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Interactions between the Solvent Acetone and the Pyrethroid Insecticide Permethrin on Activities of the Blue-Green Alga *Anabaena*

Glenn W. Stratton, Robert E. Burrell, M. Lynn Kurp, and Charles T. Corke

Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

The effect of pesticides on non-target organisms is an area of intensive research. Since many pesticides have low water solubility, they are often dissolved in an organic solvent prior to addition into experimental systems. The use of solvents in biological experiments necessitates the inclusion of solvent controls and the adaptation of a standard solvent concentration for routine use. However, there is little consensus among researchers as to which level of solvent is suitable for any given kind of experiment and little is known about the effects of solvents on biological systems. The possibility that the solvent interacts with the test compound is ignored. It is possible that one level of solvent may affect the overall response elicited by a chemical, while another concentration of the same solvent would not.

Acetone, a common solvent, is known to inhibit the replication of vaccinia and rabbitpox viruses (GHENDON and SAMOILOVA 1968, CHERNOS et al. 1972), the radial growth of fungi (R.E. BURRELL and C.T. CORKE, submitted paper), and is lethal to rainbow trout (MAJEWSKI et al. 1978). Acetone can both stimulate, and inhibit, the production of aflatoxins in Aspergillus, depending on the concentration used (BENNETT et al. 1976). Acetone was found to have no significant effect on wood-rotting fungi (BEZEMER et al. 1973).

In this study the effect of acetone on photosynthesis and nitrogen fixation by three species of the blue-green alga, Anabaena, and the interactions of acetone on the toxicity of the pyrethroid insecticide, permethrin, (3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate) were studied. Anabaena is an important freshwater blue-green alga (FOGG 1971).

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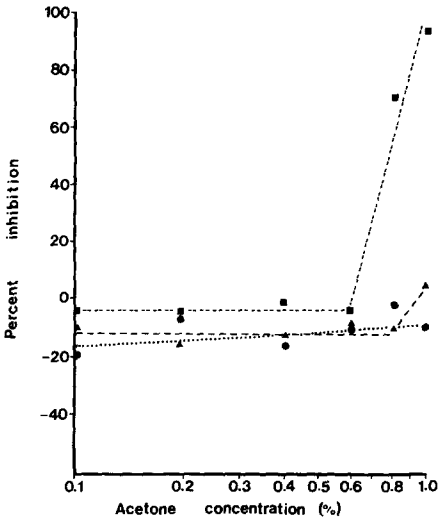

Fig. 1. The effect of acetone on $^{14}CO_2$ uptake by Anabaena. Symbols: (●) A. inaequalis, (■) A. cylindrica, (▲) A. variabilis. A negative percent inhibition is equivalent to percent stimulation.

MATERIALS AND METHODS

Anabaena inaequalis is fully described elsewhere (STRATTON et al. 1979). A. cylindrica and A. variabilis were obtained from the American Type Culture Collection (ATCC 27899 and 27892, respectively). The cultures were maintained in a liquid, inorganic nitrogen-free medium (STRATTON and CORKE 1979). All culture flasks were incubated at a temperature of 22° C and a light intensity of 7000 lux.

Photosynthesis was studied by following the uptake of $^{14}CO_2$ from $NaH^{14}CO_3$ (Amersham Corporation, Oakville, Ontario, Canada). Tissue culture flasks were used in replicates of 5, each containing 9.8 ml of cells, 0.1 ml of radioisotope and from 0.01 to 0.1 ml of acetone, or acetone plus permethrin. The final volume of mixture contained 6.5 x 10^4 cells and 0.2 µCi of radioactivity per ml. The cells were incubated for 2 hours and harvested by filtration through 0.45 µ membrane filters. The amount of radioactivity incorporated into the cells was determined using a liquid scintillation counter (STRATTON and CORKE 1979). Counts were corrected for % counting efficiency and those obtained for dark-incubated cells were subtracted from values obtained for corresponding light-incubated systems. Percent inhibition was calculated from these corrected counts.

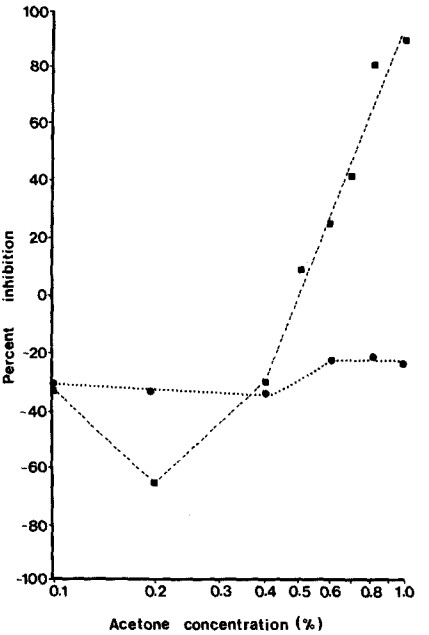

Fig. 2. The effect of acetone on acetylene reduction by Anabaena. Symbols: (●) A. inaequalis, (■) A. cylindrica. A negative percent inhibition is equivalent to percent stimulation.

Nitrogen fixation was assayed using the acetylene reduction technique (STRATTON and CORKE 1979). Tissue culture flasks were used in replicates of 5, and each contained 9.9 ml of cells and from 0.01 to 0.1 ml of acetone, or acetone plus permethrin. The final volume of mixture contained 6.5×10^4 cells per ml. After the addition of a 10% atmosphere of acetylene, the cells were incubated for 5 hours and the ethylene produced was assayed by gas chromatography. The data were expressed as nmoles of ethylene produced per 10^5 cells and percent inhibition was determined using the appropriate control. A. variabilis was not included in these studies due to its inability to fix nitrogen.

Throughout this communication the term significance refers to conclusions made following a Dunnett's test for a comparison of means at $\alpha = 0.05$ (WINER 1971).

RESULTS AND DISCUSSION

The effects of acetone on the photosynthetic activity of Anabaena inaequalis, A. variabilis, and A. cylindrica are outlined in Fig. 1. The activity of A. inaequalis was significantly altered only at acetone concentrations of 0.1 and 0.4% (v/v),

where stimulation was observed. Photosynthesis in A. variabilis was significantly stimulated by acetone concentrations below 1%. No significant stimulation of $^{14}CO_2$ uptake occurred with A. cylindrica, although inhibition was observed above 0.6% acetone. Inhibition was 75% at 0.8% and 95% at 1.0% acetone.

Levels of acetone < 0.6% are also known to stimulate the production of aflatoxins and certain pigments by Aspergillus parasiticus, while levels greater than 0.6% become inhibitory (BENNETT et al. 1976). Acetone causes a severe disruption in the intracellular ultrastructure and membrane integrity of Chlorella pyrenoidosa at levels approaching 3.33% (PARASHER et al. 1978), which could lead to an increase in the cells' permeability. Due to possible acetone-induced membrane damage and the possibility of increased permeability, Anabaena might be able to accumulate more $^{14}CO_2$, resulting in an increase in measured photosynthetic activity. Microscopic examination of A. cylindrica, after exposure to acetone concentrations greater than 0.6%, showed intracellular damage and cell lysis. Severe membrane damage and a subsequent disruption of photosystems would probably be involved.

Data for the effect of acetone on acetylene reduction are outlined in Fig. 2. The activity of A. inaequalis was stimulated by all acetone concentrations from 0.1 to 1%. The degree of stimulation was greater than that observed in photosynthetic studies. Acetylene reduction in A. cylindrica was observed to increase significantly at levels of acetone < 0.4% and decrease significantly at levels >0.5%. Greater than 95% inhibition was observed at 1% acetone, which is similar to the inhibition noted with $^{14}CO_2$ uptake. These results may be due to acetone-induced membrane damage. The inhibition of acetylene reduction by A. cylindrica, which occurs at a lower level of acetone than does the disruption of photosynthesis (0.4% vs 0.6%), may be due to the oxygen sensitivity of nitrogenase. Although less evidence of membrane damage was observed microscopically at 0.4 than at 0.6% acetone, it is possible that there was some increase in membrane permeability allowing more oxygen to enter the heterocysts, thereby inhibiting the oxygen-labile nitrogenase enzyme complex. Such a phenomenon does occur during the Hg^{2+}-induced lysis of Anabaena (STRATTON et al. 1979). Photosystems are not oxygen-labile and probably would not be disrupted until more severe membrane damage occurred.

Solvents alter the apparent toxicity of insecticides towards target organisms (DALELA et al. 1979), and acetone can interact with fungicides to alter the measured toxicity towards the mycelial growth of fungi (R.E. BURRELL and C.T. CORKE, submitted paper). Acetone interactions were also observed with Anabaena and the pyrethroid insecticide, permethrin, using both acetylene reduction and photosynthesis as assay criteria. These data are summarized in Fig. 3 and 4. The cultures were treated with varying concentrations of acetone, and the same concentrations of solvent in combination with 100 ppm (µg/ml) permethrin. Figures

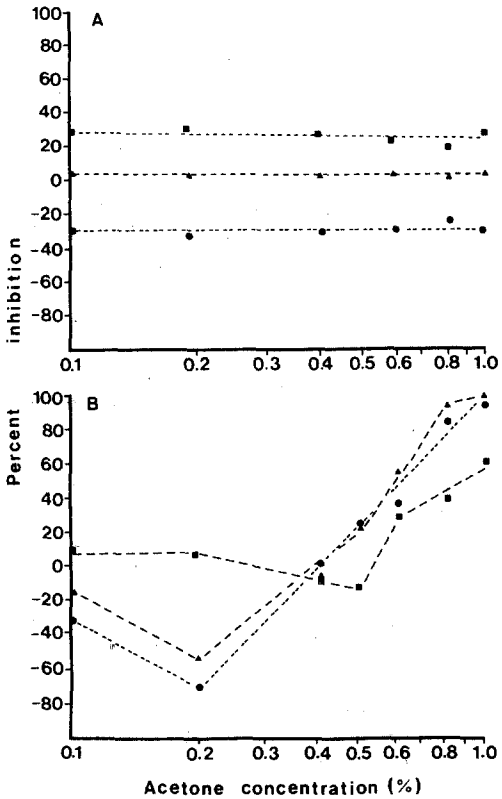

Fig. 3. Interaction of acetone and permethrin on acetylene reduction. (A) A. inaequalis, (B) A. cylindrica. Symbols: (●) percent inhibition due to acetone, based upon a control containing no solvent, (▲) percent inhibition due to the acetone plus permethrin treatments, based upon a control containing no solvent, and (■) percent inhibition due to permethrin, calculated from the acetone plus permethrin systems using the appropriate acetone controls. A negative percent inhibition is equivalent to percent stimulation.

3 and 4 contain three lines for each alga. One line (●) is the percent inhibition due to acetone, based on a control containing no solvent addition. Another line (▲) is the inhibition due to the acetone plus permethrin treatments, again based on a control containing no solvent addition. The last line (■) is the effect due solely to permethrin, which is derived by calculating the percent inhibition in the permethrin plus acetone system, based on that system containing the same level of acetone alone

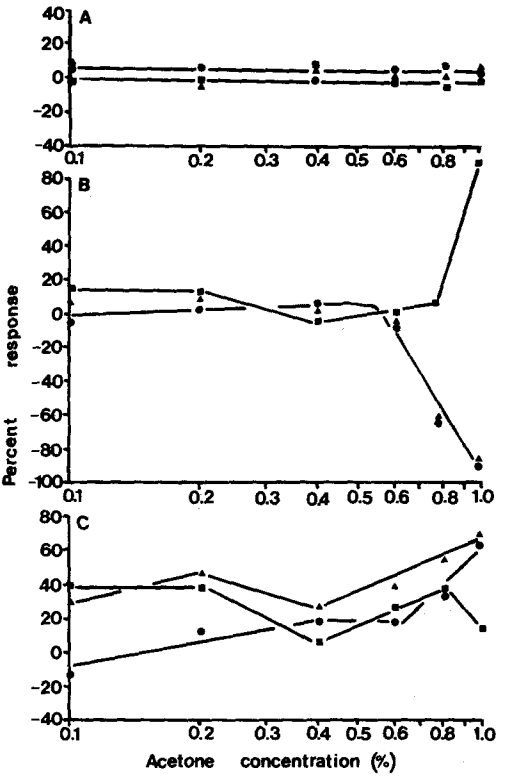

Fig. 4. Interaction of acetone and permethrin on photosynthesis. (A) A. inaequalis, (B) A. cylindrica, (C) A. variabilis. Symbols: as per Fig. 3. The response (Y axis) is expressed as percent stimulation for A. cylindrica and percent inhibition for both A. inaequalis and A. variabilis.

(acetone control). For example, the percent inhibition due to permethrin in the permethrin plus 0.2% acetone system was calculated using the 0.2% acetone control. This was repeated for all acetone concentrations. Theoretically, the line derived in this manner should be horizontal, since all systems contained the same concentration of permethrin (100 ppm) and all inhibitions were calculated using the appropriate acetone control. This expected horizontal line would be indicative of two systems interacting in a strictly additive manner. In this type of plot, antagonism is indicated by a shift of the calculated curve away from the expected horizontal line and towards the X axis, and synergism by a shift of this line away from the X axis. This has been

verified using theoretical data and the Gowing equation (GOWING 1960) which was developed to evaluate herbicide interactions. However, the response differs when the expected inhibition is negative; i.e. a stimulation is observed. In this case all data should be plotted as percent stimulation and the calculated line can then be analyzed as outlined above.

The above additive response is precisely what occurred for A. inaequalis with both acetylene reduction (Fig. 3A) and photosynthesis (Fig. 4A). Consequently, the inhibitory effect due to permethrin towards A. inaequalis, calculated from acetone control systems, would be a true indication of its toxicity over the range of 0.1% to 1.0% acetone.

With A. cylindrica acetone and permethrin interacted in a more complex manner. For acetylene reduction (Fig. 3B) acetone and permethrin interacted in an additive manner at acetone levels of 0.1 and 0.2%, but showed significant antagonism at 0.4 and 0.5%, and significant synergism at 0.6%, and greater. For photosynthesis (Fig. 4B), the same pattern was observed. The solvent and pesticide interacted in an additive manner at 0.1, 0.2, and 0.8% acetone and showed significant antagonism at 0.4 and 0.6% and significant synergism above 0.8% acetone. With A. cylindrica, percent inhibition calculations for permethrin are only a measure of inherent toxicity when acetone is used at a concentration less than 0.2%. At 1%, which is a commonly used solvent concentration, acetone and permethrin interact synergistically to increase the apparent toxicity of permethrin. This effect is more pronounced when one considers the concentration of permethrin required to cause a 50% inhibition of acetylene reduction (EC_{50}) in A. cylindrica. Detailed permethrin toxicity screenings were performed utilizing both 0.1% and 1.0% acetone. At 0.1% acetone, the EC_{50} of permethrin was >100 ppm, while at 1.0% acetone the EC_{50} was only 5 ppm. Consequently, by using 1% acetone, permethrin is erroneously concluded to be over 20 times more toxic than it actually is, due to a synergistic interaction between the pesticide and the solvent.

No data are available for A. variabilis and acetylene reduction due to this strain's inability to fix nitrogen. However, with photosynthesis (Fig. 4C) acetone and permethrin interacted in an additive manner at 0.1, 0.2, and 0.8% and induced significant antagonism at all other acetone levels assayed. Therefore, with A. variabilis a solvent concentration of 0.1-0.2% should be used in assaying permethrin toxicity.

These data illustrate three considerations which must be taken into account when dealing with the environmental toxicity testing of water-insoluble pesticides. Firstly, solvents may be extremely detrimental to a particular organism and concentrations as low as possible should be maintained in any toxicity screening test. Secondly, the solvent sensitivity of each organism should be determined, because of a possible variation in species response, as shown in this study (Fig. 1 and 2). Thirdly, solvents, even at

relatively low concentrations, can interact with the test compound to give an apparent toxicity which may not be close to the true toxicity. A study similar to that outlined for determining the proper level of solvent to use in any given experiment must be performed for every combination of culture, solvent and test compound to ensure that the toxicities obtained are those of the compound itself, and not due to interactions with the solvent.

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