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ACTION OF THE PHENYLPYRIDAZINONE HERBICIDES BASF 33650 AND BASF 44521 ON THE GREEN AND ORANGE CELLS OF *Chlorella fusca*.

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Abstract:

Autotrophically grown *Chlorella fusca* cells were treated with different concentrations of the phenylpyridazinone herbicides BASF 33650 [1-phenyl-4-methoxy-5-bromo-pyridazinone], and BASF 44521 [4-chloro-5-methoxy-2-(α,α,α,-trifluoro-m-tolyl)-3(2H)-pyridazinone] for 96 hours. The treatments induced retardation of cell division, reduction of both total chlorophyll and carotenoid contents, reduction of the Chl a : Chl b ratio, and inhibition of oxygen evolution compared to untreated control. BASF 44521 was more effective as an inhibitor of cell division than BASF 33650. On the other hand, incorporation of these herbicides in the nitrate-rich medium during regreening of orange nitrogen-deficient cells slightly inhibited growth, pigment synthesis, and the development of an oxygen-evolving capability when compared to control. Despite this slight inhibition, cultures treated at all concentrations applied could, at least partially, regreen within 32 h.

Introduction

Many phenylpyridazinone herbicides are well documented bleaching agents in plants. The bleaching induced by these herbicides was reported to be accompanied by many physiological and biochemical responses. These responses include inhibition of oxygen evolution [20], fatty-acid polyunsaturation in the chloroplast galactolipid fraction [14], inhibition of carotenoid biosynthesis [1,11], degradation of 70S ribosomes [1,9], severe disruption of the plastid ultrastructure [2], and inhibition of phytol biosynthesis [15]. However, the primary mechanism of action of these herbicides is still debatable. Controversy exists on whether the mechanism of action involves inhibition of photosynthetic electron transport [20], inhibition of the biosynthesis of photoprotective carotenoids [4], or interference with the regulated formartion of photosynthetic membranes[16]. These findings have mostly been obtained by using phenylpyridazinone herbicides containing N-methylated and N-dimethylated amine group in the side chain. In this test two phenylpyridazinone herbicides with a methoxy group instead of the substituted amine in the side chain, and with or without a trifluoromethyl substitution of the phenyl ring, namely BASF 44521 and BASF 33650, respectively, were used. Investigations involved testing the interference of these two herbicides with growth (cell number) pigment content, and oxygen evolution of green autotrophically grown and orange nitrogen-deficient *Chlorella fusca* cells, a system that proved to be ideal for such investigations [5].

Materials and Methods

Chlorella fusca 211-15 from the Collection of Algal Cultures, University of Gottingen (Germany), was grown in a nitrate-rich medium containing 8 mM KNO_3 [6] for 24 h. Organge nitrogen-deficient cells were obtained by suspending 1 g fresh weight in 200 ml of a similar, but nitrate-sparse medium containing only 0.08 mM KNO_3, with KCl (8 mM) added to maintain potassium ion and osmotic concentrations for six weeks. Both normal green and orange nitrogen-deficient *Chlorella* cells were harvested, washed with sterilized distilled water, and resuspended (10^6 Cells ml^{-1}) in a nitrate-rich medium containing the herbicides. The cultures (200 ml) of both normal green and orange nitrogen-deficient cells were continuously sparged with air at 25°C and were illuminated by a bank of fluorescent lights giving an irradience of 60 Wm^{-2}. Illumination was provided in 12 h day/night cycles for green cells and continuously for the regreening of orange nitrogen-deficient cells.

The substituted phenylpyridazinone herbicides BASF 33650 and BASF 44521 were a kind gift of BASF AG (Germany), and were recristallized twice in hexane prior to use. The herbicides were dissolved in acetone and were added to the cultures to give concentrations of 0.1, 1.0, 10, and 100 $ugml^{-1}$ (acetone concentration was kept below 0.1%).

Cell numbers were determined by using a Bright-line haemocytometer (Richert-Jung, USA). Total chlorophyll and carotenoid contents were determined after Metzner et al.[10]. Photosynthetic oxygen evolution was measured in an oxygen electrode (Rank Brothers, UK) in 4 ml of culture at 25°C and an irradience of 200 Wm^{-2}.

Results

Action on Normal Green *Chlorella* Cells:

Treatment with BASF 33650 up to a tested concentration of 10 $ugml^{-1}$ resulted in 40% inhibition of growth (expressed as cell number) of normal green *Chlorella* cells after 96 hr compared to control (Fig. 1.a). Treatment with BASF 44521 for 96 hr, on the other hand, appeared to be much more effective, resulting in 45% and 60% inhibition of growth at tested concentrations of 1.0 and 10 $ugml^{-1}$, respectively (Fig.1.f). Treatment with the higher concentration of 100 $ugml^{-1}$ of these herbicides severely inhibited growth.

Total chlorophyll content showed 50% reduction upon treatment with both herbicides up to a concentration of 10 $ugml^{-1}$, whereas treatment at a higher level of 100 $ugml^{-1}$ resulted in a more marked reduction of total chlorophyll at the end of the experiment compared to control (Fig.1.b and g.). It is worthnoting that the observed reduction of total chlorophyll was accompanied by a concomitant reduction of the Chl a : Chl b ratio (Fig.1.c and h). In addition, the total carotenoid content of the cells was reduced and their oxygen evolving capability was inhibited upon treatment with these herbicides at all levels applied. (Fig.1.d and i).

Action on Orange Nitrogen-starved *Chlorella* Cells During Regreening:

Regreening of the 6-week-old control orange nitrogen-deficient cells was complete within 32 hours after being transferred to a nitrate-rich medium. The end of regreening was manifested by the attainment of total chlorophyll

Fig.1

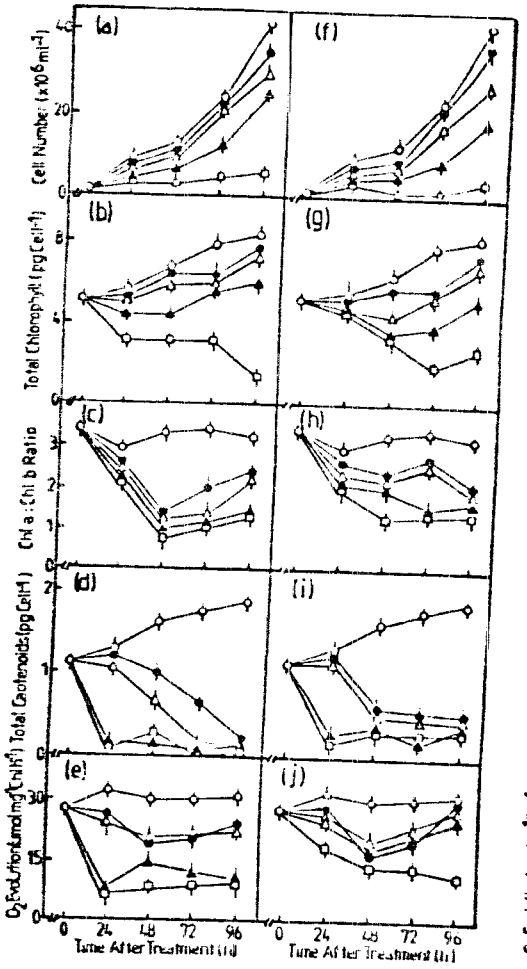

Fig.2

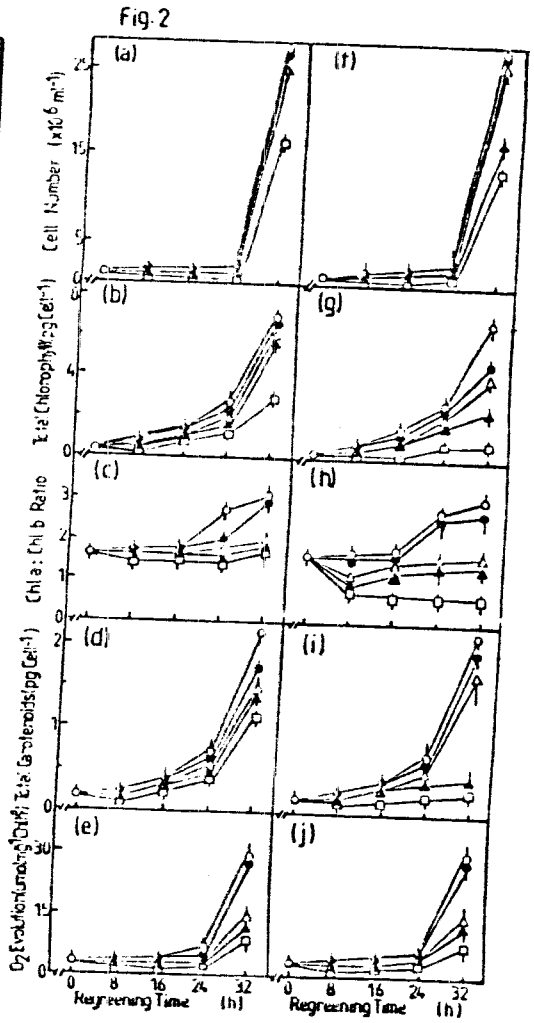

Fig.1. Effects of BASF 77650 (a-e) and BASF 44521 (f-j) on growth (cell number), pigment content, and oxygen evolution of green cells of *Chlorella fusca*. (o) control, (●) 0.1, (▲) 1.0, (▲) 10, and (□) 100 ugml⁻¹, (+ se, n=3).

Fig.2. Effects of BASF 77650 (a-e) and BASF 44521 (f-j) on growth (cell number), pigment content, and development of oxygen evolving capability during regreening of orange nitrogen-deficient cells of *Chlorella fusca*. (o) control, (●) 0.1, (▲) 1.0, (▲) 10, (□) and 100 ugml⁻¹, (+se, n=3).

content, Chl a : Chl b ratio, total carotenoid content, and the rate of oxygen evolution of levels comparable to those of control normal green cells (Fig.1).

Cell division started 24 h after transferring orange cells to the nitrate-rich medium with cell number of control cultures increasing from 0.2×10^7 to 2.7×10^7 cells ml^{-1}. Cell number increased from 0.2×10^7 to 1.6×10^7 cells ml^{-1} upon treatment with 100 $ugml^{-1}$ BASF 33650 or 10 $ugml^{-1}$ BASF 44521 (Fig.2.a and f, respectively) suggesting that BASF 44521 exerted a more pronounced effect on cell division

Total chlorophyll content (Fig.2.b and g), Chl a : Chl b ratio (Fig.2.c and h), and total carotenoid content (Fig.2.d and i) of the regreening cells were relatively reduced, compared to control, upon treatment with both herbicides in a trend that appeared to be concentration-dependent. Furthermore, the development of an oxygen-evolving capability during regreening was inhibited in cells treated with both herbicides at concentrations higher than 0.1 $ugml^{-1}$ when compared to the untreated control (Fig.2.e and j). However, non of the measured parameters was completely inhibited as a result of the treatments and partially recovered during regreening of orange cells.

Discussion

Treatment of green *Chlorella* cells with the tested herbicides up to a concentration of 10 $ugml^{-1}$ resulted in retardation of cell division, whereas treatment at a higher level of 100 $ugml^{-1}$ appeared to result in complete cessation of this process (Fig.1.a and f). However, this observed retardation of cell division was less pronounced

during regreening of orange nitrogen-starved cells in the presence of the two tested herbicides (Fig.2.a and f). Towards the end of the regreening period, cell division of control cells commenced and resulted in a 27-fold increase in cell number (Fig.2.a and f). Treatment with BASF 33650 at a concentration of 100 ugml^{-1} for 32 hr resulted in a 60% reduction in cell number compared to control (Fig.2.a) whereas treatment with BASF 44521 produced the same effect at a lower concentration of 10 ugml^{-1} (Fig.2.f). This result indicates that BASF 44521 possesses a more potent effect on cell division. Inhibition of cell division by phenylpyridazinone herbicides has previously been reported in *Chlorella* [9,13].

Treatment of green *Chlorella* cells with BASF 33650 or BASF 44521 resulted in reduction of the total chlorophyll content (Fig.1.b and g) the Chl a : Chl b ratio (Fig.1.c and h), total carotenoid content (Fig.1.d and i), and oxygen evolution (Fig.1.e and j) of these cells compared to control. These effects partially occurred upon regreening of orange cells treated with these tested herbicides, with BASF 44521 being more effective than BASF 33650, particularly at high tested concentrations (Fig.2). The observed effects come in agreement with previous reports that phenylpyridazinones inhibit pigment synthesis [9,13,14,15], and photosynthetic electron transport [3,7,20].

Orange nitrogen-starved cells are known to be photosynthetically inactive and contain non-appressed lamellae and prolamellar body-like membrane aggregations and were reported to be able to regreen and develop an active photosynthetic apparatus within 24 h upon being transferred

to a nitrate-rich medium [6,12]. Our results (Fig.2) show that despite the presence of the tested herbicides in the nitrate-rich medium and the partial inhibition of both pigment synthesis and the development of an oxygen-evolving capability during regreening, the orange cells could regreen again within 72 hr.

Although the primary mechanism of action, defined by Moreland [11], is questionable for the bleaching phenylpyridazinone herbicides, it has recently been reported that the bleaching process in *Chlorella fusca* cells is dependent on an active cell metabolism and results from the regulated metabolic destruction of the photosynthetic apparatus [16]. Thus, the bleached *Chlorella* cells recover to green, photosynthetically active and herbicide-insensitive phenotype [17-19]. In these reports it has been concluded that the bleaching process is a consequence and answer of the cell metabolism to phenylpyridazinone-induced disregulation and enebles *Chlorella* cells to survive. Our observations perhaps convey a similar message. Despite the degradation of the photosynthetic apparatus in orange cells they could surprisingly regreen and develop a functional photosynthetic apparatus even in the presence of such bleachings herbicides (Fig.2). It is, therefore, suggested that these observed responses are probably reflections of structural and/or functional characteristics of *Chlorella* cells that enable them to survive in the presence of these tested herbicides.

References

1 Bartels P.G. and C.W. Watson (1978) Inhibition of carotenoid synthesis by fluridone and norflurazon. Weed Sci., 26: 198-203.

2 Blum D.E. and J.W. McClure (1980) Developmental effects of Sandos 6706 on activities of enzymes of phenolic and general metabolism in barley shoots grown in the dark or under low or high intensity light. Plant Physiol. 56: 238-242.

3 Bohme H., K.J. Kunert and P. Boger (1981) Sites of herbicidal action on photosynthesis: A Fluorescence assay study. Weed Sci. 29: 371-375.

4 Draber W. and C. Fedtke (1979) Herbicide interaction with plant biochemical systems, in H. Geissbuhler (Ed.), Advances In Pesticide Science, Pergamon Press, Oxford, p.475- 486.

5 Grimme L.H. (1975) Ein biotést-system fur das pre-screening potentiell herbizider phytopharmaka and zur ermittleung allgemeiner toxizitantsdaten mit hydrogenase enthaltenden einzelligen grunalgen. Habil., Erlangen, Germany.

6 Grimme L.H. and R.J. Porra (1974) The regreening of nitrogen-deficient *Chlorella fusca*. I. The development of photosynthetic activity during the synchronous regreening of nitrogen-deficient *Chlorella*. Arch. Microbiol. 99: 173-179.

7 Herkzeg T., E. Lehoczki and L. Szalay (1979) The prompt effect of pyridazinone herbicides on the primary processes of photosynthesis. FEBS Lett. 108: 226-228.

8 Kummel H.W. and L.H. Grimme (1974) The inhibition of carotenoid biosynthesis in green algae by Sandoz H 6706: Accumulation of phtoene and phytofluene in *Chlorella fusca*. Z. Naturforsch., 30c: 333-336.

9 Lichtenthaler H.K. and H.K. Kleudgen (1977) Effect of the herbicide San 6706 on biosynthesis of photosynthetic pigments and prenylquinones in *Raphnus* and in *Hordius* seedling. Z. Naturforsch. 32c: 236-240.

10 Metzner H., H. Raw, and H. Senger (1965) Untersuchungen zur synchronisierbarkeit einzelner pigmentmangel mutanten von *Chlorella*. Planta, 65: 186-196.

11 Moreland D.E. (1980) Mechanisms of action of herbicides. Annu. Rev. Plant Physiol., 31 (1980) 597-638.

12 Pyliotis N.A., D.J. Goodchild and L.H. Grimme (1975) The regreening of nitrogen-deficient *Chlorella* fusca. II. Structural changes during regreening. Arch. Microbiol. 103: 259-270.

13 Sandmann G., K.J. Kunert and P. Boger (1981) Bleaching activity and chemical constitution of phenylpyridazinones. Pestic. Biochem. Physiol. 15: 228-233.

14 St.John J.B. and J.L. Hilton (1976) Structure versus activity of substituted pyridazinones as related to mechanism of action. Weed Sci., 24: 579-582.

15 Tantawy M.M. and L.H. Grimme (1981) The content of phytol in the green alga *Chlorella fusca* and the effect of the pyridazinone herbicide SAN H 6706 on phytol biosynthesis, in G. Akoyunoglou (Ed.), Photosynthesis V. Chloroplast Development, Balden International Science Service, Philadelphia, p. 997-1005.

16 Tantawy M.M. and L.H. Grimme (1982) The bleaching induced by the phenylpyridazinone herbicide metflurazon in *Chlorella* is a metabolic and reversible process. Pestic. Biochem. Physiol. 18: 304-314.

17 Tantawy M.M., Th. Braumann and L.H. Grimme (1984) Uptake and metabolism of the phenylpyridazinone herbicide metflurazon during the bleaching and regreening of process of the green alga, *Chlorella fusca*. Pestic. biochem. Physiol. 22: 224-231.

18 Tantawy M.M., Th. Braumann and L.H. Grimme (1984) A new metflurazon-resistant phenotype of the green alga *Chlorella Fusca*, in C. Sybesma (Ed.), Advances in Photosynthesis Research, Martinus Nijhoff/Dr. W. Jung Publishers, The Hage, p.77-80.

19 Tantawy M.M., Th. Braumann , B. Oelkers, W. Telle, and L. H. Grimme (1986) Influence of herbicides on the phenylpyridazinone-resistant type of the green alga *Chlorella fusca*. Minia J. Agric. Res. & Dev. 8: 109-125.

20 Trebst A. and E. Harth (1974) Herbicidal N-alkylated -ureas and ringclosed N-acylamides as inhibitors of photosystem II. Z. Naturforsch., 29c: 232-235.

21 Urbach D., M. Suchanka and W. Urbach (1976) Effect of substituted pyridazinone herbicides and of difunone (EMD-IT 5914) on carotnoid biosynthesis in green algae. Z. Naturforsch., 31c: 652-655.

الملخـــص العربى

فعـل مبـيدات الحشـائش باسـف ٣٣٦٥٠ و باسـف ٤٤٥٢١ على خلايا طحـلب الكلوريلا الخـضراء و البرتقالـيه

عوملت خلايا طحلب الكلوريلا الخضراء النقاء ذاتيا بمبيدى الفينيل بيريدازينون باسـف ٣٣٦٥٠ [١-فينيل - ٤-ميثوكسى - ٥-بروبو-بيريدازينون] و باسـف ٤٤٥٢١ [٤-كلورو - ٥-ميثوكسى - ٢- (الفا،الفا،الفا-ثلاثى كلورو-م-توليل)-٣ (هيدرو)- بيريدازينون] لمدة ٩٦ سـاعه.

تسببت المعاملات فى تثبيط الانقسام الخلوى و تقليل المحتوى الكلى من الكلوروفيلات و الكاروتينويدات و تخفيض نسبة كلوروفيل ا : كلوروفيل ب . و كذلك تثبيط تصاعد الاكسجين من البناء الضوئى . هذا و قد اظهرت النتائج ان مبيد الحشائش باسـف ٤٤٥٢١ كان اكثر فاعلية كمثبط للانقسام الخلوى عن باسف ٣٣٦٥٠.

تم ادماج هذه المبيدات فى الوسـط الغذائى الغنى بالنترات و المستخدم فى اقامة مستعمرات خلايا الكلوريلا البرتقاليه المختبره الى النيتروجين و نتج عن ذلك تثبيط نمو لعمليات النمو و تحليق الخضوب كما انخفضت قدرة الخلايا على استعادة نشاط تصاعد الاكسجين بمقارنتها بخلايا الكنترول غير المعامله . و جدير بالذكر ان على الرغم من هذا التثبيط النمو فان خلايا المزارع المعـامله التى تمت معاملتها عند جميع التركيزات المختبره استطاعت ان تخضر مرة اخرى - ولو جزئيا - فى خلال ٣٢ سـاعه.