ENHANCEMENT OF PHENYLUREA HERBICIDE ACTIVITY WITH CARBARYL AND DIAZINON

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ABSTRACT

ENHANCEMENT OF PHENYLUREA HERBICIDE ACTIVITY WITH CARBARYL AND DIAZINON

By

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Numerous herbicide-insecticide combinations were applied to the foliage of tolerant and susceptible plants to detect synergisms which might occur. More injury was obtained with a combination of the herbicide linuron (3-[3,4-dichlorophenyl] -1-methyl, 1-methoxyurea) and the insecticide carbaryl (naphthyl _N_-methylcarbamate) in tolerant carrot (_Daucus carota_ L. cv. Chantenay Long Type).

Respiration of leaf tissue was not appreciably affected by either chemical, but photosynthetic rate in the presence of both chemicals was greatly reduced when compared to tissue receiving the herbicide or carbaryl only. Carbaryl enhanced the rate of ^{14}C-linuron uptake by carrot leaves and parsnip (_Pastinaca sativa_ L. cv. Hollow Crown) leaf discs. The rate of linuron metabolism was also decreased in the presence of carbaryl.

The foliar activity of diuron (3[3,4-dichlorophenyl]-1, 1-dimethylurea) and monuron (3[3-chlorophenyl]-1, 1-dimethylurea) was also enhanced by carbaryl

in tolerant cotton when the insecticide was applied to the leaves or to the roots.

Diazinon (*O*, *O*-diethyl *O*-2-isopropyl-6-methyl-4-pyrimidinyl phosphorothioate) enhanced the toxicity of the 4 analogous phenylureas, linuron, monolinuron (3 [3-chlorophenyl]-1-methyl, 1-methoxyurea), diuron and monuron in carrots, cotton (*Gossypium hirsutum* L. cv. Polymaster) and corn (*Zea mays* L. cv. Harris Gold Cup). The foliar uptake of the ^{14}C-phenylurea herbicides was increased up to 5 fold in the presence of diazinon formulated as a wettable powder. Uptake was also considerably increased in the presence of pure diazinon. The synergism obtained with these chemicals was apparently caused by increased herbicide uptake induced both by diazinon and its carrier surfactant. Metabolism of the 4 phenylureas in cotton leaves was unaffected by the presence of diazinon.

The dimethylureas were more easily degraded by cotton than the methoxymethylureas and the monohalogenated analogs were more rapidly metabolized than the dihalogenated analogs. The selectivity of phenylurea herbicides is related to both differential uptake and metabolism in different plant species. Selectivity can be partially lost if other pesticides are added which either enhance uptake or inhibit degradation of these herbicides.

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INTRODUCTION

The application of pesticide combinations either together or at intervals is a common practice in modern crop production. There are many possible combinations of herbicides, insecticides and fungicides which may be utilized on a single crop. The activity of a combination of pesticides may be synergistic, antagonistic, additive, or similar as either material used independently. Interactions resulting from the use of combinations of pesticides may be potentially harmful or beneficial. Harmful interactions may result from increased toxicity toward desirable plant species or non-target species, increased residual life in the soil, or inactivation of the pesticide. On the other hand, beneficial interactions may result from increased or prolonged toxicity toward undesirable plant species or enhanced degradation of pesticide residues. The use of combination treatments might make it possible to decrease the rates of pesticides needed. Identification of potentially harmful combinations will make it possible to avoid them in commercial crop production.

The objectives of this study were to determine the effect of common carbamate and organophosphate insecticides on the activity of 4 analogous phenylurea herbicides with varying halogen and alkyl substitution. The

interactions were further investigated in an attempt to elucidate their bases, and to determine if they were characteristic of a family of compounds or specific for certain chemical structures.

LITERATURE REVIEW

General Review of Research with Pesticide Combinations

Many of the reports regarding herbicide-insecticide interactions have resulted from studies with cotton (*Gossypium hirsutum* L.). The combination of diuron (3 [3,4-dichlorophenyl] -1, 1-dimethylurea) and phorate (*O*, *O*-diethyl-*S*-[(ethylthio)methyl] -phosphorodithioate)resulted in severe loss of cotton seedlings reported in Texas in 1962 (62). Synergistic phytotoxicity was also observed in oats (*Avena sativa* L.) using this same combination (55). Retarded seedling growth and reduced yield of cotton were also obtained by a combination of phorate with either monuron (3 [*p*-chlorophenyl] -1, 1-dimethylurea) or diuron (32). In contrast, a combination of phorate or disulfoton (*O*, *O*-diethyl-*S*-[2-(ethylthio)-ethyl] -phosphorodithioate) with trifluralin (α,α,α,trifluro-2, 6-dinitro-*N*, *N*-dipropyl-*p*-toluidine) resulted in higher cotton yield as compared to trifluralin alone (3,43). Hassaway and Hamilton (34) reported that soil application of trifluralin and phorate did not affect cotton seed germination. However, in the presence of phorate, the trifluralin treated plants produced more secondary roots.

Severe leaf burn and subsequent yield loss were evident in rice (*Oryza sativa* L.) after application of propanil (3, 4-dichloropropioanilide) and carbaryl

(1-napthyl N-methylcarbamate) (8). Linuron (3- [3, 4-dichlorophenyl] -1-methoxy, 1-methylurea) or chloramben methyl ester (methyl 3-amino-2, 5-dichlorobenzoate) applied in combination with phorate or methomyl (methyl-N [(methylcarbamoyl)oxy] thioacetimidate) produced significantly lower seedling vigor in soybean (Glycine max (L.) Merr.) than when each pesticide was used alone (45). Recent findings of Hamill (33) showed synergistic interaction of carbofuran (2, 2-dimethyl-2, 3-dihydrobenzofuranyl-7-N-methylcarbamate) with alachlor (2-chloro-2, 6-diethyl-N methoxy-methyl acetanilide), butylate (S-ethyl diisobutylthiocarbamate) or chlorbromuron (3- [4-bromo-3-chlorophenyl] -1-methoxymethylurea) in barley (Hordeum vulgare L.).

Combinations of herbicides are often utilized to broaden the control of annual monocotyledonous and dicotyledonous weeds in agronomic and horticultural crops. Herbicide combinations may also be synergistic on a single weed species. A mixture of simazine (2-chloro-4, 6-bis [ethylamino] -s-triazine) and paraquat (1, 1'-dimethyl-4, 4'-bipyridinium ion) was more effective in controlling quackgrass (Agropyron repens Beauv.) than either simazine or paraquat alone (63). Similar results have been obtained with a mixture of ammonium thiocyanate and amitrole (3-amino-s-triazole) on quackgrass (19). Enhanced effects of mixtures of 2, 4-D

(2, 4-dichlorophenoxyacetic acid) and picloram (4-amino-3,5,6-trichloropicolinic acid) on the control of several perennial weeds have also been reported (9). Enhanced injury to soybean was also reported with combinations of chloroxuron (3 [p-(p-chlorophenoxy) phenyl]-1, 1-dimethylurea) and nitralin (4[methylsulfonyl]-2, 6-dinitro-N, N-dipropylaniline) (45).

In some cases, antagonistic responses have been observed. Picloram plus bromacil (5-bromo-3-sec butyl-6-methyluracil) appeared less effective in controlling grasses than either of the herbicides alone (72). Carbamate and growth regulator herbicides were antagonistic both in whole plants and plant segments (44). When combinations of chlorpropham (isopropyl m-chlorocarbanilate and 2, 4-D were applied to the foliage of either pigweed (Amaranthus retroflexus L.) or smartweed (Polygonum lapathifolium L.) the severe twisting effects of 2, 4-D were greatly reduced. The induced elongation of soybean hypocotyl section by 2, 4-D, dicamba (3, 6-dichloro-o-anisic acid), or picloram was inhibited in the presence of either chlorpropham or EPTC (S-ethyl dipropylthiocarbamate).

Several mechanisms have been offerred to explain the nature of pesticide interactions. Three possible sites of pesticide interaction have been proposed by Nash (55): first, at the site of absorption where one pesticide affects the penetration of the other; second, within the plant where one pesticide affects a primary pathway and the other affects the secondary pathway and third, where both pesticides affect the same pathway. Davis and his co-workers (18) showed that paraquat increased the uptake of picloram but reduced its transport in some plant species. The uptake and transport of 2,4,5-T (2,4,5-trichlorophenoxy-acetic acid) in mesquite (*Prosopis juliflora* Swartz) decreased in the presence of picloram but the uptake and transport of picloram increased in the presence of 2,4,5-T. Agbakoba (2) found that picloram increased translocation of 2, 4-D in field bindweed (*Convolvulus arvensis* L.) but the reverse was not demonstrated.

Synergism with certain pesticide combinations may occur because the insecticide inhibits the metabolic processes in the plant that are active in the degradation of the herbicide. The tolerance of rice plants to propanil is correlated with the metabolism of the chemical by the presence of the propanil hydrolyzing aryl acylamidase (51). The insecticide carbaryl inhibits the activity of this enzyme and when applied with propanil, selectivity is lost. Likewise Swanson and Swanson (70) showed that carbaryl inhibited the degradation of monuron by cotton plants. Other *in vitro* studies have since demonstrated that an enzyme called demethylase is inhibited by carbaryl (24).

Demethylase was isolated from a number of plants and was believed responsible for the successive removal of the methyl group in substituted dimethylurea herbicides. Chang et al. (13) recently reported inhibition of the metabolism of herbicides from several chemical groups by some organophosphate and carbamate insecticides.

An explanation for the antagonism observed in one pesticide combination is an alteration in nucleic acid metabolism (56). 2, 4-D applied in combination with EPTC reversed the EPTC inhibition of growth and caused an increase in nucleic acid content. Analyses showed that the EPTC-2, 4-D combination caused an increased synthesis of ribosomal RNA and t-RNA over the levels observed with EPTC alone.

Evaluation of Pesticide Combinations. Gowing (29) employed probit analysis in evaluating herbicide mixtures. This procedure consisted of plotting the logarithm of the concentration against the percent response on a probability scale and fitting a weighted regression line to the data. From this graph, a prediction of the amount of chemical for a given response can be made. Reliability of the measurement is maximum near the 50% response level.

Mathematical methods are available for testing results of pesticide combinations. The expected response for a given combination of two herbicides can be calculated as follows (30):

X = the percent inhibition of growth by herbicide A at p lb/a

Y = the percent inhibition of growth by herbicide B at q lb/a

E = expected percent inhibition by the two herbicides at p lb/a and q lb/a

$$E = X + Y - \frac{XY}{100}$$

When the observed response is greater than the expected, the combination is synergistic; when less than the expected, it is antagonistic, and if the observed and expected responses are equal the combination is additive. Colby (15) modified this formula by converting the original data to percent of control rather than percent inhibition. By doing so, the number of arithmetic operations required to obtain E is reduced. He proposed the following formula:

$$E = \frac{XY}{100}$$

where X is the growth as a percent of control with herbicide A at p lb/a, Y is the growth as a percent of control with herbicide B at q lb/a and E is the expected growth as a percent of control with herbicides A and B at p lb/a and q lb/a. Synergism is indicated when the observed growth as a percent of control is less than the expected, and antagonism when the observed growth is greater than expected.

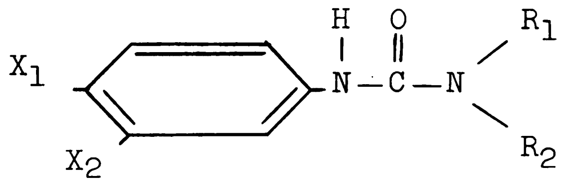
Instead of calculating the expected response of a combination, Bovey et al. (9) set a definite value to describe herbicide combinations. If a combination of 2 herbicides gave 20% or more defoliation than a single herbicide at equivalent rates, the mixture was considered synergistic and if 20% or less, the mixture was antagonistic.

Tammes (74) formulated a graphic representation of the effect of two pesticides or a pesticide and adjuvant applied jointly. Each of the components of a combination is expressed on a coordinate. In the graph, a quantitatively defined effect, for example 50% mortality, is inserted. The values are obtained by interpolation of the data of the combination on log/probit paper. The line which connects the points in the graph is called an isobole. From the isobole presentation, one can determine if the combination is synergistic, antagonistic or additive.

Some workers have analyzed pesticide combinations similar to single treatments and used tests such as Duncan's Multiple Range Test to compare the means (12, 34, 36).

Substituted Urea Herbicides

The herbicidal properties of the substituted ureas were discovered by Thompson and his co-workers (75) and by E. I. du Pont (10). The general structure of the phenylurea herbicide is:



The analogous compounds utilized in this study have the following substitution and solubility properties (38):

Herbicide	R_1	R_2	X_1	X_2	Solubility in water at 25 C (ppm)
Linuron	OCH_3	CH_3	Cl	Cl	75
Monolinuron	OCH_3	CH_3	Cl	H	75
Diuron	CH_3	CH_3	Cl	Cl	42
Monuron	CH_3	CH_3	Cl	H	230

The substituted urea herbicides are widely employed as selective pre-emergence or post-emergence treatments for annual weeds and at higher rates as soil sterilants for the control of all annual and perennial weeds. Linuron selectively controls germinating and newly established broadleaves and grasses in crops such as cotton, corn, potatoes, carrots, parsnip and soybean. Monolinuron is used on potatoes, dwarf beans, vines and other crops. At lower rates, diuron and monuron selectively control germinating broadleaf and grass weeds in cotton, sugarcane, pineapple, asparagus and citrus.

Absorption and Movements. Substituted urea herbicides are easily absorbed by the roots but they are less readily taken up by the aerial portion of the plants. Studies with monuron and diuron indicated that these herbicides moved primarily in the apoplast and moved laterally and acropetally in the transpiration stream (17).

When applied to leaf surfaces, the substituted ureas are able to penetrate the cuticular and epidermal layers. Foliar absorption of the newer methoxymethyl ureas is significantly greater than that of the dimethylureas. No basipetal movement was observed when diuron was applied to the leaves indicating that there is little or no translocation occurring in the phloem (35). Absorption into the leaf surfaces is greatly facilitated by the addition of suitable surfactants (4,39). Surfactant WK (dodecyl ether of polyethylene glycol) enhanced the deposition of high concentrations of linuron and diuron on some grasses. Environmental factors such as high temperature, high relative humidity and moisture stress increase the penetration of these chemicals into the leaves (39).

Hogue (42) demonstrated that in coriander (*Coriandrum sativum* L.) and tomato (*Lycopersicon esculentum* Mill.), the monohalogenated compounds like monuron and monolinuron were more toxic than their dihalogenated counterparts. He attributed this difference to the fact that plants took up less dihalogenated than monohalogenated herbicide. In post-emergence application, the dihalogenated herbicides were generally slightly more toxic than their monohalogenated counterparts.

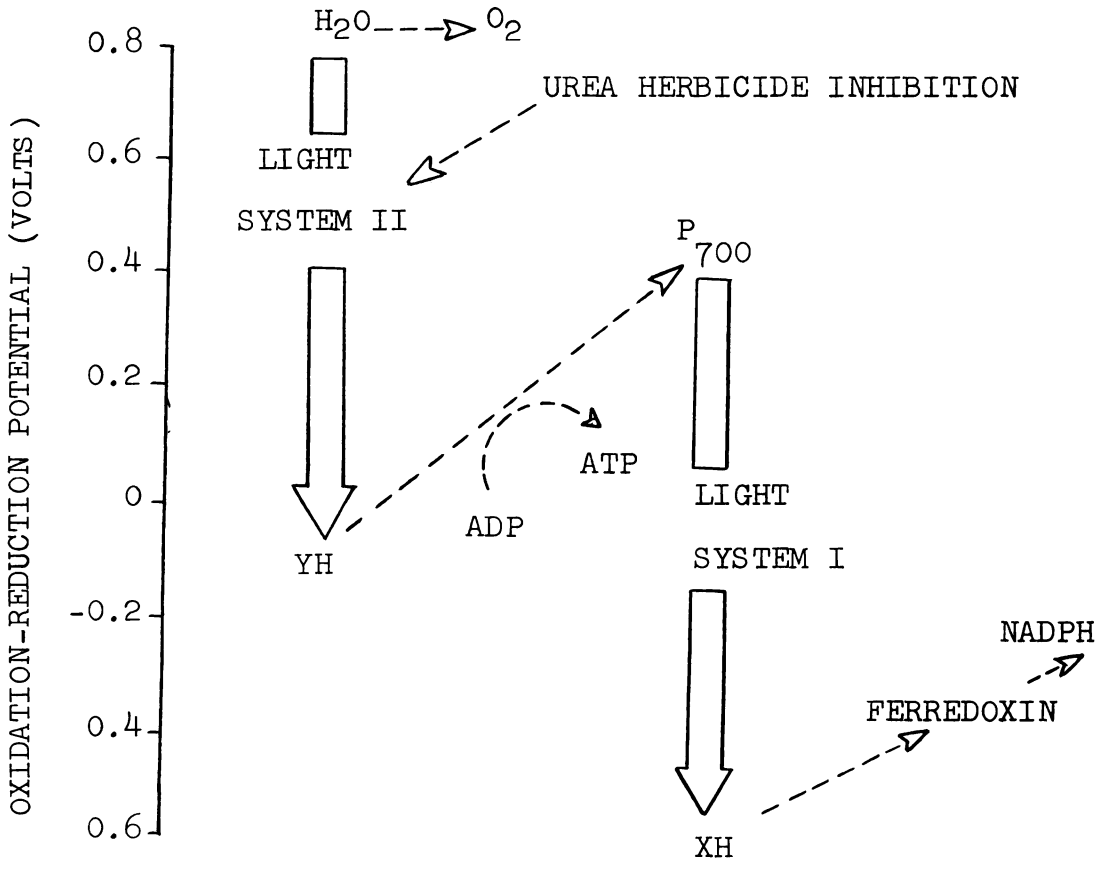
Metabolism. Fang (22) reported the formation of a monuron complex in bean leaves after foliar application of radioactive monuron. Sweetser (69) showed that monuron complexed with flavin mononucleotides and upon illumination was inactivated. Other studies indicated that 15 to 25% of linuron absorbed may be bound to a protein (56). The ability of diuron to form a complex with protein *in vitro* was also demonstrated using bovine serum albumin (11).

Another means of metabolic breakdown is by dealkylation (27). Demethylation is the initial step in the degradation scheme, followed by hydrolysis. In the case of the methoxymethylurea, linuron, the major metabolite found in plants was the demethylated derivative, 3- (3, 4-dichlorophenyl)-1-methoxyurea (56).

Further hydrolysis of the demethylated ureas produces their corresponding anilines. Onley *et al.* (58)

reported the possibility of 3, 4-dichloroaniline transformation into 3, 4-dichloronitrobenzene. Ring substitution and ring rupture or dehalogenation have not been reported.

Mechanism of Action. The substituted urea herbicides are known to be potent inhibitors of photosynthesis, particularly the Hill reaction. Wessels and van der Veen (78) and Cooke (16) found that several substituted urea herbicides were very efficient inhibitors of the Hill reaction in isolated chloroplasts. Monuron at a concentration of 1×10^{-6} M completely inhibited the Hill reaction (16). Duysens et al (21) reported that two pigment systems are involved in cytochrome oxidation during photosynthesis. Light of 560 mμ causes a reduction of the cytochrome while light of 680 mμ oxidizes the cytochrome. Diuron at 1×10^{-6} M inhibited the reduction at 560 mμ and oxidation of the cytochrome took place instead. The photosynthetic mechanism in green plants is believed to include two light reactions (7):



Although the site of action of substituted ureas is believed to be in light system II, the enzymatic events associated with this process and the specific molecular sites inhibited by the ureas are still unknown (61).

Carbaryl

Carbaryl (1-napthyl N-methylcarbamate) is one of the most widely used carbamate insecticides for fruits, vegetables, forage, cotton and many other economic crops.

The insecticidal properties and characteristics were reported by Haynes _et al_. (37).

Carbaryl is soluble in most organic solvents and has a water solubility of less than 0.1%. It is stable in light, heat and acids but is easily hydrolyzed in alkalies.

The metabolism of carbaryl has been investigated in beans (_Phaseolus vulgaris_ L.), peas (_Pisum sativum_ L.), pepper (_Capsicum frutescens_ L.) and corn (_Zea mays_ L.) (53). The comparison of the mass and ultraviolet spectra of isolated metabolites with the spectra of synthetic compounds confirmed the presence of _N_-hydroxymethylcarbaryl, 4-hydroxymethylcarbaryl and 5-hydroxymethylcarbaryl. Each plant possessed essentially the same metabolites in varying amounts.

The metabolic fate of carbamate insecticides is often similar in both plants and insects (20, 47). However, differences exist in their rate of formation, their conjugating moieties and their ultimate distribution within the organisms. Besides hydroxylation in the 4 and 5 positions, carbaryl also forms the 5, 6, dihydro-5, 6-dihydroxy derivative which conjugates in plants. Hydrolysis of the carbamate-ester linkage occurs slowly. Little is known about the plant enzymes responsible for carbamate metabolism, but in insects a mixed function oxidase system(s), present in the microsome, appears to be the main catalyst (47).

The carbamate insecticides are considered to kill insects and mammals entirely by cholinesterase inhibition (57). The symptoms accompanying their action in intact animals are typically cholinergic, involving lachrymation, salivation, myosis, convulsions and death.

Diazinon

Diazinon (O, O-diethyl O-2-isopropyl-6-methyl-4-pyrimidinyl phosphorothioate) is extensively applied to soil and crops to control phytophagous insects. It is effective for controlling resistant soil insects such as corn rootworm, wireworm and cabbage maggot and also used against many foliage insect pests of fruits, vegetables, forage, field crops and ornamentals.

Diazinon is available as wettable powder, emulsifiable and oil solutions, dust and granular formulations. It is miscible with alcohol, ether, petroleum ether, cyclohexane, benzene and similar hydrocarbons and soluble in water at .004%.

Gasser (25) first reported on the biological properties of diazinon and its effectiveness as a contact or vapor insecticide. Gunner *et al.*(31) showed that diazinon was absorbed and translocated in plants after foliar application and later appeared in roots and root exudates. Diazinon was absorbed and accumulated in higher quantities in the roots of bean plants than in any other regions (46). Translocation to the leaves occurred, but only small amounts of diazinon were present in the primary leaves after two days. Systemic activity has occurred in leaves of sugar beet seedlings grown in treated soil indicating that diazinon may accumulate in the foliage after continuous exposure of roots (59). Root treatment of cabbage (Brassica oleracea L.) and tobacco (Nicotiana tabacum L.) with water preparations of diazinon also resulted in translocation and residues in the foliage (52). Pea plants grown in diazinon treated sand also accumulated the insecticide in the leaves, especially at higher rates of treatment and shorter harvest times (49).

Metabolism of diazinon in plants has been shown to involve hydrolysis of the phosphorous pyrimidyl ester bond and subsequent metabolism of the 2-isopropyl-4-methyl-6-ol to carbon dioxide (46). Small amounts of diazoxon, (O, O-diethyl O- [2-isopropyl-4-methyl-6-pyrimidinyl] phosphate) were also detected in field grown crops (64).

It is widely accepted that organophosphate insecticides kill animals, both vertebrate and invertebrate, by inhibiting cholinesterase activity with subsequent disruption of the nervous activity caused by accumulation of acetylcholine at the nerve endings (57).

MATERIALS AND METHODS

Initial Screening of Pesticide Combinations for Enhanced Toxicity

Combinations of several common herbicides and insecticides were initially screened as foliar sprays to identify synergisms which might occur. The plant species used were carrots (*Daucus carota* L. cv. Chantenay Long Type), cucumber (*Cucumis sativus* cv. Spartan Progress), soybean (*Glycine max* (L.) Merr. cv. Harris), cotton (*Gossypium hirsutum* cv. Polymaster) and corn (*Zea mays* L. cv. Harris Gold Cup). Seeds of each species were planted in 11 x 16 cm styrofoam flats filled with muck soil. Upon emergence the seedlings were thinned to an equal number of plants in each pot. Carrots were sprayed at the 2 to 4 leaf stage, cotton, corn and cucumber at the 2-leaf stage and soybean at the first trifoliate leaf stage.

The chemicals and their rates of applications for each species are indicated in the appendices. All the formulations used were wettable powders. The amount of herbicide or herbicide-insecticide mixture required for each treatment was mixed thoroughly in a quart milk bottle. Spraying was done at 40 psi using

CO_2 as a source of pressure and water volume was equivalent to 100 gallons per acre. The potted plants were passed under the nozzle on a conveyor which moved at a fixed rate of speed. Before spraying, the soil was covered with vermiculite to prevent the herbicides from reaching the soil. Afterwards the vermiculite was removed and the treated plants were transferred into the greenhouse and arranged in a randomized block design on benches. The greenhouse was maintained at 25 ± 5 C with a daylength of 16 hr. Sunlight was supplemented with fluorescent lights. All the treatments were replicated 3 to 6 times and each experiment was conducted at least 2 times.

Injury ratings were obtained at intervals using a scale of 1 to 9 where 1 indicated no injury, 2 to 3 slight injury, 4 to 6 moderate injury, 7 to 8 severe injury and 9 death. The plants were harvested after 2 weeks and fresh weight was also obtained. The data were subjected to analysis of variance and the combinations were compared using the LSD test and Colby's formula (15). If the expected response was greater by 15% or more the combination was considered synergistic by Colby's method.

Toxicity of Phenylurea Herbicides and Herbicide-Insecticide Combinations

Seeds of cotton, carrot, corn and soybean were germinated in wooden flats containing vermiculite. After 7 to 10 days, the seedlings were transplanted into $\frac{1}{2}$ strength Hoagland's solution (40). The solution was contained in 180 ml plastic containers wrapped with aluminum foil to exclude light. The nutrient solution was changed every other day. A circular sponge which fitted the top of the container was used to hold the plants in place. Cotton was treated when the first 2 leaves were fully expanded. Corn and carrots were treated at the 3-leaf stage and soybeans were treated at the first trifoliate stage.

The following substituted urea herbicides were used: linuron (Lorox 50% WP), monolinuron (Aresin 50% WP), diuron (Karmex 80% WP) and monuron (Telvar 80% WP). The leaves were dipped in suspensions of commercial formulations of the above herbicides at concentrations ranging from 300 to 4800 mg/l. The leaves were allowed to dry at room temperature before the plants were transferred to the greenhouse. After 10 days, visual ratings and fresh weight of the shoot were taken. The data were subjected to analysis of variance and a LSD was used to compare the treatment means.

Effect of Carbaryl and Diazinon on Herbicide Activity. The 4 substituted ureas used in the above experiment were also applied to leaves singly and in combination with carbaryl (Sevin 50% WP) or diazinon (50% WP). The plants were grown in a similar manner as described above. Treatment was accomplished by dipping the leaves in a suspension of the herbicide alone and the herbicide-insecticide mixture. The concentration of the 4 herbicides used for cotton, corn and carrot was 1200 mg/l while for soybean, linuron and monolinuron were applied at 300 mg/l and diuron and monuron 600 mg/l. Carbaryl and diazinon were applied at a concentration of 2400 and 1200 mg/l respectively. The treatments were replicated 3 times and the experiments were repeated 2 times. After treatment, the plants were kept in a growth chamber with a night temperature of 20 C and day temperature of 25 C. The growth chamber was maintained with a 16-hour daylength and a light intensity of 5 watts/cm^2 (red region) and 4 watts/cm^2 (blue region).

After one week, visual injury ratings were obtained and fresh weights of the shoots were recorded. Data were subjected to analysis of variance, converted to percent of control and the combinations were assessed using Colby's formula (15).

Tests were also conducted in which one component of the combination was applied to the foliage, the other to the root media and both components applied to the root media. Six day old cotton seedlings were transplanted into nutrient solution with ½ strength Hoagland's solution. The herbicides were dissolved in the nutrient solution at a concentration of 1.0×10^{-6} M. After 2 days, the shoots were dipped in suspensions of wettable powder formulations of either carbaryl or diazinon at a concentration of 2400 and 1200 mg/l respectively.

In another experiment, the insecticide was applied in nutrient solution at a concentration of 1.0×10^{-5} M. After 2 days, the shoots were dipped in suspensions of wettable powder formulations of the 4 herbicides at a concentration of 1200 mg/l. In the third experiment, the herbicide was applied to the nutrient solution at a concentration of 1.0×10^{-6} M and the insecticide at 1.0×10^{-5} M. In all cases, visual injury ratings were taken and fresh weights of the shoots were recorded 10 days after treatment.

Effect on Respiration and Photosynthesis of Leaf Tissue. The oxygen uptake and evolution of isolated leaf discs treated with linuron alone in combination with carbaryl was followed using the method of Umbreit *et al.* (76) in a Gilson Differential Respirometer.

Discs (8.0 mm) were obtained from recently expanded parsnip (<u>Pastinaca sativa</u> L. cv. Hollow Crown) leaves using a cork borer. The leaf discs were incubated for 1.5 hr in solutions containing 1.0×10^{-5} M linuron alone or combined with 1.0×10^{-4} M carbaryl. At the end of the incubation period, the leaf discs were removed from the solution, rinsed 3 times with distilled water and dried with absorbent paper. They were then transferred into a respirometer flask containing 1.0 ml of 0.05 M phosphate buffer pH 7.0. The center well contained 20% KOH and a folded filter paper. Oxygen uptake was followed in the dark for a duration of 5 hr after which dry weight of the leaf discs was obtained.

To measure photosynthesis, the same procedure of preparation and treatment of plant material was followed. Instead of KOH buffer, the center well contained a CO_2 buffer which maintained a 0.3% CO_2 concentration in the flask (60, 70). The buffer system was made up of 10 ml of 60% diethanolamine, .5 ml 6 N HCl, 4.5 ml distilled water and 3 gm $KHCO_3$. The temperature was maintained at 25 C throughout the 5 hr run. The tissue was exposed to alternate periods of light (30 min) and darkness (25 min) and readings were obtained at each interval. In these tests, the treatments were replicated 4 times and the experiments were conducted twice.

Effect of Insecticides on the Uptake of ^{14}C-Phenylurea Herbicides

The effect of diazinon on the uptake of the 4 phenylureas in tolerant cotton and susceptible soybean was determined.

Application and Sampling. The plants were treated in the same growth stage as in the previous experiments. The concentration of the herbicides and diazinon used was 1200 mg/l. The treating suspensions were spiked with ^{14}C-labeled herbicides to give a final activity of 0.0005 $\mu c/\mu l$. The specific activity of the labeled herbicides is presented in Table 1. All the radioactive herbicides were maintained in absolute alcohol in the freezer prior to use.

Table 1. Specific activity of the carbonyl-labeled phenylurea herbicides.

Herbicide	Specific Activity $\mu c/mg$
Linuron	6.8
Monolinuron	9.4
Diuron	4.0
Monuron	0.204

Twenty microliter droplets (.01 μc) of the herbicide alone and the herbicide-insecticide combination were applied at the midrib of the leaf. Also 20 μl aliquots of each solution were counted to determine the total amount of radioactivity applied. The droplet was allowed to dry in the laboratory at 25 C before the plants were transferred to a growth chamber. The growth chamber was maintained with a 16-hour daylength with a light intensity of 5 watts/cm^2 (red region) and 4 watts/cm^2 (blue region) and a temperature of 25 C. Three or 4 replicates were sampled at 6, 12, 24 and 48 hr after treatment.

Upon sampling, the treated leaf was cut from the plant and washed 5 times with 95% ethyl alcohol to remove the herbicide remaining on the leaf surface. After washing, the leaves were frozen in a dry ice + acetone bath and dried in an oven.

The uptake of technical grade herbicide in the presence or absence of technical grade diazinon was also followed in cotton leaves. The cotton leaves were treated at the same growth stage and the manner of treatment and sampling were similar to that of the above experiments. The concentration of linuron was 4.0×10^{-4} M (.5 μc), monolinuron 2.0×10^{-4} M (.5 μc). diuron 3.0×10^{-4} M (.25 μc) and monuron 6.0×10^{-4} M (.05 μc). Diazinon was used at 6.6×10^{-5} M.

In other experiments with carrots, the uptake of linuron in the presence or absence of carbaryl was determined. The 20 μl (.01 μc) treating solution was distributed as droplets over the divided leaf surfaces. The treated leaves were handled in the same manner as in the above experiments.

Assay of Radioactivity. Total radioactivity in the treated leaves was recovered by combustion in a Model 3151 Nuclear Chicago Combustion Apparatus equipped with a light source and stirring equipment. The dried leaf samples were placed inside cellophane bags and inserted in a platinum wire basket. The flask was flushed with oxygen and the sample was completely oxidized. The flask was allowed to cool for about 5 minutes before adding 15 ml of trapping solution which consisted of ethanol: ethanolamine at a ratio of 2:1 (v/v). The solution was stirred for 10 minutes. A 1.0 ml aliquot was removed and placed in a scintillation vial containing 15 ml of cocktail mixture. The cocktail mixture was prepared by dissolving 4 grams of BBOT (2,5-bis [5-*tert* butylbenzoxezolyl (2')] thiopen) with 1 liter of toluene and 400 ml of Triton X-100. Quantitative determination of radioactivity was obtained with a Packard Tricarb Scintillation Spectrometer equipped with external standardization.

Effect of Insecticides on the Metabolism of ^{14}C-Phenylurea Herbicides

Application and Sampling. The rate of metabolism of the 4 phenylureas in the presence or absence of diazinon was followed in cotton. Plants were grown in the same manner as the previous experiments. The concentration of the herbicides used were linuron 3.0 x 10^{-4} M (0.5 μc), monolinuron 5.0 x 10^{-4} M (1.0 μc), diuron 5.0 x 10^{-4} M (0.5 μc) and monuron 6.0 x 10^{-4} M (.05 μc). The herbicides and diazinon were applied to the first true leaf of cotton with a microsyringe. The solution was allowed to dry in the laboratory at 25 C and then the plants were placed in a growth chamber under the same conditions previously described. The treated leaves were removed 2 and 5 days after treatment.

In other experiments, the metabolism of linuron in the presence or absence of carbaryl was studied in carrots. The plants were treated at the 3-leaf stage. Linuron was applied at a concentration of 3.0 x 10^{-4} M (0.5 μc) and carbaryl at 3.0 x 10^{-4} M. Twenty microliters of solution was applied with a microsyringe on each leaf. Eight leaves were used for each treatment and upon sampling 4 leaves were combined to insure high activity in the extract. The plants were placed in a growth chamber with the same condition as the above experiments and sampling was done 2 and 5 days after treatment.

Extraction and Analysis. The leaves treated with linuron and monolinuron were washed with 95% ethyl alcohol and extraction was done immediately. The leaves were macerated thoroughly using mortar and pestle and ethyl alcohol (10 ml/g sample) as solvent. The extracts were filtered using Whatman No. 1 filter paper and the residue was washed 3 times with the extracting solvent. The filtrate was then evaporated to dryness in a rotating evaporator. The residue was saved for combustion to determine the alcohol insoluble metabolites. After evaporation to dryness, the residue was redissolved in 1.0 ml of absolute ethyl alcohol. The extracts were refrigerated overnight prior to chromatography.

The procedure was essentially the same for washing and extracting the diuron and monuron from leaves except methyl alcohol was utilized as a solvent.

Chromatography of the leaf extracts was done on glass plates coated with silica gel H with a thickness of 250 microns. The plate was divided into three sections, one portion for the standard ^{14}C-compound, the second portion for the extract from plants treated with herbicide alone and the third portion for the herbicide-insecticide treatment. Fifty μl from each leaf extract was spotted in a band at the origin. In the case of linuron and monolinuron, the plates were developed in benzene: acetone (2:1; v/v) (71). After developing to a distance of 15 cm on the plate, the plate was divided into 1.0 cm sections, the silica gel was scraped, and the scrapings were placed in scintillation vials and counted for radioactivity. Rf values were calculated and used as a reference for identifying the parent compound and metabolites.

RESULTS AND DISCUSSION

Initial Screening of Pesticide Combinations for Enhanced Toxicity

Several herbicide-insecticide combinations were synergistic when applied as foliar sprays to carrot, soybean, corn, cotton and cucumber (Appendices A to F). Combinations of linuron and carbaryl enhanced toxicity on carrot as illustrated by fresh weight and injury ratings (Appendix A). Since linuron is widely used for weed control in carrots and carbaryl is used to control insects in that crop, this interaction was investigated in more detail.

The tests with other species indicated interactions between substituted urea herbicides and carbaryl, diazinon and malathion. In susceptible cucumber, diuron and chloroxuron toxicity was slightly enhanced with carbaryl, diazinon and malathion but the herbicide rates were probably too high to obtain maximum differences (Appendix B). Interactions were also evident with some of these combinations in soybean (Appendix C).

Since diazinon and carbaryl were the compounds which most consistently increased the activity of the substituted urea herbicides, they were applied in combination with 4 analogous ureas (linuron, monolinuron, diuron and monuron) on soybean, corn and cotton. In soybean, combinations of diuron with diazinon or carbaryl produced synergistic interactions (Appendix D). In corn, the presence of carbaryl and diazinon caused an increase in toxicity of linuron and monolinuron (Appendix E). Synergistic interactions were obtained in cotton with combinations of the 4 phenylurea herbicides and carbaryl (Appendix F).

The major problem encountered in the greenhouse screening tests was variability in results between experiments. The amount of enhancement in herbicide activity caused by the added insecticides varied considerably from test to test. This may have occurred because of difference in environmental conditions prior to or after spraying the plants. At this time, the greenhouse soil mix was also unsatisfactory for growing uniform plants. Insect control caused a problem in the greenhouse and the use of fumigants or other insecticides introduced a third chemical for possible interaction. Another factor may have been variable exposure of foliage to the sprays.

To overcome variability, uniform seedlings were selected and transplanted to nutrient culture. Instead of spraying the chemicals, the shoots were dipped in the chemical suspension. With this method, uniform wetting of the upper and lower leaf surfaces was obtained.

Fresh weight was utilized because there was no difference in relative toxicity using fresh or dry weight.

Toxicity of the Phenylurea Herbicides and Herbicide-Insecticide Combinations

Cotton, carrot, corn and soybean responded differently to foliar application of the 4 phenylurea herbicides (Table 2). Carrot was the most tolerant to the 4 phenylureas whereas corn was tolerant to diuron, monuron and linuron and slightly more susceptible to monolinuron. Cotton was relatively tolerant to the dimethylureas but more susceptible to the methoxymethylureas. Soybean was the most susceptible species to all of the phenylureas. Chlorosis of leaves which ultimately resulted in necrosis and death of the plant was observed in the affected species.

Table 2. Concentration of phenylurea herbicides (mg/l) required to produce 50% fresh weight reduction in 4 species.

Plant	Herbicide			
	Linuron	Monolinuron	Diuron	Monuron
Soybean	< 300	300	600	< 300
Cotton	< 300	300	2400	2400
Corn	4800	2400	> 4800	> 4800
Carrot	> 4800	> 4800	> 4800	> 4800

ED_{50} values (effective dose required to reduce fresh weight by 50%) for oat, soybean, corn and cotton 11 days after exposure to monuron and diuron in culture solution revealed that the order of susceptibility of the species to both herbicides was: oat > soybean > corn > cotton (68). Foliar application of diuron and monuron, as indicated in this experiment, showed the following order of susceptibility: soybean > cotton > corn. The tolerance of carrot to linuron is consistent with previous results (48). Sublethal concentrations of linuron by root uptake were previously reported as 0.57 ppm for corn and 0.28 ppm for soybean (56). This experiment further demonstrated the relative tolerance of corn and susceptibility of soybean to foliar application of linuron.

Effect of Carbaryl and Diazinon on Herbicide Activity. Cotton was relatively tolerant to both diuron and monuron, however, when they were combined with the insecticides carbaryl and diazinon, toxicity was greatly increased (Table 3). Diazinon had a more pronounced effect than carbaryl as indicated by injury ratings and fresh weights. Linuron plus carbaryl and monolinuron plus carbaryl produced no additional toxicity over that obtained with the herbicides applied alone. However, the toxicity of linuron and monolinuron was enhanced by diazinon.

In tolerant carrots, the combination of linuron or monuron with carbaryl was synergistic as indicated by the fresh weight (Table 4). Monolinuron or diuron plus carbaryl mixtures were about additive. Diazinon interacted with the 4 phenylureas regardless of their substitution and provided synergism in all cases (Figure 1).

Corn was also tolerant to the 4 phenylurea herbicides. Combinations of linuron or monolinuron with carbaryl both resulted in synergism (Table 5). Again, the combination of diazinon with all 4 herbicides gave extremely pronounced synergistic effects. A slight burning of the corn leaves at the margin was observed with treatments receiving diazinon alone.

Table 3. The effect of 4 phenylurea herbicides and combinations with insecticides on the growth of cotton.

Herbicide	Concn (mg/l)	Insec-ticide	Concn (mg/l)	Visual Rating	Fresh Weight (% of Control) Observed	Fresh Weight (% of Control) Expected[1]	Synergism[1]
Check	-	-	-	1.0	100		
Linuron	1200	-	-	7.7	51		
Linuron	1200	Carbaryl	2400	8.3	55	52	
Linuron	1200	Diazinon	1200	9.0	35	53*	S
Monolinuron	1200	-	-	6.3	71		
Monolinuron	1200	Carbaryl	2400	7.0	66	72	
Monolinuron	1200	Diazinon	1200	8.3	41	73*	S
Diuron	1200	-	-	1.7	100		
Diuron	1200	Carbaryl	2400	4.7	79	102	S
Diuron	1200	Diazinon	1200	8.7	41	103*	S
Monuron	1200	-	-	3.0	87		
Monuron	1200	Carbaryl	2400	5.7	59	89*	S
Monuron	1200	Diazinon	1200	9.0	29	90*	S
-	-	Carbaryl	2400	1.3	102		
-	-	Diazinon	1200	1.0	103		
LSD at 5%				1.1	28		

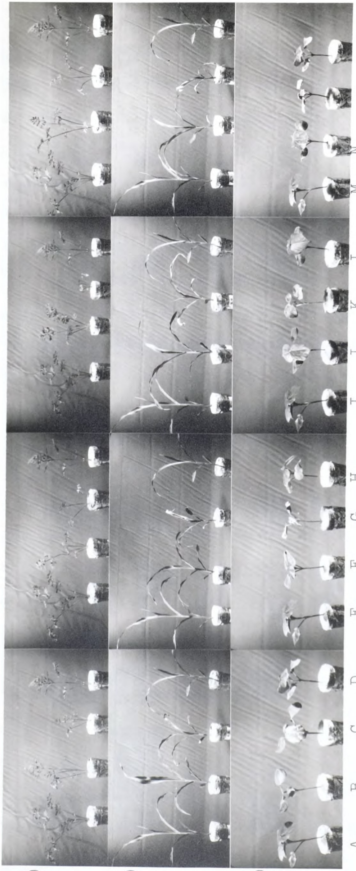
1 Calculated by Colby's method.
* Significantly different from observed value using estimated $LSD_{.05}$ of Hamill (33).

Table 4. The effect of 4 phenylurea herbicides and combinations with insecticides on the growth of carrots.

Herbicide	Concn (mg/l)	Insec-ticide	Concn (mg/l)	Visual Rating	Fresh Weight (% of Control)		Synergism
					Observed	Expected	
Check	-	-	-	1.0	100		
Linuron	1200	-	-	1.3	85		
Linuron	1200	Carbaryl	2400	4.0	56	79	S
Linuron	1200	Diazinon	1200	4.0	34	93*	S
Monolinuron	1200	-	-	3.0	54		
Monolinuron	1200	Carbaryl	2400	4.3	69	50	
Monolinuron	1200	Diazinon	1200	4.0	39	58	S
Diuron	1200	-	-	1.0	80		
Diuron	1200	Carbaryl	2400	2.7	73	74	
Diuron	1200	Diazinon	1200	8.3	11	86*	S
Monuron	1200	-	-	1.7	85		
Monuron	1200	Carbaryl	2400	2.7	41	79	S
Monuron	1200	Diazinon	1200	7.0	26	92*	S
-	-	Carbaryl	2400	1.0	93		
-	-	Diazinon	1200	1.0	108		
LSD at 5%				1.6	38		

Figure 1. Toxicity obtained with combination of diazinon and the 4 phenylurea herbicides on (a) carrot, (b) corn, (c) cotton.

A = Check
B = Linuron (1200 mg/l)
C = Linuron + Diazinon (1200 + 1200 mg/l)
D = Diazinon (1200 mg/l)
E = Check
F = Monolinuron (1200 mg/l
G = Monolinuron + Diazinon (1200 + 1200 mg/l)
H = Diazinon (1200 mg/l)
I = Check
J = Diuron (1200 mg/l)
K = Diuron + Diazinon (1200 + 1200 mg/l)
L = Diazinon (1200 mg/l)
M = Check
N = Monuron (1200 mg/l)
O = Monuron + Diazinon (1200 + 1200 mg/l)
P = Diazinon (1200 mg/l)



(a) (b) (c)

A B C D E F G H I J K L M N O P

Table 5. The effect of 4 phenylurea herbicides and combinations with insecticides on the growth of corn.

Herbicide	Concn (mg/l)	Insecticide	Concn (mg/l)	Visual Rating	Fresh Weight (% of Control) Observed	Fresh Weight (% of Control) Expected	Synergisim
Check	-	-	-	1.0	100		
Linuron	1200	-	-	1.3	88		
Linuron	1200	Carbaryl	2400	2.7	77	93	S
Linuron	1200	Diazinon	1200	6.7	28	77*	S
Monolinuron	1200	-	-	1.7	77		
Monolinuron	1200	Carbaryl	2400	2.3	46	82	S
Monolinuron	1200	Diazinon	1200	7.7	38	68	S
Diuron	1200	-	-	1.0	102		
Diuron	1200	Carbaryl	2400	1.5	102	108	
Diuron	1200	Diazinon	1200	6.7	24	90*	S
Monuron	1200	-	-	1.5	72		
Monuron	1200	Carbaryl	2400	1.3	108	77	
Monuron	1200	Diazinon	1200	7.7	17	64*	S
-	-	Carbaryl	2400	1.0	106		
-	-	Diazinon	1200	2.3	88		
LSD at 5%				0.8	41		

Soybean was the most susceptible species to the 4 phenylureas. In this experiment, diazinon at a concentration of 600 and 1200 mg/l and carbaryl at 2400 mg/l were also phytotoxic to soybean (Table 6). Since toxicity occurred from both the herbicides and insecticides, the combinations of diazinon with linuron, monolinuron and monuron were all about additive in their effects. Carbaryl plus linuron or monolinuron also produced additive responses. The only synergisms obtained were with diuron in combination with both insecticides. It should be noted that the toxicity obtained with low rates of herbicides applied alone was severe. Other synergisms may have been evident if lower rates were utilized.

Split application experiments were conducted only with cotton plants. In one study, the phenylureas were applied in nutrient solution at a concentration of 1.0×10^{-6} M and the shoot was dipped in suspensions of commercial preparations of insecticides after 2 days. Foliar applied carbaryl had little or no effect on the fresh weight reduction obtained with the 4 phenylurea herbicides by root uptake (Table 7). No significant difference was observed in the visual rating of these combinations. With the possible exception of monuron, foliar applied diazinon did not produce enhancement of the phenylureas when

Table 6. The effect of 4 phenylurea herbicides and combinations with insecticides on the growth of soybean.

Herbicide	Concn (mg/l)	Insec-ticide	Concn (mg/l)	Visual Rating	Fresh Weight (% of Control)		Synergism
					Observed	Expected	
Check	-	-	-	1.0	100		
Linuron	300	-	-	7.0	56		
Linuron	300	Carbaryl	2400	7.7	46	42	
Linuron	300	Diazinon	600	8.0	32	34	
Linuron	300	Diazinon	1200	8.0	31	35	
Monolinuron	300	-	-	7.0	54		
Monolinuron	300	Carbaryl	2400	7.0	47	41	
Monolinuron	300	Diazinon	600	8.3	37	33	
Monolinuron	300	Diazinon	1200	8.0	25	25	
Diuron	600	-	-	4.7	93		
Diuron	600	Carbaryl	2400	8.3	59	70	
Diuron	600	Diazinon	600	8.0	36	57*	S
Diuron	600	Diazinon	1200	8.0	36	41	
Monuron	600	-	-	7.0	54		
Monuron	600	Carbaryl	2400	7.3	51	41	
Monuron	600	Diazinon	600	8.3	32	33	
Monuron	600	Diazinon	1200	8.0	27	24	
-	-	Carbaryl	2400	4.0	75		
-	-	Diazinon	600	5.0	61		
-	-	Diazinon	1200	5.0	44		
LSD at 5%				0.4	11		

Table 7. The effect of foliar application of insecticides on the toxicity of root applied phenylurea herbicides on cotton.

Herbicide	Concn (M)	Insecticide	Concn (mg/l)	Visual Rating	Fresh Weight (% of Control)		Synergism
					Observed	Expected	
Check	-	-	-	1.0	100		
Linuron	$1x10^{-6}$	-	-	2.6	53		
Linuron	$1x10^{-6}$	Carbaryl	2400	2.3	47	59	
Linuron	$1x10^{-6}$	Diazinon	1200	1.0	72	67	
Monolinuron	$1x10^{-6}$	-	-	2.6	65		
Monolinuron	$1x10^{-6}$	Carbaryl	2400	2.0	56	74	S
Monolinuron	$1x10^{-6}$	Diazinon	1200	2.0	67	85	S
Diuron	$1x10^{-6}$	-	-	1.3	74		
Diuron	$1x10^{-6}$	Carbaryl	2400	1.0	86	84	
Diuron	$1x10^{-6}$	Diazinon	1200	1.0	100	96	
Monuron	$1x10^{-6}$	-	-	1.0	86		
Monuron	$1x10^{-6}$	Carbaryl	2400	1.0	91	98	
Monuron	$1x10^{-6}$	Diazinon	1200	1.0	74*	111	S
-	-	Carbaryl	2400	1.0	114		
-	-	Diazinon	1200	1.0	130		
LSD at 5%				1.1	20		

applied to roots. There was a slight stimulation in growth when either carbaryl or diazinon was applied to foliage of cotton.

In the second study, the insecticide was applied in nutrient solution and the shoot was dipped in herbicide suspensions 2 days after insecticidal treatment. Carbaryl alone was extremely toxic to the roots of cotton (Tables 8 and 9). There was no further growth of the roots after treatment and the root tips turned brown. The plant was stunted in growth although the leaves remained green. Carbaryl applied in nutrient solution produced enhanced toxicity with diuron and monuron only. Application of carbaryl to the roots of corn and peas has also been reported to reduce the growth of these plants (50). The roots grown in carbaryl-treated sand were shorter and thicker than roots grown in untreated sand and the lower portion of the root system had a dark purplish color.

Diazinon was only slightly toxic to the cotton root system (Tables 8 and 9). When diazinon was applied in solution and the herbicides were applied through the foliage, no interactions were observed (Table 8).

When both the herbicides and insecticides were applied to the roots, synergisms were obtained with combinations of either diuron or monuron with carbaryl (Table 9 and Figure 2). Root application of the 4

Table 8. The effect of root-applied insecticides on the toxicity of foliar applied phenylurea herbicides on cotton.

Herbicide (1200 mg/l)	Insecticide ($1.0x10^{-5}$ M)	Visual Rating	Fresh Weight (% of Control) Observed	Expected
Check	-	1.0	100	
Linuron	-	8.0	15	
Linuron	Carbaryl	7.3	17	6
Linuron	Diazinon	8.0	20	12
Monolinuron	-	4.0	54	
Monolinuron	Carbaryl	5.0	34	43
Monolinuron	Diazinon	4.6	44	43
Diuron	-	1.0	73	
Diuron	Carbaryl	5.6	29	28
Diuron	Diazinon	1.3	90	58
Monuron	-	3.3	59	
Monuron	Carbaryl	6.6	29	26
Monuron	Diazinon	5.3	46	47
-	Carbaryl	3.0	39	
-	Diazinon	1.6	80	
LSD at 5%		0.8	20	

Table 9. The effect of both herbicide and insecticide applied in solution on the growth of cotton.

Herbicide ($1.0x10^{-6}$ M)	Insecticide ($1.0x10^{-5}$ M)	Visual Rating	Fresh Weight (% of Control) Observed	Expected	Synergism
Check	-	1.0	100		
Linuron	-	8.7	11		
Linuron	Carbaryl	6.7	21	6	
Linuron	Diazinon	9.0	11	11	
Monolinuron	-	3.0	35		
Monolinuron	Carbaryl	4.7	33	20	
Monolinuron	Diazinon	4.3	37	34	
Diuron	-	2.3	63		
Diuron	Carbaryl	7.7	13	35	S
Diuron	Diazinon	1.0	83	60	
Monuron	-	2.0	50		
Monuron	Carbaryl	8.0	15	28	
Monuron	Diazinon	1.3	92	48	
-	Carbaryl	2.7	56		
-	Diazinon	1.0	96		
LSD at 5%		1.1	31		

Figure 2. (a) Toxicity obtained with combination of carbaryl and the 4 phenylurea herbicides applied on the roots of cotton.

A = Check
B = Linuron (1.0×10^{-6}M)
C = Linuron + Carbaryl (1.0×10^{-6} + 1.0×10^{-5}M)
D = Carbaryl (1.0×10^{-5}M)

E = Monolinuron (1.0×10^{-6} M)
F = Monolinuron + Carbaryl (1.0×10^{-6} + 1.0×10^{-5} M)

G = Diuron (1.0×10^{-6} M)
H = Diuron + Carbaryl (1.0×10^{-6} + 1.0×10^{-5} M)

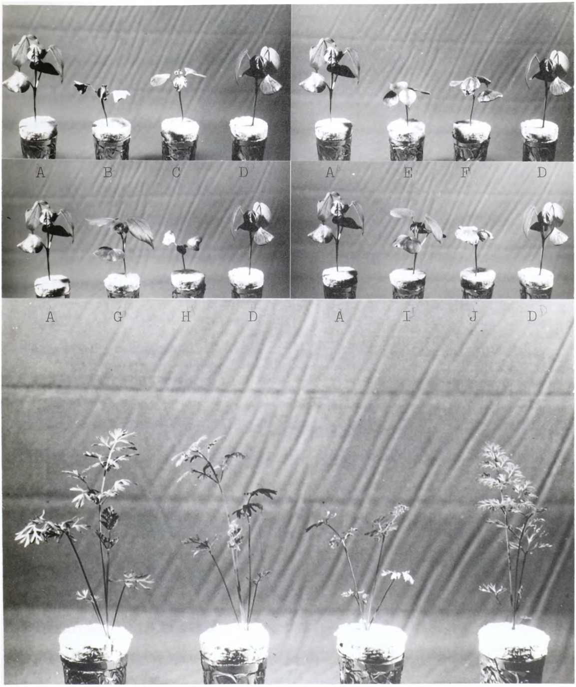
I = Monuron (1.0×10^{-6} M)
J = Monuron + Carbaryl (1.0×10^{-6} + 1.0×10^{-5} M)

(b) Toxicity obtained with foliar application of linuron and carbaryl on carrots.

A = Check
B = Linuron (1200 mg/l)
C = Linuron + Carbaryl (1200 + 2400 mg/l)
D = Carbaryl (2400 mg/l)

(a)

(b)



A B C D

phenylureas and diazinon again did not interact, in contrast to results from foliar application of both chemicals.

In general, the dimethylureas (monuron and diuron) were more affected by carbaryl than the methoxymethylureas (linuron and monolinuron) regardless of the site of application (Table 10).

The inhibition of N-demethylation of monuron in cotton leaf discs by carbaryl was previously demonstrated by Swanson and Swanson (70). Carbaryl is an effective inhibitor of demethylase, an enzyme believed to

Table 10. Summary of the interactions which occurred with insecticides and phenylurea herbicides on cotton.

Insecticide	Site of Application	Site of Phenylurea Herbicide Application	
		Foliage	Root
Carbaryl	Foliage	Monuron, Diuron	No interaction
	Root	Monuron, Diuron	Monuron, Diuron
Diazinon	Foliage	Monuron, Diuron, Linuron, Monolinuron	No interaction
	Root	No interaction	No interaction

be responsible for the demethylation of the phenylureas (24). In these experiments, carbaryl enhanced the toxicity of diuron and monuron in cotton but did not have a pronounced effect on linuron and monolinuron. It appears that demethylase is quite specific for the methyl group and that the cleavage of the methoxy group may be brought about by another enzyme apparently not affected by carbaryl.

In contrast, diazinon increases the toxicity of the dimethylureas and methoxymethylureas to cotton only if the chemicals are applied together on the foliage. This may indicate that diazinon affects some processes involved in foliar uptake only. It may also indicate that diazinon is not translocated to the leaves of cotton or it is rapidly metabolized to other compound(s).

Effect on Respiration and Photosynthesis of Leaf Tissue. These studies were conducted to determine if enhancement of linuron activity by carbaryl was a result of altered respiration or photosynthesis. Parsnip was chosen as the test plant because of the larger leaf area and the ease by which uniform leaf discs could be prepared. Furthermore, parsnip and carrot belong to the same family and respond similarly to linuron.

Linuron at a concentration of 1.0 x 10^{-5} M and carbaryl at 1.0 x 10^{-4} M caused only a slight reduction in oxygen uptake (Table 11). In the presence of both chemicals, there was no difference in respiratory rate as compared with the control. The negative effect of linuron on respiration is in agreement with reports that substituted urea herbicides did not affect respiration of leaf tissues and algae (28, 66). Various insecticides such as lindane, dieldrin, DDT, methoxychlor as well as carbaryl have been reported to cause a

Table 11. Oxygen uptake of parsnip leaf discs treated with linuron alone or in combination with carbaryl (% of control).

Chemical	Concn (M)	Time (Hr)		
		1	3	5
Carbaryl	1.0x10^{-4}	78	81	87
Linuron	1.0x10^{-5}	82	88	96
Linuron + Carbaryl	1.0x10^{-5} + 1.0x10^{-4}	95(64)[a]	97(71)	104(84)
LSD at 5%		n.s	n.s	10

[a]Expected values calculated according to Colby's method.

significant reduction in the rate of respiration of root tips of corn, oats, peas and cucumber (50). The respiratory pathway does not appear to be the site of action affected by these interacting chemicals.

Linuron at a concentration of 1.0 x 10^{-5} M or carbaryl at 1.0 x 10^{-4} M initially caused a slight reduction in oxygen evolution (Table 12). The linuron plus carbaryl mixtures caused a synergistic reduction in oxygen evolution throughout the duration of the experiment. Since the primary mode of action of the substituted phenylurea herbicides is considered to be an inhibition of photosynthesis, any factor which brings about an increase in the amount of herbicide reaching the site of action would have a significant effect on the toxicity of the herbicide. Carbaryl may have caused more movement of linuron to the site of action, in this case, the chloroplasts. Carbaryl might also increase membrane permeability allowing more linuron to be taken up by the leaves. Another possibility is that carbaryl may inhibit the metabolic processes in the plant that are active in the degradation of the herbicide.

Effect of Carbaryl and Diazinon on the Uptake of ^{14}C-Phenylurea Herbicides

The foliar uptake of ^{14}C-phenylureas alone and in combination with diazinon was monitored in cotton

Table 12. Oxygen evolution of parsnip leaf discs treated with linuron alone or in combination with carbaryl (% of control).

Chemical	Concn (M)	Time (Hr)[a]			
		2	3	4	5
Linuron	$1.0x10^{-5}$	85	99	97	88
Linuron	$1.0x10^{-6}$	96	96	95	104
Carbaryl	$1.0x10^{-4}$	82	92	97	113
Linuron + Carbaryl	$1.0x10^{-5}$ + $1.0x10^{-4}$	69 (70)[b]	53 (91 S)	54 (84*S)	45 (88*S)
Linuron + Carbaryl	$1.0x10^{-6}$ + $1.0x10^{-4}$	47 (78*S)	73 (88 S)	68 (94 S)	75 (117*S)
LSD at 5%		36	36	34	38

[a]Time represent hours after the initial 1.5 hour treatment.

[b]Expected values calculated according to Colby's formula. S indicates synergism.

*Significantly different from observed value using estimated $LSD_{.05}$ of Hamill (33).

and soybean. In both species, diazinon caused a significant increase in uptake of the 4 phenylureas. In cotton, the uptake of ^{14}C-labeled linuron and monolinuron in the presence of diazinon was rapid after 6 hr and then uptake leveled off slightly (Figure 3 and 4). Diuron was poorly absorbed by cotton and soybean leaves but the addition of diazinon caused a significant increase in the uptake of the chemical (Figure 5).

Figure 3. A comparison of uptake of ^{14}C-linuron (wettable powder) with or without diazinon (wettable powder) on (a) cotton (b) soybean. F value for the interactions of chemical x time significant at 5% level.

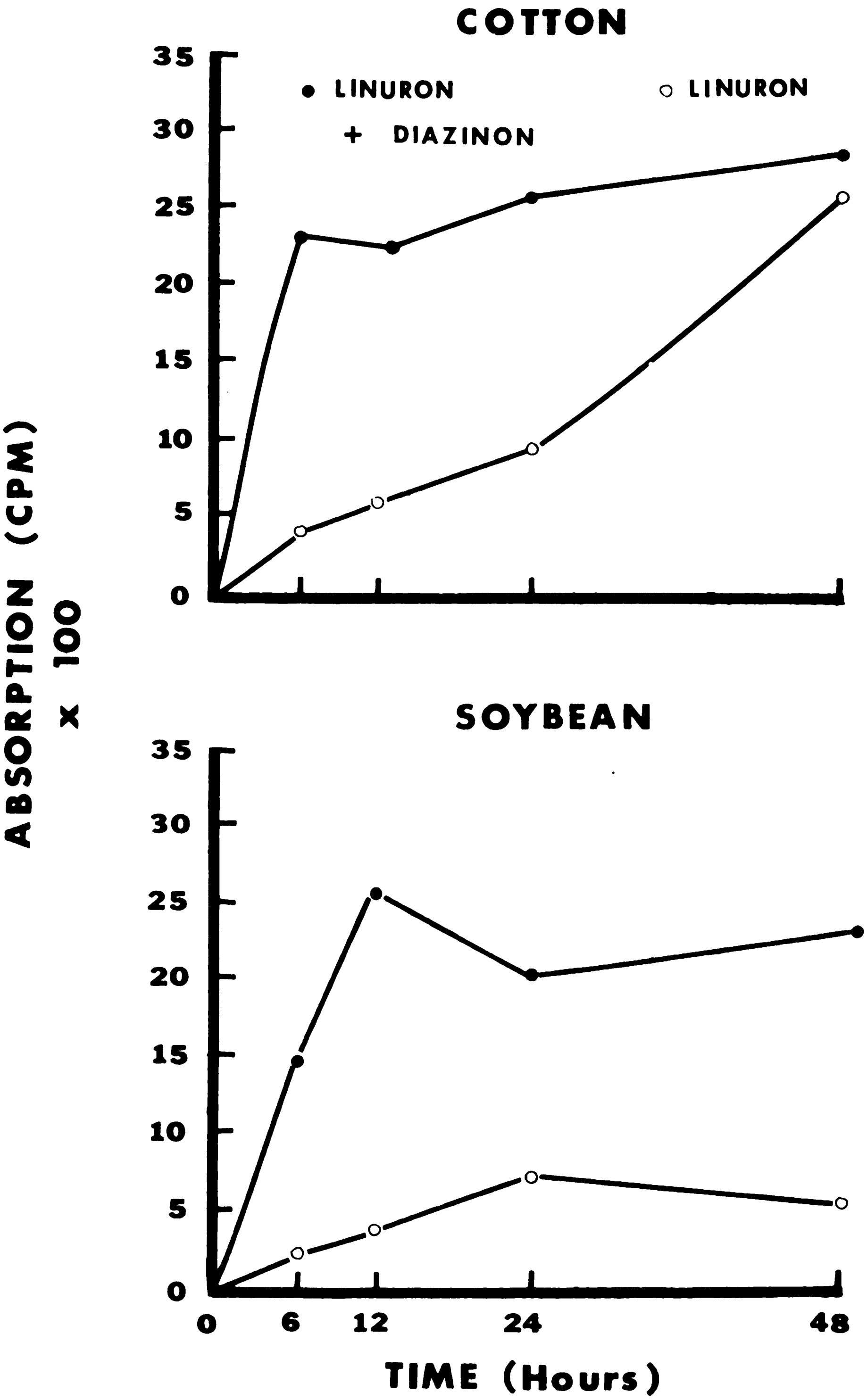

COTTON
● LINURON
+ DIAZINON
○ LINURON
SOYBEAN
ABSORPTION (CPM) x 100
0
5
10
15
20
25
30
35
0
6
12
24
48
TIME (Hours)

Figure 4. A comparison of uptake of ^{14}C-monolinuron (wettable powder) with or without diazinon (wettable powder) on (a) cotton (b) soybean. F value for the interactions of chemical x time significant at 5% level.

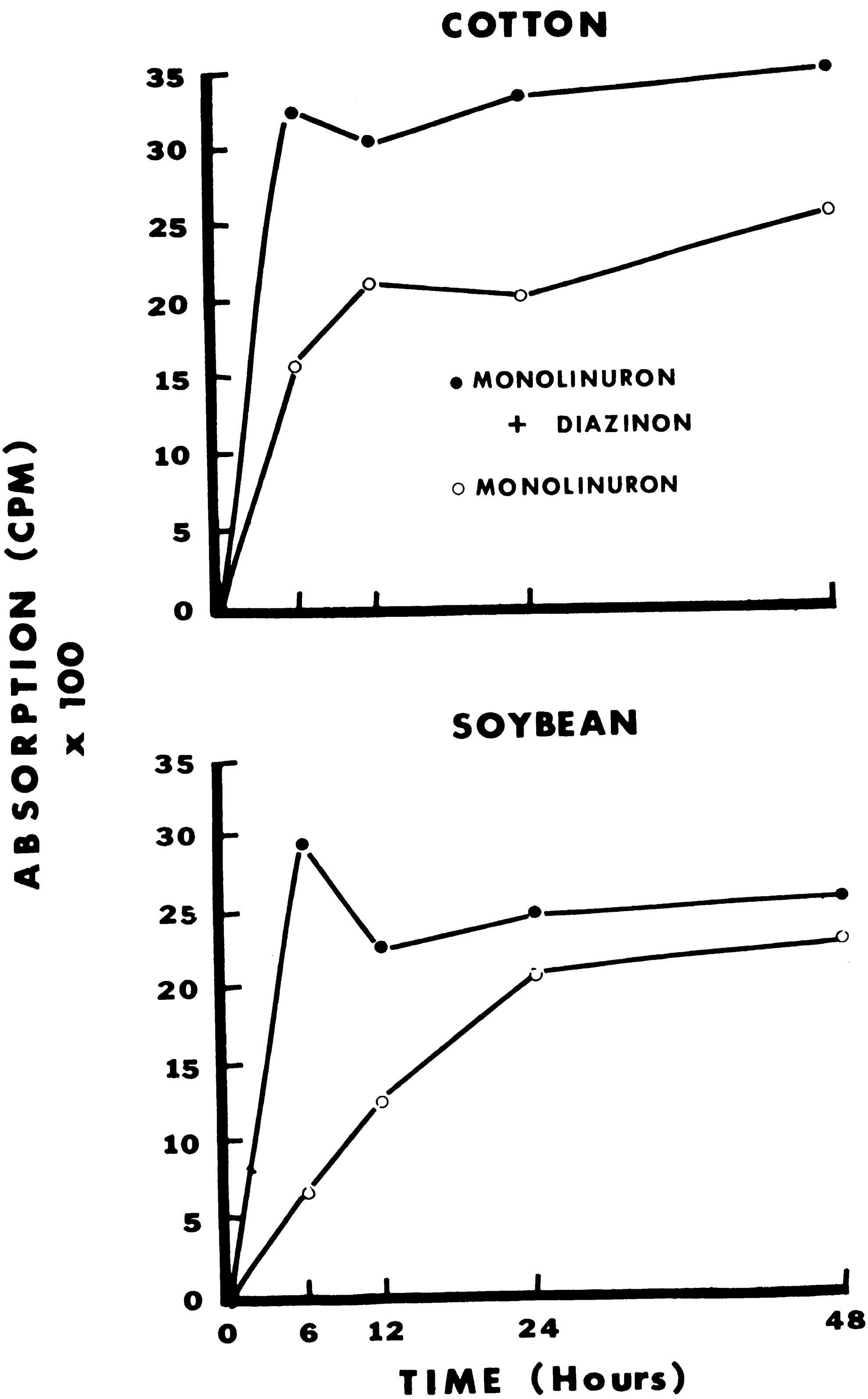
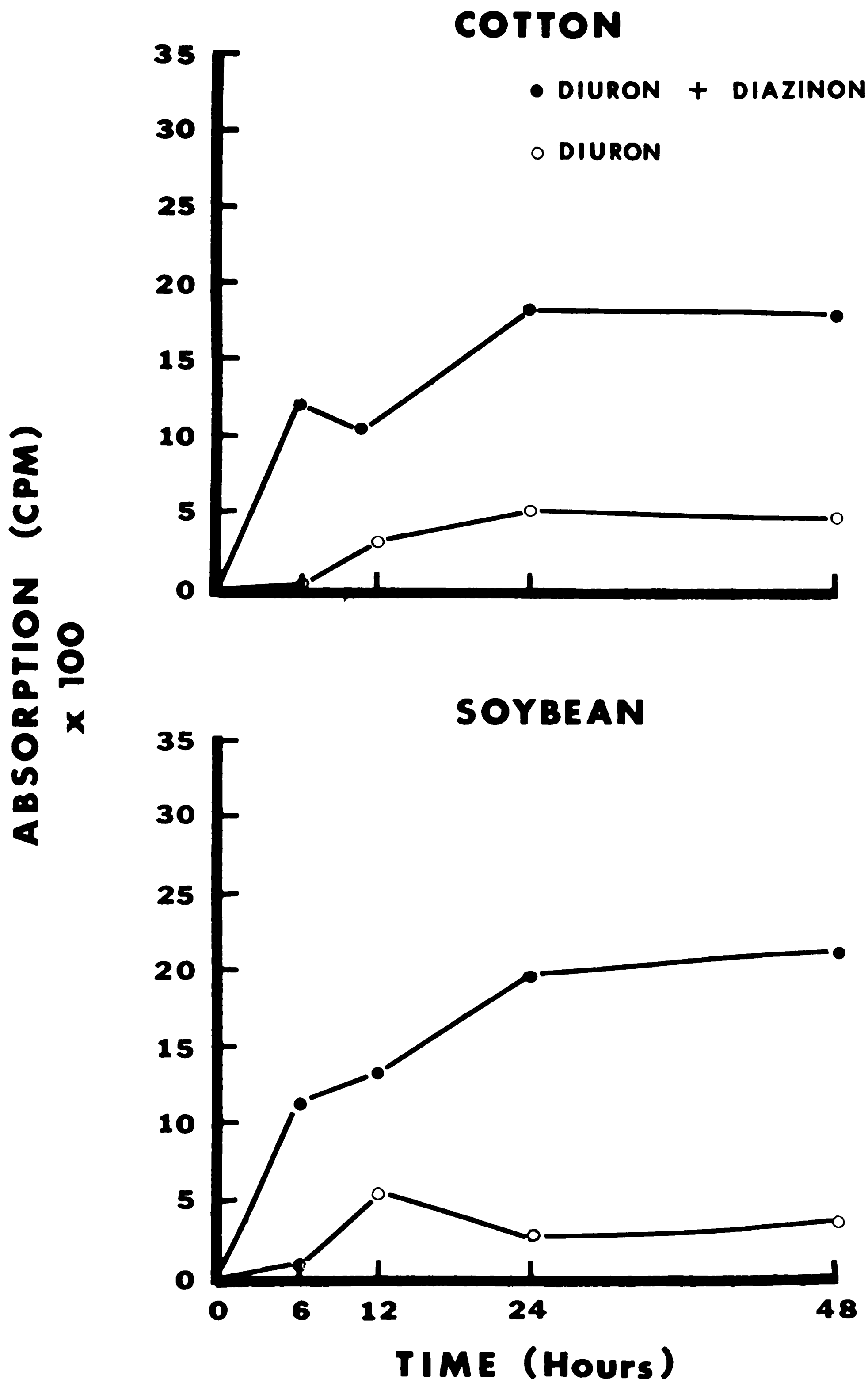

COTTON
35
30
25
20
15
10
5
0
● MONOLINURON
+ DIAZINON
○ MONOLINURON
ABSORPTION (CPM)
x 100
SOYBEAN
35
30
25
20
15
10
5
0
0
6
12
24
48
TIME (Hours)

Figure 5. A comparison of uptake of ^{14}C-diuron (wettable powder) with or without diazinon (wettable powder) on (a) cotton (b) soybean. F value for the interactions of chemical x time significant at 5% level.


COTTON
• DIURON + DIAZINON
○ DIURON
SOYBEAN
ABSORPTION (CPM) x 100
35
30
25
20
15
10
5
0
0
6
12
24
48
TIME (Hours)

Even less monuron was absorbed by cotton and soybean leaves and uptake was again enhanced by the presence of diazinon (Figure 6).

This experiment also demonstrated that the methoxymethylureas were more readily taken up by the leaves than the dimethylureas which may explain their relative toxicity. In general, both species absorbed similar amounts of the herbicide with the exception of linuron being more readily absorbed by cotton than soybean. Since uptake of the dimethylureas in the two species is similar, the superior tolerance of cotton suggests that it metabolizes these compounds.

In other experiments, pure ^{14}C-herbicides and technical grade insecticides were applied to ascertain that there was a direct interaction of the chemical and not merely a surfactant effect. Significant increases in the uptake of ^{14}C-linuron, monolinuron and diuron was obtained in the presence of pure diazinon (Table 13). With monuron, no uptake was observed with or without the insecticide.

The basis for the observed interaction between the 4 phenylurea herbicides and diazinon appeared to be caused by increased herbicide uptake induced partly by the insecticide and partly by its surfactant.

Figure 6. A comparison of uptake of ^{14}C-monuron (wettable powder) with or without diazinon (wettable powder) on (a) cotton (b) soybean.

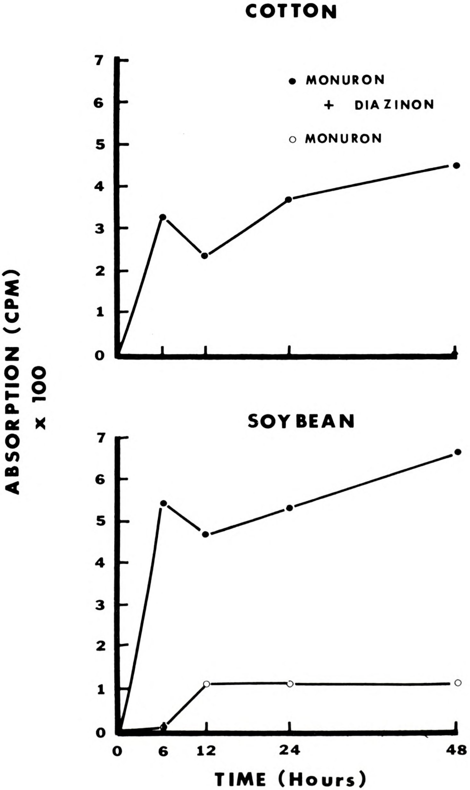

COTTON
MONURON
+ DIAZINON
MONURON
SOYBEAN
ABSORPTION (CPM) x 100
0
1
2
3
4
5
6
7
0
6
12
24
48
TIME (Hours)

Table 13. The uptake of ^{14}C-linuron, monolinuron and diuron (cpm) as affected by pure diazinon on cotton leaves.

Chemical	Time (Hr)			
	6	12	24	48
Linuron	514 a[1]	743 a	679 a	1046 a
Linuron + Diazinon	821 b	888 b	919 b	1530 b
Monolinuron	1735 a	-	1475 a	1310 a
Monolinuron + Diazinon	2270 b	-	2035 b	2030 b
Diuron	-	-	320 a	240 a
Diuron + Diazinon	-	-	365 a	525 b

[1]Means with uncommon letter are significantly different at 5% level.

The rate of uptake of ^{14}C-linuron (with wettable powder) was enhanced in the presence of carbaryl (wettable powder) in carrot leaves and parsnip leaf discs (Table 14). However, after 48 hr there was no difderence in the total amount accumulated in carrot. In this case, the interaction between linuron and carbaryl may also be brought about by other factors such as decreased metabolism or more herbicide movement into the chloroplasts.

Table 14. The uptake of ^{14}C-linuron (cpm) as affected by carbaryl in carrot leaves and parsnip leaf discs.

Plant	Chemical	Time (hr) 6	8	12	24	30	48
Carrot	Linuron	1133 a[1]	-	2086 a	2606 a		3487 a
	Linuron + Carbaryl	1600 b	-	2573 b	4280 b		2780 a
Parsnip	Linuron	-	662 a	-	1623 a	1739 a	-
	Linuron + Carbaryl	-	1737 b	-	2083 b	2419 b	-

[1]Means with uncommon letter are significantly different at 5%.

Effect of Carbaryl and Diazinon on the Metabolism of ^{14}C-Phenylurea Herbicides

Diazinon had no apparent effect on the metabolism of the 4 phenylureas by cotton leaves. The parent compound was identified by a comparison with the Rf of the standard ^{14}C-herbicide. A major portion of ^{14}C-linuron still existed as the parent compound after 5 days (Table 15). Plants receiving linuron alone had 81% linuron still unaltered as compared to 79% in the linuron plus diazinon treatment. There was essentially no change in distribution of metabolites in the two treatments.

Table 15. The effect of diazinon on the metabolism of linuron by cotton leaves[a].

Rf	Two Days		Five Days	
	Linuron	Linuron + Diazinon	Linuron	Linuron + Diazinon
0.0	7.5	8.5	8.0	8.0
0.1				
0.2	1.5	2.0	3.0	3.0
0.3	2.0	2.5	5.0	1.0
0.4				
0.5	1.0	1.0	1.0	1.0
0.6				
0.7				
0.8	84.0	83.5	81.0	79.0
0.9				
1.0				

[a]Percent of total spotted.

After 2 days, about 82 to 84% of the ^{14}C-monolinuron was still not metabolized by the cotton leaves and 5 days later 66% of the total ^{14}C remained as monolinuron (Table 16). There was also an increase in the quantity of metabolite(s) remaining at the origin. After 5 days, metabolites appeared at Rf 0.2 and 0.3 but there was no difference in the amount present between the treatments.

Because of the poor rate of absorption observed with ^{14}C-diuron and monuron, it was decided to sample only at 5 days after treatment. Cotton leaves metabolized diuron and monuron at a relatively rapid rate (Table 17). After 5 days, only 34% of the total ^{14}C

Table 16. The effect of diazinon on the metabolism of monolinuron by cotton leaves[a].

Rf	Two Days		Five Days	
	Monolinuron	Monolinuron + Diazinon	Monolinuron	Monolinuron + Diazinon
0.0	9.0	8.5	21.0	22.0
0.1				
0.2	-	-	2.0	2.5
0.3	-	-	1.5	1.5
0.4				
0.5	3.0	3.0	3.0	4.0
0.6				
0.7				
0.8	82.0	84.0	65.5	66.0
0.9				
1.0				

[a]Percent of total spotted.

remained as unchanged diuron. However, it should be noted that the quantity taken up was also less as compared to the methoxymethylureas. About 18% of the radioactivity remained at the origin. A metabolite with an Rf of 0.3 accounted for 24% and 22% for both treatments indicating again that there was no difference in metabolism caused by diazinon.

Table 17. The effect of diazinon on the metabolism of diuron and monuron by cotton leaves 5 days after treatment.

Rf	Diuron	Diuron + Diazinon	Monuron	Monuron + Diazinon
0.0	18.0	19.5		
0.1	1.5	1.0	18.0	15.0
0.2			49.0	48.0
0.3	24.0	22.0	13.0	10.0
0.4			14.0	13.0
0.5	7.5	9.0	5.0[b]	8.0
0.6	33.5[a]	34.5		
0.7				
0.8				
0.9				
1.0				

[a] ^{14}C-diuron

[b] ^{14}C-monuron

Monuron was most rapidly metabolized by cotton leaves (Table 17). After 5 days only 5% remained as the unaltered herbicide. A major percentage appeared at

Rf 0.2 but the distribution was similar for monuron alone and monuron plus diazinon. The metabolite was not positively identified, however, comparison with Rf value already published (70) showed that this Rf (0.2) corresponded to the demethylated derivative of monuron.

The relative tolerance of cotton to diuron and monuron as shown by the capacity of the plant to metabolize these dimethylureas was once again demonstrated in this experiment. As mentioned previously, demethylase is believed responsible for the N-demethylation of these compounds.

The results of this experiment confirm previous reports that monuron was more rapidly metabolized than diuron by cotton leaves (70). It also appears that the monohalogenated ureas as a group may be metabolized more rapidly than the dihalogenated ureas. Dimethylureas are more easily degraded by cotton leaves than the methoxymethylureas. It seems likely that the tolerance of cotton to the dimethylureas and susceptibility to the methoxymethylureas is due to both differential absorption and metabolism.

Metabolism of Linuron in Carrots. Carrot is tolerant to linuron. Two days after treatment with linuron alone, only 24% of the total ^{14}C was still the parent herbicide (Table 18). When linuron and

Table 18. The effect of carbaryl on the metabolism of linuron by carrot leaves[a].

Rf	Two Days		Five Days	
	Linuron	Linuron + Carbaryl	Linuron	Linuron + Carbaryl
0.0	50.0	33.5	57.5	50.5
0.1	5.0	5.5		
0.2	7.0	5.5	4.0	3.0
0.3				
0.4	3.5	2.5	4.5	2.5
0.5	6.0	4.5	6.0	3.0
0.6				
0.7				
0.8	24.0	42.5	16.5	23.0
0.9				
1.0				

[a]Percent of total spotted.

carbaryl were applied together, 42.5% still existed as the parent herbicide after 2 days. The decreased rate of metabolism may, in part, explain the effect of carbaryl in enhancing the toxicity of linuron in carrots. The major metabolite(s) remained at the origin and there was a considerable reduction in ^{14}C-compounds present due to the combination treatments. Similar trends were evident after 5 days although the differences were not as pronounced after that period of time.

The carbaryl induced inhibition of monuron and propanil metabolism demonstrated by other workers (51, 71) was hypothesized to be the basis for the increased

toxicity of these herbicides. Tolerant plants apparently have or can synthesize the particular enzyme responsible for the degradation of the herbicide. With monuron and carbaryl, synergism was evident in tolerant cotton and carrot but no interaction occurred in susceptible soybean (Tables 3,4,6). In cotton, demethylation occurs rapidly to produce nontoxic derivatives whereas in soybean, demethylation occurs much more slowly with the resulting accumulation of the toxic derivative, the monomethylurea (68). Demethylase isolated from cotton showed a much higher specific activity than that isolated from soybean (24). This indicates that plant species with more of that enzyme present will be more susceptible to phenylurea herbicides when carbaryl is introduced into the tissue.

SUMMARY AND CONCLUSIONS

Insecticide Induced Enhancement of Phenylurea Herbicide Activity

Four analogous substituted urea herbicides, linuron, monolinuron, diuron and monuron were applied alone and in combination with carbaryl and diazinon on tolerant and susceptible plant species. In tolerant carrots, carbaryl enhanced the toxicity of linuron and monuron. In tolerant corn, mixtures of carbaryl with linuron and monolinuron were synergistic. When carbaryl was applied with diuron and monuron, increased toxicity was obtained in moderately tolerant cotton. In susceptible soybean, toxicity occurred both from herbicide and insecticide treatments and all combinations appeared about additive. In all species, the addition of diazinon to foliage caused a significant increase in the foliar toxicity of the 4 phenylurea herbicides. The methoxymethylureas were more toxic to all plant species than the dimethylureas.

Foliar application of carbaryl had little effect on the growth of cotton treated with herbicides through the roots. On the other hand, root application of carbaryl enhanced the toxicity of diuron

and monuron applied to cotton leaves but did not have a pronounced effect on the activity of linuron and monolinuron. Similar results were obtained when both carbaryl and diuron or monuron were applied to the roots. The relative differences in the responses obtained with dimethylureas or methoxymethylureas and carbaryl may be attributed to the ability of the insecticide to inhibit the activity of the enzyme demethylase.

In contrast to carbaryl, diazinon increased the activity of the 4 phenylurea herbicides regardless of their substitution and in both tolerant and susceptible plants. However, the response was obtained only if the herbicide and insecticide were applied together on the foliage. This suggested that diazinon or a component of its wettable powder formulation increased the foliar penetration of the herbicides.

The nature of the linuron and carbaryl interaction was investigated in parsnip leaf discs. Respiratory rate was not significantly affected by the combination of herbicide and insecticide. However, photosynthesis, measured by oxygen evolution, was greatly reduced by the mixture of linuron and carbaryl as compared to linuron alone. This response indicated that more linuron may have reached its site of action when carbaryl was present.

Effect of Insecticides on Uptake of Phenylurea Herbicides

Formulated carbaryl enhanced the rate of uptake of ^{14}C-linuron in carrot leaves and parsnip leaf discs. In carrots, there was no significant difference in total uptake after 48 hr. It could not be ascertained from these tests if the increased rate of uptake was caused directly by carbaryl or another component of the wettable powder formulation. Increased uptake may be at least a partial cause for the enhancement of linuron activity by carbaryl.

In tolerant cotton and susceptible soybean, the uptake of ^{14}C-linuron, monolinuron, diuron and monuron (with wettable powder) was greatly enhanced by the presence of diazinon (wettable powder). Linuron and monolinuron were much more readily absorbed by the leaves of both species than diuron and monuron. This may account for the difference in toxicity between dimethylureas and methoxymethylureas. Cotton and soybean leaves absorbed comparable amounts of herbicides, except linuron which was shown to be more readily absorbed by cotton. Therefore tolerance of different species to phenylureas is not necessarily related only to the amount of herbicide absorbed.

A significant increase in uptake of pure ^{14}C-linuron, monolinuron, and diuron was obtained with the application of pure diazinon, although the magnitude of increase was not as big as that brought about by the formulated chemicals. It appears therefore, that the increase in uptake is caused partly by diazinon and partly by its surfactant.

Effect of Insecticides on Metabolism of Phenylurea Herbicides

Carbaryl caused a decrease in the rate of metabolism of linuron as shown by the presence of more parent herbicide in the carrot leaves treated with both compounds. There was a considerable difference in the amount of ^{14}C-metabolite(s) present depending on treatments. Synergism from the combination of linuron and carbaryl, therefore, may be due to both increased uptake and a decreased rate of metabolism. Other workers have demonstrated that carbaryl inhibits the activity of demethylase obtained from cotton. The demethylated products are less toxic than the parent herbicide. Since carbaryl applied either to roots or leaves enhanced the activity of diuron and monuron applied to leaves, inhibition of herbicide metabolism is a logical explanation for the observed synergism.

Diazinon had no apparent effect on the metabolism of the 4 phenylurea herbicides in cotton.

The basis for the observed synergism between diazinon and these herbicides is more likely a result of increased uptake brought about by diazinon and its surfactant.

The dimethylureas (monuron and diuron) are more easily degraded by cotton than the methoxymethylureas (linuron and monolinuron). Likewise the monohalogenated ureas (monolinuron and monuron) are metabolized more rapidly than their dihalogenated counterparts (linuron and diuron). The relative tolerance of cotton to the dimethylureas and susceptibility to the methoxymethylureas may be due to both differential absorption and metabolism. The selectivity of phenylureas in other plant species are likely also controlled by these two processes.

Implications for Field Situations

The pesticide interactions reported here were obtained from either tank mixes or applications made at short time intervals under greenhouse conditions. The study indicates that carbaryl and diazinon may enhance the activity of phenylurea herbicides, causing excessive damage in certain tolerant plants. Where insect and weed problems warrant the use of these, or related chemicals on a crop within short time intervals, further field tests should be conducted to ascertain that the applications do not

decrease the yield or quality of the crop. Some of these combinations at lower rates of application may be useful for enhancing herbicide activity on weed species without damaging crops.

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APPENDICES

Appendix A. Pesticide interactions from foliar application on carrots.

Chemical	Rate (lb/a)	Visual Rating (14 Days)	Fresh Weight (% of Control) Observed	Fresh Weight (% of Control) Expected[1]	Synergism[1]
Check	-	1.0	100		
Linuron	1.0	4.7	25		
Nitrofen	3.0	2.7	106		
Chloroxuron	3.0	1.0	107		
Carbaryl	1.0	1.0	111		
Parathion	0.5	1.0	116		
Mancozeb	2.0	1.0	115		
Linuron + Carbaryl	1.0+1.0	8.7	1	28*	S
Linuron + Parathion	1.0+0.5	5.0	32	29	
Linuron + Mancozeb	1.0+2.0	6.3	18	29	
Nitrofen + Carbaryl	3.0+1.0	1.7	111	118	
Nitrofen + Parathion	3.0+0.5	1.0	101	123*	S
Nitrofen + Mancozeb	3.0+2.0	1.3	101	122*	S
Chloroxuron + Carbaryl	3.0+1.0	2.0	92	119*	S
Chloroxuron + Parathion	3.0+0.5	1.0	100	125*	S
Chloroxuron + Mancozeb	3.0+2.0	1.0	102	124*	S
LSD at 5%		0.8	14		

[1]Calculated by Colby's method.
*Significantly different from observed value using estimated $LSD_{.05}$ of Hamill.

Appendix B. Pesticide interactions from foliar application on cucumber.

Chemical	Rate (lb/a)	Visual Rating (14 days)	Fresh Weight (% of Control)		Synergism
			Observed	Expected	
Check	-	1.0	100		
Linuron	1/4	6.0	5		
Chlorbromuron	1/4	6.6	8		
Diuron	1/2	5.6	39		
Monuron	1/2	5.0	7		
Metobromuron	1/4	4.6	26		
Chloroxuron	1/2	3.0	72		
Linuron + Carbaryl	1/4 +1	6.6	16	7	
Linuron + Diazinon	1/4 +1	6.3	4	7	
Linuron + Malathion	1/4 +1	5.0	4	12	
Chlorbromuron + Carbaryl	1/4 +1	4.6	6	12	
Chlorbromuron + Diazinon	1/4 +1	4.6	5	12	
Chlorbromuron + Malathion	1/4+1	3.0	4	13	
Diuron + Carbaryl	1/2+1	6.6	40	58*	S
Diuron + Diazinon	1/2+1	6.3	13	58*	S
Diuron + Malathion	1/2+1	5.3	16	64*	S
Monuron + Carbaryl	1/2+1	6.0	9	10	
Monuron + Diazinon	1/2+1	4.6	4	10	

Appendix B. (Continued)

Chemical	Rate (lb/a)	Visual Rating (14 days)	Fresh Weight (% of Control)		Synergism
			Observed	Expected	
Monuron + Malathion	1/2 + 1	5.0	5	12	
Metobromuron + Carbaryl	1/4 + 1	5.6	28	39	
Metobromuron + Diazinon	1/4 + 1	5.0	31	38	
Metobromuron + Malathion	1/4 + 1	5.0	21	41*	S
Chloroxuron + Carbaryl	1/2 + 1	5.6	91	107	S
Chloroxuron + Diazinon	1/2 + 1	5.0	18	107*	S
Chloroxuron + Malathion	1/2 + 1	3.6	86	119*	S
Carbaryl	1	1.3	149		
Diazinon	1	1.0	148		
Malathion	1	1.0	165		
LSD at 5%		2.4	24		

Appendix C. Pesticide interactions from foliar application on soybean.

Chemical	Rate (lb/a)	Visual Rating (14 days)	Fresh Weight (% of Control) Observed	Fresh Weight (% of Control) Expected	Synergism
Check	-	1.0	100		
Linuron	1/4	6.0	80		
Chlorbromuron	1/4	4.6	80		
Diuron	1/2	1.6	84		
Monuron	1/2	4.6	72		
Metobromuron	1/4	6.3	93		
Chloroxuron	4	3.3	103		
Linuron + Carbaryl	1/4 + 1	4.6	97	95	
Linuron + Diazinon	1/4 + 1	4.6	72	82	
Linuron + Malathion	1/4 + 1	4.6	85	92	
Chlorbromuron + Carbaryl	1/4 + 1	4.6	95	76	
Chlorbromuron + Diazinon	1/4 + 1	6.3	82	82	
Chlorbromuron + Malathion	1/4 + 1	3.0	93	92	
Diuron + Carbaryl	1/2 + 1	6.6	86	80	
Diuron + Diazinon	1/2 + 1	4.6	99	87	
Diuron + Malathion	1/2 + 1	3.3	91	97	

Appendix C. (Continued)

Chemical	Rate (lb/a)	Visual Rating (14 days)	Fresh Weight (% of Control)		Synergism
			Observed	Expected	
Monuron + Carbaryl	1/2+1	6.6	97	68	
Monuron + Diazinon	1/2+1	5.6	75	74	
Monuron + Malathion	1/2+1	3.3	86	83	
Metobromuron + Carbaryl	1/4+1	5.6	92	88	
Metobromuron + Diazinon	1/4+1	4.3	85	107*	S
Metobromuron + Malathion	1/4+1	2.3	90	88	
Chloroxuron + Carbaryl	4 + 1	4.6	85	100	S
Chloroxuron + Diazinon	4 + 1	5.3	78	108*	S
Chloroxuron + Malathion	4 + 1	3.6	94	121*	S
Carbaryl	1	1.0	95		
Diazinon	1	1.6	103		
Malathion	1	1.0	115		
LSD at 5%		1.2	16		

Appendix E. The effect of 4 substituted urea herbicides alone and in combination with insecticides on the growth of corn.

Chemical	Rate (lb/a)	Visual Rating (14 days)	Fresh Weight (% of Control) Observed	Fresh Weight (% of Control) Expected	Synergism
Check	-	1.0	100		
Linuron	1	2.5	85		
Monolinuron	1	3.5	104		
Diuron	1	4.5	98		
Monuron	1	4.8	104		
Linuron + Carbaryl	1 + 2	5.2	81	91	
Monolinuron + Carbaryl	1 + 2	4.7	91	111*	S
Diuron + Carbaryl	1 + 2	4.8	101	105	
Monuron + Carbaryl	1 + 2	4.5	108	111	
Linuron + Diazinon	1 + 1	4.0	77	87	
Monolinuron + Diazinon	1 + 1	4.5	100	106	
Diuron + Diazinon	1 + 1	3.8	96	100	
Monuron + Diazinon	1 + 1	2.7	93	106	
Carbaryl	2	2.7	107		
Diazinon	1	2.5	102		
LSD at 5%		0.9	14		