Acceptor of Light Energy in Photoactivation of Diphenylether Herbicides

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Diphenylether herbicides can be classified into two groups. One group, without the ortho-substituent on one benzene ring, is active in the light or dark, but the other group, having ortho-substituent(s), requires light for activation. The activation mechanism by light seems to be a photobiochemical process. A natural albino mutant was found to be tolerant to nitrofen (2,4-dichloro-4′-nitrodiphenylether) in light. Artificial white mutants of rice plants were also found to be tolerant to the herbicide, while yellow mutants were susceptible. Chlorotic seedlings induced by 3,4-dichlorobenzyl *N*-methylcarbamate were relatively tolerant to nitrofen. The dominant pigment(s) extracted from the yellow mutants were xanthophylls. These pigments, which are contained in the yellow mutants in large amounts, seem to make an important contribution to the photoactivation of ortho-substituted diphenylether herbicides as the acceptors of light energy.

In Japan, diphenylether herbicides are useful for preemergence weed control in transplanted rice and other crops. Nitrofen (NIP or TOK) and CNP (MO-338, 2,4,6-trichloro-4′-nitrodiphenylether) are wide spectrum herbicides which exhibit low toxicity to fish.

The common and chemical names of many diphenylether herbicides are listed in Table I. Furuya and Arai (1966) reported that nitrofen required light for herbicidal activity. Using germinating rice seeds, Matsunaka (1969) found that diphenylether herbicides could be classified into two groups depending upon their requirement for light and other properties. One group, such as nitrofen or CNP having ortho-substituent(s) on one benzene ring, required light energy to kill the plant. This group is inactive in the dark.

The ortho-substituted diphenylethers could be distinguished from meta-substituted derivatives such as HE-314, HW-40187 (HE-306), 3-chloro-4′-nitrodiphenylether, 3,5-dichloro-4′-nitrodiphenylether, and others not only from the standpoint of light requirement but also by other properties—i.e., selectivity between rice and barnyardgrass (*Echinochloa crusgalli*), inhibitory activity on root emergence, and utility in the foliar application to submerged weeds (Matsunaka, 1969). Thus, later applications, after transplantation of rice, may be possible. However, no information on their herbicidal mechanism exists.

Matsunaka (1969) also reported that in a low concentration of nitrofen (1 p.p.m.), the herbicidal activity was affected by the intensity of illumination. On the other hand, at 5 p.p.m., the effect of illumination was clear even under 1000 lux. Experimental data showed that the photoactivation of nitrofen is not a simple conversion to a toxic compound by light. Coexistence of simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine], prometryne [2,4-bis(isopropylamino)-6-methylmercapto-*s*-triazine], monuron [3-*p*-chlorophenyl)-1,1-dimethylurea], or propanil (3′,4′-dichloropropionanilide), which are well-known inhibitors of the Hill reaction in chloroplasts, with nitrofen did not affect the activity of the latter. This means that the activation of nitrofen by light would be different from the case of bipyridilium herbicides, for instance, paraquat (1,1′-dimethyl-4,4′-bipyridinium salt) by Mees (1960). Consequently, another photobiochemical activation mechanism after absorption of nitrofen into plant tissues was suspected.

In the case of photoactivation in organisms, the role of light acceptors or pigments should be mentioned. When testing the herbicidal action of nitrofen under illuminated conditions using normal rice seeds, the author found that a natural albino mutant of rice seedling was tolerant to nitrofen in light. In the present report, the susceptibility to nitrofen of white or yellow mutants, artificially created by isotopic radiation or by chemicals, and of chlorotic seedlings induced by a treatment by a herbicide, UC-22463 (3,4-dichlorobenzyl *N*-methylcarbamate), will be described. Yellow pigments, perhaps xanthophylls, are implicated as the acceptors of light during the photoactivation of nitrofen.

METHODS AND MATERIALS

The bioassay of herbicidal activity under light conditions was tested using rice seeds. Usually 20 slightly germinated rice seeds were set in 6-cm. Petri dishes with 15 ml. of test solution, and incubated in a glass chamber kept at 30° C. and illuminated by fluorescent lamps. The illumination strength at the level of the Petri dishes was 3000 lux. After incubation for five days, the fresh weight of buds and roots removed from the seeds was measured. Nitrofen recrystallized from ethanol was dissolved at first in ethanol and diluted with distilled water. Usually the final concentration of nitrofen and ethanol was 5 p.p.m. and 0.1%, respectively.

White and yellow mutants of rice plants were created by chemical treatment or radioisotopic radiation of variety Norin No. 8 in the 3rd Laboratory of Genetics (Hiratsuka, Kanagawa), and their seeds were propagated in the 2nd Laboratory of Plant Physiology (Konosu, Saitama) of this institute. In general, these mutants segregated in a ratio of three to one, three were green and one was white or yellow. By the classification of Gustaffson (1938), white mutants may be termed albina and yellow mutants xantha. Pale green mutants,

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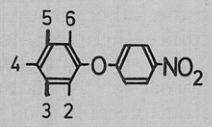
Table I. Common and Chemical Names of Two Types of Diphenylether Herbicides

Common Name	Chemical Name
Ortho-substitution	
Nitrofen (NIP or TOK)	2,4-Dichloro-4′-nitro-DPE[a]
KK-60	4-Chloro-2,4′-dinitro-DPE
C-6989	2,4′-Dinitro-4-trifluoromethyl-DPE
TCPE	2,4,4′-Trichloro-DPE
CNP (MO-338)	4′-Nitro-2,4,6-trichloro-DPE
MO-263	2,4-Dichloro-6-methyl-4′-nitro-DPE
MO-500	2,4-Dichloro-6-fluoro-4′-nitro-DPE
Meta-substitution	
HE-314	3-Methyl-4′-nitro-DPE
—	3-Chloro-4′-nitro-DPE
HW-40187 (HE-306)	3,5-Dimethyl-4′-nitro-DPE
—	3,5-Dichloro-4′-nitro-DPE
MO-600	3-Chloro-4-fluoro-4′-nitro-DPE

[a] DPE: Diphenylether.



viridis, were also used for the experiments. Tentative names of the mutants were as follows: viridis (pale green), CM-46 and CM-75; xantha (yellow), CM-123 and CM-213; and albina (pale yellow or true white), CM-9, CM-33, CM-37, CM-39, and CM-53.

In the case of chlorotic seedlings by UC-22463, rice seeds (variety Norin No. 29) were soaked in emulsions of this herbicide, in which the active concentrations were 10, 20, and 30 p.p.m. After incubation for 1 day, the seeds were transferred to 6-cm. Petri dishes containing 15 ml. of each test solution, 5 p.p.m. of nitrofen, 10 p.p.m. of UC-22463, and a combination of both herbicides. Fresh weight was measured four days after the second treatment.

The pigments contained in the buds of rice plants were extracted by 50 ml. of ethanol per 1 gram of fresh weight of buds, after grinding in a mortar with sand. After centrifugation at 1000 × G for 5 minutes, the absorption spectrum was measured using a Shimazu Model QV-50 spectrometer.

The ethanol extracts of pigments were concentrated at room temperature and chromatographed by using a thin-layer of silica gel with a solvent combination of benzene and acetone (7 to 3) (Rollins, 1963).

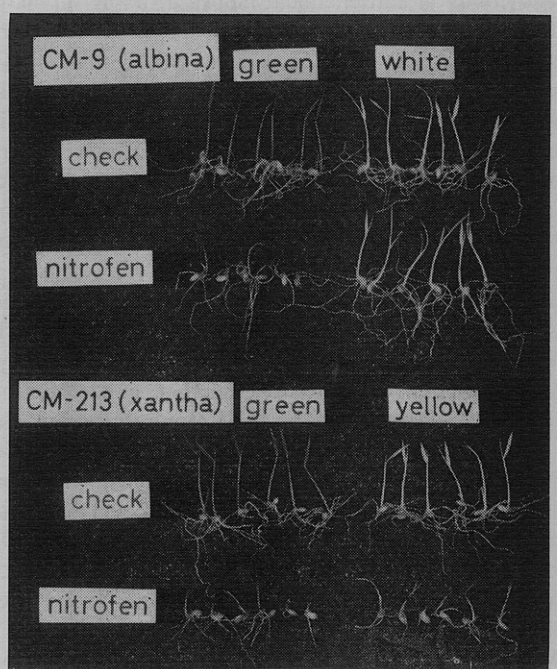


Figure 1. Susceptibility to nitrofen of white mutant (albina, CM-9) and yellow mutant (xantha, CM-213) of rice plants

RESULTS AND DISCUSSION

By use of chlorophyll-mutants of rice, the effect of nitrofen under illumination was examined. Figure 1 shows an example of the appearance of the white (albina) and the yellow (xantha) mutants treated with 5 p.p.m. of nitrofen in light. White CM-9, an example of albina mutants, was tolerant to nitrofen in light, but green CM-9 was still susceptible. On the other hand, an example of xantha, yellow CM-213, was susceptible to nitrofen similar to its green counterpart, as shown in the lower part of Figure 1.

In Figure 2, the effect of concentration of nitrofen on the growth of rice seedlings of both mutants, CM-39 (white) and CM-123 (yellow), is shown. In green seedlings of both mutants, the growth under light was almost completely inhibited even at a concentration of 2.5 p.p.m. of nitrofen. On the other hand, white mutants, CM-39, showed about 50% growth of the control even at a concentration of 20 p.p.m. The yellow mutants of CM-123 seemed to be somewhat tolerant to this herbicide, but the extent was very low and, over 10 p.p.m., it showed the same susceptibility as the green ones.

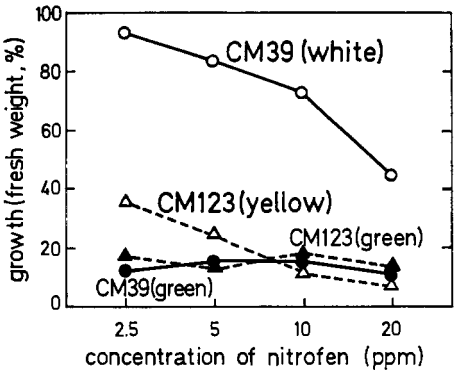


Figure 2. Susceptibility to nitrofen of white mutant (albina, CM-39) and yellow mutant (xantha, CM-123) of rice plants. Amount of test solution was 20 ml.

The meta-substituted diphenylether herbicides, but not 2,4- or 2,4,6-substituted derivatives, do not require light for their herbicidal activity. In the same series of experiments as those shown in Figure 2, the white mutant of CM-39 was found to be susceptible to HW-40187 (HE-306, 3-methyl-4'-nitrodiphenylether). The inhibitory rate of growth in this white mutant was over 80% and almost the same as that in its green counterpart at a concentration of 2.5 p.p.m. of HW-40187.

All of the other white mutants, CM-33, CM-35, CM-37, and CM-53 were also tolerant to nitrofen, but their green counterparts were susceptible as shown in Table II. Two yellow mutants, CM-123 and CM-213, were susceptible to this herbicide regardless of yellow or green colors. The pale green mutant, termed viridis (Gustaffson, 1938), was hardly distinguishable from the green plant at seedling stage. The effect of nitrofen on this mutant was examined using mixed emerged seeds in the same Petri dish. The inhibitory rate of CM-46 and CM-75 by nitrofen was the same as in the original variety, Norin No. 8.

From these results, it may be concluded that yellow or green rice plants are susceptible to nitrofen in light, but only white mutants are tolerant to this herbicide in light. Yellow pigment(s) seems to play a very important role in the photoactivation of nitrofen, since normal green seedlings may also contain a normal amount of yellow pigment. Then, the extraction of these pigments by ethanol was examined to obtain their absorption spectra and to identify them.

The absorption spectra of the extracts from three white, two yellow, and one green mutant are shown in Figure 3. The dilution rate of each sample was the same. A true white mutant, CM-53, showed no detectable peak at a wavelength longer than 400 mμ. White or pale yellow mutants, CM-9 and CM-35, contain a small amount of yellow pigment which seems to be a carotenoid. One of the yellow mutants, CM-213, contains only a yellow pigment, perhaps a carotenoid, and no chlorophylls. Another yellow mutant, CM-123, contains a large amount of yellow pigments and a small amount of chlorophylls. The green seedlings of the white mutant, CM-35, show a normal absorption spectrum as the normal original variety. It should be mentioned that the yellow pigments can activate nitrofen by themselves under an illuminated condition when the large amount is contained in the mutants.

After concentration of the ethanol extracts, the pigments were separated by thin-layer chromatography. An example of the plates (Figure 4) shows that the dominant yellow pigment in the yellow mutants was xanthophylls. The content of xanthophyll in yellow mutants was found to be at the same level as in normal green seedlings, whereas the content of carotenes was

Table II. Susceptibility of the Chlorophyll-Mutants of Rice Plants to Nitrofen

Type of Mutant	Mutant No.	Color	Per Cent Reduction of Growth upon Addition of 5 P.P.M. Nitrofen [a]
Albina	CM-9	Green	68.3
		White	− 0.4
	CM-33	Green	61.0
		White	7.8
	CM-35	Green	65.5
		White	− 6.0
	CM-37	Green	46.8
		White	2.2
	CM-53	Green	62.2
		White	17.3
Xantha	CM-123	Green	41.2
		Yellow	37.6
	CM-213	Green	61.2
		Yellow	62.8
Viridis	CM-46	Green	57.0
	CM-75	Green	64.4
Original	Norin No. 8	Green	63.9

[a] The growth amount, fresh weight per plant, without nitrofen was between 18 and 27 mg.

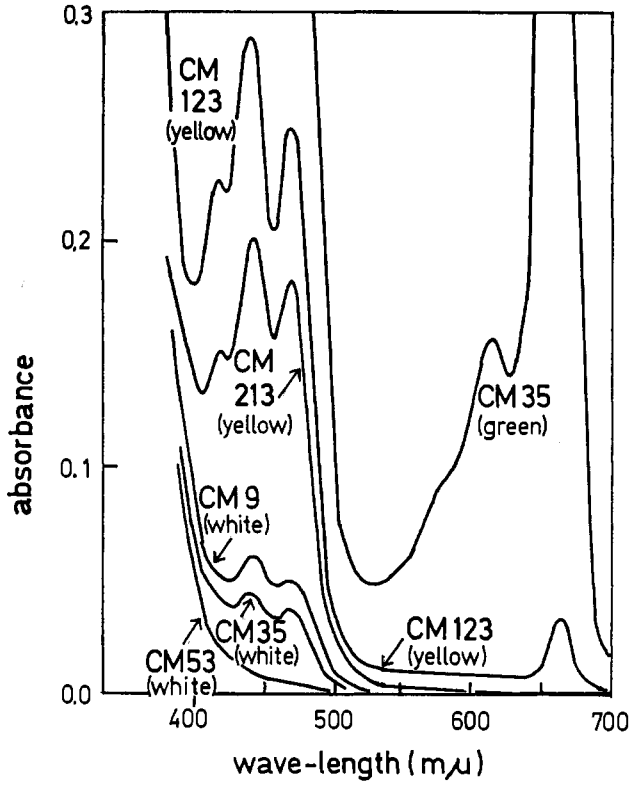


Figure 3. Absorption spectra of ethanol extracts from chlorophyll-mutants of rice

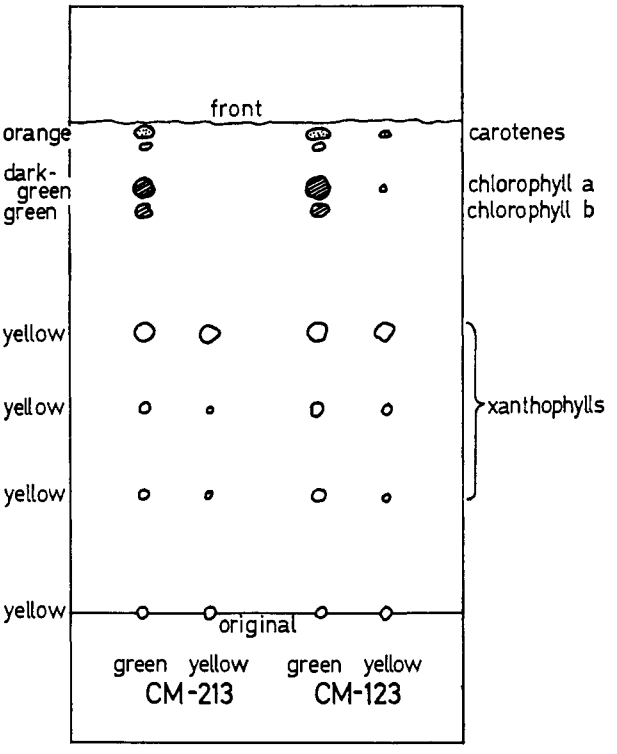


Figure 4. Thin-layer chromatogram of pigments extracted from yellow mutants. Area of the spot indicates intensity of color

Table III. Susceptibility of the Chlorotic Seedlings Induced by UC-22463 to Nitrofen

One-Day Pretreatment with UC-22463 at Concentration of: P.P.M.		Fresh Weight (Mg.) of 20 Seedlings (Buds and Roots) after: 10 P.p.m. UC-22463 only	10 P.p.m. of UC-22463 plus 5 p.p.m. nitrofen	Per Cent Reduction of Growth upon Addition of Nitrofen
0	Green	586[a]	235[a]	59.9
10	White	523	372	28.9
20	White	436	350	19.7
30	White	410	326	20.5

[a] Without UC-22463.

nil in CM-213 and significantly less in CM-123 than that of green ones.

From these data, it may be concluded that the pigments which contribute to the photoactivation of nitrofen, in other words, the acceptors of light energy in the photoactivation are chlorophylls or yellow pigments, especially xanthophylls.

Next, the susceptibility to nitrofen of chlorotic plants which caused chlorosis by a treatment of some herbicides, for instance, amitrole (3-amino-1,2,4-triazole) (Wolf, 1960) or UC-22463 (Herrett and Kramer, 1966), which produce chlorosis in certain plants, was examined. A preliminary experiment showed that UC-22463 may be more effective for this purpose than amitrole, because the former at a concentration of near 10 p.p.m. showed no effect on the fresh weight but could produce good chlorosis in rice seedlings.

The effect of nitrofen on fresh weight with or without UC-22463 is shown in Table III. The inhibition rate caused by 5 p.p.m. of nitrofen without UC-22463 was about 60%. Nitrofen mixed with UC-22463, of course, produces chlorotic plants, and, in this case, the inhibitory rate was lower than in green plants. The inhibitory rate was between 20 and 30%, which was much less than the former case without UC-22463—i.e., 60%.

As discussed in another paper (Matsunaka, 1969), when nitrofen solution was pre-illuminated without

rice plants, the herbicide did not show any activity in the dark. And pre-illumination of nitrofen with riboflavin or fluorescein also had no effect on the herbicidal activity in the dark. These data showed that the photoactivation of nitrofen was not a simple conversion to a toxic compound by light. Based upon the present data, the tentative mode of action of the ortho-substituted diphenylether herbicides may be explained as follows. The light energy absorbed by xanthophylls (or chlorophylls) may be used for the activation of the diphenylether compounds having 2,4- or 2,4,6- substituents on one benzene ring, which will be converted into toxic compounds and exhibit herbicidal action. This toxic compound will be investigated in the future.

An alternative mechanism of the herbicidal action of these herbicides can be explained as follows. The light acts upon the regulation of the hormonal level in higher plants (Briggs, 1963). The plants which have a hormonal level affected by light energy absorbed through pigments, for instance, xanthophylls, and which have different hormonal levels from that of plants in the dark may be very susceptible to the ortho-substituted diphenylether herbicides. In this case, no conversion of the herbicide into another toxic compound occurs.

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LITERATURE CITED

Briggs, W. R., *Ann. Rev. Plant Physiol.* **14,** 311 (1963).
Furuya, S., Arai, M., *Zasso-Kenkyu* (*Weed Res., Tokyo*) No. 5, 99 (1966).
Gustaffson, A., *Hereditas* **24,** 33 (1938).
Herrett, R. A., Kramer, J. A., Abstracts, Weed Society of America, p. 48, 1966.
Matsunaka, S., *Residue Rev.* **25,** in press (1969).
Mees, G. C., *Ann. Appl. Biol.* **48,** 601 (1960).
Rollins, C., *J. Chem. Ed.* **40,** 32 (1963).
Wolf, F. T., *Nature* **188,** 164 (1960).

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