PHYSIOLOGICAL EFFECTS OF DIFLUBENZURON [1(4-CHLORPHENYL)-3-(2,6-DIFLUOROBENZOYL) UREA] AND FENTIN HYDROXIDE (TRIPHENYLTIN HYDROXIDE) ON SOYBEAN [GLYCINE MAX (L.) MERR.] AND RICE (ORYZA SATIVA L.)

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KRITON KLEANTHIS HATZIOS

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ABSTRACT

PHYSIOLOGICAL EFFECTS OF
DIFLUBENZURON [1-(4-CHLOROPHENYL)-3-(2,6-DIFLUOROBENZOYL)UREA]
AND FENTIN HYDROXIDE (TRIPHENYLTIN HYDROXIDE)
ON SOYBEAN [GLYCINE MAX (L.) MERR.] AND RICE ORYZA SATIVA L.)

by

Kriton Kleanthis Hatzios

The effect of the insecticide diflubenzuron [1-4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] on photosynthesis, respiration, and leaf ultrastructure of soybean [Glycine max (L.) Merr., cv. Swift] and of the fungicide fentin hydroxide (triphenyltin hydroxide) on photosynthesis, respiration, and leaf ultrastructure of soybean and rice (Oryza sativa L., cv. Starbonnet) was examined. In addition, the effect of these two pesticides on seed quality was examined for five soybean cultivars, namely "Coker 102", "Cobb", "Tracy", "Ransom" and "Bragg".

Soybean plants at the second trifoliate leaf stage were treated with 0, 0.067, or 0.269 kg active ingredient/ha of diflubenzuron and 0, 0.56, or 2.24 kg active ingredient of fentin hydroxide/ha. Rice plants, 25 cm tall, were treated with 0, 0.56, or 2.24 kg active ingredient/ha of fentin hydroxide.

Photosynthesis and respiration were measured with an infrared CO_2 analyzer in an open flow system, prior to pesticide application

and at 4, 24, 48, and 96 hours after treatment. Leaf tissue samples from both plant species were examined for ultrasturcture changes by transmission electron microscopy 9 days after treatment.

Seeds from five soybean cultivars, obtained from plots treated with 0, 0.035, 0.069 and 0.14 kg/ha of diflubenzuron for "Coker 102", 0 and double application of 0.56 kg/ha of fentin hydroxide for "Cobb", "Tracy", and "Ransom" and 0, 0.266, 0.532 and 1.064 kg/ha of fentin hydroxide, single and in combination with 0.067 kg/ha of diflubenzuron for "Bragg", were analyzed for their chemical composition, expressed in terms of lipid, protein and carbohydrate content, by using Nuclear Magnetic Resonance (NMR), Kjeldahl, and anthrone procedures. Seed viability and seedling vigor were determined by applying a regular germination test, an accelerated aging test, and a cold test.

Neither diflubenzuron nor fentin hydroxide appeared to have any detrimental effect on photosynthesis, respiration, and leaf ultrastructure of soybean and rice with the exception that diflubenzuron at the high rate (0.269 kg/ha) stimulated respiration in a transitory manner.

In one study, fentin hydroxide at 0.56 kg/ha caused rice plants to become greener and taller than the control and the 2.24 kg/ha treated plants. This could not be repeated in successive greenhouse studies.

Both diflubenzuron and fentin hydroxide showed no negative effects on the production of healthy seedlings. Fentin hydroxide, in some instances, increased the percent of healthy seedlings following the accelerated aging stress.

No effect of either pesticide on soybean seed lipid, protein and carbohydrate content was evident with the exception that fentin hydroxide treated "Ransom" and one set of treated "Bragg" soybeans showed greater and lower carbohydrate content respectively.

PHYSIOLOGICAL EFFECTS OF DIFLUBENZURON [-1(4-CHLORPHENYL)-3-(2,6-DIFLUOROBENZOYL)UREA] AND FENTIN HYDROXIDE (TRIPHENYLTIN HYDROXIDE) ON SOYBEAN [GLYCINE MAX (L.) MERR.] AND RICE (ORYZA SATIVA L.)

By

Kriton Kleanthis Hatzios

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INTRODUCTION

Pesticides are toxic materials intended to be physiologically active only on the target organism. Success for many lies on being relatively toxic to pests and non-toxic to treated crops (28)[1].

Thus, herbicides designed to kill weeds, which are higher plants, may not affect non-vascular plants such as algae or fungi, because of differences in shape, form, and physiology existing between these two plant divisions. Similarly, fungicides may not affect higher plants and insecticides may not affect non-vascular or vascular plants.

However, selectivity is not always complete. There is documented evidence that certain herbicides affect fungi and insects, and certain fungicides and insecticides may have effects on higher plants. These interactions have been recently reviewed by Putnam and Penner (32).

Numerous pesticides affect respiration in animal and plant tissues (11) and many herbicides act on photosynthesis (8, 11). The effect on these physiological systems may or may not be involved in the pesticidal action of the compound. This effect also, may be observed only on the susceptible organism or in other instances, on both, the susceptible organism and the tolerant crop species. If observed in the tolerant species, the effect is generally transitory and its duration may be related to the rate of pesticide detoxication by the tolerant species.

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[1] References at the end of "Literature Review".

Numerous attempts have been made to relate the effects of pesticides on physiological systems to altered cellular structure (4). These relationships have not always been straight-forward as occasionally the ultrastructure may be affected without any measurable change in photosynthetic or respiratory rate.

Pesticides which cause serious deleterious effects on plant growth may affect the chemical composition of seeds in a detrimental manner (28). Failure of pesticides to control pests, resulting in growth stress on the crop may also affect seed quality. Seed quality can be divided into two basic parameters, the chemical composition of the seed and the quality of the seed in terms of germination and production of a vigorous, healthy seedling.

Production of vigorous seedlings may be controlled by the chemical composition of the seed as well as by hormones, inhibitors, and pesticide residue levels. Seeds that have been overdosed with toxic fungicides such as the organic mercurial compounds, commonly produce abnormal seedlings (17). Plants treated or accidentally subjected to certain pesticides such as 2,4-D (2,4-dichlorophenoxyacetic acid) and phenolic compounds may produce abnormal or nonviable seedlings (23). Pesticides which affect seed moisture content or microorganisms may influence the health of future seedlings.

The insecticide diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] and the fungicide fentin hydroxide (triphenyltin hydroxide), have shown promise in several tests to control insects and fungi diseases on many crops (5, 6).

Prior to registration of these two pesticides for widespread use, considerable testing is necessary to insure their effectiveness on the insects and fungi and their nontoxicity to nontarget organisms.

This study was primarily designed to determine the physiological effects of diflubenzuron and fentin hydroxide on soybean [Glycine max (L.) Merr.] and rice, (Oryza sativa L.) and to evaluate seed quality from treated plants.

CHAPTER I

LITERATURE REVIEW

I. Substituted Ureas as Insecticides

The herbicidal properties of various derivatives of urea are well-known and many of these compounds are employed in agriculture (8, 24).

In terms of insecticidal activity substituted ureas were thought to be insignificant until 1972, when van Daalen et al. (41) introduced the first substituted urea insecticide, namely DU 19111 [1-(2,6-dichlorobenzoyl)-3-(3,4-dichlorophenyl)urea]. One year later, the synthesis of other disubstituted ureas and their evaluation as insecticides were reported (44, 45). Of these compounds diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] and its dichloro-analogue (PH 6038) were found to be the most active.

With respect to their mode of action, disubstituted urea insecticides interfere with cuticle formation in insect larvae during the process of ecdysis (27) and chitinization. However, it is not entirely clear whether this is due to inhibition of chitin synthesis (30, 31) or to increased chitinase activity (16). Since these compounds are effective in early stages of insect development, their possible application to stored grain has been suggested (34).

Studies on diflubenzuron degradation showed photodegradation to be an important factor in the eventual environmental degradation of this compound (25, 35). The degradative pathways usually involved cleavage between the carbonyl and amide groups of the urea bridge (25).

Mammalian toxicity of the disubstitued ureas appears to be low (5, 41). Feeding diflubenzuron to cows did not have any adverse effect during the feeding period (26) and this insecticide has been considered as a food additive for cows to control flies larvae in manure. Diflubenzuron did not bioconcentrate in the mosquito fish Cambustia affinis, through the food chain as did DDT (25).

Diflubenzuron is chemically related to the herbicides monuron [3-(p-chlorophenyl)-1,1-dimethylurea] and dichlobenil (2,6-dichlobenzonitrile) (see Figure 1). The structural similarity raises the possibility of phytotoxicity of diflubenzuron or of its potential breakdown products.

However, the aforementioned compound DU 19111 which is chemically related to the herbicides dichlobenil and diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], appeared to be non-phytotoxic since the leaves of the French bean, tomato, sugar beet, and oats and as well a large variety of weeds remained unaffected when sprayed with a 1% acetone solution (41). The same authors also found that the germination of seeds was not inhibited after a preemergence application of DU 19111.

II. Triphenyltin Compounds as Fungicides

In general, organometalic compounds, or metal compounds in which a metal is linked with at least one carbon of an organic group, are more active as pesticides than the metal alone. Their application in agriculture has been reviewed by Kaars Sijpestein et al. (19).

The first tests on the biocidal activity of organotin compounds were done in 1886, but their insecticidal properties were only discovered in 1929 (24). Later, van der Kerk and Luijten in 1954 (42), investigated the greater fungicidal properties of trisubstituted tin compounds, i.e. R_3SnX, compared to the other three basic groups,

$RSnX_3$, R_2SnX_2, R_4Sn. In terms of structure-activity relationships, it was found that the nature of the organic group, R, which may be simple aliphatic or aromatic radicals, appeared to be more important in contrast to the nature of the fourth group, X, which may be a halide, hydroxide, OR, SH, SR, or acyl radicals and did not apparently influence activity (14, 17, 19, 22, 42).

Within the effective compound group, R_3SnX, the trialkyltin compounds proved to be more effective than triphenyltin compounds *in vitro*, but the results were the opposite under field conditions. Triphenyltin compounds appeared to be more effective *in vivo* despite their moderate fungitoxicity *in vitro* (14, 29). Triphenyltin compounds were also found to be less phytotoxic, though not sufficiently innocuous to many crops, as compared to trialkyltin compounds which appeared to be too phytotoxic at concentrations required to control fungi (14, 29).

As a consequence, particular attention was given to triphenyltin compounds for possible application in agriculture as commercial pesticides. The ultimate decomposition of organotin compounds under the action of light and air to harmless inorganic tin, coupled with its effectiveness on crops (20), promoted efforts for commercial application of these compounds.

The potential use of triphenyltin compounds in insect control has been reviewed by Ascher and Nissim (7). The action of triphenyltin compounds as insect reproductory inhibitors (20) and as antifeeding agents for some chewing insects (24) has been reported. The possible application of organotins as herbicides has also been mentioned (11, 24), but much more importance has been given to the use of triphenyltin

compounds as agricultural fungicides. Two of them fentin acetate (triphenyltin acetate) and fentin hydroxide (triphenyltin hydroxide) are used in Europe to control Phytophthora infestans on potato (Solanum tuberosum L.), Cercospora beticola on sugar beet (Beta vulgaris L.), and Septoria apii on celery (Apium graveolens L.) (7, 14, 19, 29, 39, 40). Phytotoxicity of these compounds has limited their widespread use. Tomato (Solanum lycopersicum L.), fruits, vine, greenhouse plants and ornamental plants have been reported as being sensitive (7, 14, 21).

Efforts to reduce phytotoxicity of trisubstituted tin compounds revealed that the nature of X, exerted a considerable influence. Pieters (29) reported that triphenyltin acetate caused more leaf damage than did the triphenyltin hydroxide on potatoes. Proper formulation of organotin compounds can also reduce the degree of phyotoxicity (22, 29). Kubo (21) reported that introduction of diphenyl phosphine moiety into trialkyl- or triphenyl-tin derivatives reduced phytotoxicity considerably.

Trisubstituted tin compounds are considered to exert their mode of action by inhibiting oxidative phosphorylation in mitochondria of animal tissues (1, 2, 3, 38). Kaars Sijpestein et al. (18) assumed that inhibition of oxidative phosphorylation is also responsible for the antifungal effect. Although such in vitro activity may be sufficient to explain the in vivo effects, no experiments have been done with pests (11).

The amazing increase in yield after treatments with fentin acetate, observed in sugar beet and celery (13, 36) has given rise to the hypothesis of a possible growth promoting effect of fentin acetate on these plants (7).

On the other hand, absorption and translocation studies of labeled ^{113}Sn-triphenyltin acetate by kidney beans (Phaseolus vulgaris L.) celery, and sugar beet indicated that this compound was not absorbed by plants, leaves or roots (9, 10, 15). Nevertheless, effective concentrations for growth regulation may be very low. Baumann (9, 10) ascribes the total increase in yield observed in sugar beet and celery treated with fentin acetate to the excellent fungicidal activity.

Since triphenyltin acetate is converted to triphenyltin hydroxide (19, 40) and there is well-documented evidence for the growth-regulating action of the fungicide triarimol [a-(2,4-dichlorophenyl)-a-phenyl-5-pyrimidinemeyhanol] (12, 33, 37) which structurally resembles fentin hydroxide (see Figure 1) the possibility that fentin hydroxide has growth-regulating effects on plants merits examination.

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CHAPTER 2

The Effect of Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] on Soybean [Glycine max (L.) Merr.] Photosynthesis, Respiration and Leaf Ultrastructure

ABSTRACT

The effect of the insecticide diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] on photosynthesis, respiration and leaf ultrastructure of soybean [Glycine max (L.) Merr., cv. Swift] was examined on plants treated at the second trifoliate leaf stage with 0, 0.067 and 0.269 kg active ingredient/ha of diflubenzuron. Photosynthesis and respiration were measured with an infrared CO_2 analyzer in an open flow system prior to diflubenzuron application and at 4, 24, 48, and 96 hours after treatment with diflubenzuron. Diflubenzuron had no effect on soybean photosynthesis at any rate examined. Respiration was stimulated by the high rate (0.269 kg/ha) in a transitory manner.

Tissue samples removed from both old and new leaves, 9 days after diflubenzuron application, were used for the ultrastructure study with the transmission electron microscope. The lower trifoliate leaves contained more starch grains than the upper being formed after treatment, but no aberrations or degradation of leaf ultrastructure due to diflubenzuron treatment were evident.

INTRODUCTION

1-(2,6-Disubstituted benzoyl)-3-phenylureas are a new class of selective insecticides with activity of the growth regulator type (1, 2, 3). They have been reported successful in inhibiting biosynthesis

and deposition of chitin (homopolymer of N-acetyl-D-glucosamine), which makes up the cuticle-like shell of mature insects (4, 5, 6, 7). Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] has shown promise in several tests on lepidopterous insects of soybean [Glycine max (L.) Merr.] (8).

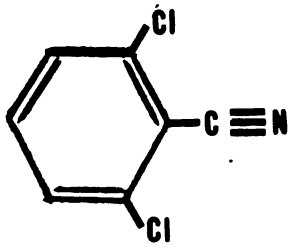
Inhibition of the Hill reaction of photosynthesis is generally acknowledged to indicate the primary site of action of the substituted urea herbicides (9, 10). Application of this knowledge to basic research has contributed markedly to the elucidation of our current information of photosynthetic pathways. Diflubenzuron is chemically related to the herbicides monuron [3-(p-chlorophenyl)-1,1-dimethylurea] and dichlobenil (2,6-dichlorobenzonitrile) (Figure 1). The latter compound has been used for the preparation of diflubenzuron (3). The aforementioned similarity raises the possibility of phytotoxicity of diflubenzuron or of its potential breakdown products.

Ultrastructural examination correlated with biochemical or physiological investigations may provide insight into possible mechanisms of pesticidal action that the latter alone could not provide (11).

The objectives of this study were to determine whether diflubenzuron affected soybean photosynthesis, respiration, and leaf ultrastructure.

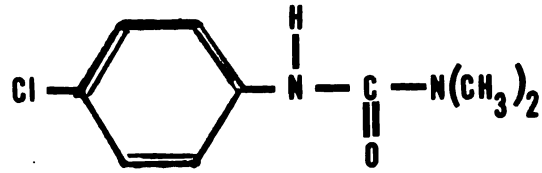
MATERIALS AND METHODS

Plant material. Soybeans [Glycine max (L.) Merr., cv. Swift] were seeded 5 seeds per 947 ml pot in greenhouse soil. After emergence the plants were thinned to 1 plant per pot. When the plants had developed to the



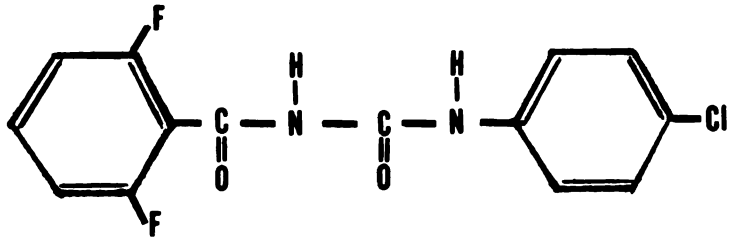
2,6 —dichlorobenzonitrile

dichlobenil



3—(p—chlorophenyl)—1,1—dimethylurea

monuron



1—(4—chlorophenyl)—3—(2,6—difluorobenzoyl)urea

diflubenzuron

Figure 1. Chemical structures of the herbicides dichlobenil and monuron and the insecticide diflubenzuron

stage where the second trifoliate leaf was about 1/3 expanded, the plants were treated with diflubenzuron.

Photosynthesis and respiration measurement. Photosynthesis and respiration were measured with an infrared CO_2 analyzer in an open flow system, 500cc/min. Measurements were made of the whole plants prior to diflubenzuron application. Two plants were then sprayed with water (controls), two with the proposed use rate or 1X rate of 0.067 kg active ingredient/ha of diflubenzuron, and two plants with the 4X rate of 0.269 kg a.i./ha of diflubenzuron. Photosynthesis and respiration were measured 4, 24, 48, and 96 hours after the diflubenzuron application. The data were calculated as the percent of the original photosynthetic and respiratory level. Data presented are the means of two experiments.

Transmission electron microscopy study. Tissue samples were removed from areas adjacent to the midvein near the apex of the center leaflet of the first trifoliate leaf and from a similar area from the center leaflet of the third trifoliate leaf 9 days after the application of diflubenzuron. The third trifoliate leaf had developed since the application of diflubenzuron.

These tissues were incubated for 2 hr at 25°C in the following fixation solution: 5%(v/v) glutaraldehyde, Sorensen's phosphate buffer, pH 7.2 (12). The material was then washed in the same buffer and fixed for one hr in 1%(v/v) osmium tetroxide. After washing, the tissues were stained in 0.5%(w/v) aqueous uranyl acetate for 2 hr. Then, the material was dehydrated in ethyl alcohol and embedded in eponaraldite (13). Thin sections were stained with lead citrate and examined in

a PHILIPS 300 Transmission Electron Microscope at 60 KV.

RESULTS AND DISCUSSION

Photosynthesis in young soybean plants was not significantly altered at the 5% level by postemergence application of diflubenzuron (Table 1). After the application of the 4X rate (0.269 kg/ha) of diflubenzuron, the normal increase in the photosynthetic rate with time was not evident; however, there were no significant differences at the 5% level. The data indicates that this substituded urea insecticide is not a photosynthetic inhibitor, and if degraded rapidly by plants its metabolites also appear to be without effect on soybean photosynthesis.

Diflubenzuron degradation studies in model systems showed the degradative pathways to be almost entirely through cleavage between the carbonyl and amide group of the urea bridge (14, 15). Some of the identified metabolites in those studies appeared to be the same to those identified in metabolism studies of the herbicides monuron (16) and the fluoro-analogues of dichlobenil (17) in plants. None of these metabolites has been reported to act as photosynthetic inhibitor. Thus far the metabolic fate of diflubenzuron in plants has not been elucidated.

Following the application of the 4X rate of diflubenzuron, there was a significant increase in the respiration rate (Table 2). Two days or 48 hr after treatment the rate had dropped to the level observed for the water control, indicating that the effect was transitory. Thus, only the high rate stimulated respiration and then only in a transitory manner.

Examination of the electronmicrographs of the leaf sections of

trifoliate leaves, both old and new, from soybean plants 9 days after treatment with 0, 0.067 and 0.269 kg/ha of diflubenzuron revealed no obvious abnormalities due to the application of this compound (Figure 2 a, b, c). The lower trifoliate leaves contained more starch than the upper (Figure 2 b and c), but no aberrations or degradation of ultrastructure due to diflubenzuron application were evident in either. The upper trifoliate leaves had developed after the treatment with diflubenzuron. Since these were normal, diflubenzuron does not appear to have any subtil morphogenic effects on soybean leaf cellular structure.

Table 1. The effect of diflubenzuron on total photosynthesis of soybean plants in the second trifoliate leaf stage.

Treatment	Hours after treatment 4	24	48	96
	(CO_2 uptake as % of original level)			
Water	93	128	134	149
0.067 kg/ha	121	123	166	124
0.269 kg/ha	116	107	105	108

Table 2. The effect of diflubenzuron on respiration of soybean plants in the second trifoliate leaf stage.

Treatment	Hours after treatment 4[a]	24	48	96
	(O_2 uptake as % of original level)			
Water	117	159	165	180
0.067 kg/ha	156	146	175	170
0.269 kg/ha	182*	182	154	156

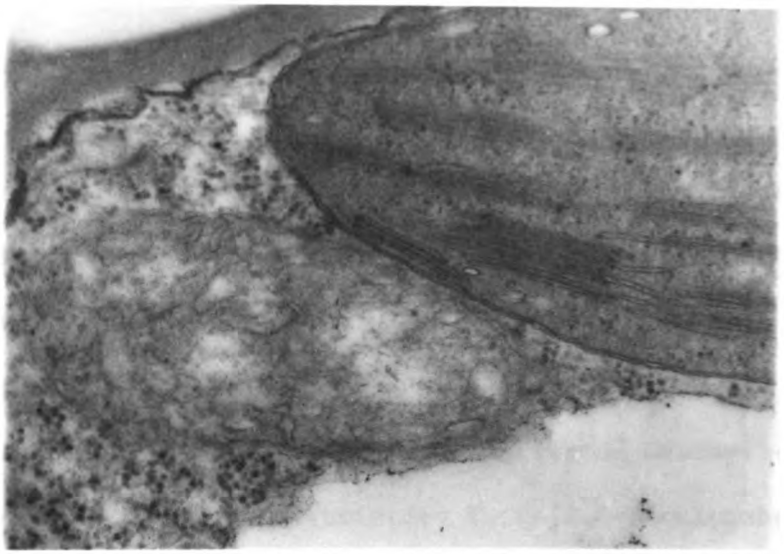
[a] An asterisk in the first column indicates significant difference from the initial level at the 5% level of significance.

Figure 2.

a. Electronmicrograph of the upper trifoliate leaf of soybean plant treated 9 days earlier with water (control). Magnification: 52,000 X, Chloroplast and mitochondria.

b. Electronmicrograph of the upper trifoliate leaf of soybean plant treated 9 days earlier with 0.269 kg/ha of diflubenzuron.
Magnification: 21,000 X, chloroplasts showing stacks of grana and starch grains.

c. Electronmicrograph of the lower trifoliate leaf of soybean plant treated 9 days earlier with 0.269 kg/ha of diflubenzuron.
Magnification: 25,000 X, starch-filled chloroplast.



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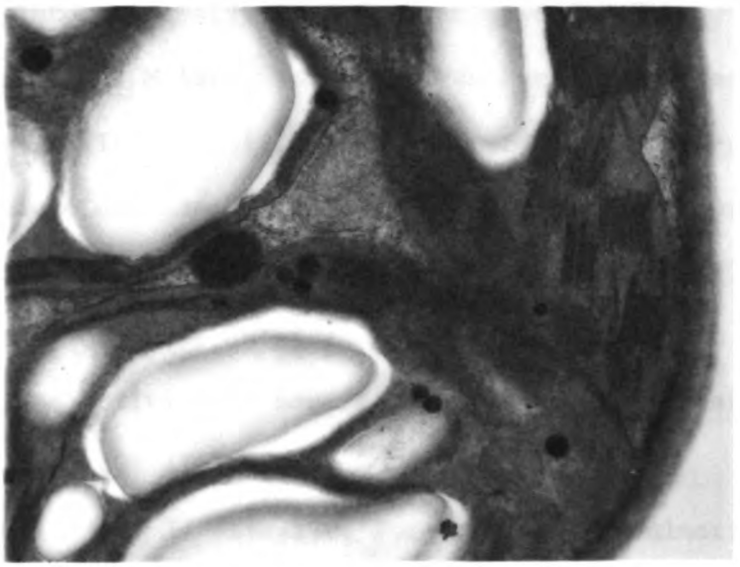

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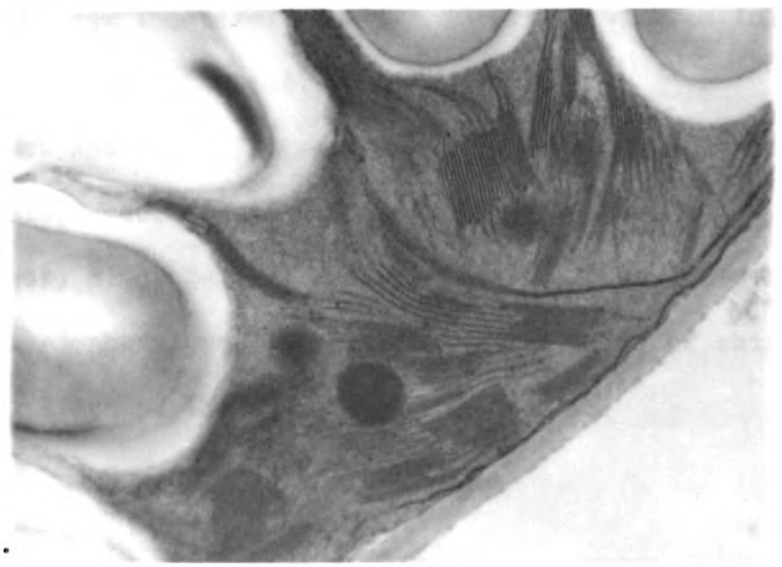

c.

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CHAPTER 3

The Effect of Fentin Hydroxide (Triphenyltin Hydroxide) on Soybean [*Glycine max* (L.) Merr.] and Rice (*Oryza sativa* L.) Photosynthesis, Respiration and Leaf Ultrastructure

ABSTRACT

Soybean [*Glycine max* (L.) Merr., cv. Swift] plants at the second trifoliate leaf stage and rice (*Oryza sativa* L. cv. Starbonnet) plants 25 cm tall were treated with 0, 0.56 and 2.24 kg/ha of fentin hydroxide (triphenyltin hydroxide) to determine the effect of this fungicide on photosynthesis, respiration, and leaf ultrastructure. Photosynthesis and respiration were measured with an infrared CO_2 analyzer in an open flow system prior to fentin hydroxide application and at 4, 24, 48, and 96 hours after treatment with fentin hydroxide. No significant detrimental effects on photosynthesis or respiration were evident in either soybean or rice through 96 hours after treatment.

Tissue samples from soybean and rice plants, 9 days after fentin hydroxide application, examined for ultrastructure changes with the transmission electron microscope showed no effect due to the fungicide treatment.

INTRODUCTION

The fungicide fentin hydroxide (triphenyltin hydroxide) has been used in Europe to control the fungi *Phytophthora infestans* on potato (*Solanum tuberosum* L.), *Cercospora beticola* on sugar beet (*Beta vulgaris* L.) and *Septoria apii* on celery (*Apium graveolens* L.) (1, 2, 3, 4, 5). Application of fentin hydroxide for the control of fungus diseases on

soybean [Glycine max (L.) Merr.] and rice (Oryza sativa L.) has also been suggested (5).

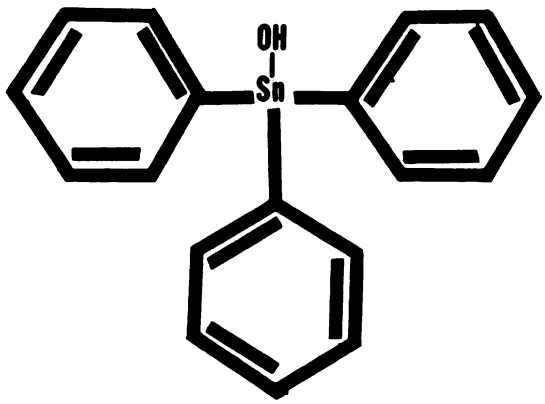
Interference with respiration due to marked inhibition of oxidative phosphorylation in rat liver mitochondria caused by triphenyltin compounds is well documented (6). The same mode of action has been assumed for the antifungal effect (7), but this is not certain since no experiments have been done with pests (8).

Phytotoxicity of triphenyltin compounds limits their application in agriculture as fungicides (1, 2, 3). On the other hand, the possible growth promoting action of triphenyltin fungicides in certain plants is of interest. Increases in yields of sugar beet and celery after treatment with fentin acetate (triphenyltin acetate) have been reported (1). Since absorption and translocation studies using ^{113}Sn-triphenyltin acetate indicated no absorption either by leaves or roots in both sugar beet and celery (9), some contribution is evident. Since triphenyltin acetate is easily hydrolyzed to triphenyltin hydroxide (3) and the growth regulating activity of the fungicide triarimol [a-(2,4-dichlorophenyl)-a-phenyl-5-pyrimidinemethanol] has been established (10, 11, 12), which structurally resembles fentin hydroxide (Figure 1), the growth promoting effect of triphenyltin fungicides bears reexamination.

The purpose of this study was to determine whether triphenyltin hydroxide affected photosynthesis, respiration, or leaf ultrastructure in soybean and rice plants.

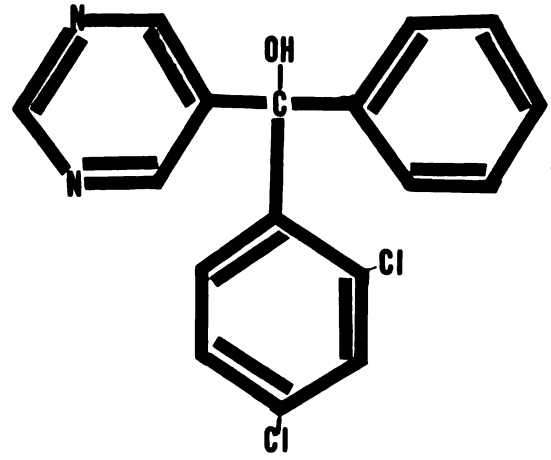
MATERIALS AND METHODS

Plant material. Soybeans [Glycine max (L.) Merr., cv. Swift] and rice



Triphenyltin hydroxide

Fentin hydroxide



α (2,4-dichlorophenyl)-α-phenyl-5-pyrimidinemethanol

Triarimol

Figure 1. Chemical structures of the fungicides fentin hydroxide and triarimol

(Oryza sativa L., cv. Starbonnet) were seeded 5 and 40 seeds per 947 ml pot, respectively, in greenhouse soil. After emergence the plants were thinned to 1 plant per pot for soybeans and 20 plants per pot for rice. When the soybean plants had developed to the stage where the second trifoliate leaf was about 1/3 expanded and the rice was about 25 cm tall, the plants were used for this study.

Photosynthesis and respiration measurement. Photosynthesis and respiration rates were measured with an infrared CO_2 analyzer in an open flow system, 500 cc/min. Measurements were made of the whole plants prior to fentin hydroxide treatment. Two plants were then treated with water (controls), two with the proposed use rate or 1X rate of 0.56 kg active ingredient/ha of fentin hydroxide, and two plants with the 4X rate of 2.24 kg a.i./ha of fentin hydroxide. Photosynthesis and respiration were measured at 4,24, 48, and 96 hours after the application of fentin hydroxide. The data were calculated as the percent of the original and respiratory level. Data presented are the means of two experiments.

Transmission electron microscopy study. Nine days after fentin hydroxide application, soybean tissue samples were removed from area adjacent to the midvein near the apex of the center leaflet of the first trifoliate leaf and from a similar area from the center leaflet of the third trifoliate leaf. The latter had developed since the application of fentin hydroxide. Tissue samples from rice leaves were obtained from an area 6 cm away from the leaf tip 9 days after the treatment with this fungicide.

These tissues were incubated for 2 hr at 25°C in the following fixation solution: 5%(v/v) glutaraldehyde, Sorensen's phosphate buffer, pH 7.2 (13). The material was then washed in the same buffer and fixed

for one hr in 1%(v/v) osmium tetroxide. After being washed, the tissues were stained in 0.5%(w/v) aqueous uranyl acetate for 2 hr. Then the material was dehydrated in ethyl alcohol and embedded in eponaraldite (14). Thin sections were stained with lead citrate and examined in a PHILIPS 300 Transmission Electron Microscope at 60 KV.

RESULTS AND DISCUSSION

The treatment with fentin hydroxide had no visual effects on soybean. However, rice plants receiving the 0.56 kg/ha or 1X rate appeared noticeably taller and greener than the control or the plants treated with the 4X rate. There were no visual differences between the control rice plants and the 4X fentin hydroxide treated plants. This effect on plant color appears similar to that observed by Shive and Sisler for triarimol on beans (12). Attempts to duplicate the result in greenhouse studies were unsuccessful. Whether this failure was due to inactivation of the compound by air or by light, it is not known.

Fentin hydroxide did not inhibit soybean photosynthesis (Table 1). However, a significant increase of the photosynthetic rate was evident 4 hr after application for the plants treated with the 4X rate of 2.24 kg/ha of fentin hydroxide as compared to the control plants treated with water. The low value for the water control 4 hr after treatment is indicative of partial stomatal closure at that measurement. Soybean respiration, rice photosynthesis, and rice respiration were not significantly affected by fentin hydroxide up to 96 hr after treatment (Tables 2, 3, and 4). Thus, the aforementioned possible effect of fentin hydroxide on rice growth and coloration did not appear to be due to a

rapid stimulation of photosynthesis or inhibition of respiration. Fentin hydroxide did not appear to have any deleterious effect on soybean or rice photosynthesis and respiration.

Electronmicrographs of control and fentin hydroxide treated rice plants are shown in Figure 2 (a, b, c). Examination of numerous sections revealed no differences between control plants and plants receiving 0.56 kg/ha of fentin hydroxide. A certain amount of grana disarray was evident in a section from rice plants treated with the 4X rate of 2.24 kg/ha (Figure 2c). This disarray is characteristic of a number of stresses and occurs during the fixation process according to Dr. G. Hooper (15). Whether the stress causing this particular effect was drought, insect, disease, temperature, or fentin hydroxide treatment cannot be assigned with any degree of certainty.

Electronmicrographs of control and fentin hydroxide treated soybean plants are shown in Figures 3 (a, b) and 4 (a, b). Fentin hydroxide treatments did not appear to have any detrimental effect on cellular ultrastructure of soybean tissues. In Figure 3b a certain amount of grana disarray is evident. Again, these are stresses induced and occur during fixation. The contributing stresses cannot be identified at this point. It is doubtful that fentin hydroxide is responsible since the 4X application rate failed to produce this effect as seen in Figure 4a, and it was observed in only one plant receiving the 0.56 kg/ha rate of fentin hydroxide.

Table 1. The effect of fentin hydroxide on total photosynthesis of soybean plants in the second trifoliate leaf stage.

	Hours after treatment[a]			
Treatment	4	24	48	96
	(CO_2 uptake as % of original level)			
Water	74 a	92 abc	96 abc	88 abc
0.56 kg/ha	99 abc	103 abc	101 abc	79 ab
2.24 kg/ha	110 c	117 c	113 c	107 bc

[a]Means with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 2. The effect of fentin hydroxide on respiration of soybean plants in the second trifoliate leaf stage.

	Hours after treatment			
Treatment	4	24	48	96
	(O_2 uptake as % of original level)			
Water	120	117	125	119
0.56 kg/ha	153	110	110	105
2.24 kg/ha	147	101	120	118

Table 3. The effect of fentin hydroxide on total photosynthesis of rice plants.

Treatment	Hours after treatment 4	24	48	96
	(CO_2 uptake as % of original level)			
Water	89	91	87	92
0.56 kg/ha	93	89	99	107
2.24 kg/ha	84	99	98	100

Table 4. The effect of fentin hydroxide on respiration of rice plants.

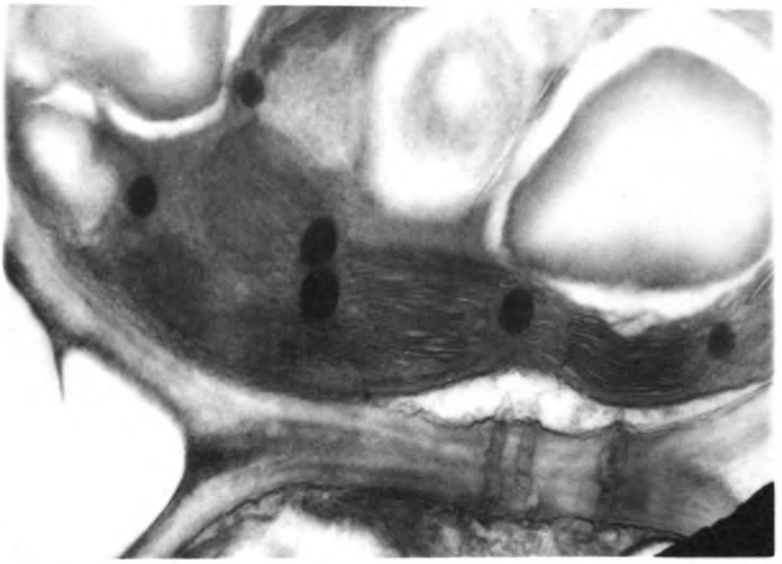
Treatment	Hours after treatment 4	24	48	96
	(O_2 uptake as % of original level)			
Water	114	99	95	104
0.56 kg/ha	107	93	88	100
2.24 kg/ha	99	114	93	122

Figure 2

a. Electromicrograph of rice leaf tissue from plant treated 9 days earlier with water (control).
Magnification: 32.000 X, dark lipid bodies, grana, starch grains, plasmadesmata.

b. Electronmicrograph of rice leaf tissue from plant treated 9 days earlier with 0.56 kg/ha of fentin hydroxide (1X rate).
Magnification: 21.000 X, chloroplasts, starch grains, plasmalemma, grana.

c. Electronmicrograph of rice leaf tissue from plant treated 9 days earlier with 2.24 kg/ha of fentin hydroxide (4X rate).
Magnification: 10,000 X, lipid bodies, starch grains, grana.



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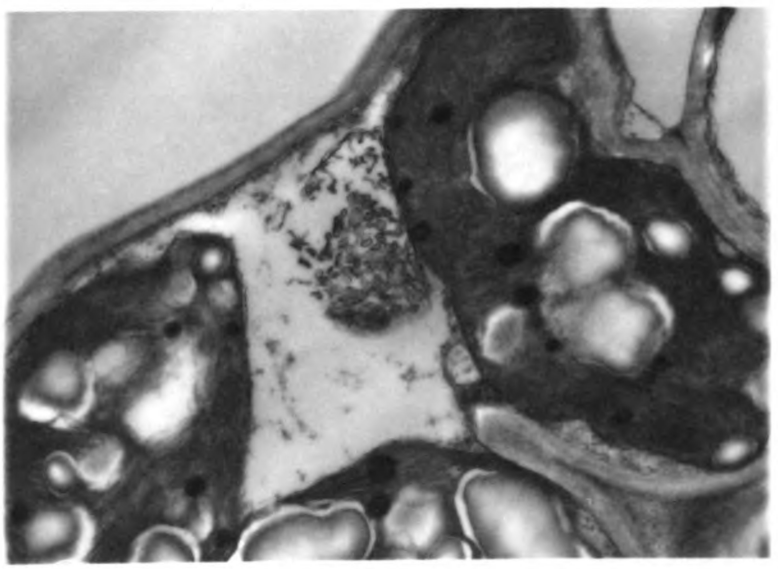

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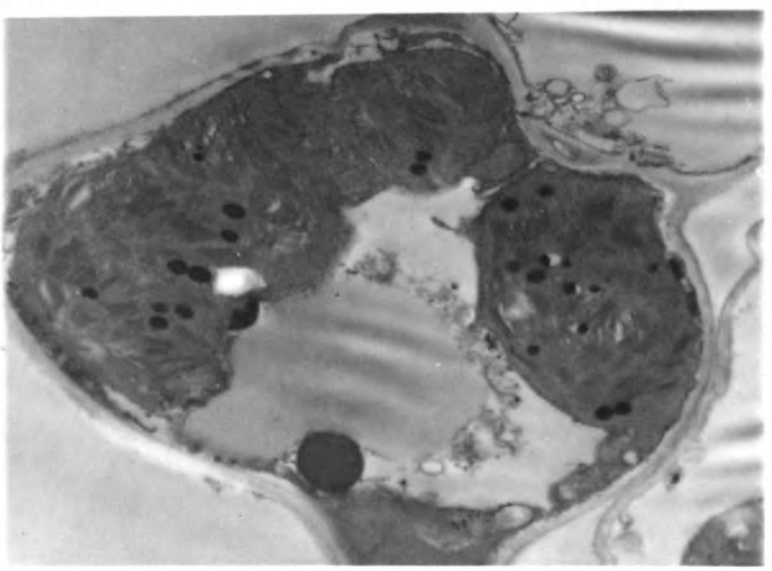

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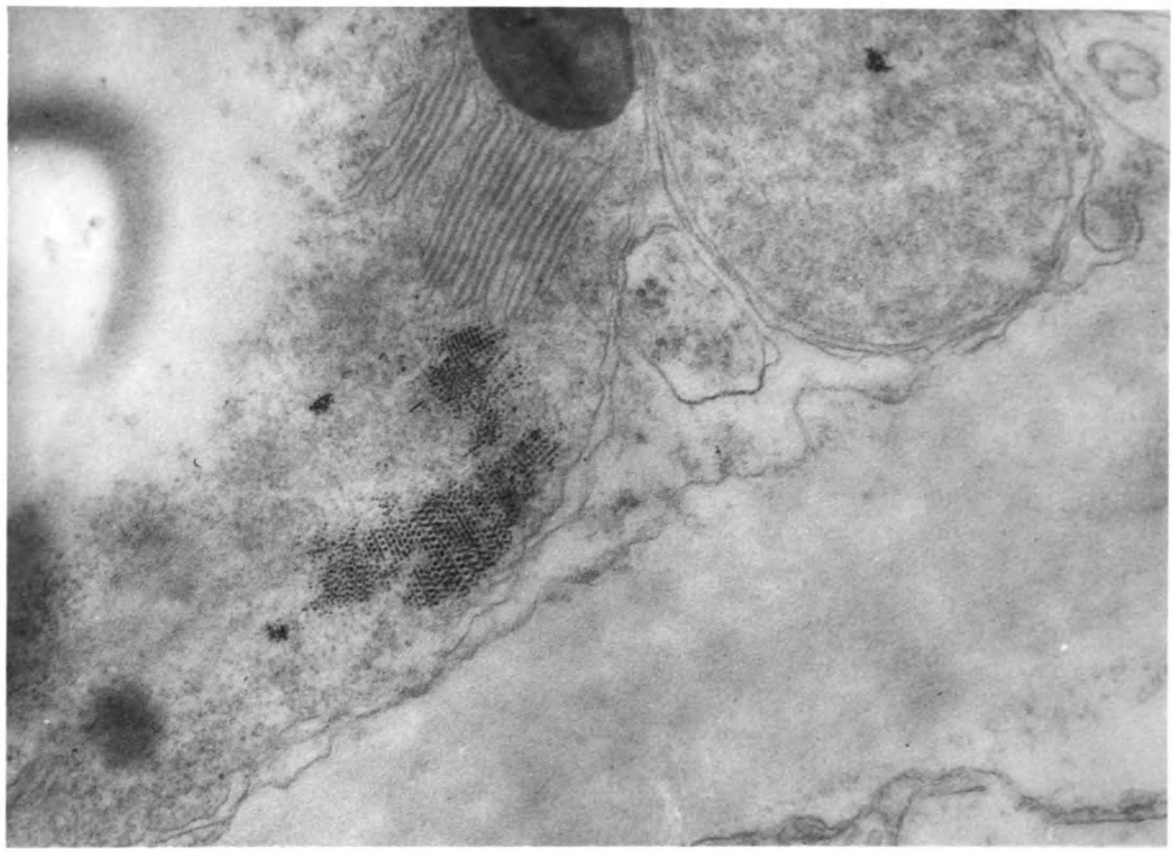
Figure 3.

a. Electronmicrograph of soybean leaf tissue from trifoliate lower leaf treated 9 days earlier with water (control). Magnification: 52.000 X, ferritin in chloroplast, lipid body, grana.

b. Electronmicrograph of soybean leaf tissue from the upper trifoliate leaf of plant treated 9 days earlier with 0.56 kg/ha of fentin hydroxide (1X rate). Magnification: 12,500 X, chloroplast.

a.



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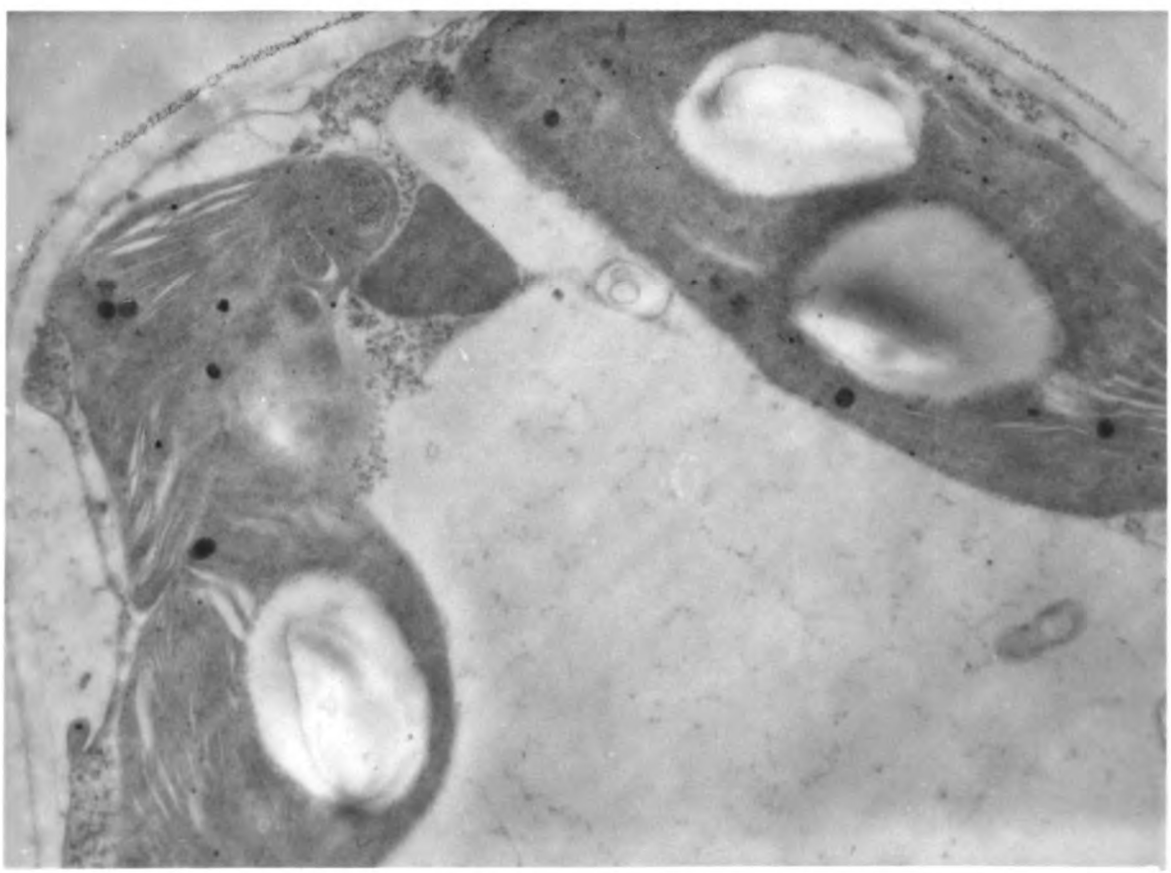


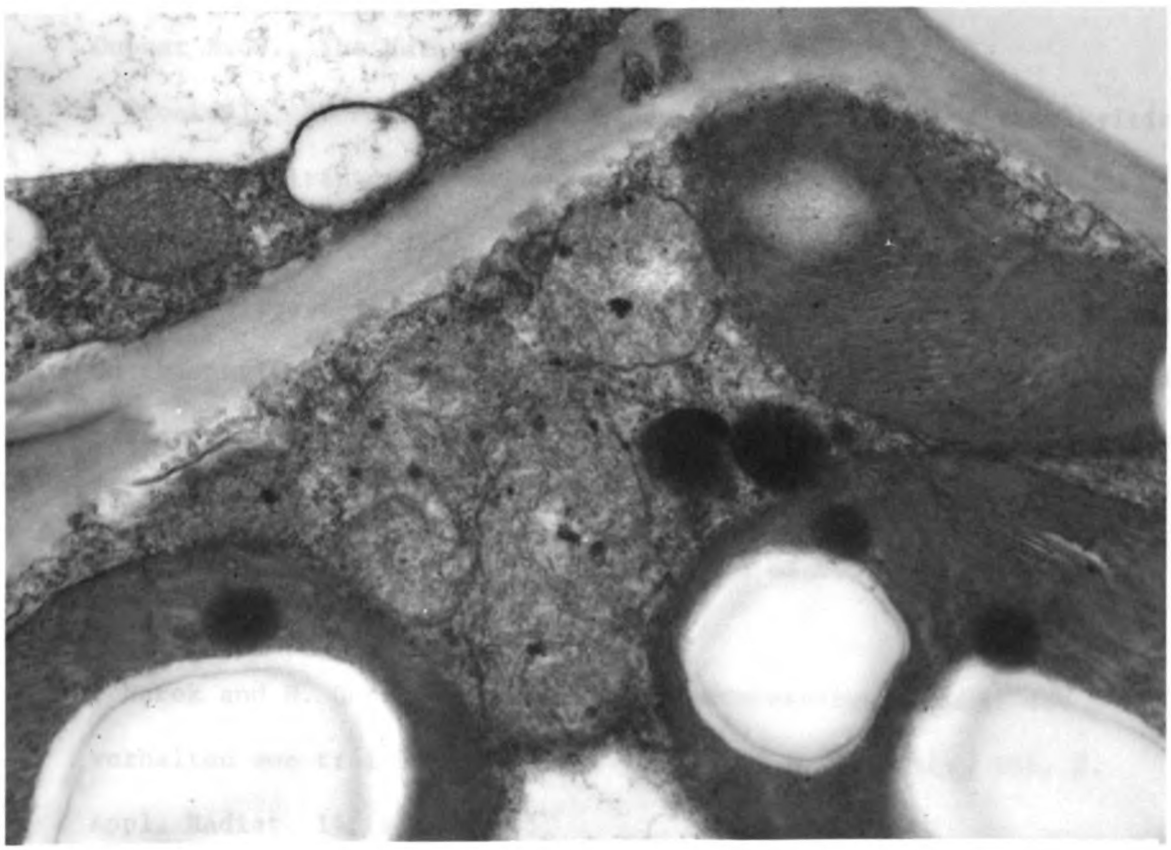
Figure 4.

a. Electronmicrograph of soybean leaf tissue from the upper trifoliate leaf of plant treated 9 days earlier with 2.24 kg/ha of fentin hydroxide (4X rate).
Magnification: 12,500 X, grana.

b. Electronmicrograph of soybean leaf tissue from the lower trifoliate leaf of plant treated 9 days earlier with 2.24 kg/ha of fentin hydroxide (4X rate).
Magnification: 21,000 X, cell wall, mitrochondria, chloroplast, grana, starch grains.

a.

b.



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CHAPTER 4

The Effect of Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] and Fentin Hydroxide (triphenyltin hydroxide) on Soybean [Glycine max (L.) Merr.] Seed Quality

ABSTRACT

Seeds of five soybean [Glycine max (L.) Merr.] cultivars, namely 'Coker 102', 'Cobb', 'Tracy', 'Ransom' and 'Bragg', obtained from plots treated with 0, 0.035, 0.069, and 0.14 kg/ha of diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] for 'Coker 102', 0 and two applications of 0.56 kg/ha of fentin hydroxide (triphenyltin hydroxide) for 'Cobb', 'Tracy', and 'Ransom' and 0, 0.266, 0.532, and 1.064 kg/ha of fentin hydroxide, single and in combination with 0.067 kg/ha of diflubenzuron for 'Bragg' were tested for their quality. Chemical composition expressed in terms of lipid, protein, and carbohydrate content was determined by Nuclear Magnetic Resonance (NMR), Kjeldahl and Anthrone procedures. No effect of either pesticide on soybean seed lipid, protein, and carbohydrate content was evident with the exception that fentin hydroxide treated 'Ransom and one set of treated 'Bragg' soybeans showed greater and lower carbohydrate content respectively.

Seed viability and seedling vigor were determined by applying a regular germination test, an accelerated aging test, and a cold test. Neither fentin hydroxide nor diflubenzuron had any effect on the production of healthy seedlings. In several seed tests fentin hydroxide increased the percent of healthy seedlings following the accelerated aging stress.

INTRODUCTION

Seed quality can be divided into two basic parameters: the chemical composition and the quality of the seed in terms of germination and production of a vigorous, healthy seedling. Chemical composition of seeds can be affected in a detrimental manner by pesticides which cause serious deleterious effects on plant growth (1). Production of vigorous seedlings may be controlled by the chemical composition of the seed as well as by hormones, inhibitors, and pesticide residue levels (2). Pesticides which affect seed moisture content or microorganisms may also influence the health of future seedlings.

Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] is a substituted urea with growth regulator insecticidal activity and has shown promise in lepidopterous insect control for soybean [Glycine max (L). Merr.] (3).

Fentin hydroxide (triphenyltin hydroxide) is a fungicide which has been extensively used in Europe to control fungus diseases on many crops (4, 5, 6, 7, 8). It has also been suggested for control of diseases on soybean and rice (Oryza sativa L.) (8).

The objectives of this study were to evaluate the influence of diflubenzuron and fentin hydroxide on soybean seed quality, examining both chemical composition and production of vigorous seedlings.

MATERIALS AND METHODS

Seed material. Seeds of five different soybean cultivars, namely 'Coker 102', 'Cobb', 'Tracy', 'Ransom', and 'Bragg', were obtained from plots

located in Florence, South Carolina. Cultivar 'Coker 102' had been treated during the growing season with 0, 0.035, 0.069, and 0.14 kg/ha of diflubenzuron, cultivars 'Cobb', 'Tracy', and 'Ransom' had been treated with 0 and double application of 0.56 kg/ha of fentin hydroxide, and cultivar 'Bragg' had been treated with 0, 0.266, 0.532, and 1.064 kg/ha of fentin hydroxide, single and in combination with 0.067 kg/ha of diflubenzuron.

Testing the seed viability and seedling vigor. The effects of these pesticide treatments on seed viability and seedling vigor were evaluated as follows. A regular germination test was run on four lots of 100 seeds each from each sample. The seeds were placed on moist paper towels at 25°C for 7 days. Only healthy germinated seeds were recorded. Viability and vigor were also tested by stressing the seeds.

In the accelerated aging test (9) four lots of 100 seeds each from each sample were subjected to 42°C for 3 days at 100% relative humidity. They were then transferred to the conditions for the regular germination test and the number of healthy seedlings 7 days later reported as percent germination. A second manner of stressing the seed, the cold test (9), was accomplished by seeding in moist unsterilized soil, 50 seeds per 16 oz cup, and placing them in a refrigerator at 10°C for 5 days. The cultures were then transferred to a control environment with a 16-hr day at 30°C and 8-hr night at 20°C for 6 days. Percent germination, emergence, and seedling height were recorded.

Testing the chemical composition of seeds. The effect of the pesticide treatments on seed composition was evaluated in this study by determining

the lipid, protein, and the carbohydrate content of the seeds. The lipid content was determined by nuclear magnetic resonance (NMR) procedures at the University of Illinois. The protein content was determined by Kjeldahl procedure and the carbohydrate content was determined by the anthrone method adapted from Yemm and Willis (10). The data for lipid and protein content are the means of two determinations. The data for the carbohydrate content are the means of four determinations.

The moisture content of the samples was 4.4±0.3. The reason for the low level of moisture was the storage of the seeds during the winter time.

RESULTS AND DISCUSSION

The germination percentages for the pesticide-treated and the untreated seeds were low for the 'Coker 102', 'Tracy', and 'Ransom' varieties (Tables 1 and 2). The varieties 'Cobb' and 'Bragg' showed higher germination levels (Tables 3 and 4). Since only normal healthy seedlings were recorded, the actual number of seeds which germinated could have been considerably higher. Neither fentin hydroxide nor diflubenzuron had any marked negative effect on the viability parameters measured. Absence of any negative effect on germination of seeds of French beans, tomato, sugar beet, and oats and as well of a large variety of weeds after preemergence application of DU19111 [1-(2,6-dichlorobenzoyl)-3-(3,4-dichlorophenyl)urea], another substituted urea insecticide, has also been reported (11). The data in the Tables 2 and 3 indicate that fentin hydroxide in some instances markedly increased the percent of healthy seedlings following the accelerated aging stress. Reduction

of microorganisms associated with the seed by the earlier fungicidal action of fentin hydroxide during the growing season may account for the observed results.

Cultivar differences were observed in the lipid and protein content of the soybean seedlings, but no effect of either pesticide tested on soybean seed lipid and protein content was evident (Tables 5 to 8).

Fentin hydroxide and diflubenzuron had no effect on carbohydrate content with the exception that fentin hydroxide treated 'Ransom' and one set of treated 'Bragg' soybeans showed greater and lower carbohydrate content, respectively (Tables 5 to 8).

Table 1. Soybean seed, cv. 'Coker 102', viability and vigor following treatment with diflubenzuron.[a]

Treatment	Regular germination test (%)	Accelerated aging (% germination)	Cold treatment		
			(% germination)	(% emerged)	(ht at 6 days) (cm/plant)
(kg/ha)					
Control	19 a	6 b	22 a	15 a	4.3 a
0.035	31 a	0 a	26 a	22 a	5.4 a
0.069	21 a	0 a	16 a	16 a	6.2 a
0.14	28 a	0 a	20 a	18 a	5.2 a

[a]Means within columns with similar letters are not significantly different at the 5% level by Duncan's Multiple Range test. Percent data involving percentage values less than 15% or greater than 85% were transformed to arcsine values for analysis of variance.

Table 2. Soybean seed, cv. 'Cobb', 'Tracy' and 'Ransom', viability and vigor following treatment with fentin hydroxide[a].

Cultivar	Treatment	Regular germination test (%)	Accelerated aging (% germination)	Cold treatment (% germination)	Cold treatment (% emerged)	Cold treatment (ht at 6 days) (cm/plant)
	(kg/ha)					
Cobb	Water	61 b	6 a	40 a	18 a	2.6 a
	0.56+0.56*	53 b	18 b	38 a	24 a	1.9 a
Tracy	Water	30 a	6 a	36 a	18 a	3.2 a
	0.56+0.56	26 a	28 c	32 a	24 a	4.1 a
Ransom	Water	20 a	0 a	21 a	10 a	1.7 a
	0.56+0.56	21 a	0 a	28 a	18 a	5.2 a

[a]Mean within columns with similar letters are not significantly different at the 5% level by Duncan's Multiple Range test. Percent data involving percentage values less than 15% or greater than 85% were transformed to arcsine values for analysis of variance.

*Double application of fentin hydroxide.

Table 3. Soybean seed, cv. 'Bragg', viability and vigor following treatment with fentin hydroxide[a].

Treatment	No. of application	Harvest intervals (days)	Regular germination test (%)	Accelerated aging (% germination)	Cold treatment (% germination)	Cold treatment (% emerged)	Cold treatment (ht at 6 days) (cm/plant)
(kg/ha)							
0.266	2	83	65 ab	5 b	44 cd	33 a	5.1 a
0.532	2	83	70 bc	3 ab	32 ab	24 a	5.1 a
1.064	2	83	68 abc	1 a	34 ab	26 a	5.0 a
0.266	3	69	71 bc	46 e	48 d	36 a	5.8 a
0.532	3	69	60 a	21 d	30 a	24 a	3.2 a
1.064	3	69	71 bc	16 c	40 bcd	32 a	4.4 a
0.532	1	97	75 bc	4 ab	28 a	21 a	5.6 a
Control	-	-	76 c	1 a	35 abc	28 a	4.7 a

[a]Means within columns with similar letters are not significantly different at the 5% level by Duncan's Multiple Range test. Percent data involving percentage values less then 15% or greater than 85% were transformed to arcsine values for analysis of variance.

Table 4. Soybean seed, cv. 'Bragg', viability and vigor following treatment with fentin hydroxide and diflubenzuron[a].

Treatment		No. of application	Harvest intervals (days)	Regular germination test (%)	Accelerated aging (% germination)	Cold treatment (% germination)	Cold treatment (% emerged)	Cold treatment (ht at 6 days) (cm/plant)
Fentin hydroxide	Diflubensuron							
0.266	0.067	2	69	73 ab	19 b	36 a	27 a	4.1 a
0.532	0.067	2	69	74 ab	12 a	44 ab	33 a	5.2 ab
1.064	0.067	2	69	76 b	21 b	53 b	38 a	5.6 bc
0.266	0.067	3	55	73 ab	17 ab	54 b	38 a	5.6 bc
0.532	0.067	3	55	67 a	21 b	44 ab	34 a	5.8 bcd
1.064	0.067	3	55	78 b	10 a	50 b	44 a	6.8 cd
0.532	0.067	1	83	78 a	12 a	47 ab	38 a	5.6 bc
Control	-	-	-	81 b	21 b	54 b	48 a	6.9 d

[a]Means within columns with similar letters are not significantly differently at the 5% level by Duncan's Multiple Range test. Percent data involving percentage values less than 15% or greater than 85% were transformed to arcsine values for analysis of variance.

Table 5. Soybean, var. 'Coker 102', composition following treatment with diflubenzuron.

Treatment (kg/ha)	Lipid content (%)	Protein content (%)	Carbohydrate content (%)
Control	20.9	39.5	14.0 a
0.035	21.4	40.7	18.3 a
0.069	21.0	41.0	17.0 a
0.14	21.0	41.9	16.0 a

[a] Means within columns with similar letters are not significantly different at the 5% level by Duncan's Multiple Range test. Percent data involving percentage values less than 15% or greater than 85% were transformed to arcsine values for analysis of variance.

Table 6. Soybean seed composition following treatment with fentin hydroxide.

Variety	Treatment (kg/ha)	Lipid content (%)	Protein content (%)	Carbohydrate content (%)
Cobb	water	22.8	39.4	17.8 c
	0.56 + 0.56	22.4	39.9	16.4 bc
Tracy	water	18.9	42.9	16.3 bc
	0.56 + 0.56	18.3	44.6	15.0 ab
Ransom	water	24.5	39.3	13.8 a
	0.56 + 0.56	23.7	40.2	16.8 c

[a] Means within columns with similar letters are not significantly different at the 5% level by Duncan's Multiple Range test. Percent data involving percentage values less than 15% or greater than 85% were transformed to arcsine values for analysis of variance.

Table 7. Soybean seed, var. 'Bragg', composition following treatment with fentin hydroxide.[a]

Treatment (kg/ha)	No. of application	Harvest intervals (days)	Lipid content (%)	Protein content (%)	Carbohydrate content (%)
0.266	2	83	23.3	37.4	13.6 a
0.532	2	83	23.0	38.4	14.0 ab
1.064	2	83	23.3	39.0	16.3 e
0.266	3	69	22.9	37.5	15.0 bcd
0.532	3	69	23.4	38.4	14.8 bc
1.004	3	69	23.0	38.4	15.1 cd
0.532	1	97	23.3	38.3	16.0 de
Control	-	-	23.4	38.4	15.7 cde

[a]Means within columns with similar letters are not significantly different at the 5% level of Duncan's Multiple Range test. Percent data involving percentage values less than 15% or greater than 85% were transformed to arcsine values for analysis of variance.

Table 8. Soybean seed, var. 'Bragg', composition following treatment with fentin hydroxide and diflubenzuron.

Treatment		No. of	Harvest	Lipid	Protein	Carbohydrate
Fentin hydroxide (kg/ha)	Diflubenzuron (kg/ha)	fentin hydroxide application	intervals (days)	content (%)	content (%)	content (%)
0.266	0.067	2	69	23.4	37.2	13.6 a
0.032	0.067	2	69	23.4	38.9	13.1 a
1.064	0.067	2	69	22.7	38.2	15.4 a
0.266	0.067	3	55	23.5	38.7	14.2 a
0.532	0.067	3	55	23.4	38.6	11.3 a
1.064	0.067	3	55	22.9	37.4	17.0 a
0.532	0.067	1	83	22.8	39.3	13.8 a
Control	-	-	-	23.1	38.3	12.4 a

[a]Means within columns with similar letters are not significantly different at the 5% level of Duncan's Multiple Range test. Percent data involving percentage values less than 15% or greater than 85% were transformed to arcsine values for analysis of variance.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

Photosynthesis, respiration, and leaf ultrastructure of soybean and rice plants appeared to be unaffected by the insecticide diflubenzuron and the fungicide fentin hydroxide. The only exception was the transitory stimulation of soybean respiration caused by diflubenzuron at the rate of 0.269 kg/ha. The positive effect of fentin hydroxide at the rate of 0.56 kg/ha on rice height and coloration, observed in one study, it is of interest but more work is needed before definite conclusions can be made.

Neither diflubenzuron nor fentin hydroxide showed any negative effect on the production of healthy seedlings. Moreover, in some seed tests fentin hydroxide increased the percent of healthy seedlings, following the accelerated aging stress.

Lipid, protein, and carbohydrate content of soybean seeds from five different cultivars were unaffected by both pesticides with the exception that fentin hydroxide treated "Ransom" and one set of treated "Bragg" soybeans showed greater and lower carbohydrate content respectively.

In conclusion, it can be said that both diflubenzuron and fentin hydroxide appeared to have no detrimental effects on the physiological functions of soybean and rice examined in this study.