BASES FOR THE INTERACTION OF ALACHLOR, BUTYLATE OR CHLORBROMURON WITH CARBOFURAN ON BARLEY AND CORN

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

ALLAN STEWART HAMILL

This is to certify that the

thesis entitled

BASES FOR THE INTERACTION OF
ALACHLOR, BUTYLATE OR CHLORBROMURON
WITH CARBOFURAN ON BARLEY AND CORN

presented by

Allan S. Hamill

has been accepted towards fulfillment
of the requirements for

PhD. degree in Crop and Soil Science

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ABSTRACT

BASES FOR THE INTERACTION OF ALACHLOR, BUTYLATE OR CHLORBROMURON WITH CARBOFURAN ON BARLEY AND CORN

By

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Numerous herbicide-insecticide combinations were screened on barley (*Hordeum vulgare* L. var. Larker) and corn (*Zea mays* L. var. Michigan 400) for phytotoxic interactions. The herbicides, 2-chloro-2',6'-*N*-(methoxymethyl) acetanilide (alachlor), *S*-ethyl diisobutylthiocarbamate (butylate) and 3-[4-bromo-3-chlorophenyl]-1-methoxy-1-methylurea (chlorbromuron) were combined with the insecticide 2,2-dimethyl-2,3-dihydrobenzofuranyl-7-*N*-methylcarbamate (carbofuran) for respiration, photosynthesis and metabolism studies in the growth chamber. The herbicides were applied preemergence and the insecticide as a seed treatment.

A germination experiment indicated all three herbicides synergistically reduced the radical length of barley seedlings and the alachlor-carbofuran treatment synergistically reduced germination.

A statistical procedure was developed for the calculation of the significant difference between the calculated expected value and the observed value, as proposed by Colby, for pesticide combinations in experiments with a completely randomized design.

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Carbofuran interacted with alachlor to synergistically reduce barley but not corn germination and growth. Alachlor was found to increase the respiration rate of barley. ^{14}C accumulation from ^{14}C-alachlor was greater in the plant roots than in the shoots. The basis for the observed interaction appeared to be greater alachlor uptake by barley plants which had received the carbofuran seed treatment. Specifically the increased accumulation of alachlor in the roots was accentuated by the reduced rate of alachlor metabolism in the roots.

Carbofuran interacted with butylate to synergistically reduce barley, but not corn root and shoot growth. The combination of these two carbamate pesticides synergistically increased respiration in barley. ^{14}C from ^{14}C-butylate preferentially accumulated in barley shoots and corn roots. The bases for these interaction effects in barley appeared to be increased absorption and decreased metabolism of butylate, as well as increased respiration in the presence of carbofuran. Although the same metabolism trend was apparent in corn, the butylate level was much lower since the absorption of butylate was reduced by the carbofuran treatment.

Carbofuran interacted synergistically with chlorbromuron to reduce the height and weight of 41-day-old corn, the root length of 3-day-old barley and the leaf area and dry weight of 7-day-old corn grown in sand culture. The chlorbromuro

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chlorbromuron-carbofuran combination reduced net photosynthesis in barley and corn and increased respiration in barley. ^{14}C from ^{14}C-chlorbromuron preferentially accumulated in barley and corn shoots. The carbofuran seed treatment reduced the level in barley shoots and corn roots and increased the content of ^{14}C in barley roots and corn shoots. The basis for this interaction appeared related to the increased accumulation of chlorbromuron in corn and barley shoots, due to reduced chlorbromuron metabolism, thus increasing the parent chlorbromuron content. These factors contributed to an extended period of exposure of the corn leaf to the herbicide causing the physiological responses measured.

Thin layer chromatography indicated a number of different herbicide metabolites in the root and shoot of each species. In many instances, these metabolites were altered in quality and/or quantity when carbofuran was present in the treatment medium.

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Allan Stewart Hamill

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INTRODUCTION

There have been many reports in recent years of agricultural chemicals interacting to give both desirable and undesirable responses on plants (1,2,5,43,57,61,67). In agriculture at least two or more chemicals, either insecticides, herbicides, fungicides or fertilizers are generally present in the plant microenvironment at the same time. Registration of pesticides requires information on the action and fate of the chemicals in the environment. However, little information is available concerning the interaction among the various agricultural chemicals that could be present simultaneously in the environment. In several instances severe damage has been reported in economic crops following the simultaneous or sequential application of pesticides (8,21,28,35). In other situations chemical combinations promoted the growth of the crop plant and still adequately controlled the weeds (2,3,15,52).

Social pressures have dictated that pesticide residues in foodstuffs be kept at minimum or zero levels. Social concern for environmental quality has been reflected in the rejection of persistant pesticides and new criteria for registration of existing and new compounds. At present, adequate information concerning the fate of pesticides in combination with other pesticides is unavailable. It would be beneficial to know in advance the effect of potential combinations on their action and persistance before widespread use.

The purpose of this study was to investigate possible herbicide-insecticide combinations with respect to changes in phytotoxicity. Several combinations that increased or decreased phytotoxicity were selected for further investigation to obtain at least in part, the basis for the interaction.

Literature Review

General features of pesticide combinations:

Pesticide combinations have become one of the more important components of weed control. Herbicide combinations have been employed for well over a decade because of the ineffectiveness of even a so-called broad spectrum herbicide to adequately control all weed species (27). Other early applications of herbicide mixtures stemmed from the need for more effective weed control in areas where other crops dictated the limited use of 2,4-dichlorophenoxy acetic acid (2,4-D) (59). Two or more narrow spectrum herbicides now may take the place of a broad spectrum herbicide lengthening the time of a weed-free environment and permitting lower application rates of the chemicals involved (16). Mixtures of agricultural chemicals have been used to a large extent on sugar beets (*Beta vulgaris* L.), cotton (*Gossipium hirsutum* L.), beans (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.) (9,29,32,34,44,45,58,59).

Combinations synergistic in their action have been found by Putnam *et al*. (56) and Nash (49). Synergistic combinations may not always be beneficial. Bowling *et al*. (8) found that some organophosphate or carbamate insecticides applied to rice (*Oryza sativa* L.) seedlings interacted synergistically with 3,4-dichloropropionanilide (propanil) to kill the rice. The herbicide propanil was used for the control of barnyard grass (*Echinochloa* spp.) and other weeds

in rice. This interaction occurred even when the chemicals were not applied at the same time. Certain pesticide combinations have shown an antagonistic response (5,55,61). In this situation, the chemicals interacted in a manner to reduce phytotoxicity. Numerous explanations for the deviation from the predicted results have been offered. Davis *et al*. (20) determined that 1,1-dimethyl-4,4 bipyridinium salt (paraquat) increased 4-amino-3,5,6-trichloropicolinic acid (picloram) uptake in some plant species while it reduced transport in others. Uptake and transport of 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) decreased in the presence of picloram, but the uptake and transport of picloram was increased by 2,4,5-T. Agbakoka *et al*. (1) reported that picloram enhanced 2,4-D movement in bindweed (*Convolvulus arvensis* L.). Arle (3) incorporated the insecticides *O*,*O*-diethyl *S*-[(ethylthio)-methyl]-phosphorodithioate (phorate) or *O*,*O*-diethyl *S*-[2(ethylthio)-ethyl]-phosphorodithioate (disulfoton) in α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine (trifluralin) treated soil which resulted in increased cotton seedling growth when compared to the trifluralin alone. He concluded that the greater number of secondary roots in the zone of incorporation accounted for the results. Smith (60) found similar results on germinating corn and wheat (*Triticum* spp.) with trifluralin and organic phosphate insecticides.

Swanson and Swanson (62) found that simultaneous treatment of cotton leaf discs with certain carbamate insecticides inhibited the further degradation of 1-(*p*-chlorophenyl)-3-methylurea (monomethylmonuron), but not the step from 3-(*p*-chlorophenyl)-1,1-dimethylurea (monuron) itself to monomethylmonuron. Chang *et al*. (12) reported insecticide inhibition of the degradation of a number of different herbicides in isolated leaf tissues. The tolerance of rice to propanil is believed to be related to metabolic degradation of propanil (43). However, in the presence of an organophosphate or organothiophosphate insecticide, rice becomes susceptible to propanil. These insecticides inhibit the enzyme which further metabolizes the first metabolic breakdown product of propanil. This enzyme is believed to be similar to acetylchlolinesterase. Another method postulated as a mechanism for the interaction of pesticide combinations was that of Beste *et al*. (6). They determined that following *S*-ethyl dipropylthiocarbamate (EPTC) plus 2,4-D treatments, sorghum (*Sorghum vulgare* Pers.) respired normally while respiration of EPTC treated plants was inhibited. These workers also postulated an interaction effect on nucleic acid metabolism as a basis for the antagonism between 2,4-D and EPTC (7). This seems logical as the carbamate inhibits cell division (4,51) and the phenoxyacetic acid promotes cell division (51).

Statistical analysis of combinations

One of the most difficult problems confronting a researcher involved in working with pesticide combinations is expression of the data, correctly indicating interactions observed. Gowing (26) applied the concepts of probit analysis to herbicide research. This involved a sequential expression of data, in graphical form, from the simple percent inhibition versus concentration to percent inhibition versus log concentration, and finally to percent inhibition in a probability distribution against the log concentration. This method permitted obtaining a value for fifty percent inhibition level for a chemical. This level was designated as a toxic unit. The mixtures of chemicals were then made up in various ratios of toxic units and the results plotted on a graph involving percent inhibition against toxic units. The expected value for the combination was a line connecting points giving a one to one ratio. Chase *et al*. (13) expanded on the probit analysis method by determining the percent inhibition as a percent of control and then using the difference between this and one hundred before plotting against log concentration. In another report, Gowing (27) compared three methods of data expression: percent response versus concentration of herbicide, percent response with log concentration, and reciprocals of response and concentration. He pointed out that all methods may be useful but caution must be used with any of them.

Tammes (63) interpolated data for two chemicals at five rates sprayed in all combinations on the same logarithm-probit diagram. He then took the fifty percent mortality probit level and plotted it on regular graph paper with the rate of one chemical required on the Y axis and that of the other on the X axis. The pictorial presentation of the isobole determined synergism or antagonism. For accurate isobole plots, a considerable amount of data must be generated.

The influence of two or more different factors can be tested by a factorial arrangement of the treatments in a suitable experimental design. Inherant in the analysis of variance of a factorial is the interaction term (42). A significant interaction term in the analysis of variance would imply that this combination was different from single treatments. This method indicates, however, that each of the treatments alone contributed in an additive manner to the combinations. Some researchers have used this approach with an accompanying Duncan's multiple range test to show that a significant interaction occurred (10,30,36). Others simply analyzed combinations, as if they were just a single treatment and applied a comparison test to the means (3, 31,35).

The quadratic equation which contains an interaction term to estimate the regression coefficients has been used by other workers (48,54,66). This equation necessitates

the use of at least three levels for each treatment which lends itself to the factorial experimental design (14). The regression line established for the two components indicates the effect of one compound on changing levels of the other. The interaction term in this equation, however, as in the factorial, is additive in nature with each term contributing in that manner.

Colby (17) approached the interaction term with the assumption that interactions occurred in a sequential fashion rather than in an additive manner. His method involved calculating an "expected" or predicted response for the combination. The easiest method for calculation of the expected value is:

$$\frac{\text{(percent of control for A)} \quad \text{(percent of control for B)}}{100}$$

This expected value for the combination is then compared to the observed percent of control value obtained for the treatment where the two chemicals were combined. Synergism is stated to occur if the observed value for compound A plus B is less than the calculated "expected" value while antagonism occurred if the observed value is greater. This formula can be extended to include a third compound if necessary. Colby (17) has attempted to determine statistical significance of the difference between the expected and observed values by Chi square analysis and analysis of variance on the logarithmically transformed percent of control values. The Chi square analysis has been

shown to be not valid for this type of data (18). The other test is not applicable for a completely randomized design experiment.

Alachlor, Butylate, Chlorbromuron and Carbofuran:

2-Chloro-2',6'-diethyl-*N*-(methoxymethyl) acetanilide (alachlor), is recommended for control of most annual grasses and certain broadleaf weeds in corn and soybeans. It is applied preemergence either to the soil surface or preplant incorporated at one to four pounds per acre. Alachlor is primarily absorbed by the germinating plant shoots and secondarily by the roots followed by translocation throughout the vegetative parts of the plant (68). Following application of alachlor to the soil, high concentrations are found in the initial emerging plant shoot and relatively smaller amounts in the reproductive sections, or younger more actively growing points. In contrast to soil application of alachlor, foliar applications to susceptible species showed some downward movement with fairly uniform distribution. Resistant species displayed significant accumulation in the cotyledons and young growing points (11). Cotton plants exposed to soil and nutrient solution containing alachlor showed stunting and inhibition of lateral root growth, but no foliar chlorosis (24). Chandler (11) found that a susceptible species, wheat, took up more alachlor via the root system than did a tolerant species, soybean. Alachlor is readily leached in lighter sandy soils but is adsorbed to colloidal particles in the soil.

The mechanism of action for this herbicide is not known, however, the compound is closely allied to 2-chloro-*N*-isopropylacetanilide (propachlor) for which some mechansim of action information is available. Jaworski (33) reported work done by Duke with cucumbers (*Cucumis* spp.) which showed that propachlor inhibited nucleic acid synthesis and protein synthesis. The protein synthesis step was hindered first. It was shown that propachlor prevented the activation of amino acids and the transfer of the aminoacyl-tRNA to the polypeptide. Duke (23) later reported the prevention of ^{14}C-leucine incorporation into protein as an early step in the mode of propachlor action. Dhillon (22) substantiated this work with the same results for germinating squash (*Cucurbita* spp.) seedlings. Propachlor was shown to be metabolized to at least three water-soluble metabolites within twenty-four hours in each of four different species. One of the metabolites has been identified as a glutathione conjugate of the herbicide (41). The structural resemblance of alachlor and propachlor point to a similar mechanism of action and possibly metabolism.

S-ethyl diisobutylthiocarbamate (butylate) is a selective incorporated preemergence herbicide for control of seeded perennial grasses and annual grasses in corn. Some broad-leaf weeds are controlled by 3 to 4 lb/A of butylate under favorable conditions. It is rapidly taken up by plant roots and transported acropetally to the whole plant. Sandy soils permit butylate leaching, which

decreases as the clay and organic matter increase. Volatilization from moist soil and microbiological breakdown substantially contribute to butylate loss from the soil and are factors contributing to a half life of one and a half to three weeks in certain soils (68). Threewitt (65) found the loss of activity of butylate for rates up to 10 lb/A to follow a parabolic curve. The length of time required for disappearance was shortened for an early spring application compared to a late winter application. However, while biological activity was reduced in the above manner, a colorimetric test showed the remaining butylate to be in a physiologically inactive state.

The mechanism of action for this herbicide is unknown. It does appear to inhibit growth in the meristematic region of the leaves of grass plants (68). A report of increased weed seed germination induced by butylate vapors perhaps by the breaking of semi-dormancy has also been postulated as a mechanism of action (64). There is little persistance of butylate in corn as it is degraded to CO_2 and natural plant compounds within 7 to 14 days (68).

Most annual grasses and broad-leaf weeds are controlled by 3-[4-bromo-3-chlorophenyl]-1-methoxy-1-methylurea (chlorbromuron) at 1 or 2 lb/A as a preemergence broadcast, banded spray or as a directed postemergence spray before the weeds are two to three inches high (68).

It is reasonable to assume that the mode of action of chlorbromuron is similar to other substituted urea herbicides and as such is a potent inhibitor of photosynthesis (38). The metabolic pathway of dimethyl substituted urea herbicides degradation in plants has been shown to proceed by demethylation to the monomethyl derivative then to phenyl urea and occassionally to aniline. The monomethyl derivative is phytotoxic. However, for the methoxy methyl urea 3-(3,4-dichlorophenyl)-1-methoxy-1-methyl urea (linuron) the first product, the methoxy derivative, is relatively non-phytotoxic. Nashed _et al_. (50) obtained evidence for the formation of the non-phytotoxic methoxy derivative and for "binding" of chlorbromuron in corn shoots and roots. Three other metabolites were also present in 3-week-old corn treated for eight days prior to harvest; the methyl, the phenylurea, and the aniline derivatives. Metabolism of chlorbromuron in corn was evident two days after treatment with the former two metabolites, first detected at four days. No metabolites of chlorbromuron could be detected in the susceptible 3-week-old cucumber treated four days prior to harvest.

The carbamate insecticide 2,2-dimethyl-2,3-dihydrobenzofuranyl-7-_N_-methylcarbamate (carbofuran) in soil treatments not only controls root-attacking pests but also foliar feeders as well. The active ingredient is absorbed by the roots and translocated throughout the plant. It is to be used as foliar contact spray at 1/8 to 1 lb/A or as a soil applied plant systemic, at 1/2 to 8 lb/A depending on the crop and formulation (53). Carbofuran is recommended for use in corn at 7 1/2 to 10 lb applied in a 7 inch band, per 13,000 linear feet.

In plants carbofuran is metabolized by hydroxylation and hydrolysis to at least four metabolites all of which, as well as existing freely, conjugate to form glucosides (39,40,46).

The cereal leaf beetle which was once considered a menace to small grain production in the United States is now considered a critical threat. The use of carbofuran as a seed treatment for small grains has successfully protected these crops for about one and one-half months after planting. The seed treatment has reduced the amount used and cost of insect control and decreased danger of environmental contamination through reduced application rates and lack of drift. For these reasons and because carbofuran has demonstrated promise for use as a seed treatment it was included in this study (47). Alachlor, butylate, and chlorbromuron are all relatively new herbicides recommended for corn. Their demonstrated potential as herbicides and the limited residue period indicate greater future use.

MATERIALS AND METHODS

Screening of Pesticide Combinations for Altered Phytotoxicity

Numerous pesticide combinations were tested in the greenhouse in completely randomized design experiments at temperatures of 25 C to 35 C and 16 hours day length. Ten barley (Hordeum vulgare L. var. Larker) or corn (Zea mays L. var. Michigan 400) seeds were planted in greenhouse potting soil (peat:loam:sand; 1:1:1) in 32 oz waxed cottage cheese containers with drainage holes in the bottom. The pots were initially watered with approximately one inch of water. Thereafter, the pots received water as needed and fertilization once a week with a 28-18-8 fertilizer applied at the rate of 100 lbs actual N per acre.

The herbicides were applied at the time of seeding with a movable table sprayer. The pressure system was activated with a CO_2 cylinder to 30 pounds pressure and the water volume was equivalent to 100 gallons per acre.

The insecticides were applied either as a granular, flowable, or seed treatment at the time of seeding or as a wettable powder 7 to 10 days after seeding.

Insecticide seed treatment was done with a sticker, glycerol:95 percent ethanol:water (1:1:1; v:v:v) at the rate of 0.5 ml for 100 g of corn seed and 2.0 ml for 100 g of barley seed. The seed, sticker and insecticide were shaken together in a container for a minimum of 4 minutes and then dried under the hood. Granular insecticide was applied with the seed at seeding. The pots were thinned to 5 plants per pot within 10 days after planting. The pots were re-randomized on the greenhouse bench on a regular basis. Barley experiments were terminated at 37 days, and corn at 41 days. The pesticide combination effects on plant growth were measured by recording individual plant height and dry shoot weight per pot. Data were subjected to analysis of variance, converted to percent of control and combinations were evaluated according to the method of Colby (17). All values stated in the tables and appendix are the means of two experiments with two or more replications per experiment.

Pesticide combination effects on germination

Alachlor, butylate and chlorbromuron each at 10^{-5} M, alone and in combination with carbofuran as a seed treatment at 4 oz/100 lb of seed were studied for combination effects on germination and seedling vigor. The herbicide solutions were made up in sterile water at pH 7.0 containing 20 ppm of streptomycin sulfate. Glass Petri dishes with two sheets of Whatman No. 2 filter paper were autoclaved and received 10 ml of the various solutions. Three replicates with 10 seeds per dish of each treatment were placed in the dark at 20 C for 3 days. Radical length and germination number were noted. Data were subjected to tests previously described.

Pesticide combination effects on photosynthesis and respiration

Ten corn or barley seeds were planted on washed sand in

6 oz styrofoam cups and covered with vermiculite. The styrofoam cup had numerous holes in the bottom and was placed in a 10 oz waxed cup with a large hole half way up the side for drainage.

The three herbicides, alachlor, butylate and chlorbromuron, at 10^{-6}, 10^{-5}, and 10^{-6} M, respectively, were prepared in modified Hoagland's solution, pH adjusted to 6.8 and added daily to the cups. Preliminary trials showed these concentrations to elicit a response similar to that observed in soil in the greenhouse. After the excess solution had drained out, the cups were re-randomized and returned to the controlled environment chambers.

The insecticide carbofuran was applied as a seed treatment at 4 oz/100 lb of seed or at 10^{-5} M in modified Hoagland's solution (Appendix A). Three replicates of each barley and corn treatment were germinated and grown at 25 C and 30 C respectively. The photoperiod was a 16 hour day and an 8 hour night with the top of the cups receiving 2000 ft candles. Two to four days after planting, the seedlings were thinned to 5 plants per cup.

Photosynthesis and respiration measurements were made 14 days after seeding for alachlor and butylate treated barley, 10 days after seeding for chlorbromuron treated barley and 7 days after seeding for all the corn treatments. The cups were Placed one at a time in a sealed clear plastic test chamber (Figure 1). The plastic chamber was located in a growth chamber similar to that in which the plants were grown, and attached to a Beckman Model IR 215 CO_2 infrared gas analyzer. Air from a compressed air tank was passed through the chamber at a rate of 500 cc per minute measured at the outlet from the analyzer. The analyzer was connected to a Sargent Model SR recorder. The analytical system was adjusted to zero on the recorder with nitrogen and to fifty percent deflection with compressed air without plants in the chamber. The plants were then placed in the plastic test chamber, the lights turned off allowing the plants to respire; thus increasing the CO_2 content of of the effluent gas until a straight horizontal line response was obtained on the recorder indicating that equilibrium had been obtained. The lights were then turned on and the plants were permitted to photosynthesize lowering the CO_2 content of the effluent gas until the recorder again gave a horizontal straight line response. A one unit change on the recorder paper was equivalent to 3.23 μg of CO_2/min. (Appendix B). Respiration of the excised apical 1.5 cm of the leaf tips of barley plants receiving butylate and chlorbromuron with or without carbofuran was also determined using a YSI Oxygen Polarograph.

Plant height was measured from the surface of the vermiculite to leaf or shoot apex. The foliage was cut off at the vermiculite surface and all aerial portions traced for determination of total area with a planimeter. The traced material was oven dried and weighed. Data were subjected to analysis of variance and Duncan's Multiple

Figure 1. Sealed plastic chamber used for photosynthesis and respiration studies.


Coca-Cola

Range Test. Statistical significance of the interaction was determined by obtaining an estimated LSD value between the observed and expected combination values, expressed as a percent of control.

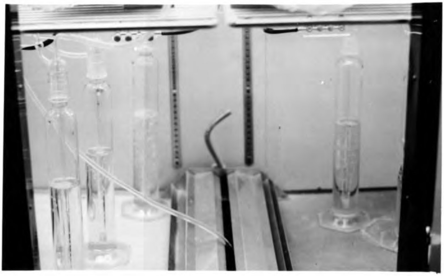
Treatment of plants with ^{14}C-herbicides

Twenty-five ml per cup of the ^{14}C-alachlor or chlorbromuron were applied 4 days after planting barley and 2 days after planting corn. The solutions were not allowed to run out the hole of the outer cup after the ^{14}C treatments had been made. Equal volumes of non-labelled herbicide were added to the cup as necessary thereafter.

The radioactive solutions were prepared at the same molar concentrations from ring-labelled alachlor (1.02 mc/mmole) and carbonyl-labelled chlorbromuron (6.5 μc/mg) as the non-labelled treatment, but contained 1 μc/l of ^{14}C-herbicide.

The volitility of butylate dictated greater caution in the ^{14}C-butylate treatments. Small glass enclosed growth chambers were constructed in an effort to trap any escaping labelled compound(s) (Figure 2). The bottoms were removed from two liter sulfuric acid bottles and the tops were fitted with three holed rubber stoppers. Glass tubing was placed in two of the holes; one piece bent and reaching to the bottom to serve as a gas exit port and the other with a serum cap seal on the outside descended downward to the cup and permitted administration of the ^{14}C-butylate to the

Figure 2. The inside of a growth chamber containing the microchambers and trapping mechanism used in the growing of barley and corn treated with ^{14}C-butylate.



cup. The third hole served as an air inlet. The outlet tube from each microchamber was connected with glass tubing to separate gas scrubbers containing an ethanolamine: ethylene glycol monomethyl ether solution (1:2,v:v). Tygon® tubing served as connectors from the scrubbers to brass fish-tank needle valves which regulated the flow of air separately from each scrubber. A small vacuum pump was connected via a manifold to the needle valves to draw the air from the growth chamber through the system. The temperature in the growth chamber was adjusted to maintain 25 C and 30 C in the glass chambers in separate barley and corn experiments, respectively. The cups were seeded as described previously, but with 7 barley or 5 corn seeds and placed in two-quart plastic bags. The plastic bags were sealed to the bottom of the glass chambers with masking tape. Wooden frames with holes cut large enough to catch the top of the cup in the plastic bag also served as tables for the microchamber; thus the top of the cup and the bottom of the chamber were on the same level. After the initial treatment, the addition of 25 ml of 10^{-5} M butylate with or without alkyl chain ^{14}C-butylate (2.9 μc/μmole) to the cups was made with a syringe through the serum cap on the center glass tube of the microchamber. The radioactive solution contained 5 μc/l.

At the date of harvest photosynthesis and respiration data were obtained and analyzed as previously described.

The plants were then removed from the cups and the roots washed free of sand and vermiculite. One or two plants from each replicate were chosen for freeze drying and radioautography according to the methods of Crafts and Yamaguchi (19). The remaining plant roots and shoots were separated, measured as before, and then freeze-dried for further analysis.

Extraction and analysis of radioactive material

All extraction procedures were done in duplicate on shoot and root samples from two separate experiments.

The samples were ground in a Wiley mill using a no. 60 mesh screen and stored in glass scintillation vials. The ground plant material was extracted for ^{14}C-labelled materials for 6 hours with 10 ml of 80 percent acetone in each vial. The vials were placed on their side in a reciprocating shaker at a speed sufficient to give complete agitation of the entire sample. Following extraction the vials were centrifuged at 455 x g for 5 minutes in a swinging bucket Sorval GLC-1 centrifuge. The supernatant was removed with a disposable pipette and stored in a scintillation vial. The pellet was extracted as before for 8 hours, the extract again centrifuged and the corresponding supernatants combined. The supernatant volume was reduced under nitrogen with the vial on a steam bath at 30 C. Upon completion of a third extraction with 10 ml of 100 percent acetone for 10 hours the homogenate was filtered through No. 1 Whatman filter paper. The filtrate was added to the reduced supernatant from before, reduced in the same manner to approximately 10 ml and placed in 10 ml conical graduated centrifuge tubes. The acetone-insoluble residue was air dried under a hood and stored in a drying oven. The volume of the combined reduced filtrate-supernatant was reduced under nitrogen to 4 ml. A preliminary experiment indicated no ^{14}C was lost with the acetone. One ml of hexane was added to the remaining water portion and mixed in with a spatula and then with a Vortex test tube stirrer. The mixture was centrifuged at 455 x g for 10 minutes, the test tubes sealed with parafilm and then placed in the freezer for about 1 hour to insure good layering of the hexane and water. After the hexane layer was removed from the test tube, one half was used for spotting on thin layer plates and the other half was counted for radioactivity content in a Packard Tri-carb Scintillation Spectrometer. One-half ml of the water fraction was added to 15 ml of a scintillation fluid consisting of 0.1 g 1, 4-bis 2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl POPOP), 5 g 2,5 diphenyloxazole (PPO), 50 g naphthalene, 380 ml toluene, 380 ml 1,4 dioxane, and 240 ml absolute ethanol. This scintillation solution was used for counting all samples.

A small portion of the residue was weighed and combusted by the Schoeninger combustion method of Wang and Willis (67), to determine the amount of ^{14}C incorporated into acetone-insoluble residue. All radioactive samples counted in the liquid scintillation counter were corrected for quenching and volume, then converted to disintegration per minute per gram dry weight of sample initially ground.

<u>Thin layer chromatography of radioactive extracts</u>

Preliminary work showed ^{14}C-labelled compounds could be separated on silica gel H thin layer plates with a thickness of 250 microns. Only those samples which contained at least 100 counts per minute in the entire sample were spotted. The hexane-soluble fractions were spotted in small circles whereas the water-soluble fractions were spotted in 1 inch wide bands. Plates spotted with ^{14}C-alachlor or ^{14}C-chlorbromuron or their metabolites were developed in petroleum ether:chloroform:95 percent ethanol (7:2:1;v:v:v), ^{14}C-butylate and metabolites in chloroform: methanol:pyridine (100:1:10;v:v:v). After development for 15 cm on the plate, the plates were scraped in 1 cm bands. The scrapings were placed in scintillation vials and counted for radioactivity as previously described.

Two scintillation counters were used. One counter was a Packard Tri-carb Scintillation Spectrometer with settings on the red and green channels - gain 9 percent and 9 percent, window A-B and C-D and discriminators at 23-70 and 30-1000, respectively. The other counter was a Nuclear Chicago Mark 1 liquid scintillator with settings on the B and C channels of E 500 for both attenuators, a window width setting of 1.2-9.9 for Channel B and 0.5-9.9 for Channel C. Quenching was corrected by the channels ratio method. An efficiency curve was ca

to dis

was calculated for each machine and all counts were converted to disintegrations per minute per gram dry weight as before.

RESULTS AND DISCUSSION

To date most published reports dealing with pesticide interaction specifically herbicide-insecticide interactions, are those resulting from the application of such combinations to rice and cotton (8,28,29,43). It would seem unlikely that such combination effects do not occur in other crops, for this reason we tested numerous pesticide combinations for possible interactions on barley and corn grown in the greenhouse (Appendix C and D). The chemical combinations chosen for further study are shown in Tables 1 and 2. Barley was used in the study because of its similarity to monocotyledonous weeds in corn.

The combination of carbofuran seed treatment with alachlor or butylate on barley gave a synergistic interaction (Colby method) for the former on dry weight and for the latter on plant height (Table 1). Chlorbromuron and carbofuran interacted antagonistically on barley dry weight. An additive response was obtained with alachlor or chlorbromuron in combination with carbofuran on plant height and for butylate with carbofuran on dry weight.

The terms synergistic and antagonistic have been extended to include interactions observed between an insecticide and a herbicide even though the insecticide is not phytotoxic in itself. Greater phytotoxicity than expected in the presence of the insecticide as defined by

Table 1. The effect of carbofuran seed treatment on the height and dry weight of 37-day-old barley which received various preemergence herbicide applications.

Treatment	Herbicide application rate	Plant height	Expected[a] value	Inter-[a] action	Dry weight	Expected[a] value	Inter-action
	(lb/A)	(Percent of control)			(Percent of control)		
No insecticide treatment							
Control		100 [b]			100 [c]		
Alachlor	1.0	98			108		
Butylate	1.5	43			34		
Chlorbromuron	1.5	111			71		
Carbofuran seed treatment [d]							
Control		89			97		
Alachlor	1.0	90	87		82	105	S
Butylate	1.5	26	38	S	30	33	
Chlorbromuron	1.5	99	99		92	69	A

[a] Derived from Colby's equation; S equals synergism, A equals antagonism.
[b] This value represents an average plant height value of 34.3 cm for 4 replications with 5 plants in each.
[c] This value represents an average dry weight value of 0.29 gm/plant for 4 replications with 5 plants in each.
[d] The rate of treatment was 4 oz/100 lb of seed.

Table 2. The effect of carbofuran seed treatment on the height and dry weight of 41-day-old corn which received various preemergence herbicide applications.

Treatment	Herbicide application rate	Plant height	Expected[a] value	Inter-[a] action	Dry weight	Expected value	Inter-action
	(lb/A)	(Percent of control)			(Percent of control)		
No insecticide treatment							
Control		100 [b]			100 [c]		
Butylate	3.0	97			103		
Chlorbromuron	4.0	110			90		
Carbofuran seed treatment [d]							
Control		91			87		
Butylate	3.0	89	89		76	91	S
Chlorbromuron	4.0	86	101	S	52	79	S

[a] Derived from Colby's equation; S equals synergism, A equals antagonism.
[b] This value represents an average plant height value of 45.6 cm for 4 replications with 5 plants in each.
[c] This value represents an average dry weight of 0.80 gm/plant for 4 replications with 5 plants in each.
[d] The rate of treatment was 4 oz/100 lb of seed.

Colby (22) is designated as synergism while reduced phytotoxicity is designated antagonism. In preliminary experiments, the carbofuran seed treatment showed no interaction with alachlor on corn and no data was recorded. Synergistic reduction of corn dry weight was obtained with the butylate and carbofuran combination while both weight and height of the corn were reduced synergistically by the chlorbromuron and carbofuran combination (Table 2). The carbofuran seed treatment combined with butylate gave only an additive effect on corn height.

To determine the influence of soil on these results a short term germination test on carbofuran treated barley seed with and without alachlor, butylate or chlorbromuron was done in the absence of soil. The germination was synergistically reduced by the alachlor and carbofuran combination as shown in Table 3. Although the other pesticide combinations did not affect the germination, all three herbicides combined with carbofuran showed a synergistic reduction of radical growth. This data indicated that the combination effects observed in the greenhouse studies did not necessarily occur in the soil.

Since germination was not completely inhibited by any of these combinations, their interaction appeared to influence certain physiological functions of the plants, therefore, their photosynthesis and respiration effects were the subject of further investigation.

Table 3. The effect of carbofuran seed treatment on seed germination and maximum root length of 3-day-old barley seedlings which received various preemergence herbicide treatments.[a]

Treatment	Herbicide concentration	Germination	Expected value[b]	Inter-action[b]	Root length	Expected value	Inter-action
	($\times 10^{-5}$ M)	(Percent of control)			(Percent of control)		
No insecticide treatment							
Control		100			100[c]		
Alachlor	1.1	94			30		
Butylate	5.5	108			86		
Chlorbromuron	3.1	100			92		
Carbofuran seed treatment[d]							
Control		86			93		
Alachlor	1.1	41	88	S	18	28	S
Butylate	5.5	91	93		47	80	S
Chlorbromuron	3.1	86	86		60	85	S

[a] The seeds were germinated under sterile conditions in the dark at 20 C.
[b] Derived from Colby's equation; S equals synergism, A equals antagonism.
[c] This value represents seedling root length of 27.5 cm.
[d] The rate of treatment was 4 oz/100 lb of seed.

The greenhouse experiments had been carried out in a completely randomized design. This design was deemed best for growing the plants in the growth chamber. It was pointed out earlier that Colby's equation makes a desirable model depicting how interactions may occur in the plant and yet is not suited to this type of design. Only the mean value for the individual treatments can be used in calculating an accurate expected value for comparison with an observed combination mean. It is not possible to determine, by analysis of variance procedures, whether the observed and expected value were significantly different. The formulas found in Figure 3 were developed to specify the difference necessary in order to label interactions antagonistic or synergistic. The first step involves the determination of an upper and lower confidence limit for the observed combination mean. The confidence limit is then substituted into the Least Significant Difference (LSD) equation to estimate the LSD between the observed and expected combination value expressed on a percent of control basis. Should the expected value lie outside this 5 percent level LSD value, the combination can be considered significantly different at the 5 percent level. Observation of the values will dictate whether it is synergistic or antagonistic. This method was used on data obtained from the growth chamber, photosynthesis, and respiration studies.

Alachlor significantly reduced the dry weight, height and leaf area per plant of 14-day-old barley plants (Table 4) but not the leaf area on a per pot basis. The

1. Confidence limits for the observed combination mean.

$$\text{Upper limit} = L_1 = CR_* + \sqrt{(C-1)\ (CR_*^2 + 1)}$$

$$\text{Lower limit} = L_2 = CR_* - \sqrt{(C-1)\ (CR_*^2 + 1)}$$

2. Estimated LSD between observed and expected combination values expressed as percent of control.

$$LSD = t_\alpha s_{\bar{d}} = t_\alpha s_{\bar{x}} \sqrt{2} \cong$$

$$\left[\sqrt{(C-1)\ (CR_*^2 + 1)}\right]\ (\sqrt{2})\ (100)$$

Where $R_* = \dfrac{\bar{x}_1}{\bar{x}_2}$

$\bar{x}_1$ = Observed combination mean

$\bar{x}_2$ = Control mean

And $C = \dfrac{(\bar{x}_2)^2}{(\bar{x}_2)^2 - (\frac{s^2}{n})\ (t_\alpha)^2}$

$\frac{s^2}{n}$ = $\dfrac{\text{Error variance}}{\text{Number of observations}}$

t_α = t value for appropriate degrees of freedom in error variance

Figure 3. Statistical equations used for the calculation of significant difference between the observed and expected values for plant response to chemical combinations.

Table 4. The effect of alachlor, carbofuran and the combination of alachlor and carbofuran on the growth of 14-day-old barley.

Treatment	Percent of control				
	Leaf area		Dry weight		Height
Control	100 b[a]	100 b	100 c	100 b	100 c
	(344 cm^2/pot)	(69 cm^2/plant)	(288 mg/pot)	(57 mg/plant)	(27 cm/plant)
Carbofuran seed treatment[b]	94 b	94 b	100 c	100 b	101 c
Alachlor, 10^{-6} M	88 b	47 a	90 b	48 a	63 b
Alachlor, 10^{-6} M + carbofuran seed treatment	62 a	39 a	76 a	48 a	53 a
Expected value	83 *[c]	45	90 *	48	64 *

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.
[b] The carbofuran seed treatment was at the rate of 4 oz/100 lb of seed.
[c] Significant difference at the .05 probability level by an estimated LSD between the observed and expected combination values expressed as a percent of control.

carbofuran treatment was not different from the control; however, when combined with alachlor, the reduction in leaf area and dry weight per pot and plant height was synergistic. This indicated that the plants receiving the combination treatment were shorter. This information supports the combination effects observed in the greenhouse and germination studies.

Photosynthesis was not inhibited but respiration was increased by alachlor compared to the control (Table 5). The carbofuran treatment increased barley respiration when combined with alachlor significantly above that of the control; however, the result was not significantly different from the alachlor treatment by itself.

Corn growth, photosynthesis or respiration were not affected by alachlor or the insecticide seed treatment-herbicide combination (Tables 6 and 7) as indicated from the preliminary greenhouse experiments. However, when carbofuran was applied in the nutrient media at 10^{-5} M, corn growth was significantly increased (Table 8), resulting in a synergistic reduction for leaf area per plant and per pot. Net photosynthesis differed between the alachlor and carbofuran treatments but neither differed from the control (Table 9).

The butylate or carbofuran treatment did not differ from the control for any of the measured parameters on barley plants (Tables 10 and 11). The combination of the two pesticides resulted in a synergistic reduction of the

Table 5. The effect of alachlor, carbofuran, and the combination of alachlor and carbofuran on respiration and photosynthesis on 14-day-old barley.

Treatment	Percent of control		
	Respiration	Net photosynthesis	Total photosynthesis
Control	100 a[a]	100 a	100 a
	(50 μg CO_2/min/gm)	(0.373 μg CO_2/min/cm^2)	(0.410 μg CO_2/min/cm^2)
Carbofuran seed treatment[b]	154 ab	102 a	108 a
Alachlor, 10^{-6} M	221 b	99 a	109 a
Alachlor, 10^{-6} M + carbofuran seed treatment	186 b	113 a	138 a
Expected value	340	101	117

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

[b] The carbofuran seed treatment was at the rate of 4 oz/100 lb of seed.

Table 6. The effect of alachlor, carbofuran, and the combination of alachlor and carbofuran on the growth of 7-day-old corn.

Treatment	Percent of control				
	Leaf area		Dry weight		Height
Control	100 a[a]	100 a	100 a	100 a	100 a
	(451 cm^2/pot)	(93 cm^2/plant)	(477 mg/pot)	(99 mg/plant)	(25 cm/plant)
Carbofuran seed treatment[b]	91 a	101 a	86 a	97 a	101 b
Alachlor, 10^{-6} M	88 a	85 a	94 a	90 a	86 a
Alachlor, 10^{-6} M + carbofuran seed treatment	92 a	86 a	95 a	88 a	86 a
Expected value	80	86	81	87	87

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

[b] The carbofuran seed treatment was at the rate of 4 oz/100 lb of seed.

Table 7. The effect of alachlor, carbofuran and the combination of alachlor and carbofuran on respiration and photosynthesis of 7-day-old corn.

	Percent of control		
Treatment	Respiration	Net photosynthesis	Total photosynthesis
Control	100 a[a] (220 μg CO_2/min/gm)	100 a (0.462 μg CO_2/min/cm^2)	100 a (0.604 μg CO_2/min/cm^2)
Carbofuran seed treatment[b]	89 a	93 a	106 a
Alachlor, 10^{-6} M	73 a	108 a	115 a
Alachlor, 10^{-6} M + carbofuran seed treatment	88 a	92 a	105 a
Expected value	65	100	122

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.
[b] The carbofuran seed treatment was at the rate of 4 oz/100 lb of seed.

Table 8. The effect of alachlor, carbofuran and the combination of alachlor and carbofuran on the growth of 7-day-old corn.

Treatment	Percent of control				
	Leaf area		Dry weight		Height
Control	100 a[a]	100 a	100 ab	100 a	100 b
	(451 cm^2/pot)	(93 cm^2/plant)	(477 mg/pot)	(99 mg/plant)	(25 cm/plant)
Carbofuran 10^{-5} M	135 b	137 b	120 b	121 b	112 b
Alachlor, 10^{-6} M	88 a	85 a	94 a	90 a	86 a
Alachlor, 10^{-6} M + carbofuran 10^{-5} M	90 a	86 a	101 ab	87 a	86 a
Expected value	118 *[b]	116 *	112	109	96

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

[b] Significant difference at the .05 probability level by an estimated LSD between the observed and expected combination values expressed as a percent of control.

Table

Table 9. The effect of alachlor, carbofuran and the combination of alachlor and carbofuran on respiration and photosynthesis of 7-day-old corn.

Treatment	Percent of control		
	Respiration	Net photosynthesis	Total photosynthesis
Control	100 a[a]	100 ab	100 ab
	(220 μg CO_2/min/gm)	(0.462 μg CO_2/min/cm^2)	(0.604 μg CO_2/min/cm^2)
Carbofuran 10^{-5} M	70 a	71 a	81 a
Alachlor, 10^{-6} M	73 a	108 b	115 b
Alachlor, 10^{-6} M + carbofuran 10^{-5} M	94 a	86 ab	103 ab
Expected value	51	76	94

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

Table 10. The effect of butylate, carbofuran and the combination of butylate and carbofuran on the growth of 14-day-old barley.

Treatment	Percent of control		
	Leaf area	Dry weight	Height
Control	100 b[a]	100 b	100 b
	(14 cm^2/plant)	(12 mg/plant)	(15 cm/plant)
Carbofuran seed treatment[b]	105 b	87 b	97 b
Butylate, 10^{-5} M	91 b	89 b	93 b
Butylate, 10^{-5} M + carbofuran seed treatment	52 a	59 a	78 a
Expected value	96 *[c]	77	91 *

a Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

b The carbofuran seed treatment was at the rate of 4 oz/100 lb of seed.

c Significant difference at the .05 probability level by an estimated LSD between the observed and expected combination values expressed as a percent of control.

Table 11. The effect of butylate, carbofuran and the combination of butylate and carbofuran on respiration and photosynthesis of 14-day-old barley.

Treatment	Percent of control		
	Respiration	Net photosynthesis	Total photosynthesis
Control	100 a[a]	100 a	100 a
	(101 μg CO_2/min/gm)	(0.178 μg CO_2/min/cm^2)	(0.260 μg CO_2/min/cm^2)
Carbofuran seed treatment[b]	136 a	89 a	96 a
Butylate, 10^{-5} M	152 a	119 a	126 a
Butylate, 10^{-5} M + carbofuran seed treatment	299 b	112 a	182 b
Expected value	206 *[c]	106	121

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.
[b] The carbofuran seed treatment was at the rate of 4 oz/100 lb of seed.
[c] Significant difference at the .05 probability level by an estimated LSD between the observed and expected combination values expressed as a percent of control.

leaf area per plant and plant height. The shorter foliage was thicker as the dry weight per plant was not reduced. Respiration was synergistically increased by the combination treatment but net photosynthesis was unchanged. The large increase in respiration contributed to the significant total photosynthesis difference, however, it was only an additive response. The data in Table 12 show that the oxygen uptake by barley leaf tips, as a measure of respiration, was synergistically enhanced by the butylate plus carbofuran treatment. Thus the respiration response measured with the oxygen polarograph supports the results obtained with the CO_2 analyzer. Previously thiocarbamate herbicides have not been considered to directly effect photosynthesis or respiration.

Application of butylate to carbofuran treated corn seed significantly decreased leaf area per pot and dry weight per pot from the butylate treatment itself, however, at no time were any of the treatments significantly different from the control plants for any of the growth characteristics measured (Table 13). Neither photosynthesis nor respiration differed significantly from the controls (Table 14). Butylate is rapidly absorbed by corn roots and translocated to the foliage (68). The results in Table 15 indicate a synergistic reduction of leaf area per plant and per pot, dry weight per pot, and plant height from the butylate and 10^{-5} M carbofuran combination treatment. However, at no time were these parameters significantly different from the

Table 12. The effect of butylate, carbofuran and the combination of butylate and carbofuran on respiration of excised barley leaf tips from 14-day-old barley plants.

Treatment	O_2 Uptake
	(Percent of control)
Control	100 a (5.49 mμ moles/min/mg)
Carbofuran seed treatment[b]	92 a[a]
Butylate, 10^{-5} M	89 a
Butylate, 10^{-5} M + carbofuran seed treatment	150 b
Expected value	82 *[c]

[a] Means followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.
[b] The carbofuran seed treatment rate was at 4 oz/100 lb seed.
[c] Significant difference at the .05 probability level by an estimated LSD between the observed and expected combination values expressed as a percent of control.

Table 13. The effect of butylate, carbofuran and the combination of butylate and carbofuran on the growth of 7-day-old corn.

Treatment	Percent of control				
	Leaf area		Dry weight		Height
Control	100 ab[a]	100 a	100 ab	100 a	100 ab
	($451\ cm^2$/pot)	($93\ cm^2$/plant)	(477 mg/pot)	(99 mg/plant)	(25 cm/plant)
Carbofuran seed treatment[b]	91 ab	101 a	86 a	97 a	101 b
Butylate, 10^{-5} M	105 b	98 a	110 b	102 a	97 ab
Butylate, 10^{-5} M + carbofuran seed treatment	87 a	94 a	87 a	94 a	91 a
Expected value	96	100	95	79	98

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

[b] The carbofuran seed treatment was at the rate of 4 oz/100 lb of seed.

Table 14. The effect of butylate, carbofuran and the combination of butylate and carbofuran on respiration and photosynthesis of 7-day-old corn.

Treatment	Percent of control		
	Respiration	Net photosynthesis	Total photosynthesis
Control	100 a[a]	100 a	100 a
	(220 μg CO_2/min/gm)	(0.462 μg CO_2/min/cm^2)	(0.604 μg CO_2/min/cm^2)
Carbofuran seed treatment[b]	89 a	93 a	106 a
Butylate, 10^{-5} M	74 a	88 a	96 a
Butylate, 10^{-5} M + carbofuran seed treatment	85 a	97 a	106 a
Expected value	66	82	102

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

[b] The carbofuran seed treatment was at the rate of 4 oz/100 lb seed.

Table

Table 15. The effect of butylate, carbofuran and the combination of butylate and carbofuran on the growth of 7-day-old corn.

	Percent of control				
Treatment	Leaf area		Dry weight		Height
Control	100 a[a]	100 a	100 a	100 a	100 a
	(451 cm^2/pot)	(93 cm^2/plant)	(477 mg/pot)	(99 mg/plant)	(25 cm/plant)
Carbofuran 10^{-5} M	135 b	137 b	120 b	121 b	112 b
Butylate, 10^{-5} M	105 a	98 a	110 ab	102 a	97 a
Butylate, 10^{-5} M + carbofuran 10^{-5} M	105 a	99 a	107 ab	100 a	96 a
Expected value	142 *[b]	134 *	132 *	124	108 *

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

[b] Significant difference at the .05 probability level by an estimated LSD between the observed and expected combination values expressed as a percent of control.

control plants. These measurements appeared to be synergistic only because of the stimulation of growth by carbofuran applied in this manner. Photosynthesis and respiration did not significantly differ from the control in this combination treatment (Table 16).

The chlorbromuron treatment did not reduce the leaf area per plant significantly from that of the control plants, but it did reduce the dry weight and height of barley plants (Table 17). The carbofuran seed treatment did not further influence the chlorbromuron response. The data in Table 18 indicate that respiration by barley was significantly increased by the combination treatment compared to the control while net photosynthesis was significantly reduced at this level of chlorbromuron treatment. The combination treatment of carbofuran and chlorbromuron did not significantly differ from the chlorbromuron treatment with respect to respiration and photosynthesis itself. Chlorbromuron, a substituted urea herbicide, might be expected to act similarly on susceptible plants as other substituted ureas. Thus, chlorbromuron should be a potent photosynthesis inhibitor. Geissbuhler (38) stated that the site of action of these herbicides can be located with reasonable certainty to that part of the photosynthetic mechanism which is connected with the process of oxygen evolution. It is reasonable to assume chlorbromuron has some influence at this point on photosynthesis. As shown in Table 19, chlorbromuron with or without carbofuran did

Table 16. The effect of butylate, carbofuran and the combination of butylate and carbofuran on respiration and photosynthesis of 7-day-old corn.

Treatment	Percent of control		
	Respiration	Net photosynthesis	Total photosynthesis
Control	100 a[a]	100 a	100 a
	(220 µg CO_2/min/gm)	(0.462 µg CO_2/min/cm^2)	(0.604 µg CO_2/min/cm^2)
Carbofuran, 10^{-5} M	70 a	71 a	81 a
Butylate, 10^{-5} M	74 a	88 a	96 a
Butylate, 10^{-5} M + carbofuran, 10^{-5} M	71 a	91 a	97 a
Expected value	52	62	78

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

Table 17. The effect of chlorbromuron, carbofuran and the combination of chlorbromuron and carbofuran on the growth of 10-day-old barley.

Treatment	Percent of control		
	Leaf area	Dry weight	Height
Control	100 bc[a]	100 c	100 b
	(14 cm^2/plant)	(12 mg/plant)	(15 cm/plant)
Carbofuran seed treatment[b]	105 c	87 b	97 b
Chlorbromuron, 10^{-6} M	83 ab	76 a	86 a
Chlorbromuron, 10^{-6} M + carbofuran seed treatment	72 a	71 a	92 ab
Expected value	87	66	84

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

[b] The carbofuran seed treatment was at the rate of 4 cz/100 lb of seed.

Table 18. The effect of chlorbromuron, carbofuran and the combination of chlorbromuron and carbofuran on respiration and photosynthesis of 10-day-old barley.

Treatment	Percent of control		
	Respiration	Net photosynthesis	Total photosynthesis
Control	100 a[a]	100 c	100 a
	(10 µg CO_2/min/cm^2)	(0.178 µg CO_2/min/cm^2)	(0.260 µg CO_2/min/cm^2)
Carbofuran seed treatment[b]	136 ab	89 bc	96 a
Chlorbromuron, 10^{-6} M	173 bc	72 ab	99 a
Chlorbromuron, 10^{-6} M + carbofuran seed treatment	193 c	62 a	103 a
Expected value	234	64	95

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.
[b] The carbofuran seed treatment was at the rate of 4 oz/100 lb of seed.

Table 19. The effect of chlorbromuron, carbofuran and the combination of chlorbromuron and carbofuran on respiration of excised barley leaf tips from 10-day-old barley plants.

Treatment	O_2 Uptake
	(Percent of control)
Control	100 a[a] (5.49 mμ moles/min/mg)
Carbofuran seed treatment[b]	92 a
Chlorbromuron, 10^{-6} M	91 a
Chlorbromuron, 10^{-6} M + carbofuran seed treatment	97 a
Expected value	84

[a] Means followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

[b] The carbofuran seed treatment was at the rate of 4 oz/100 lb seed.

not significantly change the O_2 uptake from that of the controls.

Chlorbromuron is promoted for use in corn and thus would not be expected to affect corn. The results presented in Table 20 show this to be true, except for plant height where a small but significant reduction was observed. The combination with carbofuran, on the other hand, synergistically reduced the leaf area and dry weight per pot, but did not further reduce plant height. These results are similar to those obtained in the greenhouse (Table 2). As noted in Table 21, respiration was not significantly altered from the control by the carbofuran seed treatment, the chlorbromuron treatment, or the combination of the two, whereas net photosynthesis was significantly reduced only by chlorbromuron. The synergistic plant height and dry weight reduction may have been the result of the adverse effect on net photosynthesis. The data in Table 22 show that when carbofuran was applied to corn at 10^{-5} M, the combination with chlorbromuron synergistically reduced leaf area per pot and plant height. The corn height, however, was not significantly different from that of the control, possibly because carbofuran significantly increased plant height. The leaf area per pot was reduced by the combination treatment containing carbofuran on a molar basis in the same manner as the carbofuran on a molar basis significantly increased the leaf area per pot. The dry weight reduction per pot by the combination treatment differed when carbofuran was applied as a seed treatment

Table 20. The effect of chlorbromuron, carbofuran and the combination of chlorbromuron and carbofuran on the growth of 7-day-old corn.

Treatment	Percent of control				
	Leaf area		Dry weight		Height
Control	100 b[a]	100 a	100 c	100 a	100 c
	(451 cm^2/pot)	(93 cm^2/plant)	(477 mg/pot)	(99 mg/plant)	(25 cm/plant)
Carbofuran seed treatment[b]	91 b	101 a	86 b	97 a	101 c
Chlorbromuron, 10^{-6} M	105 b	105 a	95 bc	94 a	93 ab
Chlorbromuron, 10^{-6} M + carbofuran seed treatment	77 a	109 a	64 a	91 a	86 a
Expected value	96 *[c]	107	81 *	91	94

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.
[b] The carbofuran seed treatment was at the rate of 4 oz/100 lb of seed.
[c] Significant difference at the .05 probability level by an estimated LSD between the observed and expected combination values expressed as a percent of control.

Table 21. The effect of chlorbromuron, carbofuran and the combination of chlorbromuron and carbofuran on respiration and photosynthesis on 7-day-old corn.

Treatment	Percent of control		
	Respiration	Net photosynthesis	Total photosynthesis
Control	100 a[a]	100 c	100 b
	(220 μg CO_2/min/gm)	(0.462 μg CO_2/min/cm)	(0.604 μg CO_2/min/cm^2)
Carbofuran seed treatment[b]	89 a	93 b	106 b
Chlorbromuron, 10^{-6} M	69 a	49 a	62 a
Chlorbromuron, 10^{-6} M + carbofuran seed treatment	82 a	48 a	64 a
Expected value	61	46	65

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.
[b] The carbofuran seed treatment was at the rate of 4 oz/100 lb of seed.

Table 22. The effect of chlorbromuron, carbofuran and the combination of chlorbromuron and carbofuran on the growth of 7-day-old corn.

Treatment	Percent of control				
	Leaf area		Dry weight		Height
Control	100 a[a]	100 a	100 a	100 a	100 b
	(451 cm^2/pot)	(93 cm^2/plant)	(477 mg/pot)	(99 mg/plant)	(25 cm/plant)
Carbofuran, 10^{-5} M	135 c	137 b	120 b	121 b	112 c
Chlorbromuron, 10^{-6} M	105 a	105 a	95 a	94 a	93 a
Chlorbromuron, 10^{-6} M + carbofuran, 10^{-5} M	112 b	108 a	100 a	96 a	94 ab
Expected value	141 *[b]	144	114	114	104 *

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

[b] Significant difference at the .05 probability level by an estimated LSD between the observed and expected combination values expressed as a percent of control.

versus application as a molar concentration, but again the reason is likely due to the significant dry weight increase by carbofuran itself. The data in Table 23 indicate that the chlorbromuron and carbofuran 10^{-5} M treatments both reduced photosynthesis. The combination of the two pesticides did not cause a synergistic reduction of photosynthesis. Thus, while chlorbromuron is suggested for use on corn, these greenhouse and growth chamber studies show that it can influence the early growth of the plant, through a reduced net photosynthetic rate.

Uptake, translocation and metabolism of ^{14}C-alachlor

Extraction with 80 percent acetone following the addition of ^{14}C-labelled herbicide to untreated plant material indicated none of the herbicides used in this study were bound to the ground plant tissue. Partitioning between 1 ml of hexane and 5 ml of water of the ethanol-soluble stock ^{14}C-alachlor, ^{14}C-butylate and ^{14}C-chlorbromuron indicated 92, 85, and 97 percent, respectively, partitioned into the hexane fraction.

Alachlor is absorbed mainly by the germinating plant shoot, secondarily by the roots, and then translocated throughout the plant (68). This occurred in both barley and corn (Figures 4 and 5). The greatest accumulation appeared to be in the coleoptile of corn when treated with alachlor alone, but in both cases movement throughout the plants did occur. The uptake was greater in the susceptible species, barley, compared to the tolerant corn as shown in

Table 23. The effect of chlorbromuron, carbofuran and the combination of chlorbromuron and carbofuran on respiration and photosynthesis of 7-day-old corn.

Treatment	Percent of control: Respiration	Net photosynthesis	Total photosynthesis
Control	100 a[a]	100 c	100 d
	(220 μg CO_2/min/gm)	(0.642 μg CO_2/min/cm)	(0.604 μg CO_2/min/cm^2)
Carbofuran, 10^{-5} M	70 a	71 b	81 c
Chlorbromuron, 10^{-6} M	69 a	49 a	62 a
Chlorbromuron, 10^{-6} M + carbofuran, 10^{-5} M	66 a	55 a	65 b
Expected value	49	35	50

[a] Means in a column followed by the same letter do not differ significantly at the .05 probability level according to Duncan's Multiple Range Test.

Figure 4. Barley plants (left) and radioautographs (right) of barley plants harvested 10 days after ^{14}C-alachlor was placed in the nutrient solution. Upper: ^{14}C-alachlor treated plants. Lower: ^{14}C-alachlor treated plants in the presence of carbofuran as a seed treatment.

Figure 5. Corn plants (left) and radioautographs (right) of corn plants harvested 5 days after ^{14}C-alachlor was placed in the nutrient solution. Upper: ^{14}C-alachlor treated plants. Lower: ^{14}C-alachlor treated plants in the presence of carbofuran as a seed treatment.

Figures 4 and 5 and Table 24. There was almost three times the amount of ^{14}C in the barley as in the corn although the barley was exposed for twice as long. There was only about one-third more ^{14}C label in the barley root than in the shoot while the corn root contained half again as much as the shoot. Jaworski (38) has reported that corn, a species resistant to chloroacetamides, invariably absorbed less of these chemicals than soybeans, another resistant species, which took up more than the susceptible plant species, cucumbers and oats. Therefore, it would appear that two factors may be contributing to the tolerance of corn: less alachlor is absorbed and less is translocated throughout the shoot.

The addition of carbofuran as a seed treatment appeared to increase the total alachlor uptake in both barley and corn. The amount present was greater for barley than corn and for the roots than the shoots in both species. The greater amount of ^{14}C in the roots can be seen in Figures 4 and 5 (lower). Carbofuran not only increases the uptake of alachlor into the plant but may slightly change the uptake and translocation pattern by causing ^{14}C accumulation in the root. The partitioning of the ^{14}C in the roots of barley and corn is similar, with corn having a little more in the 80 percent acetone-insoluble fraction (Table 25). The amount of ^{14}C in the hexane-soluble fraction in corn root is higher than it is in the shoot, whereas for

Table 24. The partitioning and distribution of ^{14}C-alachlor in 14-day-old barley and 7-day-old corn grown in nutrient solution containing alachlor, 10 and 5 days respectively, after ^{14}C-alachlor application.

Treatment	^{14}C in shoot: 80 Percent acetone-insoluble (dpm/g)	^{14}C in shoot: Hexane-soluble fraction (dpm/g)	^{14}C in shoot: Water-soluble fraction (dpm/g)	^{14}C in shoot: Total (dpm/g)	^{14}C in root: 80 Percent acetone-insoluble (dpm/g)	^{14}C in root: Hexane-soluble fraction (dpm/g)	^{14}C in root: Water-soluble fraction (dpm/g)	^{14}C in root: Total (dpm/g)
Barley:								
Alachlor, 10^{-6} M	5393	244	4180	9816	7462	459	4948	12869
Alachlor, 10^{-6} M + carbofuran[a]	5534	297	5757	11588	9559	507	5978	16044
Corn:								
Alachlor, 10^{-6} M	1903	16	1503	3422	3243	250	1623	5116
Alachlor, 10^{-6} M + carbofuran	2448	81	1458	3987	4047	323	1988	6358

[a] Carbofuran applied as a seed treatment at 4 oz/100 lb of seed.

Table 25. The partitioning of ^{14}C as a percent of the ^{14}C present in the shoot or root of 14-day-old barley and 7-day-old corn grown in nutrient solution containing alachlor, 10 and 5 days respectively, after ^{14}C-alachlor application.

Treatment	^{14}C in shoot			^{14}C in root		
	80 Percent acetone-insoluble (dpm/g)	Hexane-soluble fraction (dpm/g)	Water-soluble fraction (dpm/g)	80 Percent acetone-insoluble (dpm/g)	Hexane-soluble fraction (dpm/g)	Water-soluble fraction (dpm/g)
Barley:						
Alachlor, 10^{-6} M	52	3	46	57	4	39
Alachlor, 10^{-6} M + carbofuran[a]	48	3	49	60	3	37
Corn:						
Alachlor, 10^{-6} M	57	1	43	64	5	31
Alachlor, 10^{-6} M + carbofuran	62	2	36	64	5	31

[a] Carbofuran applied as a seed treatment at 4 oz/100 lb of seed.

barley it is relatively unchanged. The carbofuran treatment did not influence the fractionation with respect to species or within the barley plant or corn root, but it did increase the 80 percent acetone-insoluble fraction in the corn shoot at the expense of the water-soluble fraction. In both plant species, most of the ^{14}C was in the 80 percent acetone-insoluble fraction. The hexane-soluble portion made up only a small part of the soluble fraction.

The hexane-soluble portion contained so little ^{14}C in barley that it was not chromatographed on thin layer chromatography (TLC). The barley shoot appeared to contain twice the concentration of parent ^{14}C-alachlor in the water-soluble fraction than did the barley root (Table 26). The ^{14}C in the water-soluble fraction which co-chromatographed with the standard was considered parent herbicide in all cases. The majority of the ^{14}C was found in one metabolite in both the shoot and the root, with one other metabolite occurring in each. The carbofuran treatment appeared to decrease the proportion of parent ^{14}C-alachlor and increase the percent in the major metabolite in the shoot. The root was affected in the opposite manner with more ^{14}C as alachlor and less as the major metabolite. The minor metabolite in the barley shoots and roots appeared to be the same, following alachlor treatment, but different from either of the minor compounds found following the alachlor-carbofuran combination treatment. It is inferred in Table

Table 26. The separation on TLC of shoot and root extracts from 14-day-old barley 10 days after ^{14}C-alachlor treatment.

Plant Part	Treatment		Percent of total spotted										
		Rf	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Shoot:	Water-soluble												
	Alachlor, 10^{-6} M			70				6				23[b]	
	Alachlor, 10^{-6} M + carbofuran [a]			84					3			12[b]	
	Hexane-soluble												
	Alachlor, 10^{-6} M												
	Alachlor, 10^{-6} M + carbofuran												
Root:	Water-soluble												
	Alachlor, 10^{-6} M			84							5		10[b]
	Alachlor, 10^{-6} M + carbofuran			79					6				16[b]
	Hexane-soluble												
	Alachlor, 10^{-6} M												
	Alachlor, 10^{-6} M + carbofuran												

a Carbofuran was applied as a seed treatment at 4 oz/100 lb of seed.
b Indicates parent compound.

26 that more alachlor remains in the root in the presence of carbofuran. The data in Table 27 indicate that this is in fact what occurs in barley. Although there is an increase in parent compound in the root, there is an even greater decrease in the shoot. Since alachlor inhibited initial seedling growth in the greenhouse and in germination studies it would seem likely that the increased susceptibility of barley to the pesticide combination is in part due to increased uptake, decreased movement and also to decreased metabolism of alachlor. The alachlor treatment by itself on barley showed more parent material to be present in the shoot than in the root. Since these plants showed less toxicity than those receiving the combination, this would indicate that alachlor once in the shoot has less influence. It also suggests that if a susceptible plant root can get past the initial germination stage, following alachlor treatment, that while it is not entirely unharmed it can continue to grow.

In corn treated with ^{14}C-alachlor most of the ^{14}C was found in a major metabolite staying near or at the origin on the TLC plates (Table 28). In the water-soluble fraction from the combination treatment a different major metabolite may have been detected (Table 28). The shoot had three minor metabolites in the water-soluble fraction and the root had two. Of these five compounds, only two appeared to be the same. In the root, 8 percent of the ^{14}C in the water-

Table 27. The percent extractable ^{14}C-alachlor remaining in 14-day-old barley and 7-day-old corn shoots and roots, 10 and 5 days respectively, after ^{14}C-alachlor treatment.

	^{14}C in shoot			^{14}C in root		
Treatment	Hexane-soluble fraction	Water-soluble fraction	Total	Hexane-soluble fraction	Water-soluble fraction	Total
Barley:						
Alachlor, 10^{-6} M	0	10.6	10.6	0	4.1	4.1
Alachlor, 10^{-6} M + carbofuran[a]	0	6.0	6.0	0	5.8	5.8
Corn:						
Alachlor, 10^{-6} M	0	1.6	1.6	0	2.4	2.4
Alachlor, 10^{-6} M + carbofuran	0	4.3	4.3	0	1.2	1.2

[a] Carbofuran was applied as a seed treatment at 4 oz/100 lb of seed.

Table 28. The separation on TLC of shoot and root extracts from 7-day-old corn 5 days after ^{14}C-alachlor treatment.

Plant Part	Treatment		Percent of total spotted										
		Rf	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Shoot:	Water-soluble												
	Alachlor, 10^{-6} M			62	12		6			15			4[b]
	Alachlor, 10^{-6} M + carbofuran[a]			69	9	10							12[b]
	Hexane-soluble												
	Alachlor, 10^{-6} M												
	Alachlor, 10^{-6} M + carbofuran												
Root:	Water-soluble												
	Alachlor, 10^{-6} M		78				3			11			8[b]
	Alachlor, 10^{-6} M + carbofuran			86				5		5			4[b]
	Hexane-soluble												
	Alachlor, 10^{-6} M		35			2		9			54		
	Alachlor, 10^{-6} M + carbofuran		41			9		10			40		

[a] Carbofuran was applied as a seed treatment at 4 oz/100 lb of seed.
[b] Indicates parent compound.

soluble fraction chromatographed as the parent alachlor. This was twice as much as found in the shoot.

The addition of carbofuran to the nutrient solution caused not only a shift to a greater portion of parent material in the water-soluble fraction in the shoot than the root, but the ratio was changed from 1:2 to 3:1. The combination treatment caused a reduction in the number of detected metabolites in the shoot from four to three with little change in the major metabolite and one of the minor metabolites, but two others disappeared and a new one occurred. In the root, a new major metabolite was formed which corresponded in Rf to that of the major shoot metabolite. A reduction in the amount of the minor metabolite with an Rf of 0.7 occurred when carbofuran was present.

Carbofuran interacted with alachlor to synergistically reduce barley, but not corn germination and growth. The basis for the observed interaction appeared to be greater alachlor uptake by barley plants which had received the carbofuran seed treatment. Specifically the increased accumulation of alachlor in the roots was accentuated by the reduced rate of alachlor metabolism in the roots.

<u>Uptake, translocation and metabolism of ^{14}C-butylate</u>

Butylate is a volatile herbicide. In the initial experiment using ^{14}C-butylate, Tygon® tubing was used between the microchambers and the trapping solution. The tubing trapped 90 percent or more of the ^{14}C which evolved from the chamber during that period. This would indicate that only a very small percent of butylate was converted to CO_2 during the 10 day treatment period. Thiocarbamates are readily absorbed by both shoots and roots. EPTC has been shown to translocate from the coleoptile to the root and was also moved into the foliage from the root (38). Butylate can be absorbed by leaves or roots and is rapidly translocated from the roots to the aerial portions of the plant (68). The data in Table 29 show the latter to be the case for barley. The susceptible barley has almost a five-fold increase of ^{14}C in the shoot over that found in the tolerant corn. This is almost twice as much as in the shoot. This data is substantiated by the radioautographs shown in Figures 6 and 7 (upper). The roots for both plant species were equally labelled, while the barley shoot received more label than the corn leaves. The coleoptile for this treatment does not appear to be acting as a sink.

The concentration of ^{14}C-butylate in the barley root was increased only slightly by the addition of carbofuran. The translocation to the shoot was reduced. This indicates that carbofuran in the susceptible species not only reduced the uptake but changed the translocation pattern. Perhaps carbofuran and butylate compete for the same sites in barley and corn roots. The ^{14}C-butylate present in the tolerant corn species was reduced almost equally in the shoot and the root by the carbofuran-butylate combination. Radioautographs shown in Figures 5 and 6 (lower) support this data. The results in Table 30 show that the ^{14}C in the

Table 29. The partitioning and distribution of ^{14}C-butylate in 14-day-old barley and 7-day-old corn grown in nutrient solution containing butylate, 10 and 5 days respectively, after ^{14}C-butylate application.

Treatment	^{14}C in shoot				^{14}C in root			
	80 Percent acetone-insoluble (dpm/g)	Hexane-soluble fraction (dpm/g)	Water-soluble fraction (dpm/g)	Total (dpm/g)	80 Percent acetone-insoluble (dpm/g)	Hexane-soluble fraction (dpm/g)	Water-soluble fraction (dpm/g)	Total (dpm/g)
Barley:								
Butylate, 10^{-5} M	12409	1189	17797	31395	7689	446	4152	12290
Butylate, 10^{-5} M + carbofuran[a]	10340	1145	12685	24170	7401	502	5632	13535
Corn:								
Butylate, 10^{-5} M	3598	342	3331	7271	4268	2562	5059	12898
Butylate, 10^{-5} M + carbofuran	2215	222	2403	4839	2965	2995	2773	8731

[a] Carbofuran applied as a seed treatment at 4 oz/100 lb of seed.

Table 30. The partitioning of ^{14}C as a percent of the ^{14}C present in the shoot or root of 14-day-old barley and 7-day-old corn, grown in nutrient solution containing butylate, 10 and 5 days respectively, after ^{14}C-butylate application.

Treatment	^{14}C in shoot			^{14}C in root		
	80 Percent acetone-insoluble (dpm/g)	Hexane-soluble fraction (dpm/g)	Water-soluble fraction (dpm/g)	80 Percent acetone-insoluble (dpm/g)	Hexane-soluble fraction (dpm/g)	Water-soluble fraction (dpm/g)
Barley;						
Butylate, 10^{-5} M	39	4	57	66	4	31
Butylate, 10^{-5} M + carbofuran[a]	42	5	53	57	4	39
Corn:						
Butylate, 10^{-5} M	50	5	46	33	20	47
Butylate, 10^{-5} M + carbofuran	46	5	50	34	34	32

[a] Carbofuran applied as a seed treatment at 4 oz/100 lb of seed.

Figure 6. Barley plants (left) and radioautographs (right) of barley plants harvested 10 days after ^{14}C-butylate was placed in the nutrient solution. Upper: ^{14}C-butylate treated plants. Lower: ^{14}C-butylate treated plants in the presence of carbofuran as a seed treatment.

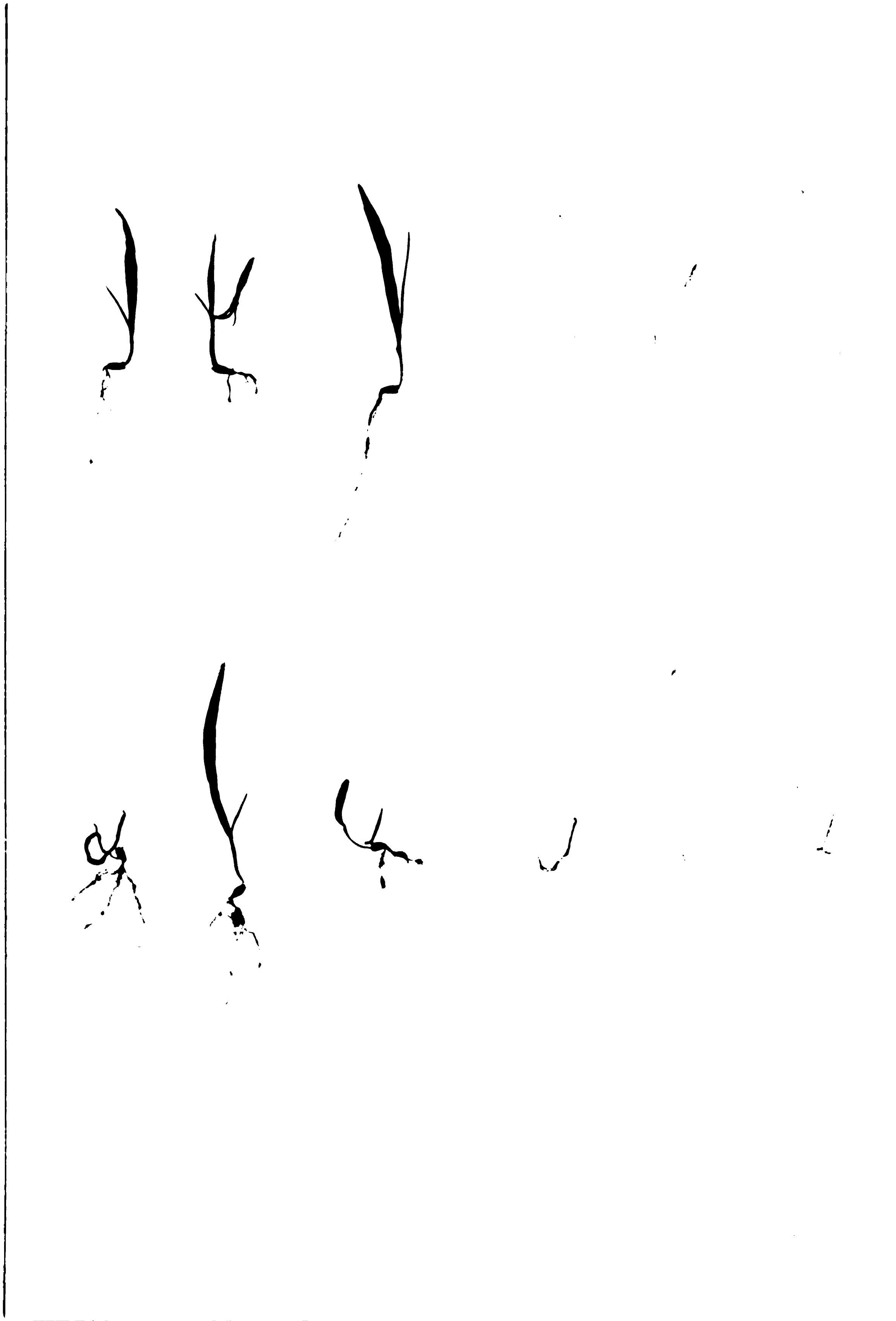
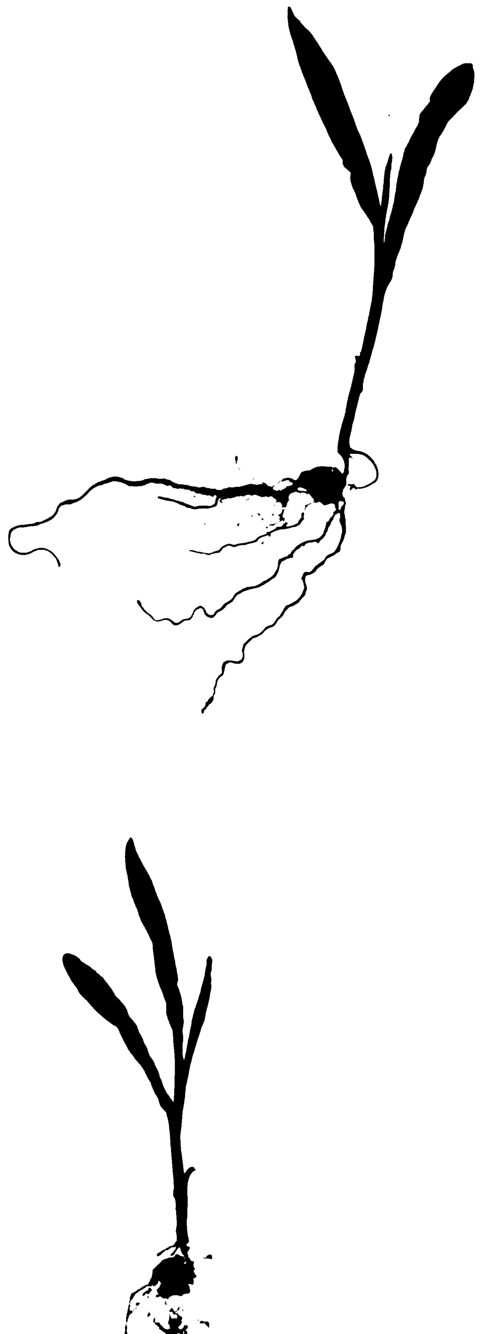


Figure 7. Corn plants (left) and radioautographs (right) of corn plants harvested 5 days after ^{14}C-butylate was placed in the nutrient solution. Upper: ^{14}C-butylate treated plants. Lower: ^{14}C-butylate treated plants in the presence of carbofuran as a seed treatment.



80 percent acetone-insoluble fraction in the barley root is almost double that in the barley shoot when treated with butylate alone. The combination treatment of carbofuran and butylate decreased the ^{14}C in the 80 percent acetone-insoluble portion, but increased the ^{14}C level in the water-soluble portion as well as the total ^{14}C in the barley root. The percent of ^{14}C in the various fractions in the shoot was altered very little by the carbofuran treatment (Table 30).

The tolerant species (corn) reacts differently. The shoot contained a relatively low concentration of ^{14}C which is almost equally divided between the 80 percent acetone-insoluble and the water-soluble fraction with the hexane-soluble portion being very small. By contrast, the hexane-soluble portion in the corn root was much larger and when the seed was treated with carbofuran it was equal to the other two portions even though the total level of ^{14}C in the root was lower.

The water-soluble fraction of the barley shoot contained a large amount of one metabolite, a small percent of a second metabolite, and about the same proportion of parent material as the second metabolite (Table 31). As might be expected, by the small influence of the carbofuran treatment on other measured parameters in the shoot, the metabolites were the same and in the same proportions. The data in Table 32 support these results in that the percent parent material was only slightly changed. In the root, the water-soluble fraction from the butylate treated plants

Table 31. The separation on TLC of shoot and root extracts from 14-day-old barley, 10 days after ^{14}C-butylate treatment.

Plant Part	Treatment	Rf 0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
		Percent of total spotted										
Shoot:	Water-soluble											
	Butylate, 10^{-5} M		86			8						6[b]
	Butylate, 10^{-5} M + carbofuran[a]		88			6						6[b]
	Hexane-soluble											
	Butylate, 10^{-5} M											
	Butylate, 10^{-5} M + carbofuran											
Root:	Water-soluble											
	Butylate, 10^{-5} M		84				6					10[b]
	Butylate, 10^{-5} M + carbofuran	64						5				32[b]
	Hexane-soluble											
	Butylate, 10^{-5} M	13			24				13			51[b]
	Butylate, 10^{-5} M + carbofuran	63			9			7				21[b]

[a] Carbofuran was applied as a seed treatment at 4 oz/100 lb of seed.
[b] Indicates parent compound.

Table 32. The percent extractable ^{14}C-butylate remaining in 14-day-old barley and 7-day-old corn shoots and roots, 10 and 5 days respectively, after ^{14}C-butylate treatment.

Treatment	^{14}C in shoot			^{14}C in root		
	Hexane-soluble fraction	Water-soluble fraction	Total	Hexane-soluble fraction	Water-soluble fraction	Total
Barley:						
Butylate, 10^{-5} M	0	3.5	3.5	1.9	3.0	4.9
Butylate, 10^{-5} M + carbofuran[a]	0	3.1	3.1	0.8	12.2	13.0
Corn:						
Butylate, 10^{-5} M	0	1.5	1.5	0	2.3	2.3
Butylate, 10^{-5} M + carbofuran	0	0	0	6.8	7.8	14.6

[a] Carbofuran was applied as a seed treatment at 4 oz/100 lb of seed.

contained metabolites similar to those in the shoot. The major metabolite was the same and the minor one was perhaps the same, but moved a little further on the TLC plate. The parent compound made up a greater proportion of the total ^{14}C than the minor metabolite. The carbofuran seed treatment greatly altered ^{14}C-butylate metabolism in the barley root. In this treatment there was half as much parent compound as there was major metabolite and the latter differed from the butylate only treatment. There was also one minor metabolite, and again it was not the same as the other minor metabolites obtained in the root or shoot. Fifty percent of the hexane-soluble fraction separated as parent material with one other metabolite being 24 percent and two others equally making up the final 26 percent. One of the minor metabolites was the same as the major water-soluble compound obtained in the root with the carbofuran and butylate combination treatment. The herbicide-insecticide combination influenced the hexane-soluble fraction in a similar manner to the water-soluble fraction. The same metabolites were found with the reduction of the ^{14}C-butylate parent fraction by the same amount that an additional metabolite increased. There appeared to be a substantial amount of ^{14}C remaining as ^{14}C-butylate in the root in the hexane-soluble fraction. Since the hexane-soluble fraction contained only a small portion of the total ^{14}C this did not greatly affect the total amount of ^{14}C-butylate present (Table 32). The three-fold increase in the parent ^{14}C-

butylate remaining in the root due to the carbofuran seed treatment may, in part, explain the synergistic action on barley.

The shoots from corn grown for 5 days in ^{14}C-butylate contained three metabolites and a very small portion of parent compound in the water-soluble fraction. Shoots from corn treated with the carbofuran and butylate combination contained only two metabolites. These were the same as the major ones from the butylate treatment. No parent ^{14}C-butylate was found in corn shoots receiving the combination treatment (Tables 32 and 33).

The water-soluble extracts from corn roots contained three metabolites from the butylate treatment (one major and two minor); similarly, the combination treatment with carbofuran contained one major and two minor metabolites, (Table 33). The proportion of parent compound was five times greater in corn roots receiving the combination treatment. In the hexane-soluble fraction from corn roots, no parent ^{14}C-butylate was found unless the plants received the carbofuran-butylate combination treatment (Table 33). Roots receiving ^{14}C-butylate contained a ^{14}C-labelled metabolite which contained 77 percent of the ^{14}C. This metabolite was reduced to 40 percent of the total ^{14}C present if the plants received the carbofuran-butylate combination treatment. Two other metabolites were found in a hexane-soluble extract from corn roots (Table 33). One staying at or near the origin in the TLC procedure increased

Table 33. The separation on TLC of shoot and root extracts from 7-day-old corn 5 days after ^{14}C-butylate treatment.

Plant Part	Treatment	Rf	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
		Percent of total spotted											
Shoot:	Water-soluble												
	Butylate, 10^{-5} M			70		24				3			3[b]
	Butylate, 10^{-5} M + carbofuran[a]			79		21							
	Hexane-soluble												
	Butylate, 10^{-5} M												
	Butylate, 10^{-5} M + carbofuran												
Root:	Water-soluble												
	Butylate, 10^{-5} M		72				13				9	5[b]	
	Butylate, 10^{-5} M + carbofuran			69		4		3					25[b]
	Hexane-soluble												
	Butylate, 10^{-5} M			17			6				77		
	Butylate, 10^{-5} M + carbofuran		38					1			40		20[b]

[a] Carbofuran was applied as a seed treatment at 4 oz/100 lb of seed.
[b] Indicates parent compound.

from 17 to 38 percent due to the carbofuran seed treatment. The butylate-carbofuran treatment substantially increased the amount of parent material in the corn root over the butylate alone (Table 32). A similar difference was used to explain the increased phytotoxicity of the combination treatment to barley. The results obtained with corn would appear to discount this hypothesis. The mode of action of butylate has been reported to be an inhibition of growth in the meristematic region of the leaves of grass plants (68). The question remains as to why carbofuran-butylate synergistically decreased barley growth when the percent of parent ^{14}C-butylate in the roots of barley and corn was increased almost equally by the carbofuran seed treatment. Kaufman et al. (37) have found methylcarbamates to inhibit phenylcarbamate metabolism. They suggested that perhaps 1-naphthyl-N-methylcarbamate (carbaryl) increased oat sensitivity to m-chlorocarbanilate (chlorpropham) rather than increased chlorpropham persistence in the soil. This may be the situation which exists between barley and corn where the sensitivity of barley respiration has been increased by the combination of carbofuran, a methylcarbamate and butylate a diisobutylthiocarbamate. Carbofuran combined with butylate to synergistically reduce barley but not corn root and shoot growth. The bases for these interactions appeared to be the greater accumulation of butylate in the roots of barley plants treated with carbofuran, due to both increased absorption of butylate and decreased metabolism of butylate. This same metabolism trend was apparent in corn, however the butylate level was much lower as the absorption of butylate by corn was reduced by the carbofuran treatment. This latter effect may have been due to competition for uptake sites in the corn root.

Uptake, translocation and metabolism of ^{14}C-chlorbromuron

About 80 percent of the ^{14}C from ^{14}C-chlorbromuron was translocated to the shoot in 6 days (Table 34). The radioautographs in Figure 8 shows the ^{14}C translocation in barley to be different than that in corn (Figure 9). The amount of ^{14}C in the corn root and coleoptile appeared to be greater than in the barley. However, the data in Table 34 indicate that the accumulation of ^{14}C in the roots of corn plants was much less than it was for the barley. In corn, two-thirds of the ^{14}C was found in the shoot versus 85 percent for barley. Geissbuhler (38) pointed out that substituted urea herbicides are readily taken up from soil and nutrient solutions by root systems and are rapidly translocated to the leaves and stem by the transpiration stream. It is important to note that different substituted urea herbicides have exhibited different mobilities in plant systems (38). It might also be considered, as shown by the corn and barley species here, that different mobilities in different plants could exist for the same substituted urea herbicide. Frank et al. (25) suggested that the selectivity of 5-amino-4-chloro-2-phenyl-3(2H)-

Table 34. The partitioning and distribution of ^{14}C-chlorbromuron in 10-day-old barley and 7-day-old-corn, grown in nutrient solution containing chlorbromuron, 6 and 5 days respectively, after ^{14}C-chlorbromuron application.

Treatment	^{14}C in shoot				^{14}C in root			
	80 Percent acetone-insoluble (dpm/g)	Hexane-soluble fraction (dpm/g)	Water-soluble fraction (dpm/g)	Total (dpm/g)	80 Percent acetone-fraction (dpm/g)	Hexane-soluble fraction (dpm/g)	Water-soluble fraction (dpm/g)	Total (dpm/g)
Barley:								
Chlorbromuron, 10^{-6} M	23340	2859	12451	35170	5307	404	534	6245
Chlorbromuron, 10^{-6} M + carbofuran[a]	20395	3658	10359	34412	5994	532	601	7129
Corn:								
Chlorbromuron, 10^{-6} M	4862	375	1894	7130	3621	317	554	4493
Chlorbromuron, 10^{-6} M + carbofuran	5950	503	1551	8004	2809	466	446	3720

[a] Carbofuran was applied as a seed treatment at 4 oz/100 lb of seed.

Figure 8. Barley plants (left) and radioautographs (right) of barley plants harvested 6 days after ^{14}C-chlorbromuron was placed in the nutrient solution. Upper: ^{14}C-chlorbromuron treated plants. Lower: ^{14}C-chlorbromuron treated plants in the presence of carbofuran as a seed treatment.

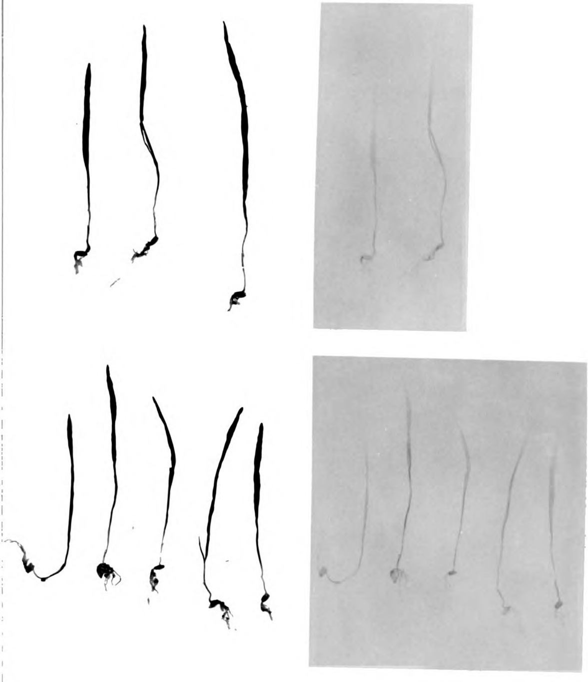


Figure 9. Corn plants (left) and radioautographs (right) of corn plants harvested 5 days after ^{14}C-chlorbromuron was placed in the nutrient solution. Upper: ^{14}C-chlorbromuron treated plants. Lower: ^{14}C-chlorbromuron treated plants in the presence of carbofuran as a seed treatment.

pyridozinone (pyrazon) for lambsquarters (Chenopodium album L.) in sugar beets is based in part on differential translocation within the two species. The proposed mechanism of action of the substituted urea herbicides is the inhibition of photosynthesis (68). Nashed et al. (50) found no aniline metabolites, which could be monitered as $^{14}CO_2$ loss in corn until 8 days after treatment with ^{14}C-chlorbromuron.

The combination of carbofuran with chlorbromuron very slightly lowered the ^{14}C concentration in the barley shoots and raised it in the root by a similar amount. In corn, the situation was reversed with the ^{14}C concentration raised in the shoot and lowered in the root. Due to the overall lower ^{14}C concentration in the corn, the shift was proportionately higher in corn.

The 80 percent acetone-insoluble portion of the root contained 60 percent of the ^{14}C label in the barley plant and the water-soluble fraction contained one-half as much (Table 35). The addition of carbofuran did not appreciably alter these values except to slightly increase the hexane-soluble fraction in the shoot. Partitioning values in the root were unaltered by the combination treatment (Table 35). In the barley root the acetone-insoluble portion contained 85 percent of the ^{14}C and the hexane- and water-soluble portions contained the remaining 15 percent.

In the corn shoot, 27 percent of the ^{14}C was found in the water-soluble fraction and 68 percent was found in the 80 percent acetone-insoluble fraction (Table 35). The

Table 35. The partitioning of ^{14}C as a percent of the ^{14}C present in the shoot or root of 10-day-old barley and 7-day-old corn, grown in nutrient solution containing chlorbromuron, 6 and 5 days respectively, after ^{14}C-chlorbromuron application.

Treatment	^{14}C in shoot			^{14}C in root		
	80 Percent acetone-insoluble (dpm/g)	Hexane-soluble fraction (dpm/g)	Water soluble fraction (dpm/g)	80 Percent acetone-insoluble (dpm/g)	Hexane-soluble fraction (dpm/g)	Water-soluble fraction (dpm/g)
Barley:						
Chlorbromuron, 10^{-6} M	60	8	32	85	7	9
Chlorbromuron, 10^{-6} M + carbofuran[a]	59	11	30	84	7	8
Corn:						
Chlorbromuron, 10^{-6} M	68	5	27	80	8	13
Chlorbromuron, 10^{-6} M + carbofuran	74	6	19	76	12	12

[a] Carbofuran applied as a seed treatment at 4 oz/100 lb of seed.

carbofuran-chlorbromuron combination treatment decreased the ^{14}C in the acetone-insoluble fraction. The hexane-soluble portion in the shoot remained virtually unchanged by the carbofuran treatment. In the root, the hexane-soluble fraction went from 8 to 12 percent with the addition of carbofuran while the 80 percent acetone-insoluble fraction remained the same. Carbofuran appeared to increase the percent of ^{14}C in the hexane-soluble fraction in the barley shoot and in corn root at the expense of the water-soluble and 80 percent acetone-insoluble fractions respectively.

In barley shoots 6 days after treatment, ^{14}C-chlorbromuron made up only 1.3 percent of the water-soluble fraction separated by TLC (Tables 36 and 37). Three ^{14}C-metabolites (Table 36) were found in the shoot, one major and two others each containing about 20 percent of the ^{14}C. In the root the proportion of label in the major metabolite was greater and less in the other two metabolites and a small amount appeared in a fourth metabolite. Due to the low amount of water-soluble ^{14}C extractable from the root the actual concentration of ^{14}C-chlorbromuron in the root was less than in the shoot (Table 37). The combination treatment of carbofuran and chlorbromuron did not change the number of water-soluble metabolites found in the barley shoot, however the Rf values were different than when chlorbromuron alone was the treatment (Table 36). The major metabolite decreased and a minor one increased by a similar amount. The other minor metabolite decreased slightly,

Table 36. The separation on TLC of shoot and root extracts from 10-day-old barley, 6 days after ^{14}C-chlorbromuron treatment.

Plant Part	Treatment	Rf	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
			Percent of total spotted										
Shoot:	Water-soluble												
	Chlorbromuron, 10^{-6} M		54			23			19				4[b]
	Chlorbromuron, 10^{-6} M + carbofuran[a]			41	19		33						8[b]
	Hexane-soluble												
	Chlorbromuron, 10^{-6} M		24		17			17		29[b]			20
	Chlorbromuron, 10^{-6} M + carbofuran			20		20				53[b]			5
Root:	Water-soluble												
	Chlorbromuron, 10^{-6} M		64				14			13	2		6[b]
	Chlorbromuron, 10^{-6} M + carbofuran		41			24		21		10[b]		3	
	Hexane-soluble												
	Chlorbromuron, 10^{-6} M		26			16	18			10		30[b]	
	Chlorbromuron, 10^{-6} M + carbofuran		18			22				55[b]			5

[a] Carbofuran was applied as a seed treatment at 4 oz/100 lb of seed.
[b] Indicates parent compound

Table 37. The percent extractable ^{14}C-chlorbromuron remaining in 10-day-old barley and 7-day-old corn shoots and roots, 8 and 5 days respectively, after ^{14}C-chlorbromuron treatment.

Treatment	^{14}C in shoot			^{14}C in root		
	Hexane-soluble fraction	Water-soluble fraction	Total	Hexane-soluble fraction	Water-soluble fraction	Total
Barley:						
Chlorbromuron, 10^{-6} M	2.3	1.3	3.6	1.9	0.5	2.4
Chlorbromuron, 10^{-6} M + carbofuran[a]	5.6	2.4	8.0	4.1	0.8	4.9
Corn:						
Chlorbromuron, 10^{-6} M	1.7	0	1.7	2.2	2.9	5.1
Chlorbromuron, 10^{-6} M + carbofuran	1.9	0.4	2.3	1.3	3.1	4.4

[a] Carbofuran was applied as a seed treatment at 4 oz/100 lb of seed.

but enough to double the ^{14}C-chlorbromuron content (Table 36). The ^{14}C-chlorbromuron in the water-soluble portion of the barley root, from plants receiving the combination treatment, did not run at the front. The data in Table 36 show that one major metabolite and two other metabolites, each approximately half the major one, were present in the barley root. The amount of ^{14}C-chlorbromuron parent material present in the root was only slightly increased by the combination treatment (Table 37).

The hexane-soluble fraction in the ^{14}C-chlorbromuron treated barley shoot contained four metabolites of almost equal concentration and the parent ^{14}C-chlorbromuron in a slightly higher proportion (Table 36). The carbofuran seed treatment resulted in a greater portion of ^{14}C present as the parent compound in the hexane-soluble fraction of the barley shoot. Furthermore, one metabolite was absent and another greatly decreased compared to the herbicide only treatment (Table 36).

The fate of the ^{14}C in the hexane-soluble barley root fraction for the herbicide treatment alone was the same as in the barley shoot except that none of the ^{14}C metabolites ran ahead of the ^{14}C-chlorbromuron on the TLC plate. The presence of carbofuran changed the ^{14}C-chlorbromuron metabolism in the root in a manner similar to that observed in the shoot (Table 36 and 37), again increasing the content of parent ^{14}C-chlorbromuron.

The data in Table 37 show that more parent ^{14}C-chlorbromuron was present in the hexane-soluble fraction than in the water-soluble fraction, in both shoots and roots of barley plants receiving the carbofuran-chlorbromuron treatment. These results contrast with those of Nashed _et al_. (50) who found no metabolites formed in the susceptible cucumber, 4 days after chlorbromuron treatment. However, they found "binding" of a portion of the original chlorbromuron. It is possible that cucumber, a dicotyledonous plant, may be unable to metabolize chlorbromuron whereas barley, a monocotyledonous plant, does to a certain extent even though both are susceptible.

No parent ^{14}C-chlorbromuron remained in the water-soluble fraction of the corn shoot 5 days after treatment (Table 38). One major and two minor metabolites were found. The carbofuran seed treatment altered chlorbromuron metabolism by causing the disappearance of one minor metabolite and the appearance of another as well as the presence of a low level of parent ^{14}C-chlorbromuron. Corn roots contained identical water-soluble metabolites to the corn shoots following ^{14}C-chlorbromuron treatment (Table 38). However, they contained less of the two minor metabolites and 23 percent of the ^{14}C was present as the parent ^{14}C-chlorbromuron. The carbofuran seed treatment decreased the major metabolite in favor of the other metabolites.

The hexane-soluble fraction from the corn shoot treated

Table 38. The separation on TLC of shoot and root extracts from 7-day-old corn, 5 days after ^{14}C-chlorbromuron treatment.

Plant Part	Treatment	Rf	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
			Percent of total spotted										
Shoot:	Water-soluble												
	Chlorbromuron, 10^{-6} M		66			12		21					
	Chlorbromuron, 10^{-6} M + carbofuran[a]		72				21			2[b]			6
	Hexane-soluble												
	Chlorbromuron, 10^{-6} M		29			23				17			31[b]
	Chlorbromuron, 10^{-6} M + carbofuran		39			17				15		29[b]	
Root:	Water-soluble												
	Chlorbromuron, 10^{-6} M		59			9			9	23[b]			
	Chlorbromuron, 10^{-6} M + carbofuran		40			24			9	27[b]			
	Hexane-soluble												
	Chlorbromuron, 10^{-6} M		30			26		14					29[b]
	Chlorbromuron, 10^{-6} M + carbofuran		56					1			33		10[b]

[a] Carbofuran was applied as a seed treatment at 4 oz/100 lb of seed.
[b] Indicates parent compound.

with chlorbromuron contained three ^{14}C-metabolites and parent ^{14}C-chlorbromuron (Table 38). The parent compound and a metabolite which occurred at the same Rf as the major one in the water-soluble fraction were present in almost equal proportions. The other two metabolites contained 23 and 17 percent of the ^{14}C. The carbofuran seed treatment increased the level of the major metabolites at the expense of the others. The number of metabolites found was not changed.

The water-soluble ^{14}C-metabolites from the ^{14}C-chlorbromuron treatment found in corn roots were similar to those found in the shoot (Table 38). However, 23 percent of the ^{14}C remained as the parent ^{14}C-chlorbromuron. The carbofuran seed treatment reduced the level of the major metabolite and increased by the same amount a metabolite with an Rf of 0.3. The level of parent ^{14}C-chlorbromuron in the water-soluble fraction was unaffected, whereas in the hexane-soluble portion there was a threefold reduction. Furthermore the carbofuran seed treatment caused the accumulation of a new metabolite, the disappearance of two minor metabolites and an increase in the major metabolite.

The amount of ^{14}C-chlorbromuron present in the corn shoot was slightly increased by the carbofuran seed treatment, while that in the root decreased by almost the same amount (Table 37). The greatest change in the root came from a reduction in the content of parent ^{14}C-chlorbromuron in the hexane-soluble fraction.

The data shown in Table 38 for the chlorbromuron treatment of corn support the results of Nashed _et_ _al_. (50) with regards to the number of metabolites found by TLC. Due to the different treatment procedures, it can only be speculated that they may be the same compounds.

Chlorbromuron markedly increased respiration and reduced photosynthesis and growth of barley. The carbofuran seed treatment more than doubled the amount of ^{14}C-chlorbromuron found in both the shoot and the root (Tables 36 and 37). Perhaps chlorbromuron was already so toxic to barley at the chlorbromuron level used in the experiment that no additional toxic response could be obtained by the carbofuran seed treatment.

Photosynthesis in corn was not further reduced by the addition of the carbofuran seed treatment to the chlorbromuron and yet synergistic reductions in leaf area and dry weight were obtained (Table 2 and 20). It might logically be proposed that if the increased amount of parent compound in the corn leaf is not causing a further reduction in photosynthesis that it is merely prolonging the time over which it occurs, and thus giving the synergistic effect observed. It would appear then if the photosynthesis reduction were not too severe and the plant could metabolize the chemical that perhaps the plant might overcome the effect of chlorbromuron.

Carbofuran interacted synergistically with chlorbromuron to reduce the height and weight of 41-day-old corn, the root length of 3-day-old barley and the leaf area and dry weight of 7-day-old corn grown in sand culture. The basis for this interaction appeared to be related to the increased accumulation of chlorbromuron and its metabolites in the corn shoots. Furthermore, carbofuran reduced chlorbromuron metabolism in corn shoots causing an increase in the parent chlorbromuron level in the corn shoot.

SUMMARY AND CONCLUSIONS

In the greenhouse, carbofuran in combination with alachlor caused a synergistic reduction of barley dry weight. The combination effect of carbofuran with chlorbromuron on barley dry weight was antagonistic. A synergistic response was obtained with carbofuran in combination with butylate or chlorbromuron on corn dry weight. Corn height was also synergistically reduced by the chlorbromuron and carbofuran combination treatment. Barley height was similarly reduced by the combination of butylate and carbofuran.

Alachlor, butylate and chlorbromuron when combined with carbofuran all synergistically reduced the radical length of germinating barley. The carbofuran combination with alachlor also reduced barley germination in a synergistic manner.

The respiration of 10 and 14-day-old barley plants grown in the growth chamber was significantly increased by each of the herbicides studied in combination with carbofuran applied as a seed treatment. The butylate-carbofuran combination treatment also synergistically increased O_2 uptake. Net photosynthesis of barley was significantly reduced only by the chlorbromuron-carbofuran combination. The results of these altered physiological processes were manifested in significantly reduced leaf area, dry weight and height. A synergistic reduction was evident on leaf area and plant height for butylate or alachlor in combination with carbofuran, as well as dry weight for the latter combination. The respiration of corn grown in a growth chamber was unaffected by the pesticide combinations, however, chlorbromuron with or without carbofuran significantly reduced net photosynthesis. This resulted in synergistically reduced leaf area and dry weight per pot and a significant reduction in plant height by the chlorbromuron-carbofuran combination treatment.

Butylate and chlorbromuron were absorbed by barley roots and translocated to the shoot where they accumulated. Alachlor was present in about equal amounts in the barley shoot and roots. The addition of carbofuran to the herbicide treatments lowered the concentration of butylate and chlorbromuron in the shoot and slightly raised the content in the root. The alachlor concentration was increased in both barley shoots and roots by the herbicide-insecticide combination. The corn shoots contained proportionately less butylate and alachlor but more chlorbromuron than the root. The carbofuran treatment lowered the butylate concentration in the shoot and raised it in the roots. This pattern was reversed for chlorbromuron while the alachlor concentration was raised in both shoots and roots.

The ^{14}C contained in the barley and corn shoots and roots was divided between the 80 percent acetone-insoluble fraction, a water-soluble fraction, and a small portion found in the hexane-soluble fraction. The corn root treated

with ^{14}C-butylate was the only treatment with a substantial amount of hexane-soluble ^{14}C.

The hexane-soluble fraction from the ^{14}C-alachlor-treated corn contained four metabolites and no parent material. The presence of carbofuran changed the concentrations of the metabolites, but not their identity. The water-soluble extract from the ^{14}C-alachlor-treated barley shoots and roots contained one major metabolite with or without the carbofuran seed treatment. The minor metabolite in the shoot was different from the minor metabolite in the root for the alachlor treatment. The minor metabolite in the root and shoot were similar for the alachlor-carbofuran treatment. The minor metabolite from the alachlor-carbofuran treatment was not the same as the alachlor treatment alone. The corn shoot contained four ^{14}C-metabolites in the water-soluble fraction from the ^{14}C-alachlor treatment. One minor compound disappeared due to the carbofuran treatment and a minor and a major metabolite remained the same. The water-soluble extract from corn roots following the ^{14}C-alachlor treatment contained three metabolites with or without the carbofuran combination. Of these metabolites, only one minor one appeared to be the same following the combination treatment, the others were different. The amount of parent ^{14}C-alachlor remaining in barley and corn, 10 and 5 days respectively, after treatment was small. The barley shoot contained a greater proportion of ^{14}C-alachlor than the root. This proportion was reduced in the shoot and raised in the root by the carbofuran combination. In corn these results were completely reversed.

Barley root extracts contained three metabolites in the hexane-soluble fraction from ^{14}C-butylate treatment. The addition of carbofuran produced, of the three metabolites found following the ^{14}C-butylate-carbofuran treatment, only one metabolite similar to that found for the butylate treatment alone. Two metabolites were present in the hexane-soluble extract from corn roots following butylate treatment. Following the ^{14}C-butylate-carbofuran treatment, three different metabolites were found.

The water-soluble extract from roots and shoots of ^{14}C-butylate-treated barley contained two ^{14}C-metabolites. In the shoot they remained unchanged on the addition of carbofuran to the treatment. In the root the combination treatment resulted in the presence of two different metabolites. The water-soluble extract from the shoots of ^{14}C-butylate-treated corn had three metabolites present. The combination treatment of butylate and carbofuran reduced the number of ^{14}C-butylate metabolites to two. The corn root contained three water-soluble ^{14}C-metabolites following the ^{14}C-butylate treatment whether or not the corn seeds had been treated with carbofuran. Only two of these metabolites may have been the same. Barley contained more parent ^{14}C-butylate than corn and for both species the roots contained more parent ^{14}C-butylate than the shoots. The amount of parent ^{14}C-butylate in the shoot was reduced for both species when the insecticide was combined with the herbicide while the amount in the root increased.

Following ^{14}C-chlorbromuron treatment four ^{14}C-metabolites were found in the barley shoots and roots in the hexane-soluble extracts. Three ^{14}C-metabolites were found in corn. When carbofuran was combined with chlorbromuron both shoots and roots of the two species contained one less metabolite in the hexane-soluble fraction.

The water-soluble extract from the shoots of chlorbromuron treated barley contained three metabolites while the root had four. The number of metabolites remained the same in the roots and shoots following the carbofuran-chlorbromuron treatment but the metabolites were not all similar. The water-soluble fraction from the corn shoots had three metabolites, at least one of which was different following the combination treatment of carbofuran with chlorbromuron. This was not the case for the root which had the same three water-soluble ^{14}C-metabolites, regardless of the presence of carbofuran.

The amount of parent ^{14}C-chlorbromuron present in the barley shoot and root doubled on the addition of carbofuran to the chlorbromuron treatment. The amount of parent ^{14}C-

chlorbromuron in the corn shoot was also increased under these conditions, while the amount in the root decreased.

Alachlor was absorbed to a greater extent by the susceptible barley plant than by the tolerant corn plant. The alachlor content increased in the root and metabolism decreased in the presence of carbofuran seed treatment These factors would appear to contribute to the decreased growth of plants receiving the combination treatment. Although the combination treatment also increased the ^{14}C content in the shoot, the increased respiration may be related to the less parent alachlor present.

Butylate was taken up by barley to a much greater extent than by corn. The addition of the carbofuran seed treatment decreased the concentration of ^{14}C present in the barley shoot, corn shoot and root. The corn root treated with the pesticide combination contained more parent ^{14}C-butylate than barley although the barley root contained more total ^{14}C. Under the conditions of the experiment the level of ^{14}C-butylate found in the roots was apparently not critical as corn remained tolerant. The decreased ^{14}C level in the barley and corn shoot coupled with a decrease in parent butylate content indicates little change in the metabolism within the shoot due to the carbofuran treatment. Thus the reduced growth of barley may be related to the observed increase in respiration. This response may be caused by an increase in susceptibility of barley to butylate, a carbamate herbicide and the carbamate insecticide, carbofuran.

The carbofuran seed treatment with ^{14}C-chlorbromuron increased the ^{14}C content of barley roots, whereas the ^{14}C content in the shoots was lowered. The combination treatment greatly increased the amount of parent ^{14}C-chlorbromuron. However, no synergistic growth reduction occurred. Respiration was significantly increased and photosynthesis significantly decreased by the chlorbromuron treatment alone resulting in decreased corn growth. The ^{14}C content as well as the parent ^{14}C-chlorbromuron content increased in corn shoots and decreased in corn roots if the seed was treated with carbofuran. Since net photosynthesis was not further reduced by the carbofuran seed treatment the slight increase in shoot content of chlorbromuron plus a decrease in ability to metabolize the herbicide, might account for a longer period of contact of chlorbromuron with the leaf tissue resulting in decreased leaf area and dry weight.

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APPENDICES

APPENDIX A

Modified Hoagland's Solution

1.	1 M KH_2PO_4	2 ml/l
2.	1 M KNO_3	2 ml/l
3.	1 M $Ca(NO_3)_2 \cdot 4H_2O$	3 ml/l
4.	1 M $MgSO_4 \cdot 7H_2O$	2 ml/l
5.	1.5 g/l $MnCl_3 \cdot 4H_2O$ 2.5 g/l H_3BO_4 0.1 g/l $ZnCl_2$ 0.05 g/l $CuCl_2 \cdot 2H_2O$ 0.05 g/l MoO_3	1 ml/l
6.	0.05 M ETDA 0.05 M $FeSO_4$	1 ml/l
or		
	26.3 g/l Sequestrene®	1 ml/l

pH 6.5-6.8 with 1 M NaOH

APPENDIX B

Method of recorder chart paper conversion to µg/CO_2/min.

Compressed air flow rate	500 cc/min
CO_2 content of compressed air	330 ppm or .033 percent
Molecular weight of CO_2	44
Standard volume	22.4 l/mole

22.4 l contains 1 mole of gas
.5 l/min contains .0223 M (g/l) of gas/min
1 mole of gas contains .033 percent CO_2
therefore .0223 M/min contains 7.359×10^{-6} M CO_2/min
or $7.359 \times 10^{-6} \times 44 = 3.23 \times 10^{-3}$ g CO_2/min
or 3.23 mg CO_2/min = 323 ug CO_2/min
therefore on SARGENT ® catalog no. S-72166 recorder
paper 1 unit change = 3.23 µg CO_2/min

APPENDIX C

The effect of various pesticide combinations on plant height and dry weight of barley (*Hordeum vulgare* L. var. Larker) grown under greenhouse conditions.

Pesticide A	Rate (lb/A)	Pesticide B	Rate (lb/A)	Plant height Percent of control Observed	Plant height Percent of control Expected	Interaction	Plant weight Percent of control Observed	Plant weight Percent of control Expected	Interaction
Preemergence		Preemergence							
Chlorbromuron	1.5	EPTC	1	80	84	0	66	89	S
Dicamba	0.5	EPTC	1	65	49	A	40	44	0
Chlorbromuron	1.5	EPTC	2	80	78	0	94	80	A
Dicamba	0.5	EPTC	2	58	46	A	41	35	0
BASF 2903-H	5	Butylate	2	67	93	S	46	62	S
BASF 2903-H	6	Chlorbromuron	3	75	93	S	24	40	S
SD 15418	3	Chlorbromuron	1.5	45	96	S	15	61	S
Trifluralin	0.5	Fluorodifen	6	100	96	0	67	57	0
Trifluralin	0.75	Fluorodifen	4	98	79	A	112	52	A
Trifluralin	1	Fluorodifen	5	101	101	0	87	72	A
Chlorbromuron	0.75	SD 15418	1	87	94	0	64	63	0
Chlorbromuron	0.75	SD 15418	3	46	50	0	15	15	0
Chlorbromuron	1.5	SD 15418	1	94	96	0	68	61	0
Chlorbromuron	2	SD 15418	2	60	74	S	19	22	0
Butylate	2	BASF 2903-H	2.5	97	90	0	68	70	0
Butylate	4	BASF 2903-H	2.5	90	102	S	65	70	0
Chlorbromuron	0.75	BASF 2903-H	5	97	92	0	72	58	A
Chlorbromuron	1.5	BASF 2903-H	5	94	94	0	66	56	0
Butylate	4	BASF 2903-H	5	91	99	0	64	62	0
		Preemergence Granular							
Chlorpropham	1.5	Disulfoton	1.5	103	107	0	105	130	S
Chlorpropham	2	Disulfoton	1.5	105	107	0	68	82	S

APPENDIX C (Continued)

Pesticide A	Rate (lb/A)	Pesticide B	Rate (lb/A)	Plant height Percent of control Observed	Plant height Percent of control Expected	Interaction	Plant weight Percent of control Observed	Plant weight Percent of control Expected	Interaction
Propachlor	3	Disulfoton	1.5	96	102	0	88	110	S
Propachlor	5	Disulfoton	1.5	86	88	0	37	61	S
Butylate	0.5	Disulfoton	1.5	97	109	S	105	105	0
Butylate	1	Disulfoton	1.5	97	96	0	94	92	0
Butylate	4	Disulfoton	1.5	101	104	0	100	101	0
Chlorbromuron	2	Disulfoton	1.5	104	105	0	98	92	0
Trifluralin	1.5	Disulfoton	1.5	99	104	0	65	68	0
Chlorpropham	2	Disulfoton	3	102	111	0	71	84	S
Propachlor	5	Disulfoton	3	96	103	0	44	62	S
Butylate	4	Disulfoton	3	108	108	0	97	104	0
Trifluralin	1.5	Disulfoton	3	100	107	0	60	69	0
Butylate	1.5	Disulfoton	1	70	47	A	86	43	A
Fluorodifen	5	Carbofuran	1	92	94	0	93	36	A
Butylate	4	Carbofuran	1	106	110	0	109	60	A
Atrazine	1	Carbofuran	1	47	35	A	12	3	0
Alachlor	3	Carbofuran	1	66	70	0	34	37	0
Chlorbromuron	3	Carbofuran	1	87	113	S	93	65	A
Propachlor	6	Carbofuran	1	97	105	0	52	58	0
Atrazine	1	Carbofuran	2	47	34	A	10	3	0
Alachlor	3	Carbofuran	2	67	69	0	36	36	0
Fluorodifen	5	Carbofuran	2	96	102	0	79	56	A
Butylate	2	Carbofuran	2	106	112	0	95	65	A
Chlorbromuron	3	Carbofuran	2	98	109	S	95	61	A
Propachlor	6	Carbofuran	2	97	91	0	48	35	A

APPENDIX C (Continued)

Pesticide A	Rate (lb/A)	Pesticide B	Rate (lb/A)	Plant height Percent of control Observed	Plant height Percent of control Expected	Interaction	Plant weight Percent of control Observed	Plant weight Percent of control Expected	Interaction
		Preemergence Wettable powder							
Alachlor	3	Carbofuran	1	35	66	S	29	49	S
Chlorbromuron	3	Carbofuran	1	87	113	S	52	86	S
Atrazine	1	Carbofuran	1	41	33	0	7	4	0
Propachlor	6	Carbofuran	1	88	98	0	56	77	S
Fluorodifen	5	Carbofuran	1	90	87	0	64	48	A
Butylate	2	Carbofuran	1	97	103	0	97	79	A
Alachlor	3	Carbofuran	2	43	63	S	22	61	S
Chlorbromuron	3	Carbofuran	2	83	100	S	37	107	S
Atrazine	1	Carbofuran	2	34	31	0	5	4	0
Propachlor	6	Carbofuran	2	88	84	0	49	59	0
Fluorodifen	5	Carbofuran	2	96	94	0	95	96	0
Butylate	2	Carbofuran	2	101	103	0	103	111	0
		Seed treatment per 100 lb seed							
Alachlor	2	Carbofuran	0.25	74	91	S	64	60	0
Propachlor	5	Carbofuran	0.25	96	81	A	65	54	A
Chlorbromuron	1.5	Carbofuran	0.25	99	99	0	92	70	A
Propachlor	4	Carbofuran	0.25	108	102	0	77	55	A
Chlorpropham	1.5	Carbofuran	0.25	100	89	A	103	102	0
Alachlor	1	Carbofuran	0.25	90	88	0	83	106	S
Alachlor	3	Carbofuran	0.25	65	61	0	39	55	S
Linuron	1.5	Carbofuran	0.25	96	93	0	54	58	0
Chlorbromuron	3	Carbofuran	0.25	100	98	0	104	96	0

APPENDIX C (Continued)

Pesticide A	Rate (lb/A)	Pesticide B	Rate (lb/A)	Plant height Percent of control Observed	Plant height Percent of control Expected	Inter-action	Plant weight Percent of control Observed	Plant weight Percent of control Expected	Inter-action
Fluorodifen	4	Carbofuran	0.25	98	83	A	65	60	0
Fluorodifen	5	Carbofuran	0.25	95	98	0	69	63	. 0
Fluorodifen	6	Carbofuran	0.25	100	91	0	103	88	A
Propachlor	3	Carbofuran	0.25	88	88	0	73	86	S
Butylate	1.5	Carbofuran	0.25	26	39	S	30	33	0
		Post emergence							
Linuron	1	Carbaryl	2.5	72	88	S	65	62	0
Trifluralin	0.75	Carbaryl	2.5	92	103	S	69	92	S
Chlorpropham	1.5	Carbaryl	2.5	98	98	0	64	54	0
Alachlor	1	Carbaryl	2.5	94	91	0	70	48	A
Fluorodifen	4	Carbaryl	2.5	99	90	0	73	54	A
Propachlor	3	Carbaryl	2.5	95	98	0	69	43	A
Amitrol	3	Carbaryl	2.5	81	80	0	34	38	0
Atrazine	0.5	Carbaryl	2.5	90	100	0	74	78	0
EPTC	1	Carbaryl	2.5	93	102	0	98	111	S
EPTC	2	Carbaryl	2.5	88	95	0	106	98	0
Chlorbromuron	0.75	Carbaryl	2.5	93	91	0	70	71	0
Paraquat	0.5	Carbaryl	2.5	82	88	0	43	55	S
Butylate	3	Carbaryl	2.5	37	42	0	14	16	0
Butylate	4	Carbaryl	2.5	103	95	0	95	93	0

APPENDIX D

The effect of various pesticide combinations on plant height and dry weight of corn (*Zea mays* L. var. Michigan 400) grown under greenhouse conditions.

Pesticide A	Rate (lb/A)	Pesticide B	Rate (lb/A)	Plant height Percent of control Observed	Plant height Percent of control Expected	Interaction	Plant weight Percent of control Observed	Plant weight Percent of control Expected	Interaction
Preemergence		Preemergence							
Chlorbromuron	5	Alachlor	3	95	92	0	84	100	S
Propachlor	4	Alachlor	3	92	92	0	113	113	0
Fluorodifen	6	Alachlor	3	89	85	0	92	102	S
Butylate	4	Alachlor	3	81	90	0	77	106	S
SD 15418	5	Alachlor	3	97	89	0	91	77	A
BASF 2903-H	8	Atrazine	3	95	95	0	93	93	0
Linuron	2	Atrazine	3	97	106	0	92	103	S
Alachlor	3	Atrazine	3	89	96	0	97	107	0
Chlorbromuron	5	Atrazine	3	101	104	0	89	107	S
Propachlor	4	Atrazine	3	98	105	0	98	121	S
Fluorodifen	6	Atrazine	3	95	97	0	91	109	S
Butylate	4	Atrazine	3	90	102	S	88	114	S
SD 15418	5	Atrazine	3	99	102	0	67	83	S
Alachlor	3	BASF 2903-H	8	84	83	0	85	87	0
Chlorbromuron	5	BASF 2903-H	8	91	91	0	81	87	0
Propachlor	4	BASF 2903-H	8	97	92	0	105	99	0
Fluorodifen	6	BASF 2903-H	8	94	85	0	85	89	0
Butylate	4	BASF 2903-H	8	90	89	0	100	92	0
SD 15418	5	BASF 2903-H	8	95	89	0	85	67	A
SD 15418	5	Butylate	4	88	95	0	62	82	S
Chlorbromuron	5	Butylate	4	98	98	0	90	106	S
Butylate	4	Fluorodifen	6	93	91	0	84	109	S
SD 15418	5	Fluorodifen	6	90	91	0	60	79	S

APPENDIX D (Continued)

Pesticide A	Rate (lb/A)	Pesticide B	Rate (lb/A)	Plant height Percent of control Observed	Plant height Percent of control Expected	Interaction	Plant weight Percent of control Observed	Plant weight Percent of control Expected	Interaction
Chlorbromuron	5	Fluorodifen	6	90	93	0	84	102	S
Trifluralin	0.75	Fluorodifen	4	90	91	0	84	83	0
BASF 2903-H	8	Linuron	2	106	92	A	95	84	A
Alachlor	3	Linuron	2	88	93	0	87	97	0
Chlorbromuron	5	Linuron	2	101	101	0	74	97	S
Propachlor	4	Linuron	2	98	102	0	107	109	0
Fluorodifen	6	Linuron	2	96	95	0	100	99	0
Butylate	4	Linuron	2	93	99	0	81	102	S
SD 15418	5	Linuron	2	92	99	0	63	74	S
Fluorodifen	6	Propachlor	4	90	94	0	88	116	S
SD 15418	5	Propachlor	4	92	98	0	77	87	0
Butylate	4	Propachlor	4	101	99	0	102	120	S
Chlorbromuron	5	Propachlor	4	77	101	S	89	113	S
Chlorbromuron	5	SD 15418	5	100	97	0	68	77	0
Chloramben	3	EPTC	3	76	78	0	67	70	0
Dicamba	0.25	EPTC	3	94	83	0	101	91	0
Dicamba	3	Chloramben	3	52	60	0	20	26	0
		Preemergence Granular							
Atrazine	3	Carbofuran	2	101	101	0	83	85	0
Chlorbromuron	5	Carbofuran	2	99	101	0	73	68	0
Atrazine	3	Carbofuran	4	87	95	0	70	80	0
Chlorbromuron	5	Carbofuran	4	87	92	0	60	63	0

APPENDIX D (Continued)

Pesticide A	Rate (lb/A)	Pesticide B	Rate (lb/A)	Plant height Percent of control Observed	Plant height Percent of control Expected	Interaction	Plant weight Percent of control Observed	Plant weight Percent of control Expected	Interaction
		Post emergence Wettable powder							
Atrazine	3	Carbofuran	2	95	95	0	75	78	0
BASF 2903-H	10	Carbofuran	2	96	98	0	86	89	0
Linuron	2	Carbofuran	2	89	91	0	49	57	0
Chlorbromuron	5	Carbofuran	2	91	94	0	64	63	0
Fluorodifen	6	Carbofuran	2	94	90	0	83	83	0
Propachlor	4	Carbofuran	2	102	96	0	95	87	0
Butylate	4	Carbofuran	2	99	93	0	90	85	0
SD 15418	5	Carbofuran	2	92	97	0	45	60	S
Atrazine	3	Carbofuran	4	92	99	0	79	84	0
BASF 2903-H	10	Carbofuran	4	98	99	0	94	96	0
Chlorbromuron	5	Carbofuran	4	100	96	0	72	67	0
Fluorodifen	6	Carbofuran	4	100	92	0	86	89	0
Propachlor	4	Carbofuran	4	100	97	0	104	94	0
SD 15418	5	Carbofuran	4	94	99	0	49	64	S
Butylate	4	Carbofuran	4	97	95	0	84	92	0
Linuron	2	Carbofuran	4	87	93	0	41	61	S
Butylate	3	Carbaryl	2.5	98	103	0	102	115	S
Alachlor	1	Carbaryl	2.5	101	103	0	108	108	0
Linuron	1	Carbaryl	2.5	101	101	0	102	126	S
Propachlor	3	Carbaryl	2.5	101	103	0	110	98	A
Chlorbromuron	4	Carbaryl	2.5	102	109	0	116	111	0
Fluorodifen	4	Carbaryl	2.5	104	109	0	119	118	0

APPENDIX D (Continued)

Pesticide A	Rate (lb/A)	Pesticide B	Rate (lb/A)	Plant height Percent of control Observed	Plant height Percent of control Expected	Interaction	Plant weight Percent of control Observed	Plant weight Percent of control Expected	Interaction
		Seed treatment per 100 lb seed							
Linuron	1	Carbofuran	0.25	92	90	0	58	56	0
Butylate	3	Carbofuran	0.25	89	89	0	76	91	S
Chlorbromuron	4	Carbofuran	0.25	87	101	S	52	80	S