THE GROWTH AND PROTEIN CONTENT OF GRAIN CROPS AFTER TREATMENT OF SEED WITH TRIAZINES

PART I
VACUUM INFILTRATION OF SIMAZINE INTO BARLEY SEED

PART II
SEED TREATMENT OF GRAINS WITH TRIAZINES

Thesis for the Degree of M. S.

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ABSTRACT

THE GROWTH AND PROTEIN CONTENT OF GRAIN CROPS AFTER TREATMENT OF SEED WITH TRIAZINES

By

Harry L. Seward

PART I

VACUUM INFILTRATION OF SIMAZINE INTO BARLEY SEED

'Larker' barley (Hordeum vulgare L.) was soaked and vacuum infiltrated in solutions of simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) to determine the feasibility of these methods for improving the protein content and/or yield. Absorption of water was closely correlated with uptake of simazine for both soaking and vacuum infiltration. More water and simazine were taken up by vacuum infiltration than by soaking. Uptake of simazine was positively correlated with the concentration of the treatment solution for both methods. The increase in uptake of simazine resulting from vacuum infiltration as compared to soaking was retained by seeds planted in soil, but released by seeds soaked in distilled water. The combustion procedure for recovery of ^{14}C simazine from seeds proved to be more effective than autoclaving after 6 hours of either soaking or vacuum

infiltration. These studies indicate that a sufficient amount of simazine may be absorbed by the seed to make seed treatment a feasible alternative to spraying.

PART II

SEED TREATMENT OF GRAINS WITH TRIAZINES

Studies were conducted to determine the effects of simazine (2-chloro-4,6-bis(ethylamino)-*s*-triazine), OHEMT (2-chloro-4(2-hydroxyethylamino)-6-methylamino-*s*-triazine, and OHEET (2-chloro-4-ethylamino-6-(2-hydroxyethylamino)-*s*-triazine), applied as seed treatments, on protein content and yield of barley (*Hordeum vulgare* L. 'Coho'), oats (*Avena sativa* L. 'Gary'), and rye (*Secale cereale* L. 'MSU Exp'). Seed treatments included vacuum infiltration with aqueous solutions, and soaking in dichloromethane solutions. Solvent controls (water infiltration and soaking in DCM) increased yield, protein content, or total protein in most of these studies. In no instance did infiltration of triazines result in increases of yield or protein content greater than the solvent controls.

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Harry L. Seward

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PART I

VACUUM INFILTRATION OF SIMAZINE INTO BARLEY SEED

INTRODUCTION

Increases in both protein content and yield of agronomic crops have been attained with spray applications of nontoxic levels of simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) (1,5,11). Further investigations have indicated that the factors necessary for obtaining a consistent maximum response are not understood (7,10,14,16,17). It is possible that a seed treatment prior to planting would assure a more accurate and consistent amount of chemical being made available to the plant.

Absorption of herbicides by seeds has been investigated to determine if sensitivity or tolerance may be related to the rate and amount of herbicides absorbed. Hansen and Buchholtz (3) reported that the amount of 2,4-dichlorophenoxyacetic acid (2,4-D) absorbed by corn (*Zea mays* L.) and pea (*Pisum sativum* L.) seeds was influenced by, but not closely correlated with, water uptake. Uptake of 2,4-D by various seeds has been reported by other investigators (2,6). Swan and Slife (15) found that soybeans (*Glycine max* L.) soaked in 3-amino-2,5-dichlorobenzoic acid (amiben) contained large amounts of the herbicide in the cotyledons, but translocated relatively small amounts to the developing seedling.

Distribution of labelled 4-iodophenoxyacetic acid in developing seedlings varied with seed type (8). Rieder, Buchholtz, and Kust (9), working with soybeans, concluded that various herbicides were absorbed at different rates, and that uptake was directly related to temperature, but not correlated with water uptake. Scott and Phillips (13) reported that seed size influenced diffusion of isopropyl m-chlorocarbanilate (chloropropham) into soybean seed.

Vacuum infiltration of growth regulators into seeds is a relatively undeveloped technique. The only reported use of vacuum infiltration on seeds was made by Schwiezer (12). Oat (Avena sativa L.) and wheat (Triticum aestivum L.) seeds were vacuum infiltrated with amino acids; however, no data is available concerning uptake of the amino acids by the seed during infiltration or translocation of the amino acids into the germinated seedlings.

The objective of this research was to investigate some of the parameters of soaking and vacuum infiltration as methods of treating grain seed with nontoxic levels of simazine to increase growth and protein content.

MATERIALS AND METHODS

Graded barley (<u>Hordeum</u> <u>vulgare</u> L. 'Larker') and aqueous solutions of ^{14}C ring-labelled simazine (2-chloro-4,6-bis-(ethylamino)-<u>s</u>-triazine) (4.9 μC/mg) were used in these studies. The procedure for vacuum infiltration (11) consisted of placing test tubes containing the seeds and solution in a desiccator and applying a vacuum (35 mm Hg of absolute pressure). All infiltrations were done at 20 C and the vacuum was momentarily released every 30 minutes. Solutions were immediately decanted upon removal from the desiccator, and the seeds were rinsed twice with distilled water. Excess water was removed by blotting with paper towels, and the seeds were dried for 24 hours at 43 C. The procedure for soaking was identical except that the seeds were not placed in a partial vacuum.

Uptake of water and labelled simazine by both soaked and vacuum infiltrated seeds was measured after ½, 1, 3, and 6 hours in a short term study, and after 3, 6, 12, and 24 hours in a second study. Lots composed of 40 seeds were weighed and placed in distilled water. Following treatment, the solutions were decanted and the seeds were centrifuged for 2 minutes at 200 x G in Filterfuge tubes (LaPINE Scientific Co., Chicago, Illinois, 60629). These tubes permit excess water to pass through a screen into a lower reservoir during centrifugation. The seeds were weighed and water uptake determined. Uptake of simazine during treatment was measured by placing seeds in a 9.0 μM ^{14}C simazine solution. Seeds were treated at a rate of 10 seeds per 2 ml in the short term study, and 10 seeds per 5 ml in the second study due to the amount of evaporation during the 24 hour infiltration.

Labelled simazine was extracted from treated seeds by placing 5 seeds in 2 ml of distilled water and autoclaving for 10 minutes at 18 psi and 121 C. A 1.0 ml aliquot was then counted. The validity of this extraction method was tested by combusting duplicate samples from the 24 hour study. Samples composed of 5 seeds were weighed and ground to pass through a 40 mesh screen. A 50 mg aliquot was combusted and the $^{14}CO_2$ was trapped in 15 ml of ethanol-ethanolamine (2:1). Three ml of this solution was counted, and the percent recovery determined by spiking a 50 mg sample of untreated seed meal with 10 μl of 9.0 μM ^{14}C simazine.

All solutions containing ^{14}C simazine were added to 15 ml scintillation solution (4 g BBOT : 1000 ml toluene : 400 ml Triton X-100). Cpms were corrected for chemical quenching by preparing quench curves. After correcting for aliquots used, dpms were converted to μg simazine. Allowances were also made for evaporation of treatment solutions which resulted in concentration of these solutions with time.

Germination of infiltrated and soaked seeds was determined on seeds treated for ½, 3, and 6 hours. Each treatment was replicated 4 times with 50 seeds per replicate. The seeds were placed in petri dishes between filter papers supported on a layer of cotton saturated with distilled water. Germination was recorded after 36 and 48 hours at 24 C.

Release of simazine to the soil was studied by measuring the amount of labelled simazine retained by seeds over time. Seeds soaked and infiltrated in ^{14}C simazine were planted in an unsterilized silt loam. Each seed was planted in an individual plastic container (with drainage), and the soil was watered to field capacity. Seeds of both soaked and infiltrated treatments were removed after 6, 18, 30, 42, 66, and 90 hours. The soil was brushed off prior to drying at 43 C for 24 hours, and the ^{14}C simazine was extracted by autoclaving. Each replicate consisted of 3 seeds.

The release of ^{14}C simazine from treated seeds was also assayed in distilled water. Treated seeds were placed in 2 ml of distilled water for each of the following times: 6, 18, 30, 42, 66, and 90 hours. A 1.0 ml aliquot of the distilled water was then removed and counted. Each replicate consisted of 5 seeds.

The effect of concentration on uptake by the seed was tested by soaking and infiltrating seeds in 2.5, 5.0, and 10.0 μM ^{14}C simazine solutions. Seeds were treated at a rate of 10 seeds per 5 ml of solution. Following rinsing and drying of the seeds, the ^{14}C simazine was extracted by autoclaving.

Uptake of ^{14}C simazine by barley plants grown in 10^{-7} and 10^{-8} M simazine solutions for 3 days was studied. Plants were grown as described by Wert (18). A 1.0 ml aliquot of the simazine solution was counted before and after the 3 day growth period in these solutions. Duplicate containers with solutions, but no plants, were also monitored. After 3 days, solutions were brought back up to volume with distilled water, and a 1.0 ml aliquot was counted. Loss of simazine from solutions was calculated and uptake of simazine expressed in ng per plant.

All experiments except for the germination study were done with 3 replications in completely randomized designs. Analyses of variance were computed, and F values calculated for single degrees of freedom. Linear correlations were calculated between relevant parameters and the following code indicates levels of significance : *P = .05 and **P = .01. Individual observations were correlated except in the case of rate studies where means were correlated.

RESULTS AND DISCUSSION

The uptake of water was increased after 30 minutes of vacuum infiltration as compared to soaking (Figure 1a). This initial increase was maintained with little further change in rate. The uptake of ^{14}C simazine was increased after 1 hour of vacuum infiltration compared with soaking, and the rate of uptake continued to increase up to 6 hours (Figure 1b). Uptake of water in the 24 hour study indicated that the same increase observed in the 6 hour study was maintained even after 24 hours of vacuum infiltration (Figure 2a). Uptake of ^{14}C simazine was similarly increased by vacuum infiltration, and this increase was maintained after 24 hours (Figure 2b). This study indicated a maximum increase in rate of simazine uptake due to vacuum infiltration after 3 hours.

After 30 minutes of treatment, the rate of simazine uptake per hour was greater for infiltration than for soaking. This may be accounted for by the effect of vacuum on the structural components of the seed resulting in increased water uptake. Apparently, vacuum infiltration predisposes some portion of the seed to more water absorption than does soaking seed (Figure 1a). This may be related to the

Figure 1a. The uptake of water by soaked and infiltrated barley seeds during 6 hours.

The F value for soaking vs. infiltration is significant at the .01 level.

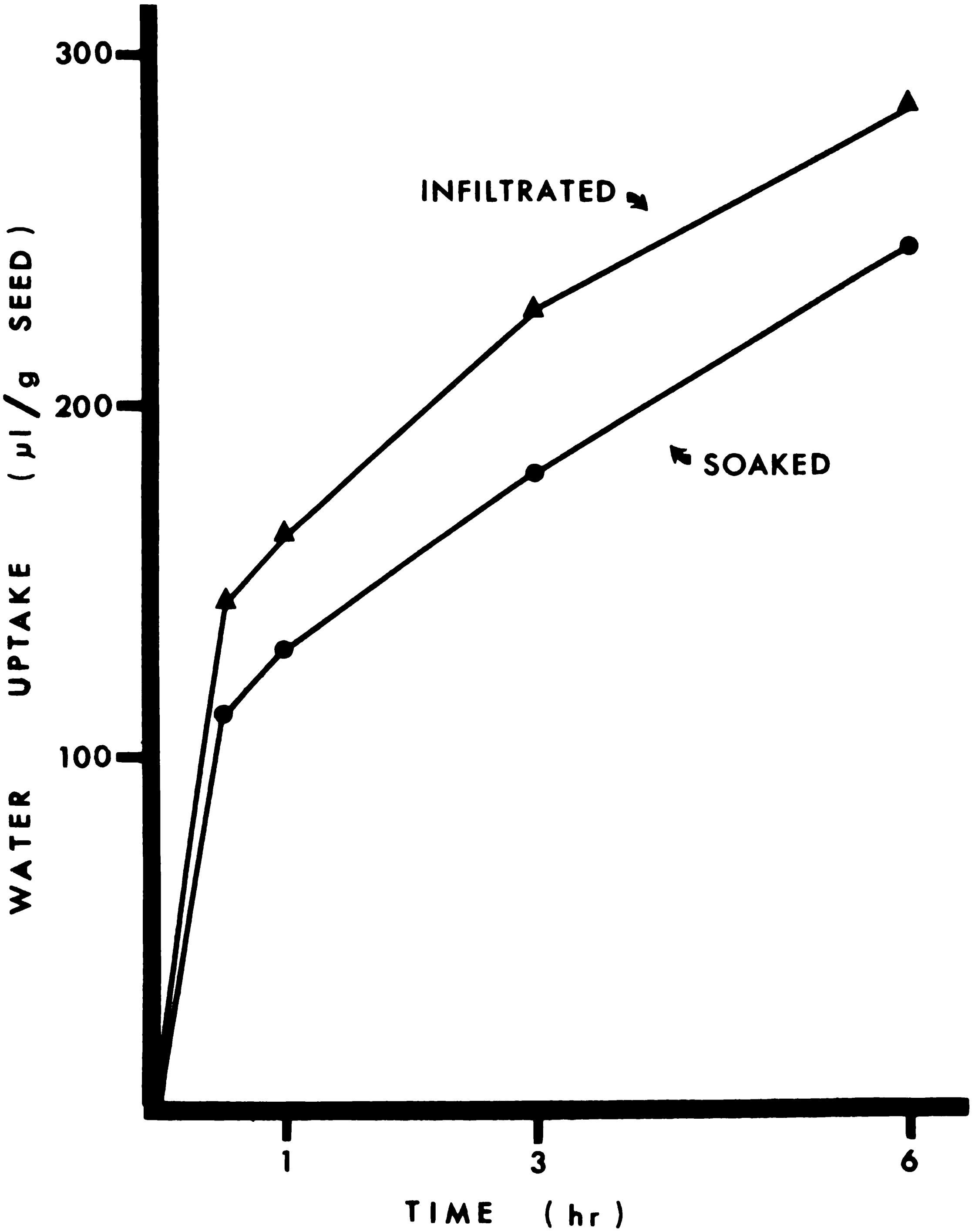
300
200
100
WATER UPTAKE (µl/g SEED)
INFILTRATED
SOAKED
1
3
6
TIME (hr)

Figure 1a

Figure 1b. The uptake of simazine by soaked and infiltrated barley seed during 6 hours.

The F value for the interaction of time and treatment method is significant at the .01 level.

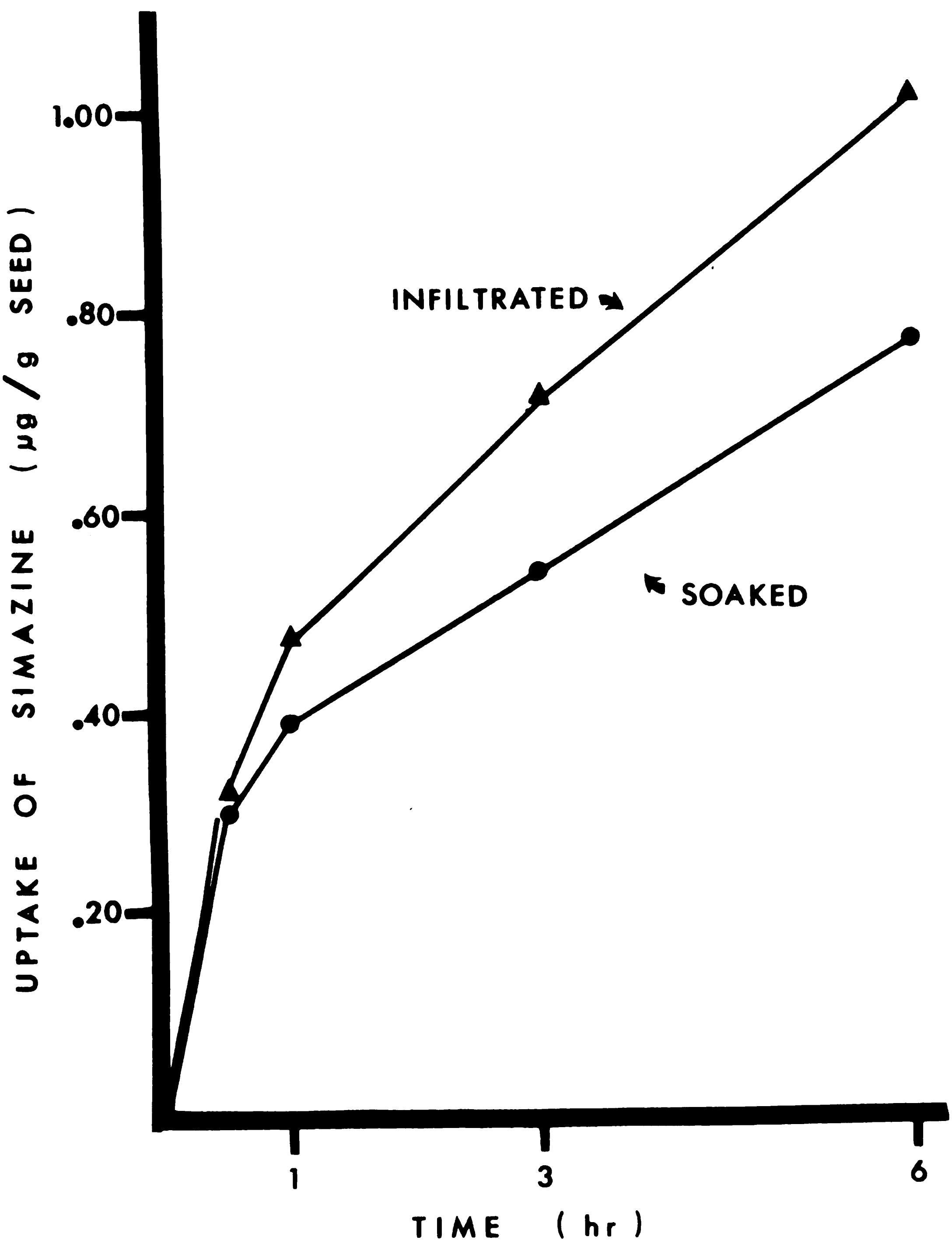
1.00
.80
.60
.40
.20
UPTAKE OF SIMAZINE (µg / g SEED)
INFILTRATED
SOAKED
1
3
6
TIME (hr)

Figure 1b

Figure 2a. The uptake of water by soaked and infiltrated barley seeds during 24 hours.

The F value for soaking vs. infiltration is significant at the .01 level.

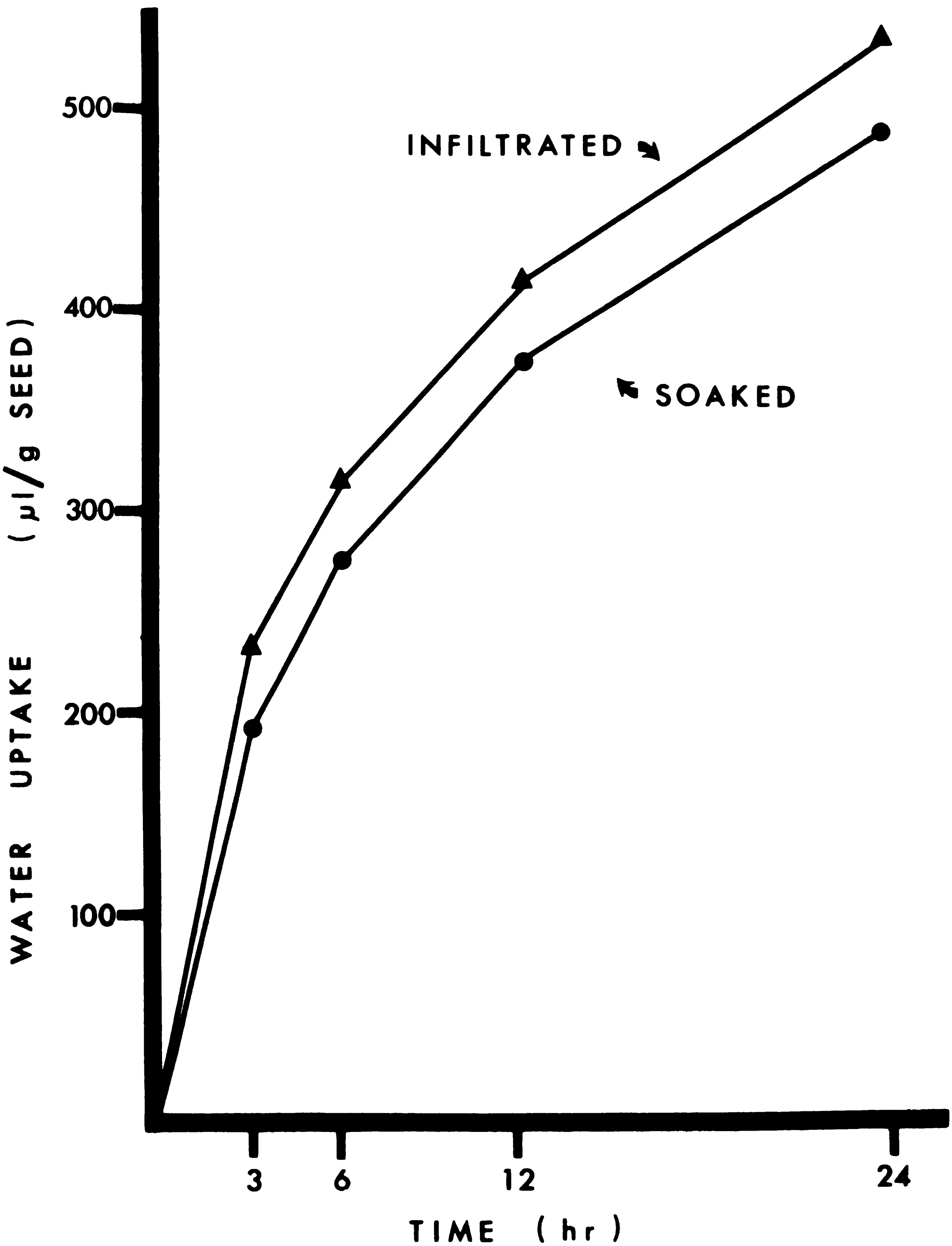
INFILTRATED
SOAKED
WATER UPTAKE (µl/g SEED)
500
400
300
200
100
3
6
12
24
TIME (hr)

Figure 2a

Figure 2b. The uptake of simazine by soaked and infiltrated barley seeds during 24 hours.

The F value for soaking vs. infiltration is significant at the .01 level.

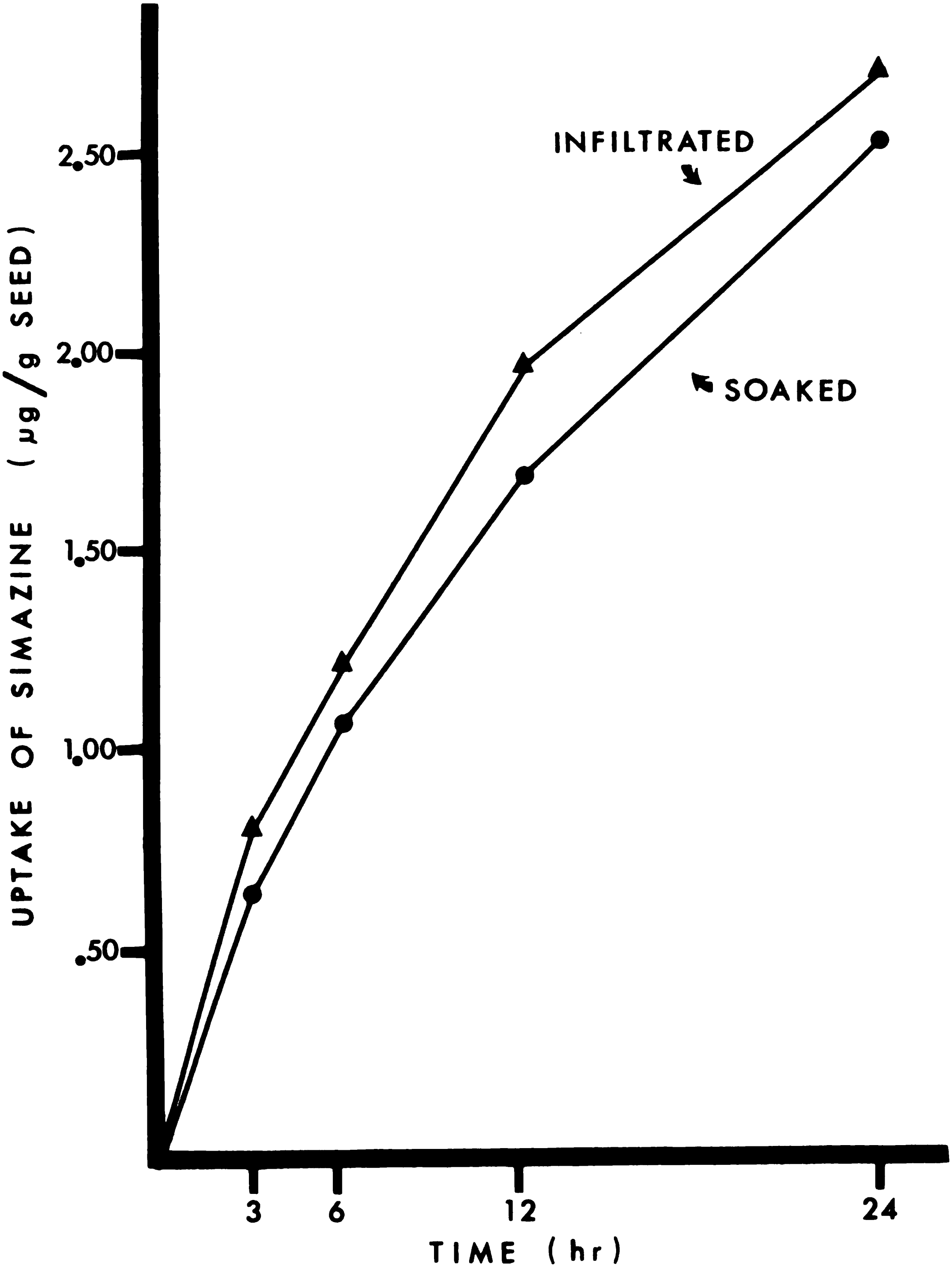
INFILTRATED
SOAKED
2.50
2.00
1.50
1.00
.50
UPTAKE OF SIMAZINE (µg/g SEED)
3
6
12
24
TIME (hr)

Figure 2b

observation of rapid release of gases from seeds during the first 30 minutes of vacuum infiltration.

While water uptake associated with vacuum infiltration attains its maximum rate after 30 minutes, simazine uptake does not attain a maximum rate of increase until some time after 3 hours. This may be explained through the possible influence of simazine on absorptive sites under conditions of vacuum infiltration, causing structural changes in some seed food reserves which further alters simazine uptake (9). Although there was an initial lag of herbicide uptake compared to water uptake, water uptake and simazine uptake were closely correlated over 24 hours for both soaked and infiltrated seeds (Table 1). Haskell and Rogers (4) also found a close correlation between water uptake and herbicide penetration of simazine into various seeds.

Table 1. A comparison of the rates of uptake of water and simazine by soaked and infiltrated seeds.

	Rate of Uptake			
	Water		Simazine	
Time interval (hr)	Soaked	Infiltrated	Soaked	Infiltrated
0-3	64.3	78.0	21.7	27.0
3-6	27.3	27.3	14.0	13.7
6-12	16.5	16.2	10.3	12.7
12-24	9.3	10.2	7.0	6.0
Correlations between water and simazine uptake r =			.986*	.980*

Uptake of simazine was found to be directly correlated to the concentration of the treatment solution for both soaking and vacuum infiltration (Table 2). This indicates two methods for controlling the amount of simazine absorbed by the seed : time and concentration of the treatment solution.

Table 2. The relationship of uptake of ^{14}C simazine with concentration of the treatment solution.

Simazine Concentration (μM)	Uptake by Seeds Soaked	Uptake by Seeds Infiltrated
	(ng/g seed)	(ng/g seed)
2.5	130	133
5.0	239	248
10.0	430	513
Correlation with simazine concentration r =	.997**	.995**

A comparison of extraction methods indicated that both show an increase in uptake of ^{14}C simazine due to vacuum infiltration (Table 3). Recovery was the same for seed treatments of 3 and 6 hours with both methods of extraction, but was reduced for seed treatments of 12 and 24 hours for extractions done by autoclaving. Increases of vacuum infiltration over soaking are not affected by this reduction in recovery. Neither method indicated a significant interaction

Table 3. A comparison between autoclaving and combustion procedures for extraction of ^{14}C simazine from soaked and infiltrated seeds.

Time (hr)	Seed Treatment	Amount Extracted[1] Autoclaved (µg/g seed)	Amount Extracted[1] Combustion (µg/g seed)	Increase of Infiltration Over Soaking Autoclaved (µg/g seed)	Increase of Infiltration Over Soaking Combustion (µg/g seed)
3	Soaked	.73	.65	.10	.16
	Infiltrated	.83	.81		
6	Soaked	1.00	1.07	.16	.15
	Infiltrated	1.16	1.22		
12	Soaked	1.27	1.69	.18	.29
	Infiltrated	1.45	1.98		
24	Soaked	1.67	2.53	.31	.17
	Infiltrated	1.98	2.70		
Coefficient of variation %		7.5	10.0		

[1]The F value for the difference between soaking and infiltration is significant at the .01 level for both extraction methods regardless of time.

of time with method of treatment (soaking vs. infiltration) and the coefficients of variation were similar.

The rate of release of simazine by seeds, as measured by decreased retention of simazine by seeds in soil, was the same for both soaked and infiltrated seeds (Table 4).

Table 4. The rate of loss of ^{14}C simazine with the time from barley seeds planted in soil; calculated from decreased retention of simazine by the seeds after different time intervals.

Time Intervals (hr)	Seed Treatment: Soaked (ng simazine/g seed/hr)	Seed Treatment: Infiltrated (ng simazine/g seed/hr)
0-6	700.0	700.0
6-18	3.0	5.0
18-30	5.0	4.0
30-42	2.5	2.5
42-66	1.6	1.2
66-90	0.4	1.2

Since the rate of release was the same, and infiltrated seeds contained a larger amount of simazine initially, more simazine was retained by infiltrated seeds than by soaked seeds in soil (Figure 3). Release of simazine by seeds in distilled water indicated a different phenomenon. Infiltrated seeds released more simazine than soaked seeds in distilled water (Figure 3). This was accounted for by an increased rate of release during the first 6 hours (Table 5).

Figure 3. A comparison of the retention of simazine by soaked and infiltrated barley seed in soil and the release of simazine by soaked and infiltrated barley seed in distilled water.

The F value for soak vs. infiltration is significant at the .01 level for both studies.

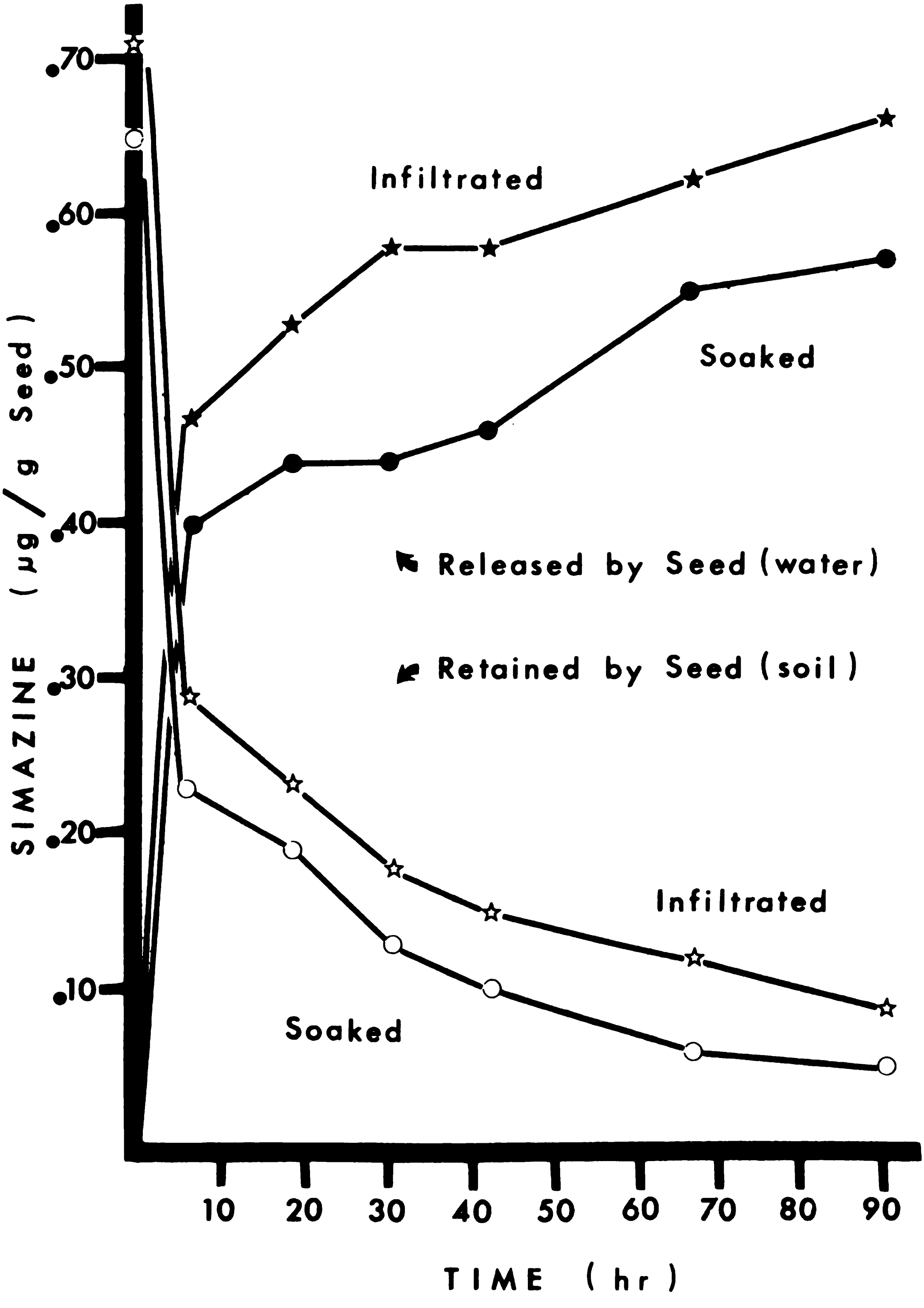
.70
.60
.50
.40
.30
.20
.10
SIMAZINE (μg / g Seed)
Infiltrated
Soaked
Released by Seed (water)
Retained by Seed (soil)
Infiltrated
Soaked
10
20
30
40
50
60
70
80
90
TIME (hr)

Figure 3

Table 5. The rate of loss of ^{14}C simazine with time from barley seeds soaked in distilled water.

Time Interval (hr)	Seed Treatment	
	Soaked	Infiltrated
	(ng simazine/g seed/hr)	
0-6	400.0	470.0
6-18	3.0	5.0
18-30	0.0	4.0
30-42	1.6	0.0
42-66	3.7	1.7
66-90	0.8	1.7

The conflicting results in the release of simazine in distilled water compared to soil may indicate that the presence of solutes affects release to the soil solution. Both studies revealed however, that simazine was readily released by both soaked and vacuum infiltrated seeds.

Germination was not affected by soaking or vacuum infiltration. The average germination over a period of 6 hours of treatment was 97% for soaked seed and 96% for infiltrated seed.

Wert (18) has observed increased plant growth of barley grown in nutrient cultures after 3 days in 10^{-7} and 10^{-9} M simazine solutions. Barley plants, similarly grown, removed 8 ng of ^{14}C simazine per plant from 10^{-8} M simazine solutions and 56 ng of ^{14}C simazine per plant from 10^{-7} M simazine solutions. Uptake was not measured in 10^{-9} M solutions because of insufficient activity. Vacuum infiltration or soaking in a 9.0 μM simazine solution for 3 hours results in uptake of approximately 30 ng of simazine per seed. This would indicate that seed treatment may provide sufficient chemical to affect the plant under field conditions and thus serve as an alternative to spraying.

These studies indicated that vacuum infiltration of barley seeds resulted in increased simazine uptake, but give no indication of the distribution of this increase in the seed. Further work should be directed toward investigating the effect of infiltration on distribution of chemicals in the seed; specifically on whether there are structural components of the seed which are absorbing water and simazine under conditions of vacuum infiltration.

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PART II

SEED TREATMENT OF GRAINS WITH TRIAZINES

INTRODUCTION

Variable results have been obtained from applications of (nontoxic levels of) *s*-triazines to agronomic crops in an effort to increase protein content and/or yield. Ries, Schweizer, and Chmiel (11) reported increases in both yield and protein content of various agronomic crops with spray applications of simazine (2-chloro-4,6-bis(ethylamino)-*s*-triazine). Increases in crude protein with no accompanying decrease in yield resulting from spray applications of simazine on forage grasses was reported by Allinson and Peters (1). Tweedy et al. (15) failed to increase protein content or yield of wheat (*Triticum aestivum* L.) and sorghum (*Sorghum vulgare* L. Moench) sprayed with simazine, atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), and terbacil (3-tertbutyl-5-chloro-6-methyluracil). Monson et al. (9) reported that spray applications of simazine on coastal bermudagrass (*Cynoden dactylon* L. Pers.) had no effect on dry matter yield, protein yield, or protein percentage. Vergara, Miller, and Avelino (16) increased percent protein in rice grain (*Oryza sativa* L.) with simazine applied in the irrigation water but also reduced yield. Foliar application and soil incorporation of terbacil and simazine produced no increase in growth or quality of cotton (*Gossypium hirsutum* L.) (14). Ries et al. (10) reported inconsistent results following application of simazine and terbacil on wheat in Michigan.

These studies indicate that the factors necessary for obtaining a consistent maximum response are not understood. It is possible that seed treatment prior to planting would assure that a more accurate and consistent amount of chemical would be available to the plant.

Treatment of seeds with biologically active compounds has been investigated by several workers (2,4,5,12). Schweizer (12) reported the use of vacuum infiltration as a method of treating grain seeds with amino acids for improvement of growth and yield. Meyer and Mayer (8) introduced the use of dichloromethane (DCM) as a solvent for chemical treatment of seeds. It was reported as having no effect on germination or respiration of lettuce (*Lactuca sativa* L. 'Grand Rapids') or peas (*Pisum sativum* L. 'Alaska').

The objective of this research was to evaluate the use of vacuum infiltration of seeds, in aqueous solutions of triazines, and soaking of seeds, in dichloromethane solutions of triazines, on the improvement of yield and protein content of several grain crops.

METHODS AND MATERIALS

Barley (Hordeum vulgare L. 'Coho'), oats (Avena Sativa L. 'Gary'), and rye (Secale cereale L. 'MSU Exp') were treated with simazine (2-chloro-4,6-bis(ethylamino)-s-triazine), OHEMT (2-chloro-4(2-hydroxyethylamino)-6-methylamino-s-trazine), and OHEET (2-chloro-4-ethylamino-6(2-hydroxyethylamino)-s-triazine).

The procedure for vacuum infiltration consisted of placing 60 g of seed in 250 ml of aqueous treatment solutions for 3 hours at 35 mm of Hg of absolute pressure with six periodic releases (12). The solutions were decanted, and the seeds rinsed twice with distilled water. Excess water was removed by blotting with paper towels, and the seeds were dryed for 12 hours at 43 C. Controls for experiments involving vacuum infiltration consisted of untreated seed, dry seed held under vacuum, and water infiltrated seed.

Another seed treatment involved soaking seeds in dichloromethane (DCM) solutions of simazine (60 g/250 ml). After soaking, the seeds were rinsed twice with DCM, blotted with paper towels, and dryed at room temperature for 24 hours. Controls included untreated seed and DCM soaked seeds (no simazine).

Field tests were initiated in the Spring of 1971 at three experiment stations located at Sodus, Entrican, and East Lansing, Michigan. Coho barley infiltrated with 20 μM simazine and OHEMT at 20, 100, 200, 400, 800, and 1200 μM concentrations was planted at all three locations. Gary oats, similarly treated, were planted at Entrican, and in addition, Gary oats soaked in 50, 100, 250, and 500 μM simazine DCM solutions with treatment times of 4 and 24 hours, were also planted.

Field plots were arranged in randomized complete block designs with 4 replications. A plot consisted of two rows 7 m long and 25 cm apart. Seed was sown at a rate of 85 kg/ha. Applications of 337 kg/ha of 12-12-12 fertilizer were made at East Lansing and Sodus. At Entrican lupines had been grown in 1970 followed by a rye cover crop; thus no supplemental fertilizer was applied.

After harvesting, the seed was cleaned, air dried, and weighed. Samples of each plot were ground to pass through a 40 mesh screen, and total nitrogen was determined by an automatic micro-kjeldahl procedure (3).

Rye (MSU Exp), infiltrated with simazine, OHEMT, and OHEET at concentrations of .05, .1, .5, 1, and 5 μM, and Coho barley, soaked in 20 and 100 μM simazine solutions with treatment times of 4 and 24 hours, were grown under greenhouse conditions. Seed samples of the 3 controls from the harvested barley field experiment at East Lansing were grown in the greenhouse to determine if the increases in mg of protein per seed among these treatments would result in increased seedling vigor as previously reported for wheat (9).

The rye experiment was grown in an unsterilized silt loam and the barley experiment in vermiculite. Seeds were sown at a rate of 10 seeds per pot and thinned to 5 seedlings. Application of 150 ml of 3 mM (N) 50% Hoaglands solution (6) per pot was made to the seedling vigor study after thinning. Weekly applications were made to the DCM barley experiment.

The rye plants were harvested after 6 weeks and the barley DCM study was terminated after 5 weeks. The seedling vigor study was harvested after 21 days. After drying for 72 hours at 43 C, plants were weighed, ground, and total N was determined.

The greenhouse rye study and the seedling vigor study were randomized complete block designs with 4 replications. The greenhouse barley study (DCM) was a split plot design with 5 replicates. The main split was for time of treatment and the minor split was for chemical treatment.

The analysis of variance was computed according to the experimental design. Where a significant F value for treatments was obtained, Duncan's Multiple Range Test was used to determine the difference between means. Where applicable nonorthogonal comparisons were made, the linear correlations were calculated between relevant parameters. The following code indicates levels of significance: *P = .05, and **P = .01.

RESULTS AND DISCUSSION

Field tests at 3 locations resulted in no effect of vacuum infiltration of 'Coho' barley with simazine and OHEMT upon yield (Table 6). Levels of OHEMT up to 1200 μM resulted in no reduction of yield. Protein content (mg/g) was increased over the untreated control by 20 and 800 μM OHEMT, and by the water infiltrated control. A set of non-orthogonal comparisons indicated that total protein (kg/ha) was increased over the untreated control by the water infiltration alone. Vacuum infiltration of simazine and OHEMT did not increase total protein per hectare.

A subsequent greenhouse study indicated that the seedling vigor of seeds obtained from the 3 control treatments at East Lansing was closely correlated with the protein content of the seed (Table 7). This is the first reported evidence that seedling vigor in barley is related to seed protein.

The yield was reduced by levels of 800 and 1200 μM OHEMT infiltrated into Gary oats planted at Entrican (Table 8). There were no increases in yield over the untreated control, and it is possible that these levels were generally too high as indicated by the toxicity of the higher levels of OHEMT

Table 6. The effect of vacuum infiltration of simazine and OHEMT on yield and protein content of barley grown at three locations.

Treatment		Yield (kg/hectare)			
Chemical	Infiltration (μM)	Sodus	Entrican	East Lansing	$\bar{X}$
Control		987	1577	2883	1816
Air		1133	1470	3300	1968
Water		1107	1623	3180	1970
Simazine	20	1013	1500	2720	1744
OHEMT	20	933	1320	2937	1730
OHEMT	100	1040	1397	2813	1750
OHEMT	200	967	1437	3467	1957
OHEMT	400	1073	1630	3147	1950
OHEMT	800	960	1343	2483	1595
OHEMT	1200	1000	1373	3183	1852
		Protein content (mg/g)			
Control		161	158	122	147 a[1]
Air		173	161	121	152 ab
Water		168	170	140	159 bc
Simazine	20	178	165	121	155 abc
OHEMT	20	177	172	127	159 bc
OHEMT	100	171	161	132	155 abc
OHEMT	200	179	163	129	157 abc
OHEMT	400	176	165	119	153 abc
OHEMT	800	178	174	134	162 c
OHEMT	1200	175	164	121	153 abc
		Total Protein (kg/hectare)			
Control		159	249	353	254 [2]
Air		202	235	379	272
Water		182	276	450	303
Simazine	20	179	247	328	251
OHEMT	20	165	229	376	257
OHEMT	100	175	225	369	256
OHEMT	200	172	237	456	288
OHEMT	400	188	267	371	275
OHEMT	800	168	234	346	249
OHEMT	1200	174	226	386	262

[1]Values in a column followed by the same letter are not significantly different at the .01 level using Duncan's Multiple Range Test.

[2]F value for comparison of control vs. water infiltration significant at the .01 level.

Table 7. The effect of seed protein (mg/seed) on seedling vigor of barley obtained from infiltration studies at East Lansing.

Seed Treatment	Repli- cate	Seed Weight (mg/seed)	Seed Protein (mg/g)	Seed Protein (mg/seed)	Seedling[1] Weight (mg/plant)
Control	I	42.9	105	4.40	28
	II	42.1	105	4.42	29
	III	43.5	141	6.13	36 a
	IV	43.9	135	5.93	37
Air	I	43.3	115	4.98	33
	II	44.1	127	5.60	30
	III	43.5	113	4.91	32 a
	IV	43.3	121	5.24	33
Water	I	42.7	123	5.25	34
	II	44.8	118	5.29	34
	III	46.0	152	6.99	41 b
	IV	42.8	157	6.80	39

[1]Values in a column followed by the same letter are not significantly different at the .05 level using Duncan's Multiple Range Test.

[2]Correlation of seed protein and seedling wt significant at the .01 level $r = .915$.

Table 8. The effect of vacuum infiltration of simazine and OHEMT on yield and protein content of oats grown at Entrican.

Treatment: Chemical	Treatment: Infiltration (μM)	Yield (hg/ha)	Protein (mg/g)	Protein (kg/ha)
Control		[1] 1180 ab	[2] 148	[1] 174 ab
Air		1264 a	153	190 ab
Water		1142 ab	159	181 ab
Simazine	20	1316 a	159	209 a
OHEMT	20	1198 ab	159	191 ab
OHEMT	100	1166 ab	158	184 ab
OHEMT	200	1111 ab	159	176 ab
OHEMT	400	961 bc	166	157 b
OHEMT	800	860 cd	163	142 bc
OHEMT	1200	623 d	163	100 c

[1]Values in a column followed by the same letter are not significantly different at the .05 level using Duncan's Multiple Range Test.

[2]F value for nonorthogonal comparison of 400, 800, and 1200 μM OHEMT vs. control significant at the .05 level.

in this experiment. This reduction in yield was accompanied by increased protein content as indicated by comparison of the untreated control versus 400, 800, and 1200 μM OHEMT. A reduction in total protein per hectare was produced by 1200 μM OHEMT. Vacuum infiltration of simazine and OHEMT did not increase total protein in this experiment. The reduction in yield and total protein per hectare associated with high levels of OHEMT indicate that vacuum infiltration in this experiment results in sufficient levels of chemical availability to affect the plant; in this case causing injury. There was no effect of water infiltration (no triazines) on yield or protein content in this experiment.

Gary oats soaked in simazine solutions (DMC) and planted at Entrican yielded the same as the DCM soaked control (Table 9). Nonorthogonal comparisons indicated that the 4 hour DCM control resulted in an increased yield over the untreated control, and the 24 hour DCM control increased protein content over the untreated control. The 4 hour DCM control increased total protein per hectare as a result of the increased yield. Seeds soaked in simazine solutions produced no increases in total protein over the DCM controls. It should be noted that no toxicity occurred even at treatment rates of 250 and 500 μM of simazine.

Barley seed soaked in simazine solutions (DCM) and grown in the greenhouse in soil, were no larger than the control (Table 10). However, soaking seeds in DCM without simazine

Table 9. The effect of soaking oat seed in DCM and simazine solution of DCM on yield and protein content.

Treatment			Protein	
Simazine (μM)	Time (hr)	Yield (kg/ha)	(mg/g)	(kg/ha)
0	-	1269 [1]	132 [1]	167 [1]
0	4	1728	146	255
50	4	1647	140	230
100	4	1604	142	225
250	4	1334	137	185
500	4	1571	139	225
0	24	1375	154	218
50	24	1426	143	203
100	24	1510	151	228
250	24	1596	156	248
500	24	1402	142	202

[1]F value for comparison of control vs. all DCM treatments is significant at the .05 level.

Table 10. The effect of soaking barley seed in DCM and simazine solutions of DCM on growth and protein content in the greenhouse (average of 1 and 24 hours).

Treatment			Protein	
Solvent	Simazine (μM)	Dry wt (mg/plant)	(mg/g)	(mg/plant)
-	-	79 b [1]	183	14.6
DCM	0	92 a	186	17.0
DCM	20	88 ab	184	16.2
DCM	100	78 b	195	15.2

[1]Values in a column followed by the same letter are not significantly different at the .05 level using Duncan's Multiple Range Test.

did produce larger plants than the control. There was no effect on either protein content or total protein by either simazine treatments or DCM. There was no difference between treatment times of 1 hour and 24 hours.

Vacuum infiltration of 3 s-triazines into rye seed increased the dry weight of plants at levels of 10^{-7} and 10^{-6} M compared to the untreated control and air infiltration control (Table 11). Protein content was increased by 10^{-7} and 10^{-6} M concentrations of simazine compared to the untreated and air infiltrated controls. OHEMT and OHEET did not increase protein content. The total protein per plant was increased by all 3 triazines at 10^{-6} and 10^{-7} M concentrations. These increases can not however, be considered as

Table 11. The effect of vacuum infiltration of 3 triazines on the dry wt and protein content of rye grown in the greenhouse.

Treatment (μM)	Chemical Simazine	OHEMT	OHEET	$\bar{X}$
		Dry wt (mg/plant)		
Control	178 [1]	178 [1]	178 [2]	178
Air	165	165	165	165
Water	195	195	195	195
0.05	223	169	189	194
0.10	194	191	215	200
0.50	189	210	217	205
1.00	234	218	218	223
5.00	189	209	202	200
		Protein (mg/g)		
Control	131 [1]	131	131	131
Air	141	141	141	141
Water	141	141	141	141
0.05	138	131	142	137
0.10	142	135	137	138
0.50	151	145	131	142
1.00	138	135	123	132
5.00	151	135	144	143
		Protein (mg/plant)		
Control	23.4 [2]	23.4 [1]	23.4 [1]	23.4
Air	23.6	23.6	23.6	23.6
Water	27.6	27.6	27.6	27.6
0.05	30.7	22.9	26.8	26.8
0.10	27.4	25.8	29.6	27.6
0.50	28.6	30.6	28.8	29.3
1.00	31.8	29.2	26.9	29.3
5.00	28.6	28.6	29.3	28.8

[1]F value for comparison of control and air versus all vacuum infiltrations significant at .05 level.

[2]F value for comparison of control and air versus all vacuum infiltrations significant at .01 level.

resulting from infiltration of triazines since none of these chemicals significantly increased yield, protein content, or total protein over the water infiltrated control.

Perhaps the most significant finding in these studies was that water infiltration and soaking seeds in DCM often increased yield, protein content, or total protein. A hypothesis for these findings is offered by May, Milthorpe, and Milthorpe (7). Grain seed soaked in water for lengthy intervals (2 to 3 cycles of 24 hours) and air dryed produced significantly increased above-ground growth and yield. A greater response occurred when the plants were subjected to water stress. No workers however, have determined whether protein content or total protein was increased by this treatment. It must be noted however, that early emergence was associated with at least some of these results. This was not the case with water infiltration of DCM soaking.

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