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Interaction Effects of Permethrin and Atrazine Combinations Towards Several Nontarget Microorganisms

Glenn W. Stratton

Environmental Microbiology Laboratory, Department of Biology, Nova Scotia Agricultural College, Truro, Nova Scotia, Canada B2N 5E3

Studies on the toxicity of pesticides towards non-target organisms are essential in accurately evaluating the potential environmental impact of these compounds. Of particular importance are effects on non-target microorganisms, since they perform a vital function in the maintenance of all biospheric nutrient cycles. Although these concerns have been the subject of numerous scientific reports, most research has dealt only with individual pesticides, and little data are available on pesticide combinations (STRATTON & CORKE 1982a,b).

Pesticide interaction studies are important for several reasons. For example, the problem of pesticide resistance has elicited a more widespread use of pesticide mixtures. Also, agricultural pesticides are rarely found alone in the environment, but are in association with other pesticides, various organic and inorganic chemicals, and other xenobiotics, such as carrier solvents, degradation products, and heavy metals (STRATTON & CORKE 1982b). However, only a few data have been published on pesticide interactions towards non-target microorganisms such as algae (LOEPPKY & TWEEDY 1969; MOSSER et al. 1974), bacteria (NAYAK & RAO 1982), and fungi (ROSLYCKY 1977).

The present study supplies detailed interaction data for combinations of atrazine (2-chloro-4-ethylamino-6-isopropylamino -1,3,5-triazine) and permethrin (3-phenoxybenzyl-(1RS)-cis,trans -3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate) when tested towards selected soil microorganisms. Atrazine is the most heavily used pesticide in the United States (DENOYELLES et al. 1982) and its residues are widely distributed in both terrestrial (CAREY et al. 1979) and aquatic (WU 1981) ecosystems. Permethrin is an important insecticide with expanding agricultural potential (ELLIOTT & JANES 1978) that is presently being evaluated for its environmental impact (STRATTON & CORKE 1982a,b). The bioassay organisms and parameters chosen included growth of the fungi Pythium ultimum and Trichoderma viride, and growth, photosynthesis and nitrogenase activity of the cyanobacterium Anabaena inaequalis. All three organisms have been used previously in toxicant interaction studies (STRATTON & CORKE 1979, 1982a,b).

MATERIALS AND METHODS

The fungi Pythium ultimum and Trichoderma viride, and the cyanobacterium Anabaena inaequalis were used as test organisms, as described elsewhere (STRATTON & CORKE 1982a,b). The fungi were maintained on Difco Potato Dextrose Agar at 25°C. The cyanobacterium was cultured in a liquid nitrogen-free medium (STRATTON et al. 1979) at a temperature of 22°C and a light intensity of 7000 lux on a 12 h light-dark cycle.

The test chemicals used were a pyrethroid insecticide, permethrin (technical grade; Chipman Chemicals Ltd., Stoney Creek, Ontario, Canada; 86.6% pure; 40:60 mixture of the cis and trans isomers) and an s-triazine herbicide, atrazine (technical grade; Ciba-Geigy of Canada Ltd., Cambridge, Ontario, Canada; >95% pure). Stock solutions of these pesticides were prepared in pesticide grade acetone (Caledon Laboratories, Georgetown, Ontario, Canada). A total concentration of 0.1% (v/v) acetone was used in all bioassay experiments, as determined using the solvent-pesticide interaction analysis technique (STRATTON et al. 1982). All the treatments outlined below were replicated five to ten times and all pesticide concentrations refer to the active ingredient.

The toxicity of permethrin-atrazine combinations was determined using a poisoned agar technique, as outlined previously (STRATTON & CORKE 1982b). Pesticide solutions were added to molten Potato Dextrose Agar (50°C), mixed for two minutes on a rotary shaker and the agar dispensed as 10 ml aliquots into petri dishes. The agar was inoculated with mycelial discs taken from the margin of fresh stock cultures and the plates incubated at 25°C until control growth reached a diameter of 50-70 mm. Growth was recorded as net colony diameter and percent inhibition calculated relative to growth on control plates (solvent only). Interaction data were analyzed as outlined below. Both permethrin and atrazine were used at levels of 0 and 100 ppm (ug per ml).

Growth of Anabaena inaequalis was assessed by measuring the absorbance of cultures with time, using a Bausch and Lomb Spectronic 20 spectrophotometer and a wavelength of 600 nm, as described elsewhere (STRATTON & CORKE 1982a). Test tubes containing 9.5 ml of nitrogen-free medium (STRATTON et al. 1979) and 0.1 ml of test chemical(s) were inoculated with 0.5 ml of an active culture containing 6.5×10^4 cells per ml, and incubated in racks inclined on a 45° angle, as outlined above. Optical densities (cell yield) were determined for 14 days and percent inhibition values calculated relative to growth in control systems (solvent only). Permethrin levels of 0, 0.5, 1.0, 2.0, and 3.0 ppm, and atrazine concentrations of 0, 0.01, 0.025, 0.05, 0.075, and 0.1 ppm, were interacted in all possible combinations and the data analyzed as outlined below.

Photosynthesis in A. inaequalis was assayed by following the uptake of $^{14}CO_2$ from $NaH^{14}CO_3$ (Amersham/Searle, Oakville, Ontario, Canada), as described previously (STRATTON et al. 1979). Each test

system contained 6.5×10^4 cells per ml, 0.1 uCi of radioactivity per ml, 0.1% (v/v) acetone, permethrin (0, 50, or 100 ppm) and/or atrazine (0, 0.05, 0.1, or 0.2 ppm). Percent inhibition values were calculated relative to activity in control systems (solvent only) and the interaction data analyzed as outlined below.

Nitrogenase activity in A. inaequalis was monitored using the acetylene reduction technique and gas chromatography, as described previously (STRATTON et al. 1979). Test systems contained 6.5×10^4 cells per ml, 0.1% (v/v) acetone, 10% acetylene in the headspace, permethrin (0 or 100 ppm) and/or atrazine (0, 50, or 100 ppm). Percent inhibition values were calculated relative to activity in control systems (solvent only) and the interaction data analyzed as outlined below.

Interaction data for permethrin and atrazine combinations were analyzed by the multiplicative survival model (GOWING 1960; MORSE 1978). This involved mathematically calculating the theoretical expected additive inhibition of a mixture of toxicants, using percent inhibition data obtained for each component toxicant tested individually, using the formula:

$$E = X + ((100 - X) / 100) \times Y$$

E = the expected additive effect of the mixture
X = the % inhibition due to component A alone
Y = the % inhibition due to component B alone

The expected inhibition was then statistically compared with the actual inhibition obtained experimentally for that mixture (Student's T test at $P = 0.05$). Synergism and antagonism were defined as an actual experimental toxicity significantly greater or less than expected, respectively, while an additive effect occurred when the actual and expected inhibitions did not differ significantly (GOWING 1960; STRATTON & CORKE 1982a,b).

RESULTS AND DISCUSSION

Interaction conclusions for combinations of permethrin and atrazine are summarized in Tables 1 to 3. Of twenty pesticide combinations tested, all but three interacted in an additive manner when tested towards growth in A. inaequalis (Table 1). These three exceptions gave an antagonistic response. Permethrin and atrazine also interacted in an additive manner when tested towards both photosynthesis (Table 2) and nitrogenase activity (Table 3) in A. inaequalis. With these two bioassay criteria all pesticide combinations tested yielded a uniform interaction response.

Results for the fungi P. ultimum and T. viride differed from those obtained with A. inaequalis. Permethrin and atrazine interacted antagonistically towards the growth of T. viride, but synergistically with P. ultimum (Table 3).

The insecticide permethrin and the herbicide atrazine interacted additively towards growth, photosynthesis, and nitrogenase activity in A. inaequalis, synergistically towards growth of P.

Table 1. Effect of permethrin-atrazine combinations on the growth of A. inaequalis.[a]

Atrazine concn (ppm)	Permethrin concn (ppm) 0	0.5	1.0	2.0	3.0
0	control	44.0±10.0[b]	52.2±8.6[b]	60.1±8.3[b]	66.0±6.6[b]
0.010	2.6±4.5[b]	50.0±6.6 (45.5±4.8)[c]	50.3±7.7 (53.4±5.0)[c]	56.0±7.0 (61.1±5.6)[c]	60.4±8.6 (66.9±3.2)[c]
0.025	6.9±5.5[b]	38.1±2.4 (47.9±6.0)[d]	52.1±3.8 (55.5±4.9)[c]	63.1±2.3 (62.9±4.5)[c]	66.7±7.2 (68.4±2.8)[c]
0.050	17.3±5.2[b]	51.2±6.5 (53.7±7.1)[c]	58.2±5.4 (60.5±5.9)[c]	69.0±4.1 (67.0±4.3)[d]	69.9±3.1 (71.9±4.8)[c]
0.075	35.7±5.7[b]	59.9±7.1 (64.0±5.3)[c]	59.2±4.1 (69.3±3.5)[d]	67.0±2.1 (74.3±5.1)[d]	79.2±3.3 (78.1±4.0)[c]
0.100	58.7±5.6[b]	72.4±5.6 (76.9±5.2)[c]	78.0±7.1 (80.3±4.3)[c]	79.9±7.7 (83.5±3.7)[c]	83.2±3.5 (86.0±3.9)[c]

[a] Table entries for each combination are the actual % inhibition ± the standard deviation, accompanied in parentheses by the expected % inhibition ± the expected standard deviation, calculated by the method of GOWING (1960).

[b] These are the individual % inhibition values used to calculate expected additive effects according to the formula of GOWING (1960).

[c] Additive interaction; actual and expected inhibitions do not differ significantly at P=0.05.

[d] Antagonistic interaction; actual inhibition significantly less than that expected at P=0.05.

Table 2. Effect of permethrin-atrazine combinations on photosynthesis in A. inaequalis.[a]

Atrazine conc (ppm)	Permethrin concn (ppm) 0	50	100
0	control	-19.3±7.8[b]	-25.7±11.8[b]
0.05	38.3±4.9[b]	29.2±6.9 (26.4±6.2)[c]	24.0±4.2 (22.4±8.3)[c]
0.10	59.9±3.7[b]	55.3±7.2 (52.2±4.3)[c]	52.8±5.0 (49.5±5.8)[c]
0.20	77.3±0.3[b]	70.7±4.1 (72.9±2.2)[c]	70.2±3.1 (71.4±2.3)[c]

[a,b,c]Refer to TABLE 1 for footnotes.

Table 3. Effect of permethrin-atrazine combinations on nitrogenase activity in A. inaequalis and growth in P. ultimum and T. viride.[a]

Permethrin concn (ppm)	Atrazine concn (ppm) 0	50	100
A. inaequalis			
0	control	46.7±12.1[b]	60.2±13.9[b]
100	-20.9±12.8[b]	28.3±10.2 (35.6±8.1)[c]	47.5±11.1 (51.9±7.3)[c]
P. ultimum			
0	control[b]	-	9.7±2.4[b]
100	1.0±2.4[b]	-	33.9±4.1 (10.7±2.7)[e]
T. viride			
0	control[b]	-	21.1±0.9[b]
100	9.6±1.2[b]	-	23.1±2.1 (28.6±0.7)[d]

[a,b,c,d]Refer to TABLE 1 for footnotes.
[e]Synergistic interaction; actual inhibition significantly greater than that expected at P=0.05.

ultimum, and antagonistically towards growth of T. viride (Tables 1, 2, 3). Few other data are available on the effects of pesticide combinations towards non-target microorganisms. For example, the herbicides atrazine and metobromuron interact synergistically towards growth of the green alga Chlamydomonas reinhardtii, with the effect being more pronounced at relatively low pesticide concentrations (LOEPPKY & TWEEDY 1969). The herbicide paraquat, either alone or in combination with linuron, diuron, atrazine, or simazine, has little effect on populations of bacteria, actinomycetes, and fungi in soil, and only transient effects on soil respiration (ROSLYCKY 1977). The interactions were not classified in the latter study. Combinations of DDT and PCBs interact antagonistically towards growth of the diatom Thalassiosira pseudonana, where DDT actually counteracts toxic effects of the PCBs (MOSSER et al. 1974). Various mixtures of selected fungicides, insecticides, and herbicides can synergize the stimulatory effects noted when these compounds are tested singly against nitrogen fixation in paddy soils (NAYAK & RAO 1982). The only exception is with combinations containing diazinon, where the stimulatory effects of component pesticides are actually reduced. No mathematical model was used to statistically analyze for synergism in that study (NAYAK & RAO 1982).

The difference in interaction response noted for permethrin-atrazine combinations with A. inaequalis, P. ultimum, and T. viride are difficult to explain. The two compounds interacted independently in the cyanobacterium, which indicates that they probably have different modes of action. Atrazine is a photosynthetic inhibitor and these data suggest that permethrin acts on some cellular process other than photosynthesis, although more research is required before definitive conclusions can be made regarding the site of permethrin toxicity in protists.

Permethrin and atrazine interacted in some complex manner with the fungi, since a species-dependent response pattern resulted. In order to identify the possible sites of permethrin-atrazine interaction, the mechanism of toxicity must be known for each component in the mixture. However, this information is unavailable for fungi. Possible explanations include interaction at adsorption sites on the cell wall, or at systems involved in the transport of these pesticides into the cell. More research is required into these aspects of toxicant interactions.

All three response patterns observed here are environmentally significant (STRATTON & CORKE 1982a,b). With additive interactions the individual effects of component toxicants are neither enhanced nor reduced, but the overall toxicity of the mixture is still greater than that of either compound alone. With synergistic and antagonistic interactions, the uptake or toxicity of individual components of a mixture is enhanced or reduced, respectively, resulting in a significant modification of toxicity patterns. Specifically, if permethrin and atrazine are present in the same system they could interact to alter their toxicity patterns

towards non-target microorganisms, and thereby modify their respective environmental impact.

Although the studies presented here deal with pure cultures of microorganisms tested *in vitro*, and although it may be difficult to extrapolate these results to the natural environment, the results identify significant deficiencies in information regarding the toxicity patterns of pesticide combinations. More research is required in this area before the *in situ* effects of pesticides can be accurately predicted.

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