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The effect of the kind of polyethylene film used for cover of low tunnels and plant shading before harvest on nitrate metabolism in butterhead lettuce

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ABSTRACT

In 2005 – 2006 the films of various colours (transparent, white, and black) made of original and recycled material were used in low tunnels and plant shades before harvest of butterhead lettuce 'Melodion'. Nitrate content, nitrate reductase activity as well as ammonium ions and free amino acid content were estimated in leaves. In the first experiment lettuce was grown in the tunnels since day of planting, then the films were taken off 12-13 days before harvest and in the second one – film shades of proper colour and origin were fixed on the low tunnel structure a week before the harvest. Nitrate metabolism was more significantly modified in the second experiment. In both experiments (tunnels and shades) with the reducing of film permeability for PAR, the decrease of nitrate reductase activity and increase of nitrates in lettuce leaves were shown. This phenomenon was accompanied by the

decrease of NH_4^+ ions and in the case of black film by a higher level of free amino acids. The film colour had a greater effect on nitrate contents and their metabolism than the material of the film.

INTRODUCTION

The use of plastic films to improve microclimatic conditions around plants is a frequent agrotechnical procedure in vegetable cultivation. Growing attention has been paid to the problems of environmental protection due to large amounts of used films and unwoven fabrics. Recently researches have been carried out on the effectiveness of using polyethylene films of various colours, including films manufactured of recycled materials in such agrotechnical procedures as mulching (Siwek et al. 2007a, Wojciechowska et al. 2007) and microclimate steering in low tunnels (Siwek et al. 2007b). The obtained results were satisfactory, particularly in respect to the yield of studied plants. Available literature includes few studies on the effect of plant shading with films of various colours and origin on vegetable quality, including the level of nitrates which, in high concentrations, can be hazardous to human health (Umar and Iqbal 2006). This problem is of particular importance in case of leafy vegetables of a short vegetation period, such as lettuce, which are capable of accumulating considerable amounts of NO_3^-. The level of such compounds largely depends on the activity of nitrate reductase, nitrate ions reducing enzyme. In green leaves this process is initiated and stimulated in the presence of light (Lillo 1994). Previous studies demonstrated that films of various colours used in celery shading significantly reduced nitrate reductase activity in petioles although their effect on nitrate content was not explicit (Siwek et al. 2006).

The aim of the present study was to determine the effect of transparent, white and black films made of original and recycled material, used in low tunnels and plant shading before harvest on nitrate reductase activity and nitrate content as well as ammonium ions and free aminoacids in butterhead lettuce ‘Melodion’.

MATERIAL AND METHODS

The study was carried out in 2005 and 2006 at the Experimental Station of Agricultural University in Kraków. The work concerned two experiments with butterhead lettuce ‘Melodion’ grown in low tunnels or shaded for 7 days before harvest. In each experiment seven treatments arranged by using the various kinds of plastic films: (1) control plants (without film), (2) transparent original film, (3) transparent recycling film, (4) white original film, (5) white recycling film, (6) black original film and (7) black recycling film. All polyethylene films of original

(Basell Orlen Polyolefins) and recycled (Gumiplast) material were manufactured by "Jagapol" Film Manufacturing Facility in Krakow.

In the autumn of each year, the field was fertilized with 35 t ha^{-1} manure, 100 kg P_2O_5 ha^{-1} (triple superphosphate) and 200 kg K_2O ha^{-1} (potassium chloride). In the spring, prior to planting, the field was fertilized with nitrogen, in doses of 50 and 75 kg N ha^{-1} for each year of the experiment respectively. Butter head lettuce seeds were sown in the greenhouse on 2 March. Seedlings were planted in spacing 30 × 25 cm on 7 April 2005 and 11 April 2006, respectively. Experimental fields each of the area of 3 m^2 (40 plants each) were randomly arranged in blocks in four replications. Tunnels of the size of 1.5 m (width), 0.75 m (height) and 10 m (length) were covered with film which was kept over lettuce from the day of seedling planting until 4 May 2005 and 10 May 2006. The harvest was carried out on 16 and 23 May, respectively. In the second experiment, shades of film of proper colour and origin were fixed on the tunnel structure of 1.5 m (width) × 1 m (height) × 10 m (length) a week before the harvest. The harvest of the lettuce grown under shades was carried out on 30 May each year. The plants were always harvested at about 9 a.m.

Immediately after harvest 12 heads (3 from each replication) were randomly collected for chemical analyses. Nitrate reductase activity (NR) was determined in accordance with the method described by Jaworski with the modifications of Rożek (1982). NO_3^- and NH_4^+ ion content in plant material was determined using ion-selective electrodes connected to ORION 920A+ ionometer (samples were 0.02M $Al_2(SO_4)_3$ $18H_2O$ extracted). Free amino acid content was determined in accordance with the procedure described by Korenman (1973). All data were subjected to analysis of variance in Statistica program and the Neumann-Keuls test was used to estimate the significance of difference at $p = 0.05$.

RESULTS AND DISCUSSION

Of all plants harvested from the tunnels in both years of the experiment, nitrate content was lowest in lettuce from tunnels covered with transparent and white films and highest in the case with black film (Fig. 1). Apart from that, NR content in lettuce heads was generally inversely related to nitrate content, particularly in the case of low tunnels covered with transparent and black films. Similar dependence was demonstrated in the experiment of plant shading with different kinds of film stretched on low tunnel structure 7 days before the harvest (Fig. 2). NR in lettuce leaves shaded with black original and recycled film was lowest, in 2005 reaching traceability limits. Furthermore, successive decrease of NR as the result of plant shading with films of decreasing light permeability was in this case significantly more drastic than in the experiment with the use of low tunnels.

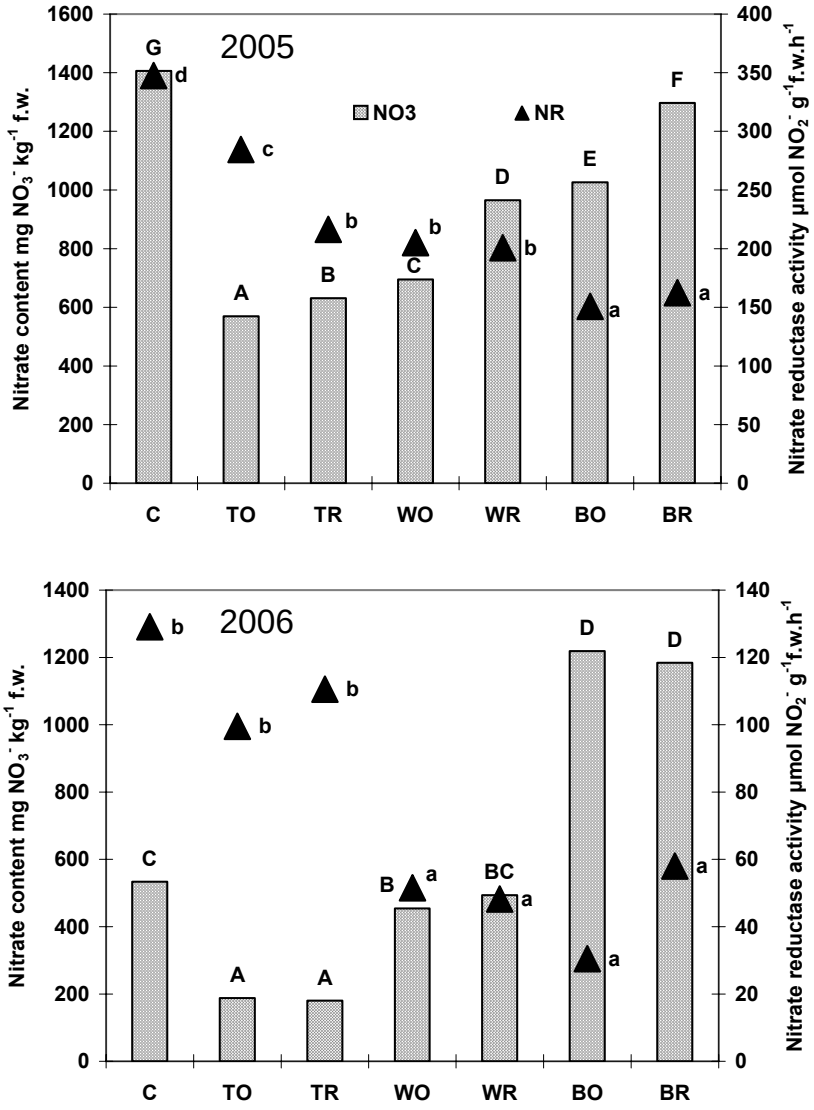

Fig. 1. The effect of plastic film on nitrate content and nitrate reductase activity (NR) in lettuce 'Melodion' grown in tunnels in 2005 – 2006: C – control (without film), TO – transparent original film, TR – transparent recycling film, WO – white original film, WR – white recycling film, BO – black original film, BR – black recycling film. Values designated with the same letters do not differ significantly according to Neumann-Keuls test (p = 0.05); small letters concern NR, capital letters – NO_3^- content.

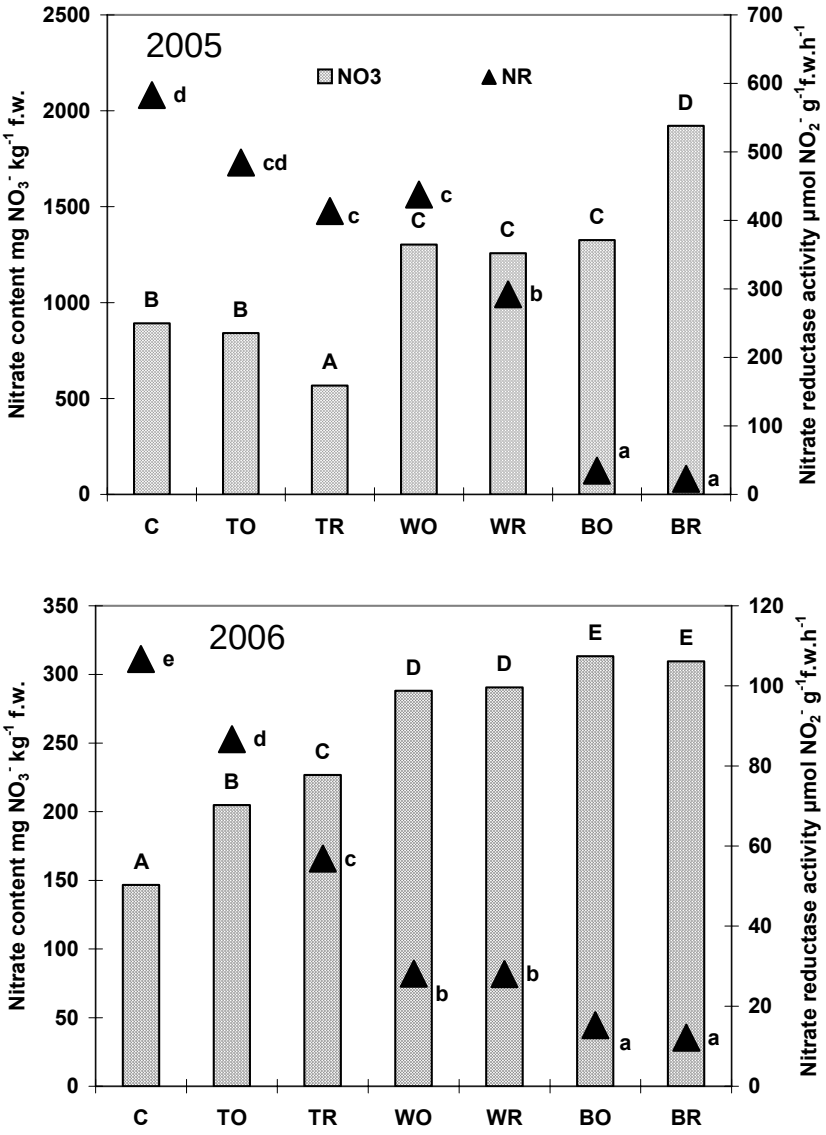

Fig. 2. The effect of plastic film on nitrate content and nitrate reductase activity (NR) in lettuce 'Melodion' grown under shades in 2005 – 2006 (for abbr. – see Fig. 1)

The main cause for the obtained dependences is the inducing effect of light on NR, both at gene transcription stage and post-translational enzyme regulation stage and the formation, during photosynthesis process, of the so-called carbon skeletons necessary for the assimilation of the reduced nitrate form (Lillo 1994, Lillo et al. 2004). Therefore, highest activity of the studied enzyme was always determined in the leaves of unshaded plants (the control). Higher NR content in the plants under shades of higher light permeability was usually accompanied by lower NO_3^- ion content, constituting substrate for the enzyme. In the leaves of lettuce a strict, negative correlation between nitrate content and photosynthetic activity was determined (Behr and Wiebe 1992). From among the films used for cover of tunnels, highest PAR transmission was determined for transparent films – original (87.1%) and recycled (83.7%), followed by white (60% and 63% respectively) and black (33.8% and 38.6%) films (Siwek et. al 2007b). On the other hand, active photosynthetic light transmission by material used for plant shading (second experiment) was the following: white transparent film: 87.1% (original) and 83.7% (recycled), followed by white (21.2% and 20.4% respectively) and black films (0% each) (Siwek et al. 2006). The differences in physical properties of the films can explain the fact that NR content in lettuce cultivated in tunnels covered with black film (Fig. 1), although 2-3 times lower than in the control, was nevertheless significantly higher in relation to the plants shaded with film of the same colour but not-permeable to light (Fig. 2).

Differences in NO_3^- content in the lettuce collected from tunnels covered with film of recycled material in comparison with the original one, regardless of the film colour, observed in 2005 (Fig. 1), as well as high NO_3^- content in the control, are difficult to interpret. In the following year the tendencies did not reoccur. The lowest of all nitrates content in the lettuce cultivated in transparent tunnels might be attributed to the higher NR activity but also high plant yield harvested from such these treatments (Siwek et al. 2007b).

The results presented in Table 1 indicate that in both years of the experiment the contents of ammonium ions and free amino acids in lettuce leaves harvested from tunnels covered with transparent and white films were significantly lower than in the other cases. The results may indicate more effective use of nitrogen in such growth conditions (favourable microclimate) through having been composed into organic compounds of a plant. Studies of De Pinheiro Henriques and Marcelis (2000) demonstrated that good light conditions significantly increase organic nitrogen content in lettuce leaves which, due to accelerated leaf surface growth, enables using light in carbon assimilation process.

Preharvest plant shading with black film, regardless of its origin, had a significant effect on the decrease of NH_4^+ ion content, the fact that was presumably related to drastic limitation of nitrate reduction (Fig. 2). However, free amino acids content remained in this case on higher level as compared to shades made of other

types of films. Similarly, in the experiment with celery, significant increase of the level of free amino acids in stalks as the effect of shading with black film for 16 days before harvest was demonstrated, the fact that might have been related to protein biosynthesis inhibition in such conditions (Wojciechowska and Siwek 2006).

Table 1. The effect of the kind of plastic film on ammonium ions (μmol NH_4^+ g^{-1} f.w.) and free amino acids (mg N 100 g^{-1} f.w.) content in lettuce 'Melodion' grown in low tunnels or under shades in 2005 – 2006

Kind of plastic film	Low tunnels				Shades			
	NH_4^+		Free amino acids		NH_4^+		Free amino acids	
	2005	2006	2005	2006	2005	2006	2005	2006
Control	27.03 d[3]	17.95 d	9.48 f	5.95 e	22.29 d	14.12 e	20.47 f	8.48 c
Transp.[1] original	21.62 b	12.01 b	6.86 c	3.47 a	20.39bcd	10.47 d	14.34 d	7.96 b
Transp. recycling	21.18 ab	13.54 c	7.24 d	3.78 b	21.48 cd	9.89 bc	9.28 a	8.84 d
Mean for transp.	21.40 A	12.77 B	7.05 B	3.63 A	20.93 C	10.18 B	11.79 A	8.40 B
White original	21.53 b	9.66 a	6.09 a	4.80 d	18.72 b	9.54 bc	11.81 c	7.08 a
White recycling	20.86 a	11.26 b	6.26 b	4.33 c	19.62 bc	9.65 bc	10.89 b	6.97 a
Mean for white	21.19 A	10.46 A	6.17 A	4.56 B	19.17 B	9.60 B	11.35 A	7.03 A
Black original	23.21 c	14.07 c	7.47 e	6.72 f	17.06 a	8.85 ab	17.58 e	13.62 f
Black recycling	23.5 c	17.52 d	7.18 d	6.90 f	16.43 a	8.24 a	17.52 e	10.04 e
Mean for black	23.36 B	15.79 C	7.33 C	6.81 D	16.74 A	8.55 A	17.55 B	11.83 C
Original[2]	23.35 X	13.42 X	7.47 X	5.23 X	19.61 X	10.75 X	16.05 Y	9.28 Y
Recycling	23.14 X	15.07 Y	7.54 X	5.24 X	19.96 X	10.48 X	14.53 X	8.58 X

[1]Transparent film
[2]Means for the film material
[3]Statistical analysis concerns each year separately; values designated with the same letters do not differ significantly according to Neumann-Keuls test (p = 0.05); small letters concern interaction material x color of film, capital letters – color of film, X,Y – the film material

Summarizing, it may be stated that film colour has a greater effect on nitrate contents and their metabolism than the material of the film. Nitrate metabolism was more significantly modified in the experiment with the use of shades kept above the plants in the final vegetation phase. With the reducing of film permeability for PAR the decreasing of nitrate reductase activity and increasing of nitrates in lettuce leaves was shown. It was accompanied by decrease of ammonium ions and in the case of black film by a higher level of free amino acids. In all experimental treatments nitrates content did not exceed the level of 2 500 mg NO_3^- per kg of fresh weight, i. e. which is the limit determined by the European Union for lettuce harvested from 1 April to 30 September (Regulation of the Minister of Health No 326 of 13 January 2003).

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WPŁYW RODZAJU FOLII POLIETYLENOWEJ ZASTOSOWANEJ DO UPRAWY W TUNELACH NISKICH I DO CIENIOWANIA ROŚLIN PRZED ZBIOREM NA METABOLIZM AZOTANÓW W LIŚCIACH SAŁATY MASŁOWEJ

Streszczenie: W latach 2005 – 2006 badano wpływ folii o różnym zabarwieniu (bezbarwna, biała i czarna) i pochodzeniu (oryginalna i recyklingowa), rozciągniętych na konstrukcji tuneli niskich oraz wykorzystanych do cieniowania roślin przed zbiorem na zawartość azotanów, aktywność reduktazy azotanowej oraz poziom jonów amonowych i wolnych aminokwasów w liściach sałaty masłowej 'Melodion'. W pierwszym eksperymencie sałata rosła w tunelach od wysadzenia rozsady, a folię zdejmowano na 12-13 dni przed zbiorem plonu, natomiast w drugim – folie rozciągano na konstrukcji tuneli niskich na 7 dni przed zbiorem. Wykazano, że przemiany azotanów były wyraźniej modyfikowane w eksperymencie drugim. W obu doświadczeniach wraz ze zmniejszającą się przepuszczalnością folii dla PAR, obniżającej się aktywności reduktazy azotanowej w liściach sałaty towarzyszyła zwiększająca się zawartość jonów azotanowych, a zmniejszająca – amonowych. W przypadku folii czarnej obserwowano ponadto większy poziom wolnych aminokwasów. Na zawartość i przemiany azotanów większy wpływ miała barwa folii niż rodzaj surowca, z którego ją wykonano.

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