Five Cycles of Selection for 2,4-D Resistance in Red Clover

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Germplasm	2,4-D rate (ml E99 L^{-1} H_2O)†	% Survival	Contrast vs. Southern 2,4-D parent bulk	Contrast vs. Marathon
WI-2,4-D cycle 4	3.94	7.88%*	< 0.01	< 0.0001
Southern 2,4-D parent bulk	3.94	2.63%		
Marathon	3.94	0.00%		
WI-2,4-D cycle 4	5.63	0.96%*	< 0.01	< 0.01
Southern 2,4-D parent bulk	5.63	0.00%		
Marathon	5.63	0.00%		
WI-2,4-D cycle 4	7.88	0.11%	Ns	Ns
Southern 2,4-D parent bulk	7.88	0.00%		
Marathon	7.88	0.00%		

† E99 – 2,4-Dichlorophenoxyacetic acid (60.1% acid equivalents)
***** % Survival significantly greater than 0.00%

2,4-D and other auxin-like mode of action herbicides are used to control broadleaf weeds in pastures. Unfortunately such herbicides also kill beneficial forage legumes in pastures. Although transgene-conferred herbicide-resistance is utilized in some crops it is unlikely that transgenic breeding approaches will ever be used in clovers due to regulatory/commercialization costs associated with transgenic crops. Therefore, breeding herbicide resistance using traditional selection approaches is attractive. Conferring herbicide resistance using traditional breeding approaches has proved successful in other crops and is also evident in herbicide tolerant weeds that have become widespread in the United States. Taylor et al. (1989) created a 2,4-D tolerant red clover germplasm for tissue culture. This population was adapted to the southern range of red clover. The objective of this study was to develop a northern range adapted 2,4-D resistant clover red clover. Seed of the southern 2,4-D resistant red clover population were obtained and a 192 parent polycross was initiated with 50% of parents being southern 2,4-D resistant and 50% being a sampling of elite northern U.S. adapted red clover germplasm. Seed was harvested separately from the northern adapted and the southern 2,4-D resistant seed-parents. The northern adapted parent seed bulk (WI-2,4-D) was used for selection, while the southern 2,4-D resistant parent seed bulk was used as a control during selection. During the first two selection cycles, protocol and 2,4-D applications rates were refined. By the 3rd selection cycle a standard protocol was utilized which consisted of fully immersing 2 month old seedlings grown in greenhouse flats for three seconds in a 2,4-D solution. Seedlings were cut 1 week prior to immersion to ensure active foliage growth on plants. 2,4-D solution rates consisted initially of: 0.99 ml E99 L^{-1} H_2O; 1.96 ml E99 L^{-1} H_2O; and 3.94 ml E99 L^{-1} H_2O. These rates are 1/8, 1/4, and 1/2 of the recommended herbicide tank mix rate for our field sprayer. Selection occurred in winter in a greenhouse and survivors were intermated in isolation during the summer. By the 5th selection cycle only the 3.94 ml E99 L^{-1} H_2O rate was retained and two new rates: 5.63 ml E99 L^{-1} H_2O and 7.88 ml E99 L^{-1} H_2O were added. During winter 2013/2014 (5 cycle) 1662, 835, and 921 cycle 4 plants were immersed in 3.94 ml E99 L^{-1} H_2O, 5.63 ml E99 L^{-1} H_2O, and 7.88 ml E99 L^{-1} H_2O herbicide. Marathon (57, 28, and 30 plants per rate) and southern 2,4-D parent bulk (76, 37, and 39 plants per rate) were used as controls. Increased 2,4-D resistance was observed in selected material compared to controls especially for the 3.99 ml E99 L^{-1} H_2O and 5.63 ml E99 L^{-1} H_2O rates. Concurrent field work has been initiated to evaluate 2,4-D resistant material in a field setting using standard herbicide application rates and equipment.

References:

Taylor S.G., D.G. Shilling, and K.H. Quesenberry. 1989. In vitro selection for 2,4-D tolerance in red clover. Theor. Appl. Genet. 78:265-270.