EFFECT OF NITROGEN FERTILIZATION ON *ACTINIDIA ARGUTA* PLANTS VIGOUR AND SOIL CHARACTERISTICS

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Abstract

This research was conducted on commercial plantation in central Poland. The effect of increasing soil N-levels on plant physiological condition and vigour of two Actinidia arguta cultivars –'Weiki' and 'Geneva' was evaluated throughout growing season of 2016. N-levels of 3, 5 and 8 mg N 100 g^{-1} d.w. were examined. Fertilization was conducted in three equal doses. Soil analysis showed decreasing pH and increasing salinity on plots where more nitrogen was supplied. Amount of available P and K was not significantly affected by time or N-levels. Nitrogen content in leaves decreased during the measured time regardless of N-level reaching approximately a half of starting values. Chlorophyll content was more dependent on fertilization as 5 and 8 mg of N inhibited chlorophyll degradation in leaves. The results revealed cultivar differences in relation to nitrogen dose. 'Weiki' cultivar was characterized by 47% higher number of 1-year old long shoots while having 23% smaller leaf area, compared to 'Geneva'. The number of long shoots was strongly dependent on soil N-fertility and time.

Key words: *Kiwiberry, chlorophyll, plant vigour, fertilization, nitrogen*

1. INTRODUCTION

Actinidia arguta (Siebold et Zucc.) Planch, ex Miq. is a dioecious vine originating from Eastern Asia bearing sweet, aromatic fruits called kiwiberry or mini kiwi. In comparison to more popular *Actinidia deliciosa* cv. 'Hayward' fruits, kiwiberry is smaller, with hairless, green or red-green edible skin and can be eaten in whole. Kiwiberry fruits are reported to be a good source of minerals – K, Ca, P, Mg, Mn, Fe (Okamoto and Goto, 2005; Latocha and Krupa, 2008; Latocha et al., 2015 a), and phenolic compounds (Okamoto and Goto, 2005; Krupa et al., 2011; Latocha et al., 2015 b). *A. arguta* fruits are also considered to have a higher vitamin C (Nishiyama et al., 2004; Okamoto and Goto, 2005; Latocha et al., 2015 b), lutein and chlorophyll content (Nishiyama et al., 2005) than *A. deliciosa* fruits. Because of the abovementioned fruit internal quality, kiwiberry is considered to be a health improving fruit. Furthermore, *A. arguta* plants are resistant to low temperatures (up to -30°C in midwinter) and can be successfully grown in colder climate zone (Krupa et al., 2011).

Nitrogen (N) is one of the essential macronutrients needed for proper plant growth, crop production and fruit colouring. N is involved in protein and nucleic acid synthesis and is important for correct structure and functioning of cell membranes. Also, N supply induces larger leaf area (Costa et al., 1997; Bojovic and Markovic, 2009; Gastal and Lemaire, 2002), as well as rapid plant growth and a higher shoots vigour (Smith et al., 1987 b). The positive effect of N on plant vigour can lead to a number of desirable changes. For example, vigour influences number of flowers in the following year and is contributing towards fruit growth and development (Cai and Wang, 2010). The influence of N fertilization on fruit growth was confirmed by Costa et al. (1997). Furthermore, there is a strong linear relation between N and chlorophyll content in leaves (Evans, 1989) as N is a structural element of chlorophyll and affects chloroplasts formation (Tucker, 1999). N deficiency severely reduces the growth of kiwifruit and can be recognized by leaves changing colour from dark-green, through light-green to yellow (Smith et al., 1987 a). On the other hand, excessive use of N fertilizers can be responsible for increased emission of nitrous oxide or pollution of surface and underground water reserves due to leaching (Ladha et al., 2005). Furthermore, excessive nitrogen nutrition causes the overall prolonged vegetation period leading to decrease of winter hardiness in perennials. A lower yield accompanied by worsened storage potential can also be caused by excessive N amount (Costa et al., 1997; Johnson et al., 1997; Pacheco et al., 2008). Controlled fertilization could minimize the environmental pollution and lower the costs of food production. Therefore, there is an ongoing trend to improve N use efficiency (NUE) by developing strategies for better fertilizer management based on soil fertility (chemical analysis) and requirement of N by the crop (Ladha et al., 2005). Studies showed that in order to minimize the N leaching losses, nitrogen fertilisers should be applied at rates that match plant demand and at times when the risk of leaching is low (Jenkinson, 2001, Cameron et al., 2013). Also, spring-applied fertilizer can be used more efficiently and thus leaching from soil can be reduced (Jenkinson, 2001). The fertilizer N-form composition is also important, as inclusion of N-NH_4- increases the N concentration of roots and annual shoots, shoot dry weight and height, leaf dry weight and the number of leaves in comparison to N-NO_3-form (Sotiropoulos et al., 2003).

Actinidia arguta has become increasingly popular in recent years and still little information regarding kiwiberry crop management can be found, including nutrition and fertilization requirements or deficiency diagnostics (leaf nutrient content and time of leaf sampling for chemical analysis). Therefore the aim of this study was to evaluate the effect of N fertilization on its dynamics in the fertilized soil and other soil quality traits, on leaf chlorophyll and nitrogen contents and on plant vigour of two the most popular *Actinidia arguta* cultivars in Europe: 'Weiki' and 'Geneva'.

2. MATERIALS AND METHODS

2.1 Experimental field, weather and soil conditions

Soil and leaf samples were collected from a commercial plantation of *Actinidia arguta* in Bodzew, Mazowieckie State, Poland (51°47'49.9"N+20°48'44.0"E) in 2016. Plants were planted in 2011 with 6:1 female to male ratio. Plots for two cultivars with three N-treatments, and three repetitions each, were tested. Examined soil N-levels were the following: 3, 5 and 8 mg N 100 g^{-1} dry weight (d.w.). Fertilisation was conducted in three equal doses (in amounts dependent on N treatment) of ammonium nitrate (34% N) starting at the beginning of April and continuing 4 and 8 weeks later. The temperature readings for 2016 season were slightly higher than long-term average with overall average difference of 1,6 °C (Figure 1). Average precipitation was also higher compared to long-term average by approximately 25% despite some precipitation fluctuations on month to month basis (Figure 2).

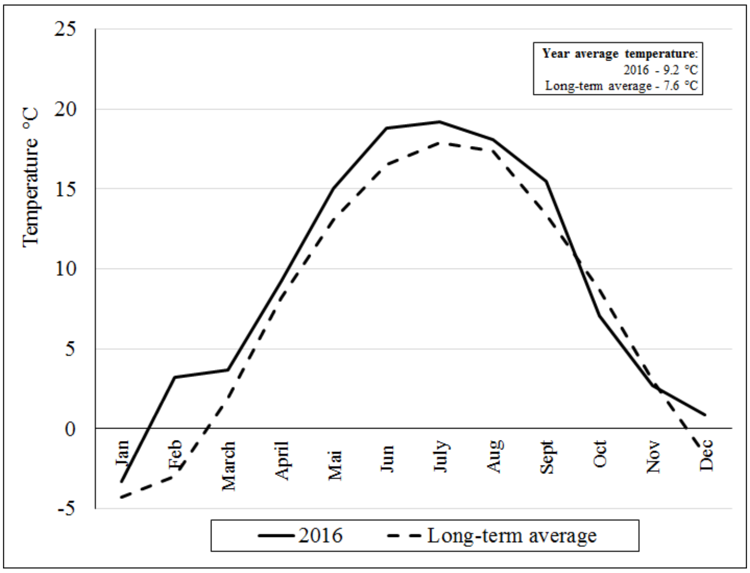

Figure 1. Average monthly and year temperatures registered in Bodzew, Mazowieckie State, Poland

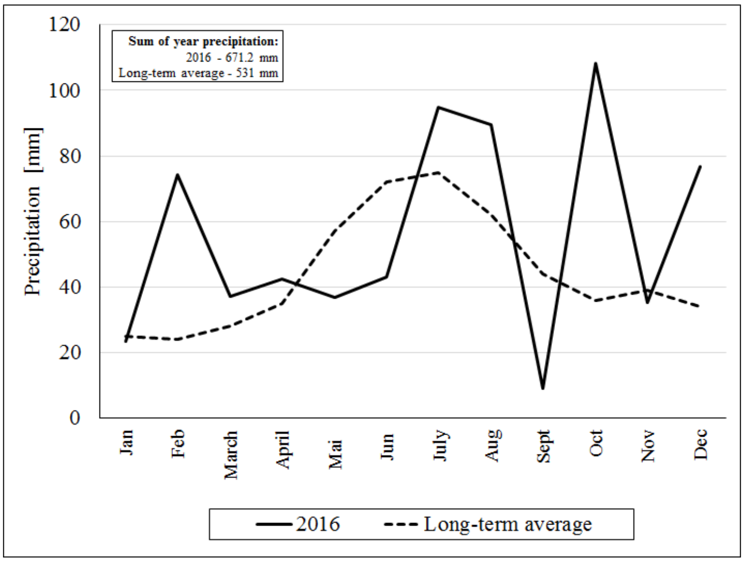

Figure 2. Average monthly and year precipitation registered in Bodzew, Mazowieckie State, Poland

2.2 Soil collection and analysis

Soil samples were collected three times during growing season, every two weeks after N fertilisation, from upper soil layer (0-20 cm) with evenly distributed punctures for every plot using soil sampler. The soil was then mixed, put in a plastic bag, marked and transferred to laboratory. Soil salinity (EC) and pH (in water extract) were measured with 1F10-220 Inolab Level 1 Multiparameter Meter (WTW, Poland). EC was expressed in mS cm^{-1}. Soil was extracted using 0.03M CH_3COOH (in a ratio 1:10; w/v) to assess available N, phosphorous (P) and potassium (K) (universal method). Chemical analyses of obtained extracts were conducted using the following methods: N-NH_4 and N-NO_3 – by distillation according to Bremner modified by Starck, P – colourimetrically with ammonium vanadium molybdate (at 440 nm with U-2900 spectrophotometer, (Hitachi, Japan)), K by flame photometry (PFP-7 Flame Photometer (Jenway Ltd., UK)) and expressed in mg 100 g^{-1} d.w.

2.3 Plant material collection and analysis

Samples of leaves for N and chlorophyll contents analysis were collected in 2016 at 5 time points during growing season starting from 02.06 and continuing through 23.06, 14.07, 04.08, 25.08. Every sample consisted of 24 leaves growing on the same height, of similar size and representing the typical appearance for particular N treatment. Leaves were gathered evenly from shoots on both sides of every plant in the row. Samples were packed into plastic bags, marked and transferred to laboratory. Leaves were dried at 60°C overnight, weighted and ground to a fine powder. Total N content in leaves was evaluated using Kjeldahl method and expressed as % of leaves dry weight. Relative chlorophyll content was measured using CL-01 dual wavelength (620 nm and 940 nm) optical chlorophyll content meter (Hansatech Instruments Ltd, United Kingdom) in 10 leaves per repetition. Leaves used for chlorophyll content evaluation were next scanned and leaf area was determined using software DigiShape (Cortex Nova, Poland) and results were expressed in mm^2. At the end of October number and diameter of 1-year old long shoots having at least 1 meter length were assessed as vigour indicator according to Cai and Wang (2010).

2.4 Statistical analysis and presentation of the data

The obtained results were elaborated by two-way factorial analysis of variance (ANOVA) using software Statistica 12 (StatSoft, Poland). The significance of the differences between means of main effects (time and nitrogen dose) was evaluated using Tukey's honestly significant difference (HSD) procedure at 5% probability level.

3. RESULTS AND DISCUSSION

3.1 Effect of N on selected soil quality traits

Soil salinity and pH in water extract are mostly influenced by the amount and composition of ions present in the soil solution. In our study an increasing N-level applied to the soil was accompanied by changes of soil pH and EC values (Table 1). There was a slight decrease of soil pH (acidification) during growing season. However, only 'Geneva' plots were characterized by significant pH decrease in relation to better soil N-fertility. Ammonium nitrate used in the experiment is known as fertiliser which belongs to N-salts acidifying soil environment what was partially documented. Blevins et al. (1977) reported the soil pH decrease caused by increasing N-levels of fertiliser as well.

Table 1. Soil pH and EC depending on soil N fertility and cultivar of *Actinidia arguta.*

Cultivar								
	'Weiki'				'Geneva'			
pH								
	Time of soil sampling (B)							
N-level (A)	T1**	T2	T3	Av^A	T1	T2	T3	Av^A
N1*	5.33	5.29	5.02	5.21 ns	5.57	5.71	5.49	5.59 b
N2	5.02	5.01	5.14	5.06 ns	5.48	5.38	5.29	5.38 ab
N3	4.99	4.87	4.86	4.91 ns	5.25	5.03	5.16	5.14 a
Av^B	5.11 ns	5.06 ns	5.01 ns	Av^B	5.43 ns	5.37 ns	5.31 ns	
EC (mS cm^{-1})								
	Time of soil sampling (B)							
N-level (A)	T1	T2	T3	Av^A	T1	T2	T3	Av^A
N1	0.30	0.39	0.45	0.38 a	0.33	0.38	0.39	0.37 a
N2	0.36	0.5	0.46	0.44 a	0.37	0.47	0.57	0.47 a
N3	0.41	0.59	0.80	0.60 b	0.44	0.74	0.68	0.62 b
Av^B	0.36 a	0.50 b	0.57 b	Av^B	0.38 a	0.53 b	0.55 b	

*N1, N2, N3 – N soil levels of 3, 5, 8 mg of N 100 g^{-1} d.w., respectively. **Soil was sampled three times (T1, T2, T3): every four weeks, two weeks after application of each of three N-doses. Values in the row (time of soil sampling - B) or column (N- level - A) marked with different letter differ significantly at $P \leq 0.05$ (Tukey HSD test); ns – not significant.

A greater influence of time and N-level on EC was noted (Table 1). EC increased considerably with an increasing N-level as well as during examined time of soil sampling. It is worth noting that increasing N nutrition can be ineffective in negating the adverse effects on yield and growth when too high salinity level is present (Papadopoulos et al., 1983). In our study the highest EC (0.60-0.62) at the highest N-level (Table 1) was only slightly above the recommended level i.e. below 0.50 (Komosa, 2012)

Available N content in soil was significantly affected by applied N dose for both cultivars (Table 2). The soil N content increased from 0.61 (N1) to 1.95 (N3) and from 1.13 to 1.87 mg 100 g^{-1} d.w. for 'Weiki' and 'Geneva', respectively. Moreover, N soil concentration increased gradually with time as subsequent dose of this nutrient was supported, which may indicate the effectiveness of N fertilization. However, these changes were statistically proved only for Geneva cultivar.

Table 2. Nitrogen, phosphorus and potassium contents depending on soil N fertility and cultivar of *Actinidia arguta.*

	Cultivar							
	'Weiki'				'Geneva'			
	N (mg 100g^{-1} d.w.)							
	Time of soil sampling (B)							
N-level (A)	T1**	T2	T3	Av^A	T1	T2	T3	Av^A
N1*	0.43	0.43	0.98	0.61 a	0.52	0.86	2.00	1.13 a
N2	1.19	1.24	1.71	1.38 b	1.00	1.09	2.48	1.52 ab
N3	1.43	2.19	2.24	1.95 b	1.09	1.38	3.14	1.87 b
Av^B	1.02 ns	1.26 ns	1.64 ns	Av^B	0.87 a	1.11 a	2.54 b	
	P (mg 100g^{-1} d.w.)							
	Time of soil sampling (B)							
N-level (A)	T1	T2	T3	Av^A	T1	T2	T3	Av^A
N1	3.44	3.4	4.17	3.67 ns	4.11	3.61	4.11	3.94 ns
N2	3.42	3.05	4.07	3.51 ns	5.66	4.44	5.49	5.20 ns
N3	3.53	3.48	4.89	3.97 ns	4.26	4.49	5.70	4.82 ns
Av^B	3.46 ns	3.31 ns	4.38 n	Av^B	4.68 ns	4.18 ns	5.09 ns	
	K (mg 100g^{-1} d.w.)							
	Time of soil sampling (B)							
N-level (A)	T1	T2	T3	Av^A	T1	T2	T3	Av^A
N1	10.4	9.2	10.4	10 .0 ns	14.9	12.0	14.8	13.9 ns
N2	11.9	9.7	9.8	10.5 ns	14.3	11.7	13.2	13.1 ns
N3	14.6	10.9	9.9	11.8 ns	13.7	12.9	14.0	13.5 ns
Av^B	12.3 ns	9.92 ns	10.0 ns	Av^B	14.3 ns	12.2 ns	14.0 ns	

*N1, N2, N3 – N soil levels of 3, 5, 8 mg of N 100 g^{-1} d.w., respectively. **Soil was sampled three times (T1, T2, T3): every four weeks, two weeks after application of each of three N-doses. Values in the row (time of soil sampling - B) or column (N- level - A) marked with different letter differ significantly at $P \leq 0.05$ (Tukey HSD test); ns – not significant.

The amount of P and K in soil was not affected by time or N fertilisation. Blevins et al. (1977) also did not note any significant change in soil K in regard to increasing N-levels. As in case of N, 'Geneva' plots were characterized by the slightly higher mean contents of P and K. No interactions between main effects (cultivar vs time of soil sampling) with respect to tested soil traits were noted.

3.2 Effect of N on plant vigour characteristics

Nitrogen, as a major component of chlorophyll and amino acids, is considered as the most important nutrient influencing plant growth and yielding (Costa et al. 1997). Leaf analysis showed significant effect of N dose on both chlorophyll and N content in leaves for both cultivars (Table 3 a,b). With better N-supply chlorophyll content successively and significantly increased in 'Weiki' leaves and a bit less clearly in leaves of 'Geneva'. Almaliotis et al. (2004) also reported significant correlation of N and chlorophyll content in leaves of peach. Relative chlorophyll content initially increased with time, starting from 16.0 and 16.0 (T1) reaching the peak of 25.6 and 22.5 near the middle (T3) term for 'Weiki' and 'Geneva', respectively. Chlorophyll determined in leaves from N2-N3 plots seemed to be less susceptible to degradation from third (T3) to fifth (T5) term in comparison to N-1 level (Table 3 a, b).

Table 3a. Chlorophyll and nitrogen contents in *Actinidia arguta* 'Weiki' leaves depending on soil N fertility

Chlorophyll content						
	Time of soil sampling (B)					
N-level (A)	T1**	T2	T3	T4	T5	Av^A
N1*	13.8	17.3	19.0	15.3	13.8	15.8 a
N2	16.5	21.8	26.7	23.4	24.5	22.6 b
N3	17.8	24.2	31.0	28.3	29.0	26.1 c
Av^B	16.0 a	21.1 b	25.6 c	22.3 b	22.4 b	
Leaf N content (% air-dry weight)						
	Time of soil sampling (B)					
N-level (A)	T1	T2	T3	T4	T5	Av^A
N1	3.56	2.31	2.41	1.93	1.52	2.34 a
N2	3.80	2.54	2.60	2.07	1.75	2.55 ab
N3	3.71	2.88	2.68	2.17	1.79	2.64 b
Av^B	3.69 d	2.57 c	2.57 c	2.06 b	1.68 a	

*N1, N2, N3 – N soil levels of 3, 5, 8 mg of N 100 g^{-1} d.w., respectively. **Leaves were sampled five times during the season of 2016, starting at the beginning of June and continuing every 3 weeks (T1-T5). Values in the row (time of leaf sampling - B) or column (N- level - A) marked with different letter differ significantly at $P \leq 0.05$ (Tukey HSD test); ns – not significant.

Table 3b. Chlorophyll and nitrogen contents in *Actinidia arguta* 'Geneva' leaves depending on soil N fertility

Chlorophyll content						
	Time of soil sampling (B)					
N-level (A)	T1**	T2	T3	T4	T5	Av^A
N1*	11.4	14.4	14.9	13.0	11.5	13.0 a
N2	18.3	22.6	25.8	26.6	25.9	23.8 b
N3	18.4	20.7	26.9	26.3	28.0	24.0 b
Av^B	16.0 a	19.2 ab	22.5 b	22.0 b	21.8 b	
Leaf N content (% air-dry weight)						
	Time of soil sampling (B)					
N-level (A)	T1	T2	T3	T4	T5	Av^A
N1	2.62	1.57	1.64	1.55	1.43	1.76 a
N2	3.04	2.25	2.23	1.86	1.76	2.23 b
N3	2.91	2.49	2.38	1.98	1.88	2.33 b
Av^B	2.86 c	2.10 b	2.08 b	1.80 a	1.69 a	

*N1, N2, N3 – N soil levels of 3, 5, 8 mg of N 100 g^{-1} d.w., respectively. **Leaves were sampled five times during the season of 2016, starting at the beginning of June and continuing every 3 weeks (T1-T5). Values in the row (time of leaf sampling - B) or column (N- level - A) marked with different letter differ significantly at $P \leq 0.05$ (Tukey HSD test); ns – not significant.

The leaf N content increased from 2.34 (N1) to 2.64 (N3) and from 1.76 to 2.33% d.w. for 'Weiki' and 'Geneva', respectively. N-fertility clearly affected leaf N status. Application of N increased leaf N content in kiwifruit, as reported by Costa et al. (1997). Smith et al. (1987 a) suggested that well developed leaves of healthy kiwifruit plants usually contain 2.2 to 2.8 % of N in dry matter. In our study leaves of plants growing at 5 (N2) and 8 (N3) mg of N 100 g^{-1} d.w. showed N content mostly in range indicated by those authors (Table 3 a, b). Leaves collected from plots where N level was set to 3 mg N 100 g^{-1} d.w. (N1), exhibited significantly lower N content. On average, 'Geneva' was characterized by a lower leaf N content what may suggests cultivar differences with respect of N uptake efficiency and/or N requirement.

Leaf N content considerably decreased during growing season, changing from approximately 3.7 to 1.7 and from 2.7 to 1.7 % d.w. for 'Weiki' and 'Geneva', respectively. Similar decreasing pattern was reported in kiwiberry plants by Decorte et al. (2015). Smith et al. (1987 b) reported N content in kiwifruit leaves collected from fruit laterals with values between 2.2 and 1.8 % d.w. The kiwifruit leaf N content decrease throughout the season was also confirmed in later research (Costa et al., 1997; Johnson et al., 1997). Montanaro et al. (2014) reported gradual increase of overall N mass (kg ha^{-1}) in kiwifruit leaves achieving peak 150 days after bud break.

The average number of 1-year old long shoots was significantly influenced by both soil N fertility and cultivar (Table 4). The number of shoots increased from 40.3 (N1) to 54.6 (N3) and from 16.0 (N1) to 40.3 for 'Weiki' and 'Geneva' cultivar, respectively. Costa et al. (1997) also reported the increase of shoot number of kiwifruit when more N was applied. The strong influence of N supply on branching was confirmed several times (for review see Gastal and Lemaire, 2002). Cultivar differences in response to N-levels occurred. Number of 'Weiki' shoots remained similar between N1 and N2 level, increasing distinctly at the highest N level. On the contrary, biggest change in number of 'Geneva' shoots was noted between N1 and N2 treatments, with much less increase for N3 level. Also, the 'Weiki' cultivar had approximately 47% more shoots, compared to 'Geneva'. The shoot diameter was not affected by N supply, whereas 'Weiki' cultivar was characterized by 16% thicker shoots, compared to 'Geneva', which was a significant difference. Irrespective of cultivar the highest leaf area values were measured at N2 and the lowest at N1 level. There was an increase in leaf area between N1 and N2 treatment by approximately 23% for 'Weiki' and 55% for 'Geneva' cultivar. The difference was not proven statistically. 'Geneva' showed greater leaf area change as a response to N fertilisation than 'Weiki'. Also, the 'Geneva' cultivar was characterized by significantly bigger leaf area by approximately 29% compared to 'Weiki'. Costa et al. (1997) revealed a significant positive effect of N on kiwifruit leaf area and number of leaves.

Table 4. Number and diameter of 1-year old long shoots and leaf area depending on soil N fertility and cultivar of *Actinidia arguta*

N-level (A)	Number of 1-year shoots			Diameter of 1-year shoots (cm)			Leaf area (mm^2)		
	'Weiki'	'Geneva'	Av^A	'Weiki'	'Geneva'	Av^A	'Weiki'	'Geneva'	Av^A
N1	40.3	16.0	28.1 a	9.7	8.2	8.9 ns	4283	4746	4510 ns
N2	41.4	36.2	40.8 b	10.1	8.8	9.6 ns	5259	7333	6296 ns
N3	54.6	40.3	45.4 b	10.0	8.6	9.3 ns	5155	6916	6035 ns
Av^B	45.4 b	30.8 a	Av^B	9.9 b	8.5 a	Av^B	4899 a	6328 b	

Leaves for area measurement were collected in the beginning of August and shoots were measured at the end of the season, in the end of October. Values in the row (cultivar - B) or column (N- level - A) marked with different letter differ significantly at $P \leq 0.05$ (Tukey HSD test); ns – not significant.

4. CONCLUSIONS

Applied nitrogen had significant impact on various characteristics of *Actinidia arguta* plants including leaf area, leaf chlorophyll and nitrogen contents, number of 1-year shoots, as well as soil pH and EC. At the lowest N-level leaf chlorophyll and nitrogen contents, as well as number of 1-year shoots decreased significantly. The range 5 to 8 mg of nitrogen per 100 g of soil weight seems to be sufficient for high plant vigour and growth. Gradual soil acidification with an increasing N-dose was observed. Therefore, soil pH should be monitored and adjusted if necessary. There was no significant effect of N-fertilization on P and K content in soil, as well as shoot diameter.

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