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Medium Composition and Its Influence on Solvent-Pesticide Interactions in Laboratory Bioassays

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Xenobiotic interactions is an area of ecotoxicology research that is often avoided. However, the increased awareness that a pollutant is present in the environment in association with other xenobiotics, degradation products, and various naturally occurring organic and inorganic compounds, has emphasized the need for interaction studies (Nat. Res. Council 1982). One area of concern deals with toxic effects of pesticides and pesticide combinations. These studies include laboratory bioassays to evaluate the effects of pesticides on both invertebrate and microbial systems. However, many pesticides are relatively insoluble in water and must be dissolved in an organic solvent prior to addition into experimental systems. Solvents are xenobiotics and have the potential to interact with pesticides in bioassays, leading to erroneous conclusions regarding toxicity (Stratton et al. 1980). This potential error must be eliminated from bioassays, but few researchers consider this problem since the role of solvents in these responses has been documented and studied only recently. For example, it has been reported that the choice of solvent can alter the toxicity of selected pesticides up to 6-fold with fungi (Stratton et al. 1982), 7-fold with freshwater teleosts (Dalela et al. 1979), and 190-fold with aquatic arthropods (Bowman et al. 1981).

Stratton et al. (1982) outlined a method for identifying and analyzing solvent-pesticide interactions in bioassays. This is a useful procedure which can be used in bioassays to choose a solvent and solvent concentration that yield a noninterfering, additive response between a solvent and pesticide. These general principles can also be applied to the analysis of other toxicant interactions, simply by substituting another xenobiotic for the solvent, or to organismal interactions (Burrell et al. 1985). However, before this technique becomes routinely used with solvent-pesticide combinations, or is applied to the above interaction problems, further data are required on the influence of various experimental parameters on the interaction conclusions obtained. Some of the variables that must be considered in the standardization of this procedure include solvent type, pH, temperature, and medium composition. The purpose of the present study is to document the effects of medium composition on acetone-captan interactions towards selected soil fungi.

MATERIALS AND METHODS

Fungal cultures of Pythium ultimum, Sclerotinia homeocarpa, and Pestalotia sp. were obtained from the Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada, and maintained on Potato Dextrose Agar (Difco Laboratories, Detroit, Mn, U.S.A.; pH 5.6 ± 0.5). The fungicide captan (3a,4,7,7a- tetrahydro- 2((trichloromethylthio))- 1H-isoindole- 1,3(2H)-dione, technical grade, 96.8% purity, Chipman Chemicals Co., Stoney Creek, Ontario, Canada) and the solvent acetone (pesticide grade, Caledon Laboratories, Georgetown, Ontario, Canada) were used as test chemicals. Solvent concentrations are given as percent (%) volume/volume and captan concentrations as ppm (mg L^{-1}) of active ingredient. The test chemicals were chosen because of their use in previous interaction studies (Stratton et al. 1980, 1982; Burrell and Corke 1980; Stratton and Corke 1981a, 1981b).

The fungitoxicity of acetone and acetone-captan mixtures was determined in petri plates using a poisoned agar technique (Stratton et al. 1982). Captan was tested against P. ultimum and Pestalotia sp. at concentrations of 0, 2.5, 5.0, 7.5, and 10.0 ppm, and against S. homeocarpa at levels of 0, 1.0, 2.5, 5.0, and 7.5 ppm. Each concentration of captan was interacted with 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% acetone. Captan was dissolved in acetone and enough of this stock solution was added to the test medium in order to reach both the captan concentration desired and an acetone level of 0.1%. Solvent concentrations greater than that were obtained by adding the appropriate volume of acetone directly to the medium. Each combination was tested in replicates of five and each assay was repeated three times. Plates were incubated at 30±0.5°C until control growth reached a diameter of 50-70 mm, at which time all plates were examined and growth recorded as colony diameter. Each individual acetone-captan combination was interacted towards test fungi using four agar media: Potato Dextrose Agar:PDA, Corn Meal Agar:CMA, Malt Extract Agar: MEA, (Difco Laboratories, Detroit, MN, U.S.A.), and V8 Juice Agar:V8A (200 ml V8 vegetable juice, 3.0 g $CaCO_3$, and 15 g agar per litre). The pH of all test media was aseptically adjusted following sterilization to 5.5 ± 0.2. Test fungi were also grown on each medium in the absence of toxicants and growth followed by monitoring the increase in colony diameter over time.

Growth curves for the three test fungi were analyzed on all four media using linear regression analysis (Stat-Pac microcomputer program, Walonick Associates, Minneapolis, MN, U.S.A.). Analyses of significant differences (P=0.05) were performed using an analysis of variance and t-test procedure (Stat-Pac program). Captan-solvent interactions were analyzed using a solvent - pesticide interaction analysis technique, which is fully documented elsewhere (Stratton et al. 1982). This involves mixing one concentration of pesticide with several levels of solvent and then determining a net pesticide effect by calculating percent inhibition in solvent-pesticide treatments with reference to activity in the corresponding solvent controls. Each net pesticide effect is

statistically compared (t-test at P=0.05) with that determined for the lowest solvent level used, which is the reference point for comparison purposes. Theoretically, the percent inhibition values calculated with each of the solvent levels used should be statistically the same, since all test systems contain the same concentration of pesticide. This trend would be indicative of an additive solvent-pesticide interaction. Data significantly greater, or less, than that obtained for the reference solvent level are indicative of synergistic or antagonistic interactions, respectively. The entire process is repeated at several pesticide concentrations in order to obtain a more accurate definition of the interaction occurring. Each of the four media was tested in separate experiments.

RESULTS AND DISCUSSION

Data obtained for the growth of all three fungi in the absence of toxicants are summarized in Table 1. P. ultimum evidenced optimal growth on V8A, S. homeocarpa on PDA, and Pestalotia sp. on both

Table 1. Effect of medium composition on growth of test fungi.[a]

Culture & Medium[b]	Growth equation[c]	S.E. for regression[d]	Growth constant[e]
P. ultimum			
V8A	Y=2.52X-2.23	5.009	0.397*
CMA	Y=2.01X-0.81	1.865	0.498*
MEA	Y=1.32X-0.65	1.633	0.758*
PDA	Y=2.03X-1.58	3.721	0.493*
S. homeocarpa			
V8A	Y=1.06X-0.83	1.105	0.943*
CMA	Y=1.01X+1.58	1.001	0.990*,**
MEA	Y=0.92X-2.84	1.730	1.087**
PDA	Y=1.13X-3.87	2.374	0.885
Pestalotia sp.			
V8A	Y=0.40X+1.31	0.798	2.500*
CMA	Y=0.34X+1.86	1.229	2.941*
MEA	Y=0.15X+1.95	1.295	6.667
PDA	Y=0.28X-0.20	1.175	3.571

a:Growth was monitored by following an increase in colony diameter (mm) over time (h). Cultures were grown at 30°C on test media in the absence of solvent or pesticide.

b:V8A: V8 juice agar; CMA: corn meal agar; MEA: malt extract agar; PDA: potato dextrose agar.

c:These are the regression equations describing the linear relationship between colony diameter in mm (Y) and incubation time in h (X). The correlation coefficient for all systems was $\geq$0.98.

d:Standard error of estimate for regression (used to specify the limit and confidence of any predictions made for Y).

e:This value, multiplied by the colony diameter "D" (mm), gives the time (h) for the culture size to double to "2D". Those values followed by the same symbol do not differ significantly at P=0.05. Smaller values indicate best growth.

V8A and CMA. All cultures grew poorest on MEA. P. ultimum was the fastest growing culture, and Pestalotia sp. the slowest. S. homeocarpa evidenced intermediate growth. These four semisynthetic media were chosen because they are commonly used in fungicide bioassays, stock culture maintenance, and fungal enumeration, and have a wide variation in their nutrient composition. Possible pH effects were corrected for by standardizing the pH of all media to 5.5±0.2. Therefore, the variations in growth outlined in Table 1 are assumed to be due to differences in the media's nutritional composition and variations in culture physiology.

Data for the effect of medium composition on acetone-captan interactions towards P. ultimum are outlined in Table 2. There was essentially no effect of medium type on the interaction responses obtained, since acetone and captan interacted synergistically with all four media. Synergism was evident at acetone concentrations >1.0 to 1.5% for 5.0, 7.5, and 10.0 ppm captan, regardless of the medium used. At 2.5 ppm captan, synergism occurred at solvent levels >0.1% with PDA, >0.5% with V8A and CMA, and >1.5% with MEA. Similarily, medium composition had no consistent effect on the magnitude of interaction responses (the difference between the net fungicide effects calculated at the reference solvent concentration and the highest solvent level used). There was, however, an effect of medium on the toxicity attributed to any given concentration of captan. Captan was most toxic when MEA was used, yielding net pesticide effects of 80-100% at the highest captan levels tested, and least toxic with V8A, yielding net pesticide effects of 20-55% at the same captan concentrations. CMA and PDA elicited intermediate effects on captan toxicity.

With S. homeocarpa, medium composition again had no effect on the type of interaction obtained (Table 3). Acetone and captan interacted synergistically in all media tested, but with some variation in the levels involved. Synergism occurred at acetone levels >0.1 to 0.5% for all but one combination when using MEA and PDA. With CMA, synergism was recorded at solvent concentrations >1.5 to 2.0% for 1.0, 2.5, and 5.0 ppm captan, and >0.1% at 7.5 ppm. An additive response occurred with 1.0 and 2.5 ppm captan in V8A, but synergism was elicited at acetone levels >0.1 to 0.5% for 5.0 and 7.5 ppm captan. Captan was most toxic when tested in MEA and CMA, and least toxic with V8A. PDA elicited intermediate toxicity values. Again, no pattern in results was obtained for interaction magnitudes, although they were clearly lower with V8A.

Acetone and captan interacted antagonistically towards Pestalotia sp., also regardless of the medium used (Table 4). With CMA and PDA, antagonism was observed at solvent levels >0.1 to 0.5% and >0.5 to 1.0%, respectively. This response occurred above 1.5% acetone with MEA, except at 10.0 ppm captan, where antagonism was evident above 0.5% solvent. With V8A, an additive interaction was recorded at 2.5 and 5.0 ppm captan, while an antagonistic response was obtained above 2.5% acetone at 7.5 ppm and >0.5% at 10.0 ppm captan. Captan was most toxic with MEA and PDA, and least toxic with V8A. V8A also elicited the lowest interaction magnitudes.

Table 2. Effect of medium composition on acetone-captan interactions towards *P. ultimum*.[a]

Acetone concn(%)	Captan concn (ppm) 2.5	5.0	7.5	10.0
V8A				
0.1	4.5(3.2)[b]	23.1(4.6)[b]	20.9(3.3)[b]	28.2(4.9)[b]
0.5	5.2(1.6)[b]	21.0(6.7)[b]	15.3(7.1)[b]	24.1(5.3)[b]
1.0	16.4(2.7)[c]	23.2(5.0)[b]	23.1(1.7)[b]	32.8(5.2)[b]
1.5	12.9(5.2)[c]	19.2(6.2)[b]	28.1(6.7)[b]	33.0(6.8)[b]
2.0	19.5(1.0)[c]	39.5(4.1)[c]	50.4(4.2)[c]	41.1(4.1)[c]
2.5	21.9(3.9)[c]	42.9(4.0)[c]	49.6(3.1)[c]	52.4(3.7)[c]
3.0	34.9(1.3)[c]	49.9(7.1)[c]	52.4(8.9)[c]	55.7(9.6)[c]
CMA				
0.1	34.9(6.7)[b]	58.3(5.2)[b]	55.9(2.1)[b]	72.4(1.6)[b]
0.5	43.8(5.6)[b]	55.2(4.4)[b]	55.3(1.2)[b]	73.0(2.3)[b]
1.0	46.7(2.6)[c]	63.2(5.2)[b]	58.7(1.1)[b]	77.1(5.4)[b]
1.5	50.9(5.2)[c]	60.0(4.7)[b]	65.1(3.5)[c]	85.2(1.3)[c]
2.0	54.7(8.3)[c]	67.5(1.4)[c]	75.4(7.5)[c]	86.7(1.2)[c]
2.5	64.3(5.1)[c]	85.7(2.4)[c]	82.6(7.1)[c]	97.5(2.0)[c]
3.0	62.5(7.7)[c]	78.1(8.4)[c]	100 - [c]	98.1(1.0)[c]
MEA				
0.1	38.7(2.5)[b]	67.6(4.4)[b]	80.0(2.9)[b]	82.4(8.3)[b]
0.5	39.9(2.4)[b]	70.0(4.2)[b]	88.5(9.7)[b]	86.8(6.2)[b]
1.0	46.8(5.1)[c]	68.8(5.1)[b]	86.3(9.2)[b]	94.2(3.2)[c]
1.5	38.3(8.5)[b]	63.3(8.5)[b]	88.1(2.4)[c]	91.5(3.5)[c]
2.0	67.8(8.6)[c]	77.9(6.3)[c]	83.3(4.3)[b]	95.8(3.4)[c]
2.5	71.2(7.7)[c]	74.2(9.9)[c]	99.5(1.0)[c]	99.0(0.8)[c]
3.0	89.6(4.9)[c]	87.0(1.8)[c]	99.2(0.5)[c]	100 - [c]
PDA				
0.1	20.4(4.7)[b]	54.9(3.1)[b]	57.1(6.1)[b]	74.6(9.3)[b]
0.5	33.6(1.6)[c]	68.4(1.9)[c]	59.3(4.6)[b]	75.9(2.4)[b]
1.0	43.2(4.2)[c]	54.8(4.4)[b]	58.3(3.7)[b]	74.1(6.9)[b]
1.5	42.4(4.1)[c]	59.1(4.5)[b]	65.1(3.1)[c]	76.4(9.2)[b]
2.0	53.6(9.7)[c]	64.2(4.3)[c]	76.3(6.9)[c]	88.0(4.2)[c]
2.5	56.3(6.8)[c]	71.7(8.1)[c]	86.2(6.5)[c]	91.7(2.9)[c]
3.0	77.1(5.9)[c]	91.7(3.0)[c]	88.2(1.7)[c]	93.5(2.7)[c]

a:Table entries (net fungicide effect) are mean % inhibition values, followed by the standard deviation in brackets, calculated from growth in control systems containing only the appropriate level of solvent. V8A:V8 juice agar, CMA:corn meal agar, MEA:malt extract agar, PDA:potato dextrose agar.

b:These values do not differ significantly (P=0.05) from that calculated for the lowest concentration of acetone and are indicative of an additive interaction response. Each medium is considered separately.

c:These values are significantly greater (P=0.05) than that calculated for the lowest concentration of acetone and are indicative of a synergistic interaction response.

d:These values are significantly lower (P=0.05) than that calculated for the lowest concentration of acetone and are indicative of an antagonistic interaction response.

Acetone and captan interacted synergistically towards P. ultimum and S. homeocarpa, and antagonistically towards Pestalotia sp. (Tables 2, 3, and 4), which is consistent with data obtained from previous solvent-pesticide interaction studies using these cultures (Burrell and Corke 1980; Stratton et al. 1982). The response patterns were dependent upon both the acetone concentration and captan level used, which is also consistent with data published previously on solvent-pesticide combination effects towards fungi (Burrell and Corke 1980; Stratton et al. 1982), algae (Stratton and Corke 1981b), and cyanobacteria (Stratton et al. 1980). This indicates the importance of testing for interactions over a range of toxicant levels. The significance of solvent-pesticide interactions in laboratory bioassays has been well documented (Dalela et al. 1979; Bowman et al. 1981; Stratton et al.

Table 3. Effect of medium composition on acetone-captan interactions towards S. homeocarpa.[a]

Acetone concn(%)	Captan concn (ppm)			
	1.0	2.5	5.0	7.5
V8A				
0.1	10.1(5.5)[b]	7.5(6.2)[b]	3.3(3.1)[b]	3.4(1.0)[b]
0.5	8.0(7.4)[b]	2.1(4.7)[b]	2.7(1.9)[b]	13.6(2.7)[c]
1.0	5.5(4.2)[b]	7.9(4.5)[b]	11.4(2.8)[c]	13.2(3.2)[c]
1.5	5.3(5.9)[b]	6.8(1.9)[b]	13.4(1.1)[c]	15.5(3.6)[c]
2.0	6.2(3.3)[b]	12.1(5.9)[b]	15.3(4.2)[c]	22.9(1.7)[c]
2.5	6.6(6.4)[b]	10.9(4.2)[b]	17.1(5.1)[c]	18.9(2.3)[c]
3.0	3.6(2.5)[b]	11.8(4.8)[b]	15.7(2.8)[c]	28.4(3.4)[c]
CMA				
0.1	18.1(3.2)[b]	28.9(2.5)[b]	32.8(6.3)[b]	27.1(1.2)[b]
0.5	11.4(2.5)[b]	32.7(4.5)[b]	33.3(2.2)[b]	47.2(1.6)[c]
1.0	16.4(3.4)[b]	42.7(5.9)[c]	38.2(3.4)[b]	53.4(9.7)[c]
1.5	17.8(3.5)[b]	34.1(1.7)[b]	37.4(8.1)[b]	52.2(4.7)[c]
2.0	21.4(4.3)[b]	33.9(7.0)[b]	40.2(2.5)[c]	83.1(2.4)[c]
2.5	31.9(7.6)[c]	45.4(3.9)[c]	53.6(6.7)[c]	98.9(1.0)[c]
3.0	58.0(2.3)[c]	67.0(3.0)[c]	92.0(2.8)[c]	97.3(2.1)[c]
MEA				
0.1	18.1(4.5)[b]	25.6(3.1)[b]	16.2(4.8)[b]	33.2(3.6)[b]
0.5	12.0(2.9)[b]	31.7(3.5)[b]	37.4(2.1)[c]	38.9(4.2)[b]
1.0	26.8(2.3)[c]	33.1(1.8)[c]	38.5(5.5)[c]	53.9(5.4)[c]
1.5	33.1(3.0)[c]	36.3(4.1)[c]	47.9(6.6)[c]	68.1(6.9)[c]
2.0	37.8(1.9)[c]	39.5(7.1)[c]	64.2(9.9)[c]	95.2(5.6)[c]
2.5	47.5(3.4)[c]	49.7(5.1)[c]	78.9(9.1)[c]	94.6(2.1)[c]
3.0	42.6(2.4)[c]	58.8(1.9)[c]	94.6(2.6)[c]	100 - [c]
PDA				
0.1	9.6(2.4)[b]	10.5(6.5)[b]	20.0(2.7)[b]	11.2(6.2)[b]
0.5	18.0(1.5)[c]	24.6(5.4)[c]	26.1(4.0)[b]	27.9(3.2)[c]
1.0	15.6(6.1)[b]	25.6(8.7)[c]	30.4(6.8)[c]	33.3(4.8)[c]
1.5	29.7(2.9)[c]	25.8(5.6)[c]	40.4(3.2)[c]	34.4(3.1)[c]
2.0	29.1(1.9)[c]	26.8(6.7)[c]	43.6(1.5)[c]	38.2(8.3)[c]
2.5	26.2(7.5)[c]	50.0(3.8)[c]	50.1(1.9)[c]	51.4(5.7)[c]
3.0	31.8(2.1)[c]	55.4(2.1)[c]	61.2(4.1)[c]	97.4(1.0)[c]

a,b,c:Refer to footnotes in Table 2.

1982), and in order to eliminate erroneous conclusions regarding pesticide toxicity it is essential that the solvent system chosen be one which interacts additively with the test pesticide. The biological mechanisms responsible for these interactions are still unclear, but are probably related to the solvent's mode of action in biological systems and an organism's biochemical characteristics. Research into this aspect of toxicant interactions is needed before these observations can be adequately explained.

Medium composition is one of the least standardized variables in microbial bioassays and is therefore a potential cause of variation among results obtained from different laboratories. Although this factor did not affect the overall interaction response observed for combinations of acetone and captan, it did alter the

Table 4. Effect of medium composition on acetone-captan interactions towards *Pestalotia* sp.[a]

Acetone concn(%)	Captan concn (ppm) 2.5	5.0	7.5	10.0
V8A				
0.1	3.7(9.2)[b]	10.7(2.5)[b]	7.3(1.3)[b]	25.8(9.8)[b]
0.5	2.0(4.9)[b]	6.9(7.9)[b]	7.4(3.5)[b]	15.8(8.0)[d]
1.0	1.8(3.5)[b]	10.6(9.4)[b]	7.8(4.2)[d]	11.9(7.9)[d]
1.5	-2.4(2.9)[b]	3.4(4.2)[b]	1.0(2.5)[b]	10.0(3.9)[d]
2.0	3.3(6.0)[b]	5.1(2.4)[b]	1.7(4.8)[b]	3.7(2.7)[d]
2.5	-7.2(3.9)[b]	6.1(4.4)[b]	1.9(5.1)[d]	3.9(2.8)[d]
3.0	-2.9(3.7)[b]	4.9(7.1)[b]	0.1(2.8)[d]	3.5(7.2)[d]
CMA				
0.1	30.6(7.6)[b]	52.1(4.2)[b]	74.4(5.2)[b]	65.9(3.0)[b]
0.5	23.4(8.9)[d]	49.8(5.1)[d]	56.5(1.7)[d]	58.1(7.6)[d]
1.0	16.3(5.7)[d]	35.3(3.9)[d]	54.2(5.9)[d]	44.2(5.7)[d]
1.5	17.4(5.9)[d]	33.1(5.7)[d]	36.7(2.9)[d]	47.5(6.3)[d]
2.0	18.6(4.2)[d]	29.2(6.1)[d]	35.6(6.3)[d]	29.6(2.6)[d]
2.5	11.1(4.5)[d]	26.5(4.9)[d]	27.9(3.3)[d]	25.0(6.7)[d]
3.0	9.2(3.6)[d]	19.3(3.7)[d]	22.2(6.9)[d]	22.5(7.2)[d]
MEA				
0.1	50.9(5.4)[b]	72.7(4.5)[b]	70.9(2.6)[b]	84.6(9.8)[b]
0.5	46.5(9.5)[b]	69.2(2.6)[b]	72.7(6.4)[b]	83.6(4.5)[d]
1.0	44.4(8.5)[b]	68.5(5.7)[b]	77.7(5.1)[b]	64.4(4.1)[d]
1.5	45.4(7.5)[d]	67.8(8.7)[d]	67.3(2.6)[d]	55.6(9.1)[d]
2.0	39.9(2.5)[d]	55.8(2.7)[d]	61.5(2.7)[d]	52.3(2.1)[d]
2.5	36.4(6.4)[d]	47.7(8.5)[d]	53.8(1.7)[d]	42.3(2.5)[d]
3.0	29.9(4.1)[d]	46.3(9.4)[d]	40.7(6.9)[d]	57.4(4.5)[d]
PDA				
0.1	62.8(2.7)[b]	51.7(5.4)[b]	78.5(5.1)[b]	80.2(3.5)[b]
0.5	66.5(2.1)[d]	45.9(3.5)[b]	71.1(4.4)[d]	79.7(1.1)[d]
1.0	54.6(1.7)[d]	45.7(3.8)[d]	60.6(4.1)[d]	74.1(1.4)[d]
1.5	46.6(1.0)[d]	43.5(2.6)[d]	45.8(9.8)[d]	62.7(3.2)[d]
2.0	27.3(3.5)[d]	27.0(3.7)[d]	35.5(6.8)[d]	55.1(2.5)[d]
2.5	30.9(9.8)[d]	21.7(4.2)[d]	34.9(5.7)[d]	55.0(4.1)[d]
3.0	28.3(6.2)[d]	19.3(4.5)[d]	32.7(2.9)[d]	62.5(3.4)[d]

a,b,d:Refer to footnotes in Table 2.

solvent concentration at which synergism or antagonism was first observed. Interaction magnitudes were little affected, but tended to be lower in V8A. Therefore, from a solvent-pesticide perspective the choice of medium is of little importance, as long as an interaction screening procedure (Stratton et al. 1982) is used to ensure that the solvent level chosen elicits an additive response. However, medium type had a pronounced effect on captan toxicity. The fungicide was more toxic when tested with MEA, the medium in which the cultures grew slowest, and less toxic with V8A, the medium usually eliciting the best growth. Since it is desirable to use the most sensitive test systems in bioassays, a medium which allows stressed, but adequate growth may be preferable. A detailed explanation of these results requires more research into the mechanisms responsible for toxicant interactions. The data presented here will be useful in standardizing microbial bioassay media for pesticide ecotoxicity testing.

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