Ecological Features of *Ambrosia* spp Seeds from Genetically Modified Soybean Fields

Asif Tanveer[1], R. Nadeem Abbas[1], Stefano Benvenuti[2], Ilaria Marotti[3], Pietro Catizone[3], Giovanni Dinelli[3]

[1]Dep. of Agronomy, University of Agriculture, Faisalabad, Pakistan, drasiftanveeruaf@hotmail.com
[2]Dep. of Agronomy and Agroecosystems Management, University of Pisa, Italy, sbenve@agr.unipi.it
[3]Dep. of Agroenvironmental Science and Technology, University of Bologna, Italy, giovanni.dinelli@unibo.it

Sole reliance on a single herbicide over a long period of time to control weeds results in weed population that are resistant to that herbicide. Glyphosate is a non- selective post- emergence herbicide that controls many annual and perennial narrow-leaved and broad-leaved weeds. *Ambrosia artemisiifolia* (annual ragweed) and *Ambrosia trifida* (giant ragweed) are important weeds which cause great damage in field crops, in particular in the USA, where glyphosate resistant biotypes have been observed in genetically modified (Roundup Ready) soybean fields. Glyphosate resistant weeds are of major economic importance all over the world because of large scale use of glyphosate. The present study was conducted to investigate seed ecology (seed morphological characteristics, dormancy, germination and emergence) of suspected resistant (SR) and susceptible (S) biotypes of *A. artemisiifolia* and *A. trifida* collected in Roundup Ready (RR) soybean fields from USA.

Methodology

Seeds of suspected resistant (SR) *A. artemisiifolia* and *A. trifida* were collected from US RR-soybean fields repeatedly treated with glyphosate. Farmers claimed an incomplete control of both biotypes and soybean yield reduction due to heavy infestation (approximately 5-20 plant m^{-2}). Seeds of susceptible (S) populations were collected from areas (never treated with glyphosate) adjacent to RR soybean fields. For each biotype the weight of 100 seeds was determined. The main morphological seed features, such as the length of major and minor axes (cm), perimeter (cm), surface area (cm^2), elongation, roundness and compactness factors, were determined by image analysis (Kodak Digital Science 2-D software). For dormancy trials, seeds were stratified in wet sterilized sand for 1, 2, 3 and 4 weeks at 4°C and germinated at 25°C (12 h light / 12 h darkness). For germination trial, seeds (stratified in wet sterilized sand for 4 weeks at 4°C) were germinated on moist filter paper in Petri dishes at 10, 15, 20 and 25°C (12 h light/ 12 h darkness). Germinated seeds (defined as cotyledon appearance) were counted daily. Germination counts were stopped when final germination percentages were reached (2-4 weeks as a function of temperature). Minimum temperatures for seed germination were calculated using the "x intercept" method previously utilized on other species (Wiese and Binning, 1987). Germinated seeds were counted and removed daily from Petri dishes. For emergence trials, pre- germinated seedlings of *A. artemisiifolia* and *A. trifida* were planted in boxes filled with sand at the depth of 1, 2, 4 and 6 cm and 1, 3, 6 and 9 cm, respectively. The boxes were placed at a constant temperature of 25 °C (12 h light / 12 h darkness). A seedling was considered as emerged when the shoot appeared from the sand surface. All the experiments were conducted according to a randomized complete design with 4 replications (each replicate of 50 seeds) and were repeated twice.

Results

The seed weight of *A. artemisiifolia* (0.43 ± 0.01 g) and *A. trifida* (5.36 ± 0.44 g) S biotypes was statistically different (P<0.05) as compared to that observed for SR biotypes of annual (0.28 ± 0.02 g) and giant ragweed (4.39 ± 0.24 g). The dimensional parameters (seed major and minor axes, perimeter and surface area) confirmed for both species that seeds were bigger in S biotypes than in SR ones (data not shown). The shape of S biotype seeds differed from that of SR biotypes, being the later more elongated and less spherical.

Seeds of SR and S biotypes of *A. artemisiifolia* exhibited a similar dormancy, while differences in seed dormancy between *A. trifida* S and SR biotypes were observed (Table 1). In particular, when exposed for 7 and 14 days at low temperature (4 °C) in a wet environment, the S biotype of *A. trifida* showed significantly more dormant seeds than SR biotype. In contrast, SR and S biotypes of *A. trifida* did not differ in seed dormancy when exposed at low temperature for time intervals of 21 and 28 days.

As regards temperature requirement for germination after seed stratification at low temperature for 4 weeks, the optimum temperature was 25°C for both S and SR biotypes of annual ragweed. In addition, in the whole range of tested temperatures, no germination differences between S and SR biotypes were observed (Table 1). Also for both S and SR biotypes of giant ragweed the optimum germination temperature was 25°C. No differences in germination between S and SR biotypes of giant ragweed were found at 20 and 25 °C, while at 10 and 15°C the germination of SR biotype was 2.65-4 times lower than S biotype (Table 1). Germination data at different

temperatures were employed to calculate the minimum (or threshold) temperature for germination: S and SR biotypes of *A. artemisiifolia* showed similar values (5.7 and 4.4°C, respectively), whereas the threshold temperature of *A. trifida* S biotype (5.4°C) was half as compared to that of SR biotype (9.4°C)

Finally, no significant differences in emergence as a function of seed depth were observed between S and SR biotype of *A. artemisiifolia:* the great proportion of emergence was observed at 1-cm seed depth, while for deeper sowing a strong reduction of emergence was found. At seed depth greater than 2 cm, no emergence occurred (Table 1). At 1- and 3-cm depth similar emergences were observed for both S and SR biotypes of *A. trifida,* while at 6-cm depth the emergence of S biotype (16.7%) was 2.5 times higher than SR biotype (6.7%). At seed depth greater than 6-cm no emergence was observed for both S and SR biotypes (Table 1).

Conclusions

The present study evidenced that seed morphological features and weight of giant and annual ragweed SR biotypes (smaller seed with elongated shape) differed from those observed for the respective S biotypes (bigger seeds of spherical shape), sampled in non-agricultural areas. Considering that SR biotypes were sampled in RR soybean fields with sod seeding management, it is plausible that small seeds have been selected for their best adaptation to this particular agro-technique (emergence from soil surface and avoidance of seed predators).

No substantial differences were observed in the seed ecology of S and SR biotypes of *A. artemisiifolia*. This evidence suggests that the failed glyphosate control under field conditions could be due to a physiological resistance phenomenon: investigations are in progress in order to verify this hypothesis. In contrast the seed ecology of *A. trifida* SR biotype was different as compared to S biotype. In particular, the seeds of SR biotype were more dormant and exhibited macro-thermal characteristics with respect to S biotype. Consequently, SR biotype of giant ragweed may avoid glyphosate control for their ecological features due to the delayed dynamic of emergence. On the other hand investigations are in progress in order to establish the respective role of physiological resistance and ecological features in determining the resistant response of this biotype towards glyphosate. On the whole, obtained results are in agreement with Di Tommaso (2004) who demonstrated that ragweed from agricultural areas evolved biotypes characterized by different seed ecology with respect to biotypes from urban areas where anthropic disturbs were less frequent.

Table 1: Seed dormancy, germination at different temperatures, threshold temperature and emergence (see "Methodology" section) of *A. artemisiifolia* (AA) and *A. trifida* (AT) S and SR biotypes. Values are means ± SD.

	AA (S)	AA (SR)	AT (S)	AT (SR)
Non-dormant seeds (%) after 7 d	14.0 ± 4.0	13.3 ± 1.2	26.0 ± 4.0	20.0 ± 2.0
Non-dormant seeds (%) after 14 d	21.3 ± 3.1	18.0 ± 2.0	27.0 ± 3.1	19.3 ± 1.2
Non-dormant seeds (%) after 21 d	30.0 ± 9.2	33.3 ± 4.2	30.0 ± 9.2	33.3 ± 4.2
Non-dormant seeds (%) after 28 d	38.7 ± 6.1	40.0 ± 5.3	44.7 ± 3.1	44.7 ± 5.0
Germination (%) at 10°C	5.3 ± 3.1	2.7 ± 1.2	10.7 ± 3.1	2.7 ± 1.2
Germination (%) at 15°C	18.0 ± 2.0	15.3 ± 2.3	24.7 ± 3.1	9.3 ± 4.2
Germination (%) at 20°C	32.0 ± 3.5	28.0 ± 4.0	37.3 ± 4.2	34.0 ± 2.0
Germination (%) at 25°C	38.7 ± 6.1	40.0 ± 5.3	44.7 ± 3.1	44.7 ± 5.0
Threshold temperature (°C)	5.7	4.4	5.4	9.4
Emergence (%) at 1-cm depth	53.3 ± 5.8	56.7 ± 5.8	86.7 ± 5.8	86.7 ± 5.8
Emergence (%) at 2-cm depth	6.7 ± 3.3	3.3 ± 5.8	nd	nd
Emergence (%) at 3-cm depth	nd	nd	50.0 ± 10.0	53.3 ± 5.8
Emergence (%) at 4-cm depth	0.0	0.0	nd	nd
Emergence (%) at 6-cm depth	0.0	0.0	16.7 ± 5.8	6.7 ± 5.8
Emergence (%) at 9-cm depth	nd	nd	0.0	0.0

nd= not detected

References

Di Tommaso A. 2004. Germination behavior of common ragweed population across a range of salinities. *Weed Sci*. 52: 1002-1009.
Wiese A.M. and Binning L.K. 1987. Calculating the threshold of temperature of development for weeds. *Weed Sci,* 35, 177−179.