Porcine Hemoglobin Hydrolysate as a Biostimulant for Lettuce Plants Subjected to Conditions of Thermal Stress

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SUMMARY. The effects of different kinds of biotic and abiotic stress on crops can be lessened through exogenous application of different biostimulant products. Although some of these products come from enzymatic hydrolysates derived from animal remains, the literature does not contain references to the use of enzymatic hydrolysates obtained from animal hemoglobin, specifically porcine blood. With the aim of evaluating the effectiveness of a product obtained from the enzymatic hydrolysis of porcine hemoglobin (PHH) as a biostimulant that lessens the effects of thermal stress, two experiments were carried out in which lettuce plants (*Lactuca sativa*) were subjected to short-term episodes of intense cold and heat. After these episodes, different doses of the PHH product were administered into the growing medium. Moreover, in the heat episode experiment, one group of plants was first subjected to a heat episode and then administered a commercial biostimulant with the aim of comparing its efficacy with the PHH product. The biometric measurements carried out on the lettuce plants several days after being subjected to the episodes of cold and heat and then either administered or not administered the indicated treatments show that at the highest tested dose, the PHH product promoted a reaction that lessened the harmful effects caused by the intense cold and heat treatments.

In the last decade, a considerable number of products referred to generically as biostimulants have appeared on the market for application to crops. With greater or lesser effectiveness, these products lessen some of the negative effects derived from adverse conditions associated with water, salt, thermal, and mechanical stress, as well as the effects of insect infestations, fungal diseases, nutrient imbalance, and metal toxicity that can affect crops (Boehme et al., 2005). Others have biostimulating effects under conditions that are not especially adverse; they improve performance,

organogenesis, fruit formation, and photosynthetic activity (Liñán, 2004). Some of these products also contain fertilizers that complement their characteristic biostimulating effect (Cadahía et al., 1998).

Many different kinds of biostimulants are available. Some are synthesized from acid hydrolysis of organic aldehydes, while others come from enzymatic fermentation caused by the action of proteolytic yeasts on a specific substrate. Still others are obtained through the hydrolysis of proteins in an acid or basic medium. A large group of biostimulants come from cultures of bacteria, algae, and macrophytes whose proteins can be subjected to hydrolysis,

which releases varying amounts of amino acids (Dimitrieva et al., 2003) and cytokinins (Liu and Huang, 2000). Others are obtained from raw materials of animal origin, such as remains from industrial abattoirs that are subjected to hydrolysis treatments (Liñán, 2004). Certain organic substances in the group of humates and lactates also have bioactive properties, and their effect is enhanced when they are applied in conjunction with specific microorganisms, such as the gram-negative rhizobacteria *Bacillus subtilis* (Boehme et al., 2005). Finally, certain saturated alcohols, such as triacontanol, increase photosynthetic capacity (Syltje, 1988; Xing et al., 2002). Other organic products, such as benzothiadiazole, induce resistance to powdery mildew (*Sphaerotheca macularis*) in strawberry (*Fragaria xananassa*) (Anttonen et al., 2003) and increase the defense mechanisms of plants against different kinds of stress (Kornienko and Kul'nev, 2004). With regard to the effect of certain biostimulants on resistance to water stress, the literature reveals that the cytokinin content of some algae extracts and humic substances produces an increase in endogenous levels of cytokinins. This effect has been related to drought resistance in certain species of Gramineae (Zhang and Ervin, 2004). In terms of tolerance to thermal stress, the literature shows it is related to variations in the activity of antioxidant enzymes (Liu and Huang, 2000).

Animal blood, especially in desiccated form, has traditionally been used as an organic fertilizer for ornamental plants, edible mushrooms, and fruit trees. It has considerable macronutrients and its nitrogen content is especially high (9% to 16% of total dry matter); one fraction of its nitrogen content is released quickly while another is released gradually (Bensa, 1974; Bossard, 1960). Although some

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
0.0929	ft ²	m ²	10.7639
3.7854	gal	L	0.2642
9.3540	gal/acre	L·ha ⁻¹	0.1069
0.2276	inch ² /oz	cm ² ·g ⁻¹	4.3942
1.1209	lb/acre	kg·ha ⁻¹	0.8922
28.3495	oz	g	0.0353
28,350	oz	mg	3.5274 × 10 ⁻⁵
1	ppm	µg·g ⁻¹	1
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

industrial patents refer to products containing fresh animal blood that accelerate seed germination, as well as the use of desiccated blood as an organic fertilizer, no references have been found in the literature or in industrial patents on the use of animal blood or blood derivatives as a raw material for obtaining biostimulants for cultivated plants, except for the product under study. In particular, the use of porcine blood derivatives subjected to hydrolysis as a biostimulant of cultivated plants may represent a resurgence of this raw material.

This article provides an agronomical evaluation of a product obtained through enzymatic hydrolysis of porcine red blood cells (PHH) under controlled pH and temperature conditions and used as a biostimulant for lettuce plants subjected to thermal stress.

Materials and methods

DESCRIPTION OF PHH. The product being evaluated is known commercially as Pepton 85/16 (APC Europe, S.A., Catalonia, Spain). It is of natural origin, water-soluble, and obtained through enzymatic hydrolysis of porcine red blood cells under alkaline conditions. It is available in microgranular form. PHH has a high total content of L- α amino acids (84.83%) and free amino acids (16.52%), a high organic-nitrogen content (12%) and a low mineral-nitrogen content (1.4%), a medium potassium content (4.45%), a high iron content (4061 ppm), a very low heavy-metal content, and is free from pathogenic microorganisms (Table 1).

EXPT. 1: EFFECTS OF PHH ON LETTUCE PLANTS SUBJECTED TO AN EPISODE OF INTENSE HEAT. On 30 Apr. 2004, 50 lettuce seedlings of cultivar Arena (Vilmorin Clause et Compagnie, Paris), a Batavia type, were transplanted

into 1-L pots full of sieved soil with a sandy-loamy texture used for cultivation. The lettuce plants were grown in a glass-roofed greenhouse at the Institut de Recerca i Tecnologia Agroalimentàries (IRTA) Center's Santa Creu property in Cabriels (Maresme County, Catalonia, Spain). Cultivation followed good farming practices and the plants were watered through fertigation twice per day, starting on the day they were transplanted until 17 May 2004 (2-min fertigation sessions). From that date until the end of the experiment, they were watered four times per day (2-min fertigation sessions). They were always watered with a nutrient solution recommended for lettuce (Sonneveld and Straver, 1994) and applied using one drip tube per pot with a 2 L·h⁻¹ nominal flow rate. The composition of the nutrient solution was: 1.25 mmol·L⁻¹ ammonium, 11.0 mmol·L⁻¹ potassium, 4.5 mmol·L⁻¹ calcium, 1.00 mmol·L⁻¹ magnesium, 19.00 mmol·L⁻¹ nitrate, 1.12 mmol·L⁻¹ sulphate.

On 3 June 2004, a batch of 40 lettuce plants was moved from the greenhouse to a chamber for growth under controlled conditions. The ambient temperature was gradually increased over a 3-h period from 25 to 42 °C, where it remained stable for 2 h. The other 10 replicates of the group of 50 remained in the greenhouse. The plants subjected to the intense heat episode were considerably dehydrated. The temperature was then reduced to 25 °C in 1 h. The plants were then immediately returned to the greenhouse they had been taken from to join the 10 replicates that were not subjected to the heat episode.

The plants were distributed in the following different treatment groups:

- No stress control treatment (NSC): Made up of the 10 replicates

that were not subjected to the heat episode described above and were not administered the biostimulant product. Each plant was a replicate.

- Stress control treatment (SC): Made up of 10 replicates that were subjected to the heat episode, but were not administered the biostimulant product after the episode.

- Commercial biostimulant treatment (CB): Made up of 10 replicates that were subjected to the heat episode and then immediately administered a dose of a commercial biostimulant, Siapton (Isagro España, S.L., Madrid, Spain), produced from the hydrolysis of protein material of animal origin with an amino-acid content of 7.9%. The CB has a medium total content of L- α amino acids (54.35%) and free amino acids (7.90%), a medium-high organic-nitrogen content (8.70%), a low mineral-nitrogen content (1.2%), a very low potassium content (0.03%), and very low iron content (<14 ppm). The CB has a very low heavy-metal content and is free from pathogenic microorganisms (Table 2). The CB dose applied was in accordance with commercial recommendations, equivalent to 4 L·ha⁻¹, in an aqueous solution with 250 mL of CB for every 100 L of solution; the density was 15 plants/m².

- Low-dose PHH treatment (PHH-LD): Made up of 10 replicates that were subjected to the heat episode and then immediately administered a dose of PHH equivalent to 2 kg·ha⁻¹ in an aqueous solution with 250 g of PHH for every 100 L of solution; the density was 15 plants/m². Therefore, the dose of PHH administered to each plant was 13.25 mg. In terms of the overall amino-acid content, this dose was equivalent to the dose administered to the CB treatment group.

- High-dose PHH treatment

Table 1. Chemical composition of porcine hemoglobin hydrolysate (PHH).^z

Total organic matter	92.0%	Total amino acids	84.83%	Leucine	10.99%
Total nitrogen	13.4%	Free amino acids	16.52%	Lysine	7.19%
Organic nitrogen	12.0%	Alanine	6.90%	Methionine	0.71%
Ammonia nitrogen	1.0%	Arginine	5.22%	Phenylalanine	5.93%
Nitric nitrogen	0.4%	Aspartic acid	9.93%	Serine	3.88%
Potassium	3.32%	Cysteine	2.25%	Threonine	2.47%
Phosphorous	0.17%	Glutamic acid	7.25%	Tryptophan	IP ^x
Calcium	0.43%	Glycine	4.06%	Tyrosine	1.92%
Magnesium	195 ppm ^y	Histidine	6.34%	Valine	6.79%
Iron	4061 ppm ^y	Isoleucine	0.15%	Proline	2.84%

^zBased on dry weight.

^y1 ppm = 1 µg·g⁻¹.

^xIP = inappreciable.

Table 2. Chemical composition of Siapton (Isagro España, S.L., Madrid, Spain) a commercial biostimulant produced from the hydrolysis of protein material of animal origin.^z

Total organic matter	57.1%	Total amino acids	54.35%	Leucine	2.10%
Total nitrogen	10.0%	Free amino acids	7.9%	Lysine	2.11%
Organic nitrogen	8.7%	Alanine	6.51%	Methionine	0.61%
Ammonia nitrogen	0.8%	Arginine	0.53%	Phenylalanine	1.24%
Nitric nitrogen	0.4%	Aspartic acid	2.36%	Serine	0.46%
Potassium	0.02%	Cysteine	0.02%	Threonine	0.04%
Phosphorous	0.03%	Glutamic acid	7.03%	Tryptophan	0.04%
Calcium	0.29%	Glycine	15.12%	Tyrosine	0.48%
Magnesium	< 1 ppm ^y	Histidine	0.46%	Valine	1.54%
Iron	<4 ppm ^y	Isoleucine	0.98%	Proline	8.32%
		Hidroxypoline	4.42%		

^zBased on dry weight.

^y1 ppm = 1 µg·g⁻¹.

(PHH-HD): Made up of 10 replicates that were subjected to the heat episode and then immediately administered a dose of PHH equivalent to 4 kg·ha⁻¹ in an aqueous solution with 250 g of PHH for every 100 L of solution; the density was 15 plants/m². Therefore, the dose of PHH administered to each plant was 26.50 mg. In terms of the overall amino-acid content, this dose was equivalent to twice the dose administered to the CB treatment group.

Therefore, the experiment consisted of five different treatments: each plot contained one lettuce plant and there were 10 replicates per treatment (n = 10).

Growth was monitored until 14 June 2004 (i.e., 10 d after the heat episode). At the end of the experiment, the following parameters were measured for each of the plants in the experiment:

- Fresh weight of the aerial part of the plants (grams)
- Dry weight of the aerial part of the plants (grams)
- Specific leaf area [SLA (the quotient of the surface area of a circle taken from the centre part of an adult leaf divided by its respective dry weight)] (cm²·g⁻¹).
- Relative growth rate [RGR (the quotient of the increase in dry weight of individual plants during the 10-d period after the heat episode, divided by the plant's respective dry weight at the beginning of the 10-d period and by the number of days in this period)] (g·g⁻¹ per day).

The aim of this article is to evaluate the agronomical effects of the tested products and doses on plant growth. We have therefore used plant-growth analysis parameters such as RGR and SLA (Lambers et al., 1998) instead

of the standard parameters used in ecological studies, such as chlorophyll fluorescence and electrolyte leakage (Sakai and Larcher, 1987). Specifically, the SLA and RGR parameters for fast-growing herbaceous species such as lettuce are frequently high and can be modified considerably when the plants are subjected to stress (Cambridge and Lambers, 1998).

EXPT. 2: EFFECTS OF PHH ON LETTUCE PLANTS SUBJECTED TO AN EPISODE OF INTENSE COLD. On 23 July 2003 a total of 50 'Arena' lettuce seedlings were transplanted into 1-L pots full of sieved soil with a sandy-loamy texture. The lettuce plants were grown in a glass-roofed greenhouse at the IRTA Santa Creu property in Cabrils. Cultivation followed good farming practices and the plants were watered through fertigation on a daily basis with the same nutrient solution used in Expt. 1, and applied using one drip tube per pot with a 2 L·h⁻¹ nominal flow rate.

On 13 Aug. 2003, 40 lettuce plants were moved from the greenhouse to a refrigeration chamber set initially at ambient temperature. After 1 h, the temperature was lowered to -3 °C and maintained for 4 h. After this cold period, the temperature was gradually raised to 30 °C over a 2-h period. The plants were then moved back to the greenhouse. Ten of these plants were administered an aqueous solution containing 60 mg of PHH in 150 mL of water. This treatment was called PHH-60. Another 10 plants were administered 120 mg of PHH in 150 mL of water (PHH-120), and yet another 10 plants were administered 240 mg of PHH in 150 mL of water (PHH-240). The doses of PHH used in this experiment were established

based on the results of preliminary tests (unpublished data) on the effects of PHH on lettuce plants subjected to cold episodes. Each of the 10 remaining plants was administered only 150 mL of water. This treatment was called SC. The 10 plants that were not subjected to the cold episode were also administered 150 mL of water. This treatment was called NSC. The plants remained in the greenhouse for 13 d after administration of the treatments. The same parameters as in Expt. 1 were measured for each of the plants in Expt. 2.

All statistical analyses were carried out using the General Linear Model of SAS (SAS Institute Inc., Cary, N.C.). A randomized design was used when comparing the effects of the treatments on lettuce parameters with individual plants taken as samples. Tukey's mean-separation test for comparison between treatments was used.

Results and discussion

EXPT. 1: EFFECTS OF PHH ON LETTUCE PLANTS SUBJECTED TO AN EPISODE OF INTENSE HEAT. The analysis of variance of each parameter measured allowed us to detect statistically significant differences between treatments (Table 3).

With regard to fresh weight, the results of the PHH-HD treatment were significantly different from those of the SC, CB, and PHH-LD treatments, but not from those of the NSC treatment. Furthermore, the CB treatment had the lowest mean fresh weight of all the treatments under study and was significantly different from all the others. Treatments PHH-LD and SC were not significantly different from one another. These data show that, in terms of the fresh weight of the aerial

part of the lettuce plants, the PHH-HD treatment lessened the effects of the intense heat. However, the lower dose (PHH-LD) was not enough to lessen these effects. The tested dose of biostimulant CB had a depressive effect on the lettuce plants, given that their fresh weight was significantly lower than the ones that had not been administered a biostimulant after exposure to intense heat (SC). On the other hand, an equivalent dose of PHH had a biostimulating effect under test conditions (Table 3).

The results of the means-separation test for the dry-weight variable were similar to those mentioned above, although in this case the mean dry weight of the PHH-HD treatment was significantly higher than that of the NSC treatment. Likewise, the mean dry weight of the PHH-HD treatment was significantly different from and greater than those of the other treatments. The dry weight of the PHH-LD treatment was not significantly different from that of the NSC treatment, but it was significantly different from that of the CB treatment, which was significantly lower than all the others. Therefore, as in the foregoing case, at the tested dose, biostimulant CB reduced the biomass of the aerial part of the lettuce compared to the plants that received the SC treatment, which were not administered a biostimulant after being subjected to the episode of intense heat. Indeed, at the tested dose, CB had an undesirable effect on the lettuce plants (Table 3).

Mean SLA values for treatments PHH-HD, PHH-LD, and NSC were not significantly different from each other, but were significantly greater than those of the SC and CB treatments. This result shows that, in the treatments in which PHH was administered, the product prevented or corrected the effect of the reduction in leaf area in reference to the biomass unit caused by the heat episode the lettuce plants were subjected to. On the other hand, the tested dose of biostimulant CB did not produce this prevention or correction (Table 3).

Mean RGR values for treatments PHH-HD and NSC were not significantly different from each other. The RGR for treatment PHH-LD was significantly lower than those for the previous two treatments, and RGR values for treatments CB and SC were significantly lower than the others, but

Table 3. Means and mean-separation statistics corresponding to the parameters fresh weight, dry weight, specific leaf area, and relative growth rate of 'Arena' lettuce plants subjected to five different treatments: 1) control plants not subjected to a heat episode (NSC), 2) plants subjected to a heat episode (SC), 3) plants subjected to a heat episode and then administered a dose of biostimulant (CB), 4) plants subjected to a heat episode and then administered 13.25 mg^z of porcine hemoglobin hydrolysate (PHH-LD) and 5) plants subjected to a heat episode and then administered 26.50 mg of porcine hemoglobin hydrolysate (PHH-HD) (n = 6).

Treatments	Fresh wt (g) ^z	Dry wt (g)	Specific leaf area (cm ² ·g ⁻¹) ^z	Relative growth rate (g·g ⁻¹ per day)
NSC	299.50 a ^y	16.15 b	383.14 a	0.0965 a
SC	229.83 b	14.23 c	320.51 b	0.0355 c
CB	167.67 c	11.78 d	313.47 b	0.0287 c
PHH-LD	217.83 b	15.52 bc	366.30 a	0.0696 b
PHH-HD	273.83 a	17.87 a	359.71 a	0.0953 a
Significance	***	***	***	***

^z1 mg = 3.5274 × 10⁻⁵ oz; 1 g = 0.0353 oz; 1 cm²·g⁻¹ = 4.3942 inch²/oz.

^yMean separation in column by Tukey's multiple range test at $P \leq 0.001$ (***).

were not significantly different from each other.

A significant regression line was obtained between RGR and SLA values ($RGR = 0.0010 \cdot SLA - 0.2794$; $R^2 = 0.866$; $P \leq 0.05$). The specific conditions of the heat episode the lettuce plants were subjected to probably did not significantly alter the positive correlation between these two variables generally found in these plants (Garnier and Laurent, 1994).

The fresh weight, dry weight, SLA, and RGR of lettuce plants subjected to PHH-HD treatment were 19%, 25%, 12%, and 168% higher than the respective values for plants subjected to the heat episode without subsequent addition of the biostimulant (SC treatment).

EXPT. 2. EFFECTS OF PHH ON LETTUCE PLANTS SUBJECTED TO AN EPISODE OF INTENSE COLD. The results

and corresponding mean separation tests for each treatment and variable analyzed are shown in Table 4.

The mean fresh and dry weights of the lettuce plants subjected to the episode of intense cold and then administered any of the three test doses of PHH (PHH-60, PHH-120, or PHH-240) were significantly greater than those of the plants that were subjected to the episode of intense cold but were not administered the product (SC treatment). The mean fresh and dry weights of the plants that were administered the highest dose of PHH (PHH-240) were not significantly different from those of the NSC treatment (i.e., the plants that were not subjected to the episode of intense cold). Therefore, with regard to the parameters involving the fresh and dry weight of the aerial part of the lettuce plants, the high dose of PHH

Table 4. Means and means-separation statistics corresponding to the parameters fresh weight, dry weight, specific leaf area and relative growth rate of 'Arena' lettuce plants subjected to five different treatments: 1) control plants not subjected to a cold episode (NSC), 2) plants subjected to a cold episode (SC), 3) plants subjected to a cold episode and then administered 60 mg^z of porcine hemoglobin hydrolysate (PHH-60), 4) plants subjected to a cold episode and then administered 120 mg of porcine hemoglobin hydrolysate (PHH-120) and 5) plants subjected to a cold episode and then administered 240 mg of porcine hemoglobin hydrolysate (PHH-240) (n=10).

Treatments	Fresh wt (g) ^z	Dry wt (g)	Specific leaf area (cm ² ·g ⁻¹) ^z	Relative growth rate (g·g ⁻¹ per day)
NSC	101.90 a ^y	4.93 ab	433.50 a	0.0972 a
SC	88.08 c	3.57 c	408.05 b	0.0392 c
PHH-60	97.06 b	4.64 ab	440.06 a	0.0658 b
PHH-120	96.85 b	4.49 b	447.99 a	0.0612 b
PHH-240	102.74 a	5.10 a	440.04 a	0.1040 a
Significance	*	*	*	*

^z1 mg = 3.5274 × 10⁻⁵ oz; 1 g = 0.0353 oz; 1 cm²·g⁻¹ = 4.3942 inch²/oz.

^yMean separation in column by Tukey's multiple range test at $P \leq 0.05$ (*).

neutralized the effects of this drop in temperature.

The SLA values of the PHH-240, PHH-120, PHH-60, and NSC groups were not significantly different from each other, but were significantly greater than the SLA values of the SC treatment.

Mean RGR values of treatments PHH-240 and NSC were not significantly different from each other, but were significantly greater than the RGR values of the other treatments. Moreover, the RGR values of treatments PHH-120 and PHH-60 were not significantly different from one another, but were significantly greater than the RGR values for the SC treatment. In this experiment, the correlation between SLA and RGR was not statistically significant. The specific cold conditions applied probably altered the positive correlation usually found between these variables.

The fresh weight, dry weight, SLA, and RGR of lettuce plants subjected to PHH-240 treatment were 17%, 43%, 8%, and 165% higher than the respective values for plants subjected to the cold episode without subsequent addition of the biostimulant (SC treatment).

The proline and 4-hydroxyproline content of certain biostimulants such as CB is responsible for lessening the damage caused by abiotic stress (Filippini and Bonfiglioli, 2005). The proline and 4-hydroxyproline content of CB is 8.32% and 4.42%, respectively (Table 2). The proline content of PHH is 2.84% (Table 1). Nevertheless, the response of the PHH tested in Expt. 1 (stress caused by intense heat) was better than the response of the CB. Other compounds besides proline may therefore have been responsible for the palliative effects of the heat episode attributed to the hydrolysate derived from porcine blood studied here. Some results from the literature suggest that arginine plays a role in protection from injuries caused by freezing (Sagisaka and Araki, 1983). In fact, the arginine content of the PHH product was quite high (5.22%) (Table 1). After a low temperature episode, absorption of minerals by plants is altered. In Expt. 2, the potassium, phosphorous, and iron supplements contained in treatments

PHH-60, PHH-120, and PHH-240 may have contributed to lessening the effects of the low temperature related to the absorption of minerals. However, PHH's mode of action on plants subjected to different kinds of abiotic stress should be investigated.

In conclusion, the high dose of PHH tested in Expt. 1 (PHH-HD) helped correct the negative effects on lettuce growth caused by the increase in temperature. The high test dose of PHH in Expt. 2 (PHH-240) lessened the harmful effects of the cold, given that the parameters recorded in lettuce plants subjected to treatments PHH-HD and PHH-240 were not significantly different from the corresponding parameters of plants not subjected to episodes of heat and cold.

Literature cited

Anttonen, M., A. Hukkanen, K. Tiilikkala, and R. Karjalainen. 2003. Benzothiadiazole induces defense responses in berry crops. *Acta Hort.* 626:177–182.

Bensa, M. 1974. La pianta in relazione al terreno, p. 101–160. In: *Floricoltura industriale*. Edizione Edagricole, Bologna, Italy.

Boehme, M., J. Schevtschenko, and I. Pinker. 2005. Effect of biostimulators on growth of vegetables in hydroponical systems. *Acta Hort.* 697:337–344.

Bossard, R. 1960. *Cultures florales*. J.-B. Baillièrre et Fils, Paris.

Cadahia, C., E. Eymar, and J.J. Lucena. 1998. Materiales fertilizantes utilizados en fertirrigación, p. 81–121. In: C. Cadahia (ed.). *Fertirrigación: Cultivos hortícolas y ornamentales*. Ediciones Mundi Prens, Madrid, Spain.

Cambridge, M.L. and H. Lambers. 1998. Specific leaf area and functional leaf anatomy in western Australian seagrasses, p. 88–99. In: H. Lambers, H. Poorter, and M. van Vuuren (eds.). *Inherent variation in plant growth*. Backhuys Publ., Leiden, The Netherlands.

Dimitrieva, G., Yüksel, G.U., Minskaya L.A., and T.K. Kalenik. 2003. The nature of a biostimulator activity for cell growth exerted by associative bacterium of laminaria algae. *Proc. Microbiological Investigations on the Far East* 31:41–44.

Filippini, L. and M. Bonfiglioli. 2005. L'impiego dei biostimulanti per la difesa delle colture dagli stress abiotici. *Relazione*

tra sostanze che incrementano l'accumulo di prolina nei tessuti vegetale e autodifesa delle piante. *Phytomagazine* 36:27–31.

Garnier, E. and G. Laurent. 1994. Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. *New Phytol.* 128:725–736.

Kornienko, A. and A.I. Kul'nev. 2004. On increasing efficiency of protection against diseases and stress factors. *Sakharnaya Svekla* 4:34–35.

Lambers, H., H. Poorter, and M. van Vuuren. 1998. Research variation in plant growth rate—Introduction, p. 1–4. In: H. Lambers, H. Porter, and M. van Vuuren (eds.). *Inherent variation in plant growth*. Backhuys Publ., Leiden, The Netherlands.

Liñán, C. 2004. *Vademecum de productos fitosanitarios y nutricionales*. Ediciones Agrotécnicas Sociedad Limitada, Madrid, Spain.

Liu, X. and B. Huang. 2000. Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. *Crop Sci.* 40:503–510.

Sagisaka, S. and T. Araki. 1983. Amino acid pools in perennial plants at the wintering stage and at the beginning of growth. *Plant Cell Physiol.* 24:479–494.

Sakai, A. and W. Larcher. 1987. Frost survival of plants. Response and adaptation of freezing stress. *Ecol. Studies* 62. Springer-Verlag, Berlin.

Sonneveld, C. and N. Straver. 1994. Nutrient solutions for vegetables and flowers grown in water or substrates. *Voedingso-plossingen glastuinbouw Serie, 8*. Glasshouse Crops Res. Sta. Rpts. Naaldwijk, Aalsmeer, The Netherlands.

Syltje, P.W. 1988. How Agrispon works: A paper describing current understandings of the mode of action of the metabolic plant and soil stimulant Agrispon. *Appropriate Technol. Ltd.*, Dallas, Texas.

Xing, C., Y. Hong, C. Rhong, Z. Li, D. Bo, W. Qing, and H. Guang. 2002. Isolation and characterization of triacontanol-regulated genes in rice: Possible role of triacontanol as a plant growth stimulator. *Plant Cell Physiol.* 43(8):869–876.

Zhang, X. and E.H. Ervin. 2004. Cytokinin-containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. *Crop Sci.* 44:1737–1745.