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EFFECT OF NITROGEN FORM, TYPE OF POLYETHYLENE FILM COVERING THE TUNNEL AND STAGE OF FRUIT DEVELOPMENT ON CALCIUM CONTENT IN SWEET PEPPER FRUITS

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Abstract. In this study we hypothesized that the effect of nitrogen form well as light intensity on Ca content in the sweet pepper fruit may depend on stage of fruit maturity. Thus the effect of nitrogen form (100% N-NO_3 or 50% N-NO_3 + 13% N-NH_4 + 37% N-NH_2), type of polyethylene film covering the tunnel (two types of film, Ginegar or Gemme 4S, characterized by various PAR transmissions) and stage of fruit maturity on Ca and dry matter content in sweet pepper fruits were determined. Sweet pepper plants were cultivated in rockwool slabs. The chemical analyses were performed on sweet peppers harvested in five stages of growth and ripening, beginning with fruits of 2–4 cm diameter. Plants supplied with nutrient solution containing nitrate N form accumulated more Ca. The influence of film type covering the tunnel was unsystematic. Irrespective of N form and film type the contents of Ca and dry matter varied depending on fruit growth and ripening stages. Systematic increase in Ca accumulation occurred until reaching the stage of mature-green sweet peppers, while during further ripening the level of Ca decreased. The dynamics of dry matter accumulation were opposite to that of Ca. Additional determination of Ca was performed in mature green fruits, coming from nitrate N and Gemme 4S film (transmitted more light) treatment, both normal and with visible symptoms of blossom-end rot (BER). The analysis was conducted separately in the upper and lower part of fruits. Higher amount of Ca was found in the upper part in normal and BER fruits. Since BER was developed in fruits with Ca content in the lower part equaled to 0.60 $mg \cdot g^{-1}$ d.m., but in 2007 no symptoms of BER were noted in fruits contained only 0.38 $mg \cdot g^{-1}$ of Ca, other co-factors, not only low Ca level in fruit, should be considered in the prediction of BER.

Key words: *Capsicum annuum* L., light intensity, soilless culture, BER

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INTRODUCTION

The content of Ca in sweet pepper fruits depends on numerous environmental and cultivation factors including light intensity, temperature, humidity, plant condition, form of nitrogen fertilization and the presence of other mineral nutrients. Rarely Ca deficiency in fruits in greenhouse production is related to the absence of Ca in the root zone. The most common cause of Ca deficiency is its impaired distribution within the plant [Ho and White 2005]. Ca transport across the plant occurs mainly through xylem, hence Ca distribution within the plant depends on transpiration rate and factors affecting this process. For that reason, symptoms of blossom-end rot (BER) can be observed even on plants with optimal level of Ca in the leaves. Saure [2001] state that there is no conclusive evidence for the role of Ca in the development of BER caused by environmental stress. In the induction of BER by salt stress, free radicals produced in large quantities in these conditions are mainly responsible for BER [Aktas et al. 2005]. Some authors [Nonami et al. 1995] suggest that there are no critical Ca levels in fruits which would allow to predict the incidence of BER.

It is well known that the form of nitrogen in the nutrient solution as well as light intensity can affect Ca content in the sweet pepper fruit [Ho et al. 1993, Sarro et al. 1995, Bar-Tal et al. 2001, Kowalczyk 2006]. However, the question is if these effects depend on its stage of maturity. Thus the aim of the study was to determine the effect of nitrogen form, type of polyethylene film covering the tunnel (two types of foil with various PAR transmissions) and stage of maturity of sweet peppers fruits on the content of Ca and dry matter in fruits.

MATERIAL AND METHODS

The experiment was conducted in year 2007 and 2008 (in both years from the end of March till the end of September), on sweet pepper cv. ‘Spartakus F_1’ cultivated in a plastic tunnel. The tunnel was divided into two parts (I and II), which were covered with different polyethylene film of different PAR transmission. Part I was covered by Ginegar film and part II by Gemme 4S film. Light intensity for Ginegar film was 200–220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in cloudy days and 650–750 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in sunny days. Gemme 4S film transmitted approximately 30% more light, i.e. 300–320 and 950–1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in cloudy and sunny days, respectively. Plants were grown in rockwool slabs. In each part of the tunnel, two independent cultivation sets were arranged with nutrient solution differing in applied nitrogen form, i.e. nitrate (100% N-NO_3) or nitrate-ammonium-urea (50% N-NO_3, 13% N-NH_4, 37% N-NH_2). N-NO_3 was introduced into the nutrient solution in calcium nitrate and potassium nitrate, on the other hand the ammonium nitrate and urea were sources of N-NH_4 and N-NH_2, respectively.

Each cultivation set consisted of 3 channels (replicates) with 63 plants per channel. Fertigation was carried out in a non-recirculating system. Volume and composition of nutrient solution was regulated depending on light conditions and growth stage of plants. The content of available mineral nutrients in the solution was: N – 155, P – 55, K – 210, Ca – 150, Mg – 30 $mg \cdot dm^{-3}$; pH 5.5–5.8, EC 2–2.25 $mS \cdot cm^{-1}$ and, until 2 to 4 fruits of 2 cm diameter were set, N:K ratio was maintained at 1:1.1. The content of Ca in nutrient solution was adjusted to 180–190 mg·dm^{-3} until ripening of fruits from the first node. Plants were pruned to two stems and the apex of the main stem was removed above 15th node. The plant density was 2 plants m^{-2}. Plants were pruned to two leader stems.

During the vegetation, fruits from 9th and 10th nodes were harvested and the content of Ca and dry matter was determined. Analyzes were performed in the following fruit development and ripening stages:

1. fruit buds – fruit of 2–4 cm diameter;
2. maturing green fruit;
3. mature green fruit;
4. turning fruit;
5. fully red fruit.

Only intact fruits without visible symptoms of physiological diseases were taken into analysis.

Six to seven sweet pepper fruits of similar size were chosen for each analysis. After washing in distilled water and removing seeds, fruits were homogenized. Ca content was determined using ICP-OES technique after mineralization in $H_2SO_4/HNO_3/HClO_4$ mixture [Ostrowska et al. 1991]. Dry matter content was determined in the oven at 65°C to constant weight.

The results were subjected to three-way ANOVA with the factors being nitrogen form, type of polyethylene film covering the tunnel and fruit stage of maturity. Significance of differences between means were analyzed by Duncan range test at $P = 0.05$ [SAS 1996].

Additional determination of Ca was performed in mature green fully developed fruits, coming from nitrate N and Gemme 4S film (transmitted more light) treatment, both normal and with visible symptoms of BER. The analysis was conducted separately in the upper and lower part of fruits, cut in the half of fruit length. The analysis was performed on 15 normal and 15 BER fruits.

RESULTS

In both years of cultivation a relatively low content of Ca was found in sweet pepper fruits (0.43–1.23 mg·g^{-1} d.m.; table 1), irrespective of nitrogen form, type of polyethylene film covering the tunnel as well as growth and ripening stage. Fruits from plants grown on nutrient solution containing nitrate form of N, regardless of its maturity stage and type of polyethylene film, accumulated significantly higher amount of Ca than plants supplied with nitrate-ammonium-urea form of N. The effect of polyethylene film covering the tunnel on Ca content in both years was unsystematic. In 2008 more Ca was found in fruits from plants cultivated in a part of the tunnel which was covered by lower PAR transmission film. It was, however, an opposite to the results found in 2007. In both years, a significant interaction between nitrogen form and film type and stage of fruit growth on Ca content in sweet pepper fruits was observed. In 2007, turning fruits (stage 4) harvested from the tunnel covered with film transmitting less PAR light

Table 1. Effect of nitrogen form, type of polyethylene film covering the tunnel and stage of fruit development on calcium and dry matter content in sweet pepper fruits in year 2007 and 2008

Tabela 1. Wpływ formy azotu nawozowego, rodzaju folii pokrywającej tunel oraz fazy dojrzałości owoców na zawartość Ca i suchej masy w owocach papryki słodkiej uprawianej w 2007 i 2008 r.

Film type Rodzaj folii	Nitrogen form Forma azotu	Fruit stage of maturity Faza wzrostu owocu	2007		2008	
			Dry matter (%) Sucha masa (%)	Ca ($mg \cdot g^{-1}$d.m.) Ca ($mg \cdot g^{-1}$ sm)	Dry matter (%) Sucha masa (%)	Ca ($mg \cdot g^{-1}$d.m.) Ca (($mg \cdot g^{-1}$sm)
I*	NO_3	1**	8.03	0.52	7.35	0.88
		2	6.83	0.69	6.93	1.06
		3	7.06	0.74	7.14	1.14
		4	9.18	0.47	9.38	0.98
		5	10.34	0.56	10.37	0.86
	NO_3/ NH_4/NH_2	1	8.18	0.43	7.21	0.87
		2	6.99	0.60	6.79	0.92
		3	7.35	0.72	7.03	1.23
		4	9.53	0.53	9.82	0.90
		5	10.47	0.51	10.28	0.73
II	NO_3	1	8.47	0.47	7.77	0.85
		2	6.96	0.63	6.81	1.01
		3	7.45	0.71	6.91	1.16
		4	9.50	0.53	10.08	1.08
		5	10.70	0.62	10.75	0.66
	NO_3/ NH_4/ NH_2	1	8.52	0.45	7.51	0.59
		2	7.11	0.55	6.88	0.91
		3	7.91	0.60	6.95	0.83
		4	9.90	0.49	10.04	0.89
		5	10.64	0.56	10.66	0.79
Means for Średnia dla:	I		8.40b	0.57	8.23b	0.96a
	II		8.72a	0.56	8.44a	0.88b
	NO_3		8.45b	0.59a	8.35	0.97a
	NO_3/NH_4/NH_2		8.66a	0.54b	8.31	0.87b
	1		8.31c	0.47a	7.46c	0.80c
	2		6.97e	0.62c	6.85d	0.98ab
	3		7.44d	0.69d	7.00d	1.09a
	4		9.53b	0.50a	9.83b	0.96b
	5		10.54a	0.56b	10.51a	0.76c
LSD for NIR dla:	Film type – Rodzaj foli (A)		0.143	n.s. – n.i.	0.130	0.048
	Nitrogen form – Forma azotu (B)		0.143	0.034	n.s. – n.i.	0.048
	Fruit stage of maturity Faza wzrostu owocu (C)		0.227	0.054	0.207	0.076
	A × B		n.s. – n.i.	0.048	n.s. – n.i.	n.s. – n.i.
	A × C		n.s. – n.i.	0.076	0.293	0.107
	B × C		n.s. – n.i.	0.077	n.s. – n.i.	n.s. – n.i.
	A × B × C		n.s. – n.i.	0.113	n.s. – n.i.	0.151

*I – Ginegar film; II – Gemme 4S film; **1 – fruit buds – fruits of 2–4 cm diameter; 2 – maturing green fruits; 3 – mature green fruits; 4 – turning fruits; 5 – fully ripening fruits

Values marked in columns with different letter differ significantly at $P < 0.05$; n.s. – non significant

*I – folia Ginegar; II – folia Gemme 4S; **1 – wielkości 2–4 cm; 2 – dorastające zielone; 3 – wyrośnięte zielone; 4 – zapalone; 5 – całkowicie wybarwione

Średnie wartości w kolumnach oznaczone różnymi literami różnią się istotnie przy $P < 0.05$; n.i. – różnice nieistotne (part I) and fed nitrate-ammonium-urea form of N contained more Ca than fruits from plants supplied with nitrate N. On the other hand, in part II of the tunnel (covered with polyethylene film of higher PAR transmittance and lower dispersion) no significant differences were found in Ca content between turning fruits from plants fertilized with various nitrogen forms. Similarly, no nitrogen form effect was found for Ca content in mature green fruits (stage 3) from part I of the tunnel as well as in fruit buds (stage 1) from plants grown in part II of the tunnel.

Ca content varied depending on fruit growth and ripening stage. Until the stage of green mature fruits (stage 3), a systematic increase in the accumulation of Ca was observed, while during subsequent stages of ripening (coloration) Ca content decreased to the level similar to that in fruit buds (figs. 1–2). These relations were found in both parts of the tunnel, irrespective of nitrogen form.

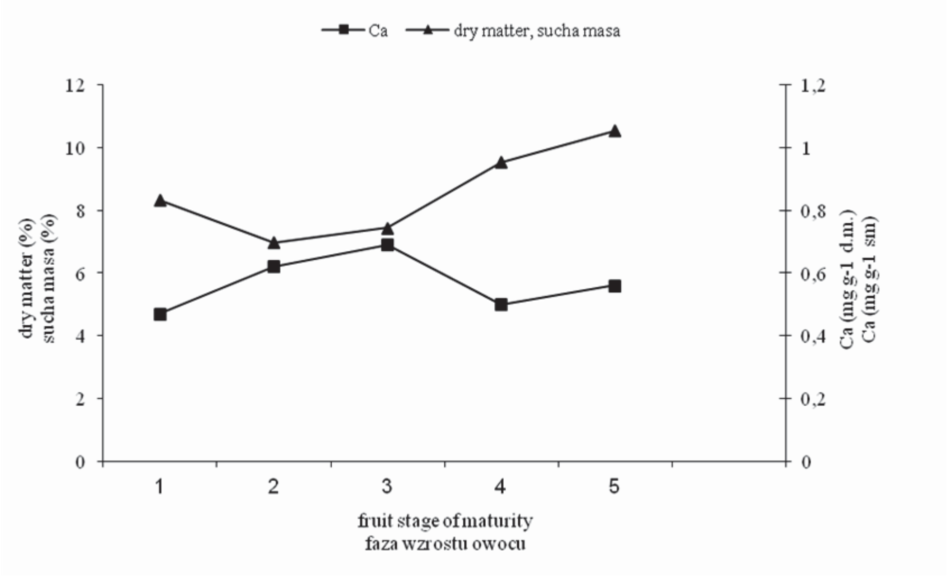

Fig. 1. Calcium and dry matter content in sweet pepper fruits in different fruit stage of maturity (year 2007)

Rys. 1. Zawartość wapnia i suchej masy w owocach papryki słodkiej będących w różnych fazach dojrzałości (2007 rok)

A significant effect of tested factors on dry matter content in fruits was found, with dry matter varying from 6.79 do 10.75% (tab. 1). Fruits from plants grown in the tunnel part covered with film of higher PAR transmittance accumulated more dry matter and this relation was not affected by nitrogen form or fruit growth stage. Only in 2007 did fertilization with various N forms influenced the content of dry matter as plants supplied with nitrate-ammonium-urea form of N contained more dry matter, irrespective of film type. In both years the lowest dry matter content was found in green maturing fruits (stage 2; fig. 1 and 2). In further stages of fruit development (stage 3–5) a systematic

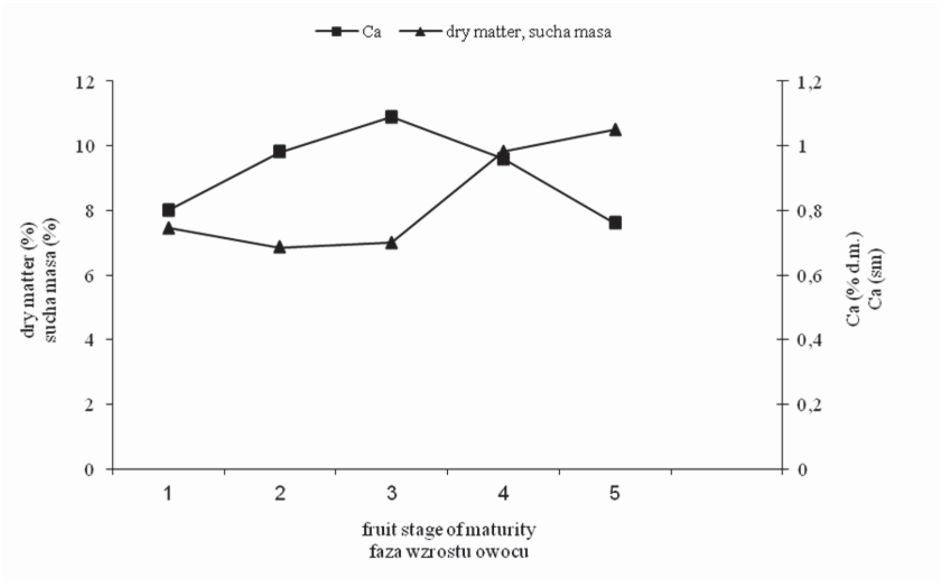

Fig. 2. Dry matter and Ca content in sweet pepper fruits in different fruit stage of maturity (year 2008)

Rys. 2. Zawartość wapnia i suchej masy w owocach papryki słodkiej będących w różnych fazach dojrzałości (2008)

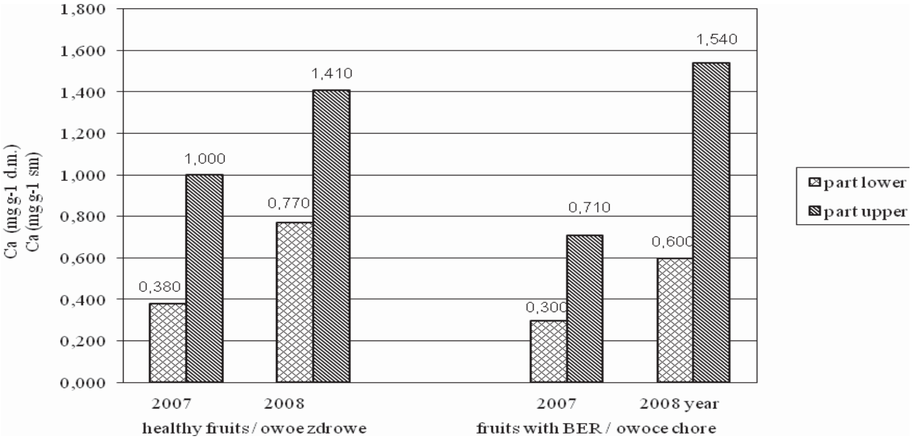

Fig. 3. Ca content in healthy fruits or fruits with visual symptoms of BER in lower and upper part of fruits

Rys. 3. Zwartość Ca w części dolnej i górnej owoców papryki; owoce zdrowe oraz owoce z objawami suchej zgnilizny wierzchołkowej increase in dry matter content was observed with the highest level being in fully mature fruits. In 2008, a significant interaction between type of film and stage of fruit growth was noted for dry matter content.

In both years, in mature green fruits, higher amount of Ca (2 to 3 times more) was found in the upper than in the lower part of the fruit, regardless if they were with and without visual symptoms of BER (fig. 3). In 2007 fruits with BER contained less Ca than normal fruits in both parts of the fruit. Whereas in 2008 fruits with BER contained more Ca than normal ones in the upper part (1.54 *vs.* 1.41 $mg \cdot g^{-1}$ d.m.), and less in the lower part (0.60 *vs.* 0.77 $mg \cdot g^{-1}$ d.m., respectively). It is worth to note that in 2007, Ca content in the lower part of fruits without BER was 0.38 $mg \cdot g^{-1}$ d.m., whereas in 2008, a lower part of fruits with BER contained twice as much Ca (0.60 $mg \cdot g^{-1}$ d.m.).

DISCUSSION

Nitrogen form used for fertilization is an important factor influencing the uptake of mineral nutrients by plants [Sarro et al. 1995]. It is generally known that ammonium ion antagonistically affects Ca uptake. As reported by Bar-Tal et al. [2001], an increase in nitrate N : reduced N ratio in root zone in the range of 0.25 to 4.0 caused higher uptake of Ca and K by sweet pepper plants (quadratic increase). In the present experiment, fruits from plants fertilized with nitrate form of N contained more Ca than fruits from plants fed the mixture of various N forms, irrespective of polyethylene film covering the tunnel. Similar results were also found for leaves of sweet pepper [Kowalska et al. 2010].

The type of polyethylene film covering the tunnel significantly, regardless on N form, affected Ca level in sweet pepper fruits only in 2008, when higher amount of Ca was found in fruits of plants cultivated in the part of tunnel covered by film of lower light transmittance and higher dispersion. Supposedly, these light conditions could have weaken the transpiration rate of leaves and, therefore, its competitiveness with fruits in relation to Ca accumulation. According to Ho et al. [1993] high light intensity, when combined with ammonium nutrition, increased the number of fruits with symptoms of BER.

Changes in Ca accumulation in sweet pepper fruits throughout their growth and ripening were similar to that observed in tomato by Ho and White [2005]. For both nitrogen forms and film types, the lowest content of Ca was found in early stages of growth, *i.e.* in fruit buds and mature turning fruits. By Spurr [1959] and Ho and White [2005] the induction of BER occurs within 2 weeks after fruit setting when the relative growth rate of fruits is the highest due to, among others, an intensive cell proliferation in the distal part of the fruit. Rapid fruit growth in the distal part is not followed by sufficient production of xylem, which transports Ca within the plant [Ho and White 2005].

An increase in dry matter content of sweet pepper fruits in subsequent growth stages was also shown by Marcussi et al. [2004]. The highest dynamics of dry matter accumulation was observed between 80th and 100th day after transplantation. Such an increase in dry matter content reflects accumulation of solid compounds in the fruit, including sugars and proteins. More lights (part II of a tunnel) increased photosynthesis resulting in higher accumulation of these compounds.

Numerous studies indicate that insufficient accumulation of Ca in fruits results in the development of BER in tomato and sweet pepper fruits [Marcelis and Ho 1999, Ho and White 2005, Michałojć i Horodko 2006]. Ca is transported mainly through xylem, by apoplastic flow. Thus, the level of Ca uptake and its distribution in the plant directly depend on transpiration rate. Leaves, having much higher transpiration rate than flowers and fruits, are main receivers of Ca. Therefore, in the studies conducted by Bar-Tal and Aloni [2005], increasing Ca content in irrigation water was not effective in reducing the occurrence of BER in sweet pepper fruits. In the present experiment, plants of sweet pepper received sufficient amount of Ca since the content of Ca in indicator parts of plant (leaves located at the mid-height of plants), determined at two growth stages, *i.e.* in flowering and fruiting stages, ranged from 3.85 to 5.90% d.m. (as published elsewhere by Kowalska et al. 2010], being the values within the range accepted for sweet pepper [Pilbeam and Morley 2007]. Despite optimal Ca level in leaves, its content in fruits was relatively low, varying from 0.69 to 1.09 mg·g^{-1} d.m. and 0.51 to 1.07 mg·g^{-1} d.m. in normal fruits and affected by BER, respectively.

Regardless of the health condition of fruit, Ca accumulation in its distal (lower) part was always less than in the upper one. It reflects the distribution of Ca within the fruit. Nonami et al. [1995] suggest that no critical levels of Ca in fruits can be proposed for predicting of BER as its symptoms develop with various levels of Ca in fruits. The results obtained in the present study may confirm this statement. In 2008, BER was developed in fruits with Ca content in the lower part equaled to 0.60% mg·g^{-1} d.m., while in 2007 no symptoms of BER were noted in fruits contained only 0.38 mg·g^{-1} of Ca. Other co-factors, not only generally accepted low Ca level in fruit, should be considered in the prediction of BER. In order to determine the critical levels of Ca for which symptoms of BER in fruits could occur, a distal part of the fruits should be analyzed rather than whole fruit. Moreover, according to Ho and White [2005] cellular Ca instead of total Ca should be determined.

CONCLUSIONS

1. Nitrate form of N increased Ca content in sweet pepper fruits when compared to nitrate-ammonium-urea N.
2. Higher transmittance of polyethylene film covering the tunnel lowered Ca content in sweet pepper fruits and increased dry matter content.
3. A systematic accumulation of Ca in sweet pepper fruits occurs from 2–4 cm fruit stage until the development of maturing green fruit, after which a gradual decrease in Ca level occurs.
4. Determination of total Ca in the sweet pepper fruit seems to be insufficient for prediction of BER.

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WPŁYW FORMY AZOTU NAWOZOWEGO I RODZAJU FOLII POKRYWAJĄCEJ TUNEL NA ZAWARTOŚĆ WAPNIA W OWOCACH PAPRYKI BĘDĄCYCH W RÓŻNYCH FAZACH ROZWOJU

Streszczenie. W badaniach przyjęto hipotezę, że wpływ formy azotu nawozowego i intensywności światła na zawartość wapnia w owocach papryki zależy od fazy ich rozwoju. W doświadczeniu badano wpływ formy azotu nawozowego (100% N-NO_3 lub 50% N-NO_3 + 13% N-NH_4 + 37% N-NH_2), rodzaju folii pokrywającej tunel (dwa rodzaje folii, tj. Ginegar i Gemme 4S o różnej przepuszczalności dla promieniowania PAR) oraz fazy dojrzałości owoców papryki słodkiej na zawartość w nich Ca i suchej masy. Rośliny uprawiano w rynnach wypełnionych wełną mineralną. Owoce analizowano w pięciu fazach wzrostu i wybarwienia, rozpoczynając od fazy owocu wielkości 2–4 cm. Więcej Ca zgromadziły owoce z roślin zasilanych pożywką azotanową. Wpływ rodzaju folii pokrywającej tunel był niesystematyczny. Zawartość Ca i suchej masy w owocach zmieniała się w zależności od fazy wzrostu i stopnia ich wybarwienia, bez względu na formę azotu nawozowego i rodzaj folii pokrywającej tunel. Do fazy owocu wyrośniętego zielonego następowało systematyczne gromadzenie Ca, po czym w okresie ich wybarwiania obniżenie zawartości tego składnika. Dynamika gromadzenia suchej masy przebiegała przeciwnie. Dodatkowo oznaczano zawartość Ca w owocach wyrośniętych, zielonych, zdrowych oraz z objawami suchej zgnilizny wierzchołkowej. Do analizy wybrano owoce z jednej części tunelu, tj. z tunelu pokrytego folią Gemme 4S (folia o większej przepuszczalności światła) i zasilanego pożywką zawierającą formę azotanową azotu. Analizę prowadzono oddzielnie w części górnej (przyszypułkowej) owocu i części dolnej. Więcej Ca gromadziło się w części górnej owocu niż w części dolnej. Zależność ta dotyczyła tak owoców zdrowych, jak i z objawami suchej zgnilizny wierzchołkowej. Zawartość wapnia w dolnej części owocu z objawami suchej zgnilizny wierzchołkowej wynosiła 0,60 mg·g^{-1} s.m., natomiast w 2007 r. nie obserwowano objawów tej choroby w owocach zawierających jedynie 0,38 mg Ca·g^{-1} s.m. Inne czynniki nie tylko niski poziom Ca powinny być rozpatrywane przy przewidywaniu wystąpienia suchej zgnilizny wierzchołkowej owoców.

Słowa kluczowe: *Capsicum annuum* L., intensywność światła, uprawy bezglebowe, sucha zgnilizna wierzchołkowa owoców

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