL per plant) of radio labeled herbicide solution containing [^{14}C]-2,4-D and commercial 2,4-D mixed to a final herbicide concentration of 3 g·L^{-1} (equivalent to a 600 g a.i.·ha^{-1} delivered at 200 L·ha^{-1} spraying volume). Every plant received a total activity of 18.4 MBq mmol^{-1}. Five plants from each population were harvested at 12, 24, 48, and 96 h after treatment (HAT). Unabsorbed herbicide was rinsed from the treated leaves using 2 ml of an acetone/water (1:1 v/v) solution. The rinse solution was mixed with 15 mL of scintillation fluid (Ultima Gold^{TM}, Perkin-Elmer, Packard Bioscience BV). Washes were analyzed by liquid scintillation spectrometry (LSS) (Beckman LS 6000 TA scintillation counter; Beckman Instruments, CA, USA). Plants were separated into three parts; treated leaf, shoot and root, each of which was dried at 70 °C for 48 h and combusted in a sample oxidizer (OX 500; R. J. Harvey Instrument, Tappan, NY, USA). The trapped [^{14}C]-CO_2 was determined by LSS. Foliar absorption (%) was calculated as: (radioactivity recovered from plant parts) ⁄ (total radioactivity recovered) x 100. Translocation (%) was calculated as: (total radioactivity in treated leaf, shoot or root)⁄(total radioactivity in all tissues) x 100.

To assess translocation of 2,4-D, two treated plants for H-S013, D-R703 and F-R213 populations were removed from pots 48 HAT. Roots were rinsed and whole plants were dried (65 °C for 48 h) and pressed against a 25 by 12.5–cm phosphor storage film (PerkinElmer Life and Analytical Sciences, Shelton, CT) for 6 h, and scanned using a phosphor imager (Cyclone, Perkin-Elmer, Packard Bioscience BV).

2.5. Contact angle and microroughness assays

To assess any effects of leaf surface on herbicide deposition, 2,4-D was applied as one drop of 0.5 µL in the adaxial surface of the fourth leaf. Immediately after, individual droplets were photographed using a laboratory-built device consisting of a dissection microscope (Leica MZ6; Leica Microsystems Ltd., Heerbrugg, Switzerland) plus a high-definition digital camera with macro objective (Leica Dililux 4.6; Leica Camera AG, D35606 Solms, Germany). Thirty drops for each population (from different plants) were photographed and contact angle of the drops were analyzed using image processing software (Image J 1.31v; US National Institutes of Health, Bethesda, MD, USA). The same procedure was followed for the microroughness determination, where an acetone/water (1:1 v/v) solution was used instead of the herbicide.

2.6. Ehylene production

Experiments were conducted to evaluate the amount of ethylene produced by R (F-R213 and D-R703) and S (H-S013 and S-S012) plants in response to 2,4-D treatment. Two seedlings were sown in a 145 ml pot (BeltaLab, Barcelon, Spain) and once established, were reduced to one per pot. Plants were sprayed, as described above, with commercial 2,4-D at 0, 150, 300 and 600 g a.i.·ha^{-1}. Treatments were replicated six times. Prior to each treatment, the soil mixture was covered with a layer of perlite to avoid deposition of the herbicide on the substrate. Immediately following treatments, the pots were closed with a specific hermetic top and the two holes beneath the pot were sealed with vaseline and Parafilm. Ethylene was measured by withdrawing a 1 ml gas sample from the head-space with a syringe and injecting it into a gas chromatograph (GC; Agilent Technologies 6890, Wilmington, Germany) equipped with an alumina column F1 80/100 (2m x 1/8 x 2.1, Teknokroma, Barcelona, Spain) and a flame ionization detector (FID) [25]. This experiment was repeated twice; in October 2014 and again in February 2015 (the later only with S-S012 as a S population).

2.7. Statistical analysis

Data from dose-response experiments were analyzed using a non-linear regression model (1). The herbicide rate required for 50% growth reduction of plants (GR_{50}) was calculated with the use of a four parameter logistic curve of the type:

$$y = \mathrm{c} + \frac{(d-c)}{1+\mathrm{EXP}[b(\log(x)-\log(GR50)]} \quad (1)$$

where c = the lower limit, d = the upper limit and b = the slope at the GR_{50}. In this regression equation, the herbicide rate (g a.i.·ha^{-1}) was the independent variable (x) and the plants' dry weight expressed as percentage of the untreated control was the dependent variable (y). The resistance index (RI) was computed as $GR_{50}(R)/GR_{50}(S)$. Data from [^{14}C]-2,4-D uptake and translocation experiments were subjected to analysis of variance (ANOVA). The requirement of homogeneity of variance was checked by visual inspection of the residual plots and residuals were analyzed using Shapiro–Wilk Test. Where variances were not homogeneous, Generalized linear models (GLM) were used. The binomial distribution (Logit-link) was used in all GLM, because this distribution resulted in normally distributed residues. Population means were compared using a post-hoc Tukey´s pairwise procedure at P = 0.05. Data from the cross resistant experiment (efficacy) and ethylene production assay (μLC_2H_4·g^{-1}·h^{-1}) were subjected to analysis of variance (ANOVA) and means were separated using Tukey´s pairwise comparison at 0.05 probability level. Repetitions from the ethylene experiment (October and February) were not pooled due to statistical differences between experiments.

All statistical analyses were carried out with the use of the R programming language [26]. *drc* packag [27] for the non-linear regression and *multcom* [28] for the post hoc Tukey´s test were employed

3. Results

Both R and S plants showed morphological damage after 2,4-D application. Plant growth was reduced, and leaves were curled. R plants produced new growth within a few days of herbicide application. S and R plants treated with 600 g a.i.·ha^{-1} and 4800 g a.i.·ha^{-1} of 2,4-D, respectively, died 14 days after application. The GR_{50} for 2,4-D were the same for the two S populations (66.3 vs 68.6 g of a.i.·ha^{-1}). F-R213 and D-R703 plants were 12-fold and 15-fold more resistant to 2,4-D than H-S013 plants, respectively. There was very little control of F-R213 plants with tribenuron-methyl at 600 g a.i.·ha^{-1} (thirty-two times the field rate), and GR_{50} was 25.2 g a.i.·ha^{-1}, 286-fold more resistant than H-S013 plants. Tribenuron-methyl at 18.7 g a.i.·ha^{-1} (field rate) controlled the population D-R703 (Figure 1), and it showed a very low RI (Table 1). Differences between S populations in the response to tribenuron-methyl were minimal (Figure 1).

The D-R703 and F-R213 populations were also resistant to dicamba and aminopyralid at the field rate (144 and 9.9 g a.i.·ha^{-1}, respectively; Figure 2). The effectiveness of auxinic herbicides on the R population increased when they were applied at two times the field rate, but other than dicamba on F-R213, they failed to control the populations (Figure 2).

There were no significant differences between R (D-R703 and F-R213) and S (H-S013) plants in the quantity of [^{14}C]-2,4-D absorbed, with between 65 to 70% of the herbicide applied absorbed at 12 HAT. F-R213 and D-R703 plants translocated much less [^{14}C]-2,4-D than H-S013 plants with, significantly less translocation to the shoots and roots compared to the susceptible population (Table 2). Percentages of recovered radioactivity ranged from 89 to 96% in H-S013 plants and from 85 to 98% in the R plants. Images obtained from the qualitative studies at 48 HAT confirmed the above

results (Figure 3). Data from the contact angle and microroughness assays did not reveal any kind of differences between R and S plants (data not shown).

No differences in ethylene production among populations were detected in untreated (0 g a.i·ha^{-1}) or plants sprayed at 150 g a.i·ha^{-1} of 2,4-D. There were differences between R and S populations starting at 300 g a.i.·ha^{-1} of 2,4-D, with maximum differences at the field rate (600 g a.i.·ha^{-1}), when S plants produced between five and eight times more ethylene than R plants (Figure 4). Even though statistical differences in ethylene production occurred between repeated trials (October and February), similar patterns between R and S populations were confirmed in both experiments (Figure 4).

4. Discussion

Resistance to both tribenuron-methyl and 2,4-D in F-R213 plants was confirmed in our study. Multiple resistant corn poppy populations have also been previously detected in Italy and Greece [5,29]. Resistance to both auxinic and ALS inhibitor herbicides have been reported in other dicot weeds such as: *Gallium spurium* L. [13], *Sisymbrium orientale* L. [4], *Kochia scoparia* L. [14], *Limnocharis flava* L. and *Raphanus raphanistrum* L. [5]. Resistant factors obtained to tribenuron-methyl and 2,4-D were similar to those observed in other studies [29,30].

The resistant plants were also resistant to dicamba and aminopyralid. Resistance to multiple synthetic auxins was also observed in *Lactuca serriola* L. [31], *Sinapis arvensis* L. [11], and *K. scoparia* [14]. New discoveries of proteins involved in auxin mode of action have indicated that specific alterations in nuclear receptors might contribute as a potential resistance mechanisms in auxinic herbicide resistant dicotyledonous weeds [2]. Similar to the results presented in this study, cross-resistance between 2,4-D and dicamba was also found in a F-box receptor mutant of *Arabidopsis thaliana* L. [32]

There was no difference in absorption of 2,4-D, however, reduced [^{14}C]-2,4-D translocation was observed in 2,4-D resistant corn poppy populations. Reduced synthetic auxin translocation has previously been reported for resistant populations of *Galeopsis tetrahit* L. [16] and *L. serriola*. [3]. Alteration to the auxin efflux carriers (PIN-FORMD, PIN; ATP-binding cassette, ABC) could explain the lack of translocation observed in 2,4-D resistant corn poppy plants. Members of the PIN and ABC efflux carrier families have been considered the main mechanism involved in active and long-distance auxin transport [33]. Recent studies conducted with *A. thaliana* suggested that ABCB4 transporter (ABC family) is the target of 2,4-D [34]. In addition, a mutation in *A. thaliana* in another efflux carrier of ABC family, ABCG9, has been reported to provide increased tolerance to 2,4-D without affecting endogenous auxin Indole-3-acetic-acid (IAA) transport [35].

Results from the ethylene experiments are consistent with previous studies conducted with other species. A three-fold increase in ethylene was induced in quinclorac-sensitive *G. spurium* plants compared with quinclorac-resistant plants [13]. Sensitive and resistant *K. scoparia* plants demonstrated greater than four-fold difference in ethylene production when they were treated with dicamba and sampled 24 HAT [36]. The stimulation of ethylene biosynthesis through the expression of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase has been described as one of the first phases after 2,4-D and F-box proteins binding [37]. Therefore, our results suggest that in R plants 2,4-D may not be binding this nuclear receptor.

Overall, these results suggest that 2,4-D does not promote the signaling pathway in R plants because its receptor is not activated, either due to its alteration or as a consequence of reduced translocation involving any of the known auxin transporter families. The first step toward uncovering this mechanism could be seeking an

alteration in these specific proteins affecting the auxinic nuclear reception or auxin efflux carriers (a specific transporter belonging to PIN or ABC families). A comprehensive understanding of the resistance mechanisms in corn poppy biotypes, especially in those with multiple resistance to auxinic and ALS inhibitor herbicides, is needed to further understand the risk of resistance evolution to others modes of action. This information will be crucial to assist in the design of integrated weed management strategies.

Abbreviations used

2,4-D [2,4-dichlorophenoxyacetic acid]; ABA [Abscisic acid]; ABC [ATP-binding cassette]; ACCase [Acetyl-coenzyme A carboxylase]; ACCsynthase [1-aminocyclopropane-1-carboxylic acid synthase]; ALS [Acetolactate synthase]; GR_{50} [Herbicide rate causing 50% mortality]; HAT [Hours after treatment]; MCPA [4-Chloro-2-ethylphenoxyacetate]; NTSM [Non-target-site mechanisms]; PIN [PIN-FORMD proteins]; R [Resistant]; RI [Resistant Index]; ROS [Reactive oxygen species]; S [Sensitive]; TSM [Target-site mechanisms]; WAT [Weeks After Treatment].

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Table 1

Estimated GR_{50} and resistance index (RI) values to tribenuron-methyl and 2,4-D for H-S013, S-S012, D-R703 and F-R213 corn poppy (*Papaver rhoeas*) populations.

Herbicide	Field dose	Population	GR_{50} (g a.i.·ha^{-1}) ± SE	RI
Tribenuron-methyl	18.75 g a.i.·ha^{-1}	H-S013	0.08 ± 0.02	--
		S-S012	0.10 ± 0.02	1.1
		D-R703	0.17 ± 0.04	2
		F-R213	25.22 ± 6.4	286
2,4-D	600 g a.i.·ha^{-1}	H-S013	68.60 ± 10.2	--
		S-S012	66.36 ± 20.4	0.9
		D-R703	1039.70 ± 402.0	15
		F-R213	816.60 ± 96.0	12

Table 2

Absorption (percentage of recovered radioactivity) and translocation (percentage of penetrated radioactivity) of [^{14}C]-2,4-D in H-S013, F-R213 and D-R703 populations of corn poppy (*Papaver rhoeas*) at different times. Data are means and means followed by different letters indicate significant differences in each time and location (Absorption, treated leaf, shoots and roots) (P<0.05).

Population	12 h	24 h	48 h	96 h
Foliar absorption (% recovered radioactivity)				
H-S013	70.98 a	78.06 a	62.71 a	65.81 a
D-R703	65.67 a	69.55 a	69.26 a	71.98 a
F-R213	65.83 a	78.22 a	70.54 a	76.98 a
Remained in the treated leaf (% penetrated radioactivity)				
H-S013	93.79 a	83.60 a	78.36 a	70.04 a
D-R703	97.34 b	96.45 b	98.56 b	96.87 b
F-R213	99.08 b	96.26 b	98.29 b	97.49 b
Translocation to the shoots (% penetrated radioactivity)				
H-S013	4.25 a	12.77 a	15.05 a	22.22 a
D-R703	2.23 ab	2.27 b	0.77 b	2.44 b
F-R213	0.32 b	2.69 b	0.55 b	1.04 b
Translocation to the roots (% penetrated radioactivity)				
H-S013	1.95 a	3.61 a	6.57 a	7.73 a
D-R703	0.41 b	1.26 ab	0.65 b	0.34 c
F-R213	0.58 b	1.04 b	1.14 b	1.46 b

Figure 1. Dose-response regression curves of susceptible (H-S013 and S-S012), and resistant (D-R703 and F-R213) corn poppy (*Papaver rhoeas*) populations to 2,4-D (A) and tribenuron-methyl (B) (log scale). Data were expressed as percentage of the mean dry weight of untreated control plants.

Figure 2. Efficacy of aminopyralid (AMI), dicamba (DIC) and 2,4-D at the field rate: 9.9, 144 and 600 g a.i.·ha^{-1} (1x) and two-fold the field rate: 19.8, 288 and 1200 g a.i.·ha^{-1} (2x) on H-S013 (black), D-R703 (dark grey) and F-R213 (grey) corn poppy (*Papaver rhoeas*) populations. Columns with different letters indicate significant differences (P<0.05) for each product and dose.

Figure 3. Digital image (upper panel) and autoradiographic image (lower panel) depicting [^{14}C]-2,4-D translocation throughout plants tissues of H-S013, D-R703 and F-R213 populations of corn poppy (*Papaver rhoeas*), 48 HAT. Arrows in the upper image indicate the leaf where [^{14}C]-2,4-D droplets were applied.

Figure 4. Ethylene production (µL $C_2H_4 \cdot g^{-1} \cdot h^{-1}$) in susceptible (H-S013 and S-S012), and resistant (D-R703 and F-R213) corn poppy (*Papaver rhoeas*) populations after foliar application of 2,4-D at different concentrations. The experiment was repeated twice, in October 2014 (A) and February 2015 (B). Ethylene was measured 16 h after treatment (HAT). * indicate significant differences (P<0.05) between R and S plants for each application dose.

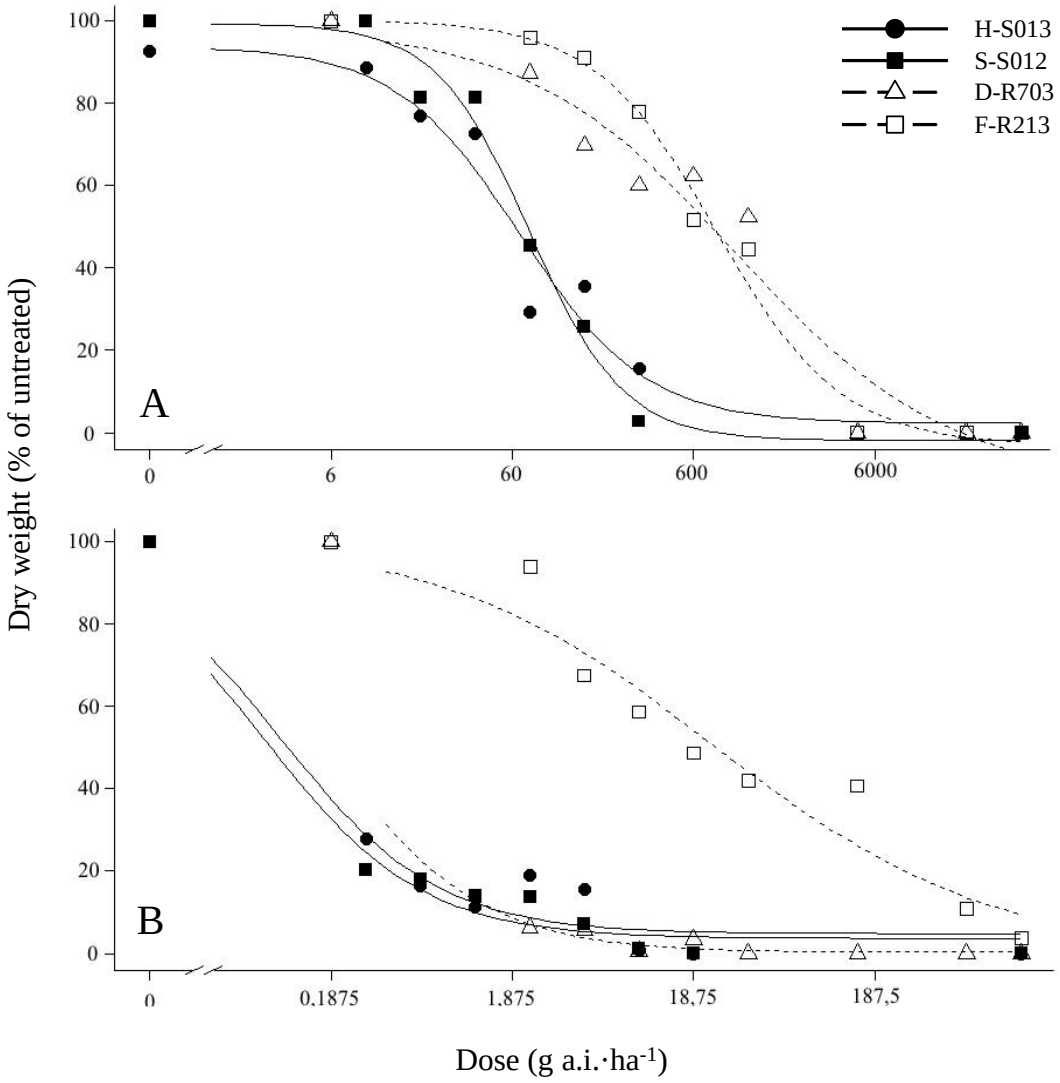

H-S013
S-S012
D-R703
F-R213
Dry weight (% of untreated)
100
80
60
40
20
0
A
0
6
60
600
6000
100
80
60
40
20
0
B
0
0,1875
1,875
18,75
187,5
Dose (g a.i.·ha-1)

Fig. 1

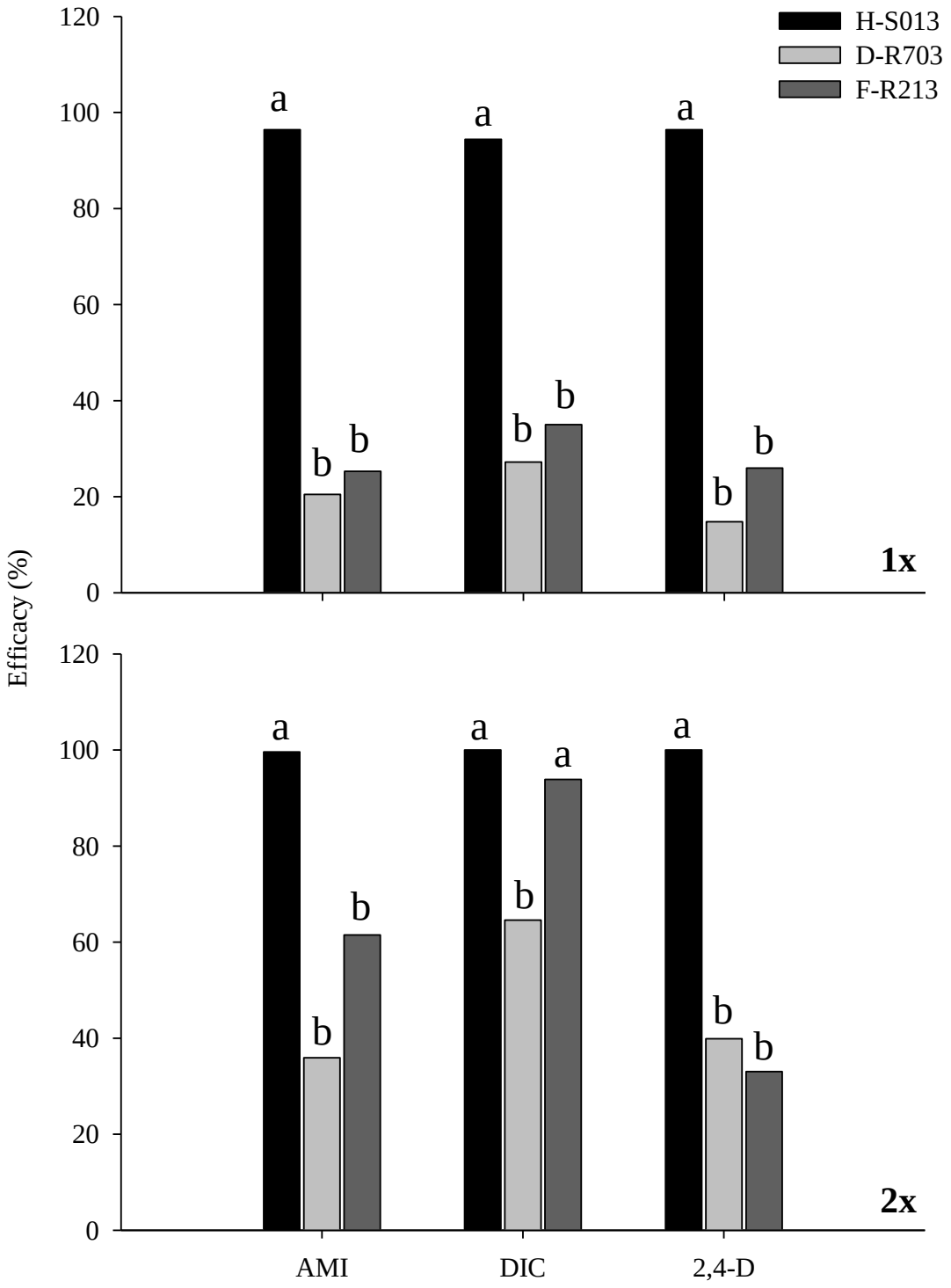

H-S013
D-R703
F-R213
Efficacy (%)
120
100
80
60
40
20
0
a
b
b
a
b
b
a
b
b
1x
a
b
b
a
b
a
a
b
b
2x
AMI
DIC
2,4-D

Fig. 2

Fig. 3

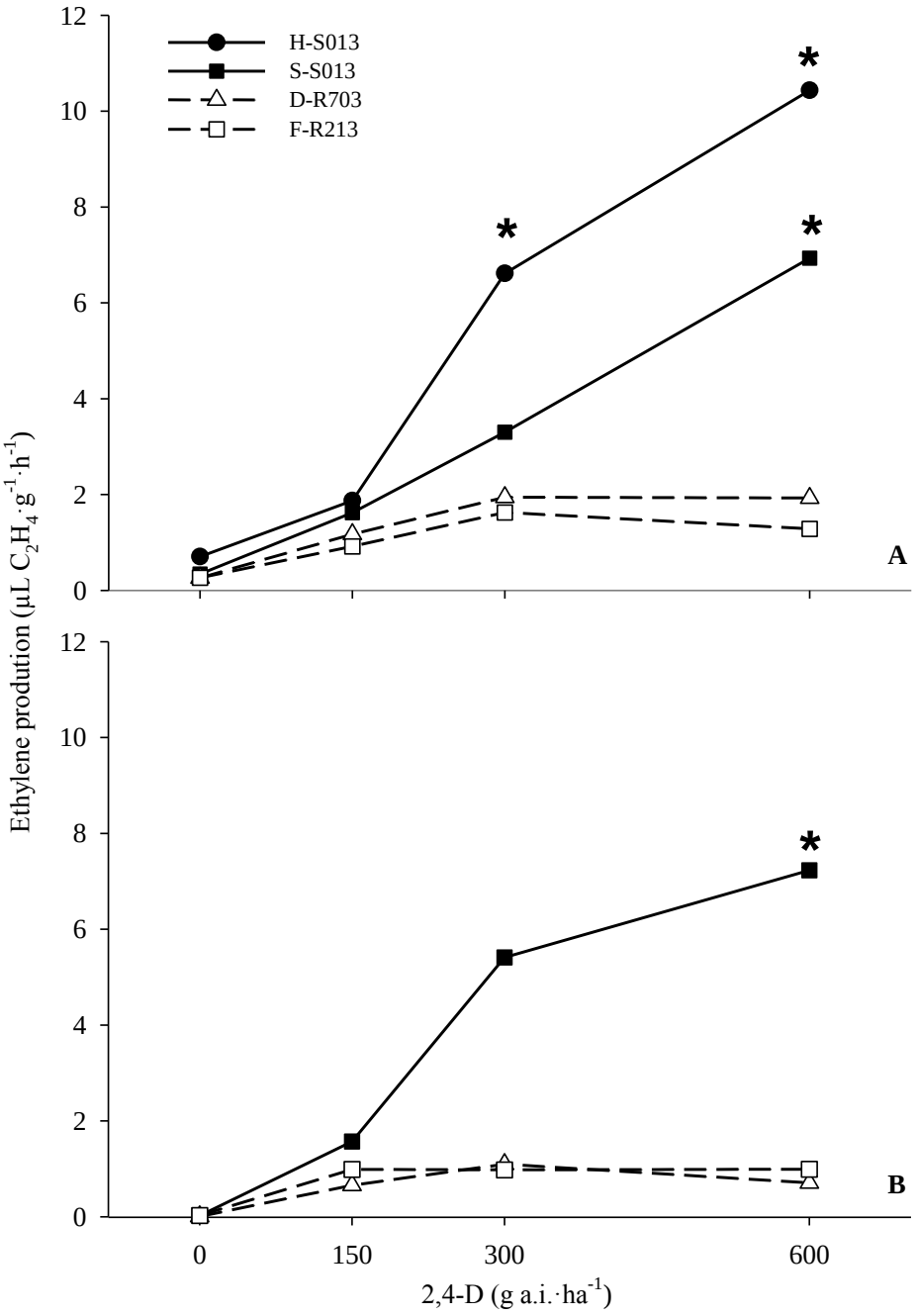
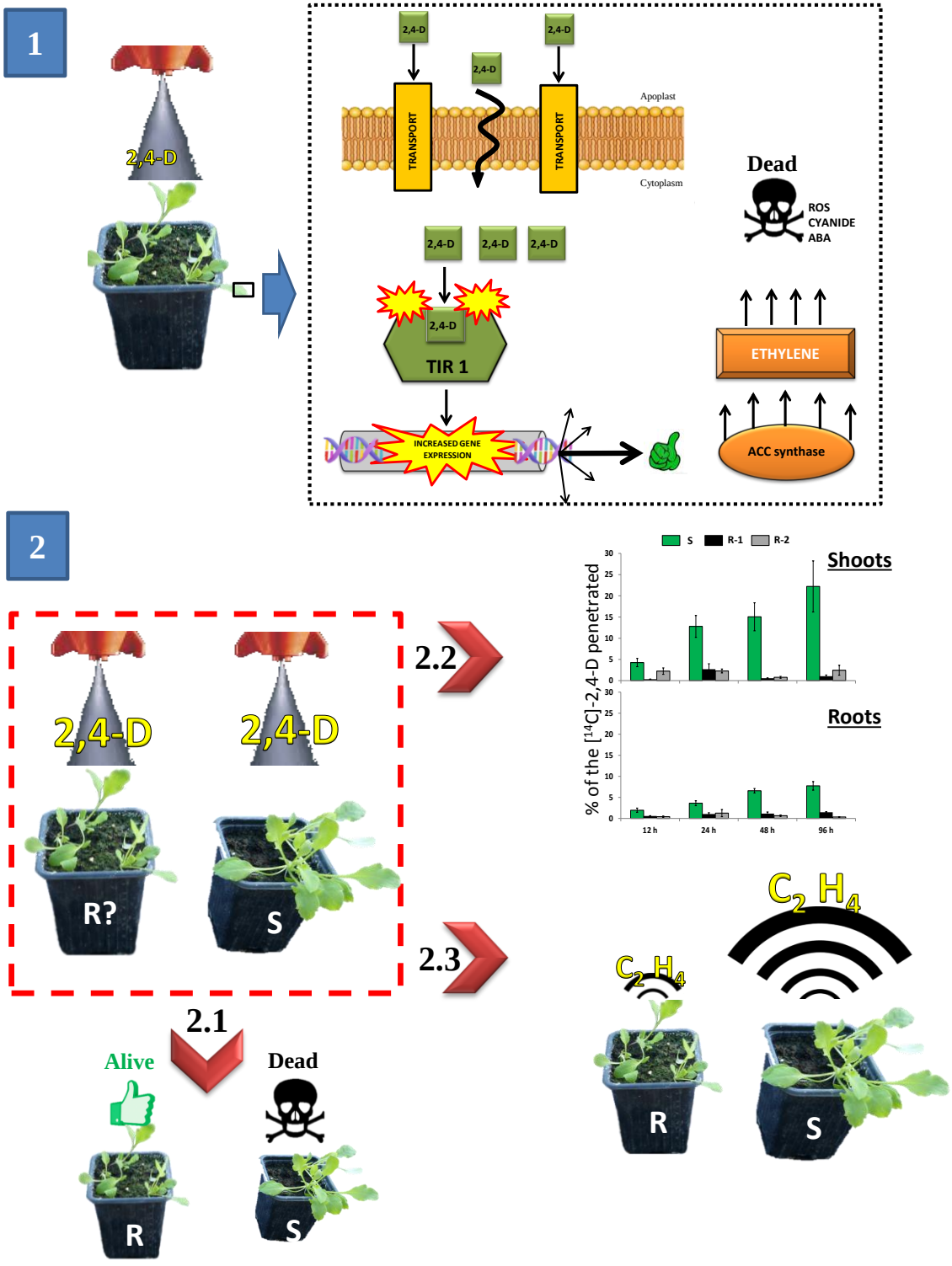
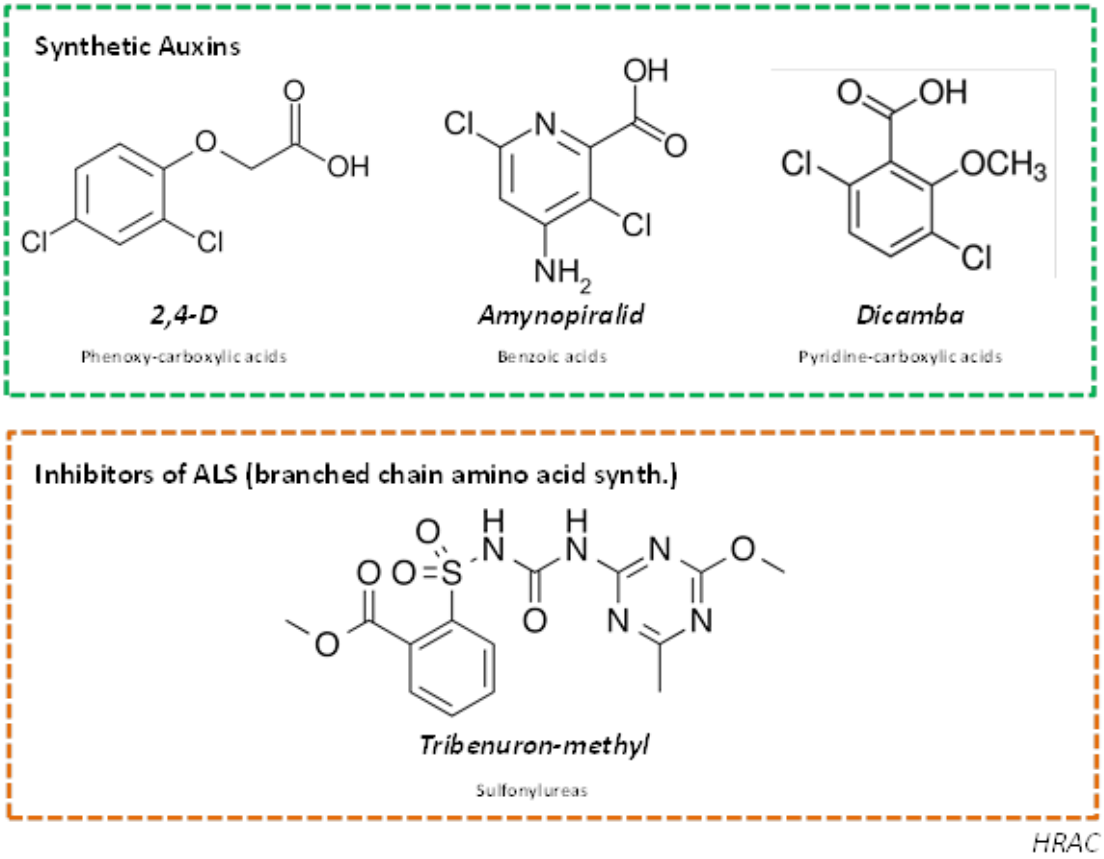

H-S013
S-S013
D-R703
F-R213
Ethylene prodution (µL $C_2H_4 \cdot g^{-1} \cdot h^{-1}$)
2,4-D (g a.i.·ha^{-1})
A
B
0
2
4
6
8
10
12
150
300
600
*

Fig. 4


1
2,4-D
2,4-D
2,4-D
2,4-D
TRANSPORT
TRANSPORT
Apoplast
Cytoplasm
2,4-D
2,4-D
2,4-D
2,4-D
TIR 1
INCREASED GENE EXPRESSION
Dead
ROS
CYANIDE
ABA
ETHYLENE
ACC synthase
2
2,4-D
2,4-D
R?
S
2.2
2.3
2.1
Alive
Dead
R
S
S
R-1
R-2
Shoots
Roots
% of the [14C]-2,4-D penetrated
12 h
24 h
48 h
96 h
C2 H4
C2 H4
R
S

Graphical abstract 1



Graphical abstract 2