Synergistic Activity of the Herbicide Safener Dichlormid with Herbicides Affecting Photosynthesis

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Dichlormid, a safener for thiolcarbamate herbicides, was tank-mixed with several herbicidal inhibitors of photosystem II, or with the herbicide acifluorfen, and applied postemergence to *Ipomoea hederacea* plants. Dichlormid had no visible effects on the plants when applied alone, but interacted synergistically with the herbicides in the combination treatments. Dichlormid strongly decreased the ascorbic acid levels in the *Ipomoea hederacea* cotyledons. Ascorbate is known to protect plant tissue from photooxidative damage. The herbicides which interacted synergistically with dichlormid are believed to generate their phytotoxic action *via* the production of excess singlet oxygen. It is suggested that the decreased ascorbate levels in the *Ipomoea hederacea* cotyledons after dichlormid treatment result in an impaired singlet oxygen scavenging system and consequently lead to increased plant damage in the presence of singlet oxygen generating herbicides.

Introduction

Recently a synergistic interaction was reported between the inhibitor of photosystem II metribuzin and picolinic acid *t*-butylamide (PABA) [1–2]. In particular, the hard to control weed *Ipomoea hederacea* was very sensitive to a combination of metribuzin and PABA, whereas either compound, when applied alone, had very little or no damaging potential. The studies on the mode of synergistic interaction revealed an inhibition of metribuzin degradation. This inhibition was presumably not caused by a direct interference with degrading enzymes, but rather indirectly *via* a decrease of the cellular free ascorbic acid levels.

The synergism with PABA was not only observed with the triazinone herbicides metribuzin and ethiozin, but also with several other herbicidal inhibitors of photosystem II, and with herbicides leading to other types of oxygen toxicity. The latter herbicides included in particular compounds, such as acifluorfen, which are now known to damage plants by singlet oxygen generation *via* excessive tetrapyrrole accumulation [3]. It is therefore tempting to suggest that the decreased ascorbic acid content after PABA application is the basis for the extended synergism found with compounds other than metribuzin and ethiozin, where an inhibition of herbicide degradation is unlikely to occur. The decreased ascorbic acid content could form the basis for an increased oxygen toxicity and the resulting synergism effect, because of the function of ascorbic acid as an oxygen scavenger [4].

The compounds which were found to be active metribuzin synergists included the dichloroacetamide herbicide safeners, especially the safener dichlormid [5, 6]. We therefore investigated the synergism in *Ipomoea hederacea* plants after a postemergence combination treatment of dichlormid with certain herbicides which interfere with photosynthesis and produce singlet oxygen. We have also investigated the effect of pre- and postemergence applications of dichlormid on the ascorbic acid levels in *Ipomoea hederacea* cotyledons.

Abbreviations and common names: metribuzin, 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5-(4H)-one; ethiozin, [4-amino-6-(1,1-dimethylethyl)-3-(ethylthio)-1,2,4-triazin-5(4H)-one; dichlormid, N,N-diallyl-2,2-dichloroacetamide; acifluorfen, 5-[2-chloro-N(trifluoromethyl)phenoxy]-2-nitrobenzoic acid; atrazine, 6-chloro-N-ethyl-N′-(1-methylethyl)-1,3,5-triazine-2,4-diamine; propanil, N-(3,4-dichlorophenyl)propanamide; PABA, picolinic acid *t*-butylamide.

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Materials and Methods

Plants were grown in vermiculite in the greenhouse for the synergism experiments. The growth conditions were 15,000 lx supplemental illumination with Osram HQI bulbs for 14 h and a 24 °C and 18 °C day and night temperature regime with 70 and 90% relative humidity, respectively. The plants were 7 days old when treated and were sprayed with the herbicides and dichlormid at 1000 l/ha spray volume.

The plants for the ascorbate analyses were similarly grown in vermiculite, but in a controlled environment chamber at 20,000 lx with similar temperature and humidity conditions. The extraction and analysis of ascorbate were done essentially as described by the supplier of the ascorbate test combination [7]. The extraction medium was 15% w/v metaphosphoric acid. Ascorbate was estimated by a color reaction with 3-(4,5-dimethyl-thiazolyl-2)-2,5-diphenyltetrazoliumbromide (MTT) in the presence of 5-methylphenaciniummethylsulfate (PMS) at pH 3.5. The difference between the measurements obtained before and after ascorbate oxidase treatment yielded the ascorbate content of the sample.

Results and Discussion

Dichlormid, a safener for certain herbicides, has been found to interact synergistically with three herbicides (atrazine, metribuzin and propanil) which inhibit photosynthetic electron flow in photosystem II, and with one herbicide (acifluorfen) which induces plant damage *via* tetrapyrrole-catalyzed singlet oxygen generation (Table I). Interestingly, the photosystem II inhibiting herbicides have also been suggested to induce their herbicidal damage *via* singlet oxygen generation [8, 9]. Singlet oxygen-catalyzed damage in plant tissue appears therefore to be the common phytotoxic mechanism of the herbicides listed in Table I.

Metribuzin is detoxified by a deamination reaction in *Ipomoea hederacea,* and this detoxification reaction has been shown to be inhibited by the synergist PABA [1, 1a]. A similar inhibition has been shown in experiments with dichlormid. However, the other herbicides found to interact synergistically with dichlormid (Table I) are detoxified by different mechanisms which are not likely to be inhibited by the same compound. Also, the level of synergism obtained is generally much lower than in the case of metribuzin.

The synergist PABA caused a decrease of the ascorbate levels in *Ipomoea hederacea* plants [1]. Dichlormid causes similar decreases, but the required doses are much higher than in the case of PABA. This is true either after a postemergence application of 8 kg/ha in an experiment similar to the synergism experiment recorded in Table I (Fig. 1), or after a preemergence application of lower

Table I. Synergistic interactions in *Ipomoea hederacea* plants of postemergence applications of combinations of dichlormid (8 kg/ha) with different herbicides acting at the chloroplast level. The injury ratings were taken 7 days after treatment and are given with their standard deviations.

Herbicide	Rate [g/ha]	Injury [%] Herbicide	Herbicide + dichlormid
Acifluorfen	250	95 ± 4	100 ± 0
	125	85 ± 6	100 ± 1
	60	68 ± 5	97 ± 3
	30	58 ± 5	94 ± 3
Atrazine	1000	73 ± 5	94 ± 3
	500	45 ± 17	85 ± 6
	250	35 ± 19	78 ± 5
	125	10 ± 8	70 ± 8
Metribuzin	125	35 ± 13	68 ± 13
	60	5 ± 6	55 ± 10
	30	13 ± 25	20 ± 8
	15	0 ± 0	8 ± 5
Propanil	1000	80 ± 8	85 ± 5
	500	40 ± 20	60 ± 20
	250	25 ± 6	53 ± 13
	125	3 ± 5	28 ± 10
Control	–	0 ± 0	0 ± 0

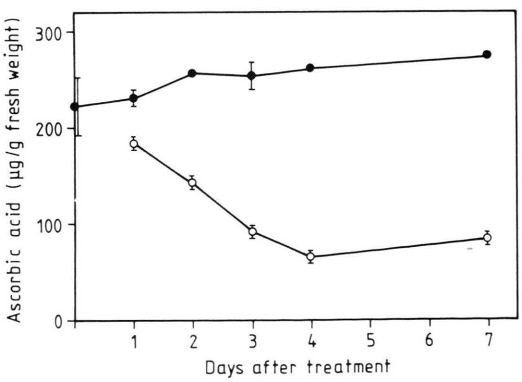

Fig. 1. Decrease of the ascorbic acid content in cotyledon leaves of *Ipomoea hederacea* after postemergence treatment with dichlormid of 7 days old plants. ● Control; ○ treated.

doses (Fig. 2). However, a strong and permanent decrease of the ascorbate levels was obtained in both cases, suggesting a causal relationship between the decrease of the ascorbate levels and the synergistic response.

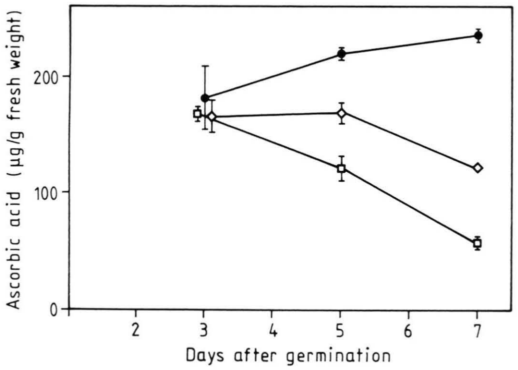

Fig. 2. Decrease of the ascorbic acid content in cotyledon leaves of *Ipomoea hederacea* plants after preemergence treatment with dichlormid. ● Control; ◇ 2 kg/ha dichlormid; □ 4 kg/ha dichlormid.

As mentioned before, a common mechanism of synergism *via* inhibition of herbicide degradation is considered unlikely for all the herbicides studied. Only in the case of metribuzin has an inhibition of herbicide degradation been shown. However, the mechanism of phytotoxic action *via* singlet oxygen generation is common to all the herbicides found here to interact synergistically with either dichlormid or PABA and could possibly be the reason for the unspecific synergism with several widely different compounds. It is therefore suggested that an impaired oxygen scavenging system suffering from unusually low ascorbate levels is the cause of the synergistic interactions observed in the combinations with herbicides that generate excess singlet oxygen.

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