**Supplementary Table S1.** Effect of potential 2,4-D synergists on seedling radicle elongation when applied alone. Compounds potentially synergising 2,4-D were incorporated into 0.6% (w/v) agar at a range of concentrations and the rate of radicle elongation of seedlings grown on this agar was measured over 7 d. The concentration causing a 50% decrease in radicle elongation (ED50) was estimated from the dose-response curve using the drc package in R. The resistance index (RI) is the ratio of ED50 values of the R population vs. population S1 within a treatment, and the p-value shows the significance of the RI. There were three replicates of five seedlings for each compound and concentration.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Concentration inhibiting radicle elongation by 50% (ED50) |  |  |
| Compound | Population S1 | Population R2 | Population R3 | RI | p-value |
| ABA | 1.7 ± 1.1 μM | 6.2 ± 0.9 μM | nd | 3.5 ± 2.4 | 0.289 |
| ACC | 0.2 ± 0.0 μM | 0.4 ± 0.1 μM | nd | 2.1 ± 0.7 | 0.126 |
| Adenosine | 0.3 ± 0.0 mM | 0.1 ± 0.1 mM | nd | 0.4 ± 0.3 | 0.052 |
| BAP | 3.2 ± 1.2 nM | 56 ± 7 nM | nd | 18 ± 7 | 0.018 |
| 1-Butanol | 0.1 ± 0.0% (v/v) | 0.1 ± 0.0% (v/v) | nd | 1.0 ± 0.3 | 0.145 |
| Cyclanilide | 5.9 ± 4.6 μM | nd | 1.0 ± 0.7 μM | 0.2 ± 0.2 | <0.01 |
| EGTA | 1.0 ± 0.3 mM | nd | 0.7 ± 0.1 mM | 0.7 ± 0.2 | 0.274 |
| IAM | 0.3 ± 0.0 μM | nd | 0.1 ± 0.0 μM | 0.4 ± 0.1 | <0.01 |
| DL-Methionine | 0.3 ± 0.1 mM | 3.0 ± 0.5 | nd | 8.9 ± 3.8 | 0.047 |
| Methyl anthranilate | 59 ± 88 μM | nd | 2.5 ± 1.2 | 0.04 ± 0.07 | <0.01 |
| Tryptamine | 0.2 ± 0.1 μM | nd | 0.3 ± 0.1 | 1.8 ± 0.5 | 0.171 |

Abbreviations: ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; BAP, 6-benzylaminopurine; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; IAM, indole-3-acetamide.

**Supplementary Table S2.** Dose of MCPA, mecoprop or a 1:1 mix required to kill 50% of individuals (ED50) in a dose-response experiment performed in the glasshouse. The resistance index (RI) is the ratio of ED50 values of each population vs. population S1 within a herbicide treatment, and the p-value shows the significance of the RI (bold font indicates a significant difference between a population and S1 within a treatment). Different letters across rows indicate a significant difference among treatments within each population.

|  |  |  |  |
| --- | --- | --- | --- |
|  | MCPA | Mecoprop | 1:1 mix |
| Population | ED50 (g ha-1) | RI | p-value | ED50 (g ha-1) | RI | p-value | ED50 (g ha-1) | RI | p-value |
| S1 | 185 ± 279a | 1 | n/a | 214 ± 61a | 1 | n/a | 142 ± 136a | 1 | n/a |
| S2 | 68 ± 67a | 0.4 ± 0.3 | 0.080 | 163 ± 93a | 0.8 ± 0.3 | 0.446 | 108 ± 184a | 0.8 ± 1.5 | 0.900 |
| R1 | 287 ± 50a | 1.5 ± 2.7 | 0.844 | 344 ± 81a | 1.6 ± 0.6 | 0.282 | 293 ± 47a | 2.1 ± 1.5 | 0.485 |
| R2 | **10 ± 29c** | **0.1 ± 0.3** | **<0.001** | **365 ± 48a** | **1.7 ± 0.3** | **0.041** | 180 ± 55b | 1.3 ± 0.8 | 0.755 |
| R3 | 902 ± 289a | 4.9 ± 8.5 | 0.647 | **1371 ± 480a** | **6.4 ± 2.4** | **0.035** | 1348 ± 853a | 9.5 ± 10.9 | 0.986 |
| R4 | 1970 ± 932a | 10.6 ± 27.0 | 0.726 | 2676 ± 1746a | 12.5 ± 9.8 | 0.253 | 1569 ± 401a | 11.0 ± 7.8 | 0.211 |
| R5 | 507 ± 90a | 2.8 ± 4.5 | 0.689 | **507 ± 94a** | **2.4 ± 0.7** | **0.047** | 482 ± 97a | 3.4 ± 3.6 | 0.508 |
| R6 | 1102 ± 377a | 5.9 ± 16.4 | 0.765 | 536 ± 158a | 2.5 ± 1.4 | 0.283 | 820 ± 115a | 5.8 ± 5.7 | 0.410 |
| R7 | 1180 ± 132a | 6.4 ± 11.8 | 0.630 | **1064 ± 167a** | **5.0 ± 1.1** | **0.001** | 1123 ± 212a | 7.9 ± 6.8 | 0.322 |
| R8 | 2135 ± 947a | 11.5 ± 19.5 | 0.595 | 5550 ± 10072a | 25.4 ± 37.2 | 0.519 | 2465 ± 1139a | 17.4 ± 17.1 | 0.348 |
| R10 | 1134 ± 150b | 6.1 ± 12.1 | 0.677 | 6672 ± 11506a | 31.1 ± 44.6 | 0.507 | 861 ± 161ab | 6.1 ± 5.2 | 0.341 |
| R11 | 1184 ± 276a | 6.2 ± 17.3 | 0.767 | **897 ± 178a** | **4.2 ± 1.3** | **0.021** | 808 ± 80a | 5.7 ± 3.6 | 0.210 |



**Supplementary Fig. S1.** Effect of a foliar spray of tryptamine applied alone, or in combination with 2,4-D, on 2,4-D-resistant wild radish. Plants were sprayed with 178 g ha-1 technical-grade tryptamine, 250 g ha-1 formulated 2,4-D amine (Amicide Advance 700) or with both tryptamine and 2,4-D amine applied at the same rates as for the standalone treatments. Untreated plants were sprayed with the carrier solution used for all three herbicide treatments (5% DMSO plus 0.2% Tween 20). Photographs were taken at 21 d after treatment.