Using Biological Test to Confirm Enhanced Biostimulation of Atrazine Degraders in an Atrazine Adapted Soil

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Abstract

The repeated application of xenobiotic herbicides to soils tends to cause enhanced degradation of such compounds by native soil microorganisms (biostimulation). Two field experiments were conducted in 2002 and 2003 at the Teaching and Research Farm, Federal University of Technology Akure (7 16 N, 5 12 E), Nigeria, to evaluate atrazine applied at varying rates for weed control effectiveness and phytotoxicity to *Corchorus olitorius* sown to succeed maize in rotation in an atrazine-adapted soil. The results show that atrazine at all the rates considered did not provide season-long weed control in the 12-week late maturing maize. Also, the atrazine residue from maize cropping was no longer phytotoxic to the extent of adversely affecting the growth and yield of *Corchorus olitorius*. There was no consistent correlation between increasing dose of atrazine and the growth and yield parameters of *Corchorus olitorius* in the two years. Therefore, since the atrazine did not provide a season-long weed control in an atrazine adapted soil owing to enhanced microbial degradation, it is suggested that the vegetable may be adopted as the rotational crop of choice after a maize crop in which atrazine has been used for selective weed control.

Key words: Phytotoxicity, biostimulation, atrazine adapted soil, *Corchorus olitorius*

Introduction

Biostimulation in natural environments involves providing some encouragements to microbial degraders to work by the facile addition of substrates or nutrients to the microbial habitat which consequently invigorates the biodegradation of target compounds (Singh, 2008). This explains the enhanced degradation of herbicides resulting from their repeated application. The prolonged availability of substrates through repeated application is likely to favour an acclimatization lag phase which has been reported as a period during which selection and proliferation of a small population of the indigenous degradative microorganisms occurs, as well as genetic mutation, gene rearrangement, and acquisition of genetic material by horizontal gene transfer prior to the mineralization of the herbicides (Alexander, 1994). Degradation of atrazine in soils follows first order kinetics (Radosevich *et al.,* 1995) and its complete degradation is expected to be achieved within one cropping season in fields with records of repeated atrazine application. The half-life of atrazine has been established to be 66 days (Ng *et al.*, 1995; Sorenson *et al.*, 1994) but studies in tropical soils with higher microbiological activity have however confirmed a shorter half-life due to enhanced microbial degradation of the herbicide and there are even indications that atrazine applied in humid environments persists for a much shorter period after application than in temperate climates (Akinyemiju *et al.* 1986). In Nigeria, atrazine has been observed to be less effective as it failed to provide season-long control of weeds even at above the usual field application dose of 3.0 kg a.i. ha^{-1} which is probably due to its dissipation from the soil environment with time after application (Aladesanwa and Adejoro, 2009).

The length of time that atrazine remains active in the soil has been a topic of concern as this could relate to phytotoxic after-effects that may prove injurious to succeeding crops or plantings following maize in rotation. Studies by Aladesanwa *et al.* (2001) and Aladesanwa (2005, 2007) demonstrated clearly that celosia, okra and long-fruited jute were significantly affected by atrazine residues when these vegetables were sown 12 weeks after atrazine was applied to soils in the screen house at the usual soil application dose of 3.0 kg a.i. ha^{-1}. In contrast, Akinyemiju *et al.* (1986) reported that atrazine at the foregoing rate was no longer phytotoxic to tomato seedlings under field conditions 8 weeks after application in the humid tropical environment. Similar conflicting results were also obtained by Aladesanwa and Adejoro (2009); Adejoro and Aladesanwa (2012) when amaranth and celosia were respectively sown to succeed maize on a field treated with atrazine. The present study seeks to gain an insight into the behavior of atrazine in tropical soils following repeated application as well as the effects of its residues on the growth and leaf yield of a sensitive leafy vegetable (*Corchorus olitorius*) as biological test plant sown to succeed maize in the soil.

Materials and Methods

A preliminary field trial involving application of atrazine in maize at 1.0, 2.0, 3.0 and 4.0 kg a.i. ha^{-1} including a control, where no weeding occurred during the experimental period, was laid out each in 2002 and 2003 in a randomized complete block design (RCBD) with four replications. The experiment was conducted at the Crop Experiment Station in the Teaching and Research Farm of the Federal University of Technology, Akure (7 16 N, 5 12 E) located in the rain forest vegetation zone of Nigeria. The field in both years had two years of previous atrazine application. The average annual rainfall is about 1,300mm with a mean temperature of 27 C and the climate is of the sub-humid type. The physico-chemical properties of the soil in the experimental site show the following: 310, 500, 190 g kg^{-1} of clay, silt and sand respectively, 11.1 g kg^{-1} organic carbon, 1.2 g kg^{-1} total nitrogen, 20 mg kg^{-1} available phosphorus and 0.33 cmol kg^{-1} exchangeable potassium. The pH of the soil was 6.8. Atrazine (80WP), a wettable powder formulation, was applied pre-emergence at the above rates with a knapsack sprayer fitted with Polijet nozzles calibrated to deliver 250 l ha^{-1} of the spray solution at a pressure of 2.5 kg cm^{-2}. Weed assessment was conducted twice at 4 and 12 weeks after atrazine treatment (WAT) to determine the weed species diversity and fresh weight using two fixed 50 50 cm quadrats along a diagonal in each plot from which weed samples were collected and bulked, weighed and separated into individual species.

Following maize harvest, soils from the various treated plots were filled in plastic plots and transferred to the screen house for phyto-toxicity evaluation using *Corchorus olitorius* as the test plant. The seeds of *C. olitorius* were subjected to hot water treatment to break dormancy and sown into the pots but the seedlings were later thinned to two after emergence. Watering was done at two-day intervals; no fertilizer was applied and the emerged weeds were hand-pulled from the pots. Growth parameter (plant height and number of leaves $plant^{-1}$) were taken on weekly basis beginning from the fourth week up to 8 weeks after planting. At harvest, yield parameters as well as leaf area were determined.

Data collected from the experiments in both years were subjected to an analysis of variance using the Minitab statistical package while treatment means were separated using the Tukey test. Simple linear correlation and regression analysis between increasing dose of atrazine (X) and weed fresh weight at 4 and 12 WAP as well as cumulative fresh weight of weed and growth and yield parameters (Y) of the *C. olitorius* was performed with a scientific calculator (Casio *fx*-7400G PLUS POWER GRAPHIC Model).

Results

The effects of the weeding treatments on the weight of weeds removed at 4 and 12 WAT as well as weight of the total weeds removed from individual treatment plots are shown in Fig. 1 and 2. In both years, the total weight of weed removed increased appreciably at 12 WAT in the atrazine treated plots compared to what obtained at 4 WAT. In 2002, the atrazine treated plots were not significantly different ($P < 0.05$) from one another in terms of weights of weeds removed at 12 WAT while in 2003, none of the atrazine treatment significantly reduced weight of weeds removed over the unsprayed plot at 12 WAT. The total weight of weeds removed from the weedy check plot was not significantly higher than the weight of weeds removed from atrazine treatments at 3.0 and 4.0 kg a.i. ha^{-1} in 2002 and 2003. The highest weed weights were recorded when atrazine was applied at 1.0 kg a.i. ha^{-1} at both times (4 and 12 WAT) of weed harvest both in 2002 and 2003 cropping seasons.

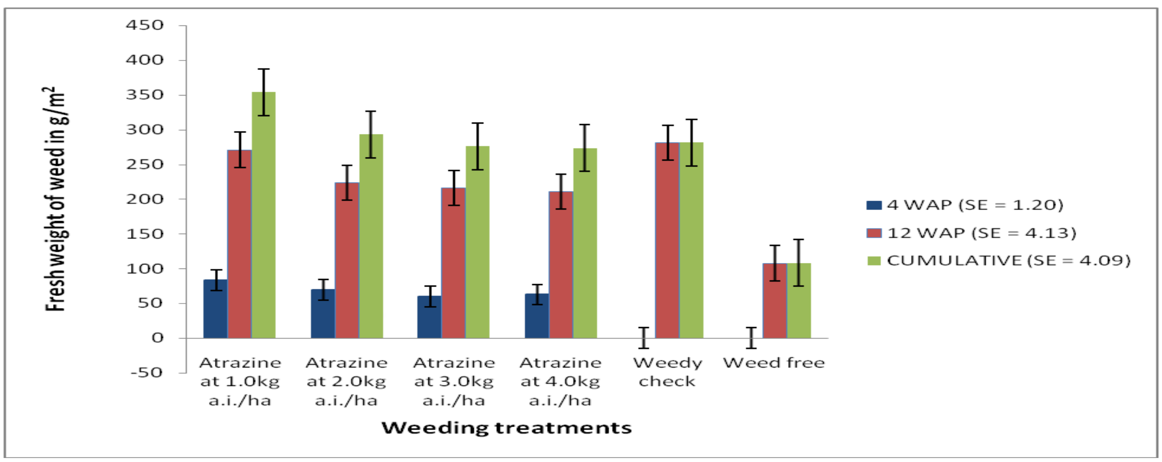

Fig. 1: Effects of atrazine on the cumulative fresh weight (g / m^2) of weeds removed in 2002
SE = Standard error

The residual effects of atrazine application on the growth and yield of long-fruited jute (*C. olitorius*) in 2002 and 2003 are presented in Tables 1 and 2 respectively. Over 90% of *C. olitorius* seed germination was recorded in all the treatment pots in both planting seasons (record not shown). In the 2002 late planting season, only the leaf area exhibited significant differences across treatments of all the growth and yield parameters considered. However, none of the atrazine treatments significantly reduced leaf area compared to the weedy check. Only atrazine application at the rate of 4.0 kg a.i ha^{-1} reduced plant height compared to the weedy check but the reduction was only 2 percent and not significant ($P<0.05$). During the 2003 early planting season, only total biomass showed significant ($P<0.05$) differences among the various weeding treatments (Table 2). Significant differences did not also occur in total biomass across the atrazine treatments

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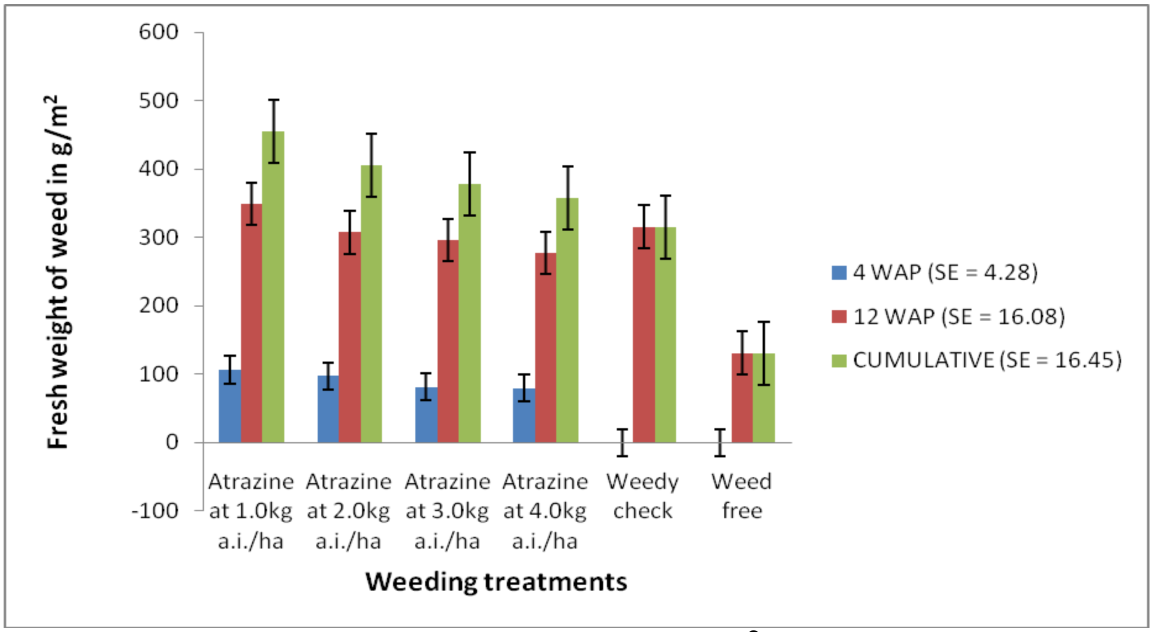

Fig. 2: Effects of atrazine on the cumulative fresh weight (g / m^2) of weeds removed in 2003
SE = Standard error

The plant height and leaf area exhibited negative relationship with increasing dose of atrazine application but marketable yield and total biomass of *C.olitorius* increased as the dose of atrazine increased in 2002 (Table 3). The regression of growth and yield parameters (Y) against increasing residual dose of atrazine (X) in 2003 showed that all the growth and yield parameters had positive correlation with increasing atrazine dose (Table 3).

Table 1: Growth and yield parameters of the *Corchorus olitorius* as influenced by atrazine residues from late season maize in 2002

Residual treatment	Plant height (cm)	Leaf area (cm^2)	Edible yield (g /m^2)	Marketable yield (g/m^2)	Total biomass (g/m^2)
Atrazine at 1 kg a.i./ ha	36.40^a	24.79^a	68.55 ^{a}	149.30^a	177.00 ^{a}
Atrazine at 2 kg a.i./ ha	38.95^a	20.53^{ab}	73.10 ^{a}	164.60^a	191.50 ^{a}
Atrazine at 3 kg a.i./ ha	37.16^a	18.32^{ab}	61.70 ^{a}	156.35^a	179.20 ^{a}
Atrazine at 4 kg a.i./ ha	35.53^a	10.96^b	68.10 ^{a}	157.00^a	181.40 ^{a}
Weedy control	36.25^a	21.20^{ab}	61.80 ^{a}	150.65^a	178.90 ^{a}
Weed-free control	39.80^a	21.11^{ab}	67.80 ^{a}	170.50^a	187.10 ^{a}
S.E. (15DF)	2.25	2.70	9.24	20.00	9.00

Means in a column followed by the same letter (s) are not significantly different according to Tukey Test (P = 0.05), SE = Standard error, ns = no significant difference

Table 2: Growth and yield parameters of the *Corchorus olitorius* as influenced by atrazine residues from early season maize in 2003

Residual treatment	Plant height (cm)	Leaf area (cm^2)	Edible yield (g /m^2)	Marketable yield (g/m^2)	Total biomass (g/m^2)
Atrazine at 1 kg a.i./ ha	50.83^a	30.54^a	188.00^a	484.20^a	549.80^{abc}
Atrazine at 2 kg a.i./ ha	48.67^a	25.99^a	194.45^a	480.30^a	535.50^{bc}
Atrazine at 3 kg a.i./ ha	44.70^a	23.23^a	222.50^a	534.20^a	618.01^{ab}
Atrazine at 4 kg a.i./ ha	47.28^a	33.17^a	255.60^a	572.40^a	716.50^{ab}
Weedy control	41.92^a	20.79^a	170.83^a	398.90^a	435.50^c
Weed-free control	49.39^a	26.71^a	254.70^a	646.10^a	724.50^a
S.E. (15DF)	4.84	4.79	48.76	146.72	39.72

Means in a column followed by the same letter (s) are not significantly different according to Tukey Test (P = 0.05), SE = Standard error, ns = no significant difference

Table 3: Linear correlation and regression analysis between increasing dose of atrazine (X) and growth or yield parameters (Y) of *C. olitorius* (n=4)

Growth and yield parameters	2002		2003	
	Correlation coefficient (r)	Regression equation	Correlation coefficient (r)	Regression equation
Plant height (cm)	-0.39	Y= 38.11-0.44X	+0.73	Y= 51.50 - 1.45X
Leaf area (cm^2)	-0.97	Y= 29.58- 4.37X	+0.15	Y= 26.95 + 0.51X
Edible yield (g/ m^2)	-0.35	Y= 71.05- 1.28X	+0.97	Y= 157.43+23.09X
Marketable yield (g/ m^2)	+0.30	Y= 153.10+ 1.49X	+0.94	Y= 438.15+31.85X
Total biomass (g/ m^2)	+0.02	Y= 182.05+ 0.09X	+0.91	Y= 459.30+58.26X

Discussion

The appreciable increase in the total weight of weeds removed at harvest in the atrazine treated plots compared to what obtained at 4 WAT suggests that the atrazine at all the rates considered did not provide season-long weed control in the 12-week late maturing maize which could be attributed to enhanced atrazine degradation resulting from two years atrazine application history of the soil under consideration. Reduced residual weed control with atrazine in s-triazine adapted soils has been confirmed under greenhouse conditions (Krutz *et al.*, 2007) while simazine cross-adaptation has been verified as the cause of reduction in herbicide residual weed control under field conditions (Krutz *et al.*, 2008a). Earlier reviews had indicated that residual atrazine activity failed to provide expected weed control in Hawaii (Shaner *et al.*, 2010) while Viator *et al.* (2000) attributed poor atrazine performance to enhanced degradation rather than weed resistance to the herbicide. Enhanced atrazine degradation has also been identified as the cause of appreciable loss of weed control in Colorado and Mississippi (Shaner and Henry, 2007; Krutz *et al.*, 2008b). Similar cases were also recorded in some sugarcane fields (Griffin *et al.*, 2000; Jones and Griffin, 2009.).

Pre-emergence atrazine application in maize at all the rates in this study appeared to be no longer phytotoxic to *C. olitorius* sown to succeed maize in rotation. This conforms with the report of Akinyemiju *et al.* (1986) that atrazine applied at the usual dose of 3.0 kg a.i. ha^{-1} was no longer phytotoxic to tomato seedlings under field conditions 8 weeks after application in the humid tropical environment. This, however, was not supported by Aladesanwa (2007) who observed significant reductions in the total leaf area, fresh weight and dry weight of *C. olitorius* plants grown in a screen house as indication of the responses to atrazine residues. Several factors could be responsible for these different observations. First, the soil used in this study had previously received two years of consecutive atrazine applications. Shaner *et al.* (2010) had observed that the soils in Hawaii soils with the most rapid degradation rate were those where the growers were dissatisfied with the residual activity of atrazine.

These Hawaiian soils degraded atrazine approximately 10-fold faster than soils that did not have a history of atrazine use. Second, the presence of maize plants in this study could also have contributed to the poor carry-over effects of atrazine on *C. olitorius* plants. Alvey and Crowley (1996) observed that planting soils with maize increased the survival of an atrazine degrading consortium and enhanced the transformation of atrazine to hydroxyatrazine. The genes and enzymes responsible for atrazine catabolism by soil bacteria have also been linked with enhanced s-triazine degradation in corn production systems (Krutz *et al.*, 2008a, b). The inconsistency in the manner with which increasing atrazine dose is related to the growth and yield parameters of *C. olitorius* between the 2 years could be attributed to seasonal variations. Walker *et al.* (1982) reported that variations in weather pattern between seasons will have a major influence on persistence because of the effects of temperature and soil moisture content on degradation rates. In general terms, the rates of degradation of many herbicides are about 20 times faster in moist soils at 25 C than in dry soil at 5 C (Walker *et al.*, 1982).

Thus a warm, moist, well-aerated, fertile soil with optimal pH will be most favourable to the microorganisms which play important roles in the breakdown of atrazine (Klingman and Ashton, 1975).The increase in the yield parameters of *C. olitorius* with increased atrazine rate in this study might be as a result of NH_4-N availability to the test plant because one of the major degradation products of atrazine is NH_4-N (Sene *et al.*, 2010). This may also serve as an indication that atrazine underwent complete degradation within the 12 weeks of application on the field.

Conclusion

The failure of atrazine at all the rates tested in this study to provide season-long weed control in maize may be attributed to enhanced atrazine degradation. It is, therefore, suggested that possible alternatives be sourced for atrazine among the triazine family. For instance, it has been reported that the non-symmetrical triazine herbicide metribuzin is not cross-adapted with atrazine. Metribuzin, therefore, could serve as a viable alternative for soils exhibiting enhanced atrazine degradation. Also, further studies should be conducted to identify other cost-effective solutions for areas experiencing enhanced atrazine degradation, most especially in the rain forest vegetation zone of southwestern Nigeria.

References

Adejoro S. A, Aladesanwa R. D. 2012. Employment of chemical test and bioassay to evaluate soil residual activity of atrazine in southwestern Nigeria. Applied Tropical Agriculture.Vol 7: 1 and 2

Akinyemiju, O. A, Ogunyemi S. O, Ojo I. O. 1986. Persistence of atrazine in a humid tropical soil. Nigerian journal of agronomy 1 (1), 22-25.

Aladesanwa, R.D., Adenawoola, A.R., and Olowolafe, O.G. 2001. Effects of atrazine residue on the growth and development of celosia (Celosia argentea) under screenhouse conditions in Nigeria. Crop Protection: Vol 20:4. Pp. 321 – 324(4)

Aladesanwa, R.D. 2005. Screenhouse evaluation of atrazine for soil residual activity on growth, development and nutritional quality of okra (Abelmoschus esculentus Moench) in Southwestern Nigeria. Crop Protection 24, 927-931.

Aladesanwa, R. D. 2007. Effects of atrazine residue and poultry manure on the growth and development of long-fruited jute (Corchorus olitorius L.) under screenhouse conditions in southwestern Nigeria. Bulletin of Science Association of Nigeria Vol. 28 (2007) 64- 67

Aladesanwa, R. D. and Adejoro, S. A. 2009. Weed control in maize Zea mays L.) with atrazine and its soil residual activity on the growth and yield of amaranth (Amaranthus cruentus (L.) Sauer) in Southwestern Nigeria. Weed: Management, Economic Impacts and Biology: Nova science Publishers, Inc. New York. Pp 87-99.

Alexander, M. 1994. Acclimation, in *Biodegradation and Bioremediation*, (ed: Singh A. and Ward O. P) pp. 16–40, Academic Press, San Diego, Calif, USA

Alvey , S., Crowley, D.E. 1996 Survival and activity of atrazine mineralizing bacterial consortium in rhizosphere soil. Environ Sci Technol 30:1596–1603

Griffin, J. L., Viato, B. J.and Ellis, J. M. 2000. Tie- vine (morningglory) control at layby. Sugar Bull. 78:23-35.

Jones, C. A., and Griffin, J. L. 2009. Red morningglory (*Ipomoea coccinea*) control and competition in sugarcane. J. Amer. Soc. Sugar Cane Technol. 29:25-35.

Klingman, G.C. & Ashton, F.M. 1975. *Weed Science: Principles and Practices*, Wiley, New York.

Krutz, L. J., Zablotowicz, R. M., Reddy, K. N., Koger, C. H., and Weaver, M. A. 2007. Enhanced degradation of atrazine under field conditions correlates with a loss of weed control in the glasshouse. Pest. Manag. Sci. 63: 23-31

Krutz, L. J., Shaner, D. L. Accinelli, C. Zablotowicz, R. M. and Henry, W. B. 2008a. Atrazine dissipation in s-triazine-adapted and nonadapted soil from Colorado and Mississippi: Implications of enhanced degradation on atrazine fate and transport parameters. J. Environ. Qual. 37:848–857.

Krutz, L. J., Burke, I. C. Reddy, K. N.and Zablotowicz, R. M. 2008b. Evidence for cross-adaptation between s-triazine herbicides resulting in reduced efficacy under field conditions. Pest. Manag. Sci. 64:1024–1030.

Ng, H.Y.F., Gaynor, J. D. Tan, C. S. and Drury, C. F. 1995. Dissipation and loss of atrazine and metolachlor in surface and subsurface drain water: a case study. Water Res. 29:2309-2317.

Radosevich, M., Traina, S.J, Hao, Y. and Tuovinen, O.H. 1995. Degradation and Mineralization of Atrazine by a Soil Bacterial Isolate Applied and environmental microbiology, Jan. 1995, p. 297–302 Vol. 61, No. 1

Sene, L., Converti, A., Secchi, G. A. R. and Simao, R. C. G. 2010. New aspects on atrazine biodegradation. *Brazilian Archives of Biology and Technology. 53(2):487-496.*

Shaner, D. and Henry, W. B. 2007. Field history and dissipation of atrazine and metolachlor in Colorado. J. Environ. Qual. 36:128–134.

Shaner, D. L., Krutz, L. J., Henry, W. B. Hanson B. D., Poteet, M. D.and Rainbolt, C. R. 2010. Sugarcane soils exhibit enhanced atrazine degradation and Cross adaptation to other atrazine: *Journal of American Society of Sugar Cane Technologists*, Vol. 30: 1-10

Singh, D. K. 2008. Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments, *IndianJournal of Microbiology*, vol. 48, no. 1, pp. 35–40.

Sorenson, B. A., Koskinen, W. C., Buhler, D. D., Wyse, D. L., Lueschen, W. E.and Morgan, M. D. 1994. Formation and movement of 14C-atrazine degradation products in a clay loam soil in the field. Weed Sci. 42:618–624.

Viator, B. J., Griffin, J. L. and Richard, Jr. E. P. 2002. Evaluation of red morningglory (*Ipomoea coccinea)* for potential atrazine resistance. *Weed Techno*l. 16:96–101.

Walker, A., Briggs, G.G., Greaves, M.P., Hance, R.J. & Thompson, A.R. 1982. Herbicides in soil. In H.A.Roberts (Ed.), *Weed Control Handbook*: *Principles* (7^{th} edition, pp.87-105). Oxford, Blackwell Scientific Publications

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