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Weather Conditions and Biostimulants Influence Nitrogen Acquisition from Different Sources by Soybean Plants

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Abstract: This study aimed to determine the influence of weather conditions (air temperature, precipitation and insolation) on the quantity of nitrogen taken up by soybean plants whose cultivation included an application of two biostimulants: Asahi and Improver, which have been approved for sale. An isotopic dilution method was used which involved an application of mineral fertilisers enriched with the isotope ^{15}N (5%) to detect the quantity of nitrogen fixed from the atmosphere, acquired from the soil and taken up from the fertiliser. Microplots of 1 m^2, organised to form larger units, were planted with soybean. The impact of meteorological conditions on the amount of nitrogen fixed by plants as influenced by the experimental biostimulants was estimated using regression trees based on the C&RT algorithm in STATISTICA 13.3. This procedure yielded regression trees which revealed that, irrespective of the test biostimulant, the quantity of nitrogen fixed from the atmosphere was mainly influenced by the air temperature in July, as indicated by the first and most significant branching of the tree. The poorest fixation of atmospheric nitrogen in plants was observed when the average 24-h air temperature in July was higher than 20.9 °C, the quantities being 20.61, 31.33 and 30.49 kg, respectively, in the control, Asahi- and Improver-treated plots. The superior nitrogen uptake from fertiliser, from 10.64 (for the control) to 14.98 kg (in the Improver-amended units), was found when the air temperatures recorded in July and June did not exceed, respectively, 20.9 and 13.15 °C, and the daily rainfall in July was up to 5.65 mm. The regression tree model associated with the quantity of nitrogen acquired by soybean plants from soil indicates that, just like atmospheric nitrogen and nitrogen taken up from fertiliser, the average daily air temperature in July was the major factor determining the first branching of the tree. When this temperature went beyond 20.9 °C, the lowest uptake of nitrogen from soil was found for control plants.

Keywords: biostimulants; weather conditions; nitrogen fixation; *Glycine max* (L.) Merr.; fertiliser; isotope ^{15}N; legume

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1. Introduction

Nitrogen (N) applied with fertiliser or farmyard manure, and not taken up by crop plants may be released into the atmosphere as nitrogenous greenhouse gases [1] or leach into groundwater [2,3]. Mineral nitrogen should be replaced with alternative sources in order to promote the development of more sustainable agriculture. Legumes meet such criteria because they are able to fix atmospheric nitrogen which is then left in the soil to be used by subsequent crops [4]. The cost-free increase in the yield of the following crop may be higher by even 5–15% [5]. As a result, cultivation of leguminous plants is of importance from the standpoint of soil enrichment in sustainable agricultural systems, in particular in the zone of temperate climates [6–8]. A need exists to develop methods of quantitative prediction of biological nitrogen fixation by leguminous plants so as to assist in making decisions regarding the development and management of sustainable agriculture programs.

In nature, biological fixation of atmospheric nitrogen is only carried out by microorganisms (bacteria), which are prokaryotic organisms. Seed inoculation with *Rhizobium* contributes to a substantial increase in NPK uptake (by as much as 39%) in soybean shoots, and improves soil fertility [9]. Due to the aforementioned advantages, there has been observed in recent years increasing interest in biological methods of enhancing yield quality and quantity. The extent of N fixation by leguminous crops is affected by meteorological conditions during the growing season and growth stage [10,11]. Also, progressing climate change determines soybean yielding and nitrogen uptake associated with it. Cultivation of soybean in Poland has become possible due to climate warming and progress in soybean breeding in Europe. Higher air temperatures during the growing season and monthly precipitation sums in July and August contribute to growing interest in the cultivation of this leguminous species [12].

Analysis of the weather conditions that have the greatest influence on vegetative and reproductive development is an important step to alleviate their impact on soybean yields. In turn, selection of sowing time depending on the cultivation region may minimise losses resulting from adverse atmospheric conditions [13]. An appropriately chosen sowing time translates into light availability, and temperature during soybean plant growth and development determines the appropriate progress of phenological phases [14,15]. Relationships between soybean yielding and meteorological conditions are reported in many papers. However, few authors raise the subject of the effect of meteorological conditions on atmospheric nitrogen uptake. The majority of soybean cultivars have substantial thermal requirements and need a long growing season, which makes it more difficult to grow this species in some regions.

Plant germination, growth and flowering, biological processes taking place in the plant, as well as the course and length of all the plant's phenological phases may be affected by air temperature [16]. In order to germinate, the soybean plant needs temperatures of 7–8 °C followed by 20–25 °C during the period from emergence to flowering, and 22–25 °C during flowering [17]. According to Camara et al. [18], the average daily (24 h) temperature during the growing season should not drop below 15 °C because at lower temperatures plant growth is stunned and no new leaves, stems or pods are formed, whereas temperatures of less than 10 °C may prevent plants from flowering. Too-low temperatures and drought during flowering may cause the dying and abortion of flowers, pod buds and pods [19,20]. Lewandowska [12] claims that the temperature must be at least 10 °C during the growing season of soybean if the plants are to grow and develop optimally. Such a temperature from emergence to full flowering extends the duration of soybean growth stages and the crop is capable of reaching maturity before the first frosts in autumn. Seedling injury due to low temperature (5 °C) was much lower when the seedlings had germinated and emerged at a higher temperature (20 °C).

Another critical period occurs at the flowering stage when biological minimum air temperatures are believed to range from 17 to 18 °C, the optimum range being 22–25 °C [21]. According to Gass et al. [22] soybean plants affected by slight cold during the reproductive stage develop small seedless or deformed pods usually located at the tips of the stem, which is due to flowers remaining closed during cold weather, and lack of pollination. Moderate stress during this growth stage may lead to plants aborting flowers, which results in no pod set or formation of sterile pods along the main stem. Soybean displays lower warmth-related needs ranging from 8 to 14 °C (the level of biological minimum) during maturation, the optimum range being 14–19 °C [23]. Also temperature significantly affects the plant's symbiotic ability and nitrogen fixation efficiency. An optimum temperature range is from 14 to 28 °C. At a lower soil temperature, bacteria find it more difficult to infect root hairs, which results in poorer nodule formation. Moreover, low temperatures affect the efficiency of nitrogen fixation by curbing photosynthesis and transpiration efficiency. By contrast, temperatures which exceed 28 °C inhibit nitrogen fixation although this depends on bacteria strain [24,25]. Unfavourable meteorological conditions during the soybean growing season activate numerous plant protection systems. Under such conditions, plants tend to preserve energy and water resources, and rely on their own reserves for the maintenance of their important functions. An appearance of stressors during the course of the growing season is followed by physiological changes occurring in plants which close their stomata so as to prevent moisture loss and delay the process of photosynthesis [26].

In modern plant cultivation, apart from agrotechnology and meteorological conditions, biostimulants are one of the factors which may beneficially affect yield quality and quantity. Regardless of their composition and origins, growth regulators are supposed to enhance basic biochemical processes taking place in the plant and soil, and, as a result, positively affect plant growth and development, and alleviate the impact of adverse environmental conditions. Products containing stimulating substances do not serve to replace fertilisation but are meant to positively affect the utilisation of fertiliser-derived nutrients predominantly as a result of a more vigorously developed rooting system of plants [27]. Kozak et al. [28] claim that, on average, the highest soybean seed yield (2.53 t ha^{-1}) is obtained following an application of Asahi SL at the budding stage. Compared with control, plants treated with Asahi SL at this stage produced seeds with higher magnesium contents, higher potassium contents being also recorded when the plants were sprayed with the biostimulant at the stage of leaf formation and budding. By contrast, Kocira [29] reports that an application of biostimulant based on seaweed extract and free amino acids reduced the number of microorganisms inhabiting soybean seeds. In this context, thorough monitoring of plants and knowledge of microorganisms inhabiting the plants make it possible to respond promptly and protect the plants with an application of, e.g., biostimulants.

The objective of the research reported here was to determine the effect of meteorological conditions and biostimulants on the quantity of atmosphere-derived nitrogen as well as nitrogen acquired from mineral fertiliser and soil reserves on soybean cv. Abelina grown under the conditions of Central Europe.

2. Materials and Methods

2.1. Description of the Climate of the Study Area

The Polish territory is where a boundary between the areas of Western and Eastern Europe extends, which results in influences of various types of oceanic and continental climates. Also, the mesoclimate of this area differs due to surface conformation. To the east, continental features, which are associated with the duration of winter and temperatures of the winter months, become more pronounced. According to the division of Poland into climatic regions by Woś [30] the study area is part of the Central Mazovian region, and includes the central part of the Mazovian Lowland and the Warszawa Basin. The region is characterised by a large number of very warm and cloudy days, particularly with very warm, cloudy weather without rainfall. Very warm days without precipitation are frequent, too. Compared with other regions, there are slightly fewer very cold days with a freeze. The average annual air temperature ranges from 6.7 to 6.9 °C, and is 15 °C in the summer period. There are 50 to 60 frosty days with 110 to 138 days with a freeze. Annual precipitation falls within a range of 550 to 650 mm and snow cover is present for 80 to 87 days.

2.2. Description of the Experiment

A field experiment was conducted at Łączka, eastern Poland (52°15′ N, 21°95′ E) in 2017–2018. The experiment was arranged as a randomised block design with three replicates to examine the effect of the following levels of the experimental factor (biostimulants): A1—Control (no biostimulant), A2—Asahi, A3—Improver. In each study year, the experiment was set up in a different field of the same locality. Asahi contains sodium p-nitrophenolate, sodium o-nitrophenolate and sodium 5-nitroguaiacolate whereas Improver contains potassium p-nitrophenolate, potassium o-nitrophenolate and potassium 5-nitroguaiacolate. Both the biostimulants contain components that activate metabolic processes, support natural life processes of plants and enhance plant resistance to stressors. Under stress conditions, active substances of the products help the plant overcome symptoms of stress and adapt to these conditions.

Seeds of soybean cv. Abelina were supplied by Saatbau Polska Sp. z o.o., which is the sole supplier of soybean seeds awarded with a certificate confirming that every batch of the produced soybean seed on offer is free of GMO contamination. The producer provides seeds that are ready for sowing as they are treated and coated with nodulating bacteria (*Bradyrhizobium japonicum*). The experimental soil was classified as Haplic Luvisol [31]. Selected soil properties are presented in Table 1. The soil had average organic carbon, total nitrogen and phosphorus contents, a high potassium content and a low magnesium content in terms of plant-available forms.

Table 1. Selected soil properties in the layer 0–0.25 m prior to set-up of the experiment in 2017 and 2018.

Year	pH (in KCl)	C_{org} g kg^{-1}	N_t g kg^{-1}	Fe_t g kg^{-1}	B_t g kg^{-1}	P_{av} (mg kg^{-1})	K_{av} (mg kg^{-1})	Mg_{av} (mg kg^{-1})
2017	7.0	9.5	0.77	998	0.71	55.5	132.7	26.3
2018	7.1	9.1	0.75	995	0.65	57.0	130.5	26.1

Manual sowing of soybean and maize was performed on the 4–5 May at a row spacing of 0.22 m and a depth of 0.04 m. A total of 75 maize seeds were planted per 1 m^2. No herbicides were applied and weeds were removed by hand. In late September, the whole soybean and maize test plants were manually harvested at the stage of full maturity (BBCH 99) by digging them out with a spade pushed to a depth of 0.25 m. This research involved an application of isotope dilution analysis of mineral fertilisers enriched with the isotope ^{15}N (5%) and a simultaneous cultivation of a control crop—maize (*Zea mays* L.)—unable to form a symbiotic relationship with nitrogen-fixing bacteria. Soybean was grown in 1 m^2 microplots in the study years. Biostimulants were applied as assumed in the methodology after soybean plants developed the trifoliolate leaf at the third stem node at the BBCH 13–15 stage, and at the beginning of flowering (BBCH 61). The rates were 0.6 and 1.0 dm^3 ha^{-1} for Asahi SL and Improver, respectively. Nitrogen fertiliser rates applied to soybean and maize corresponded to 30 N·ha^{-1} (3 g N m^{-2}) introduced into the soil as ammonium sulphate $(NH_4)_2SO_4$. Phosphorus and potassium application rates were established to match the soil availability of both the nutrients (Table 1) and amounted to 30 kg P and 90 kg K per 1 ha, respectively. In each study year, the soybean crop was preceded by maize, and it was grown in the conventional soil cultivation system.

2.3. Chemical Analyses

Plant samples of soybean cv. Abelina were collected to determine the following parameters: dry matter content (DM) using the gravimetric method at 70 °C, and enrichment in the isotope ^{15}N using an emission spectrometer NOI-6e. The percentage of nitrogen derived from the atmosphere—NDFA, mineral fertiliser—NDFF, and soil—NDFS in soybeans was computed using the formulas mentioned by Azam and Farooq [32] as well as Kalembasa et al. [33]:

(a) the percentage of nitrogen derived from the atmosphere:

$$\%NDFA = \left[1 - \frac{at\%^{15}\ Nwzbog.fx}{at\%^{15}\ Nwzbog.nfx}\right] \times 100 \quad (1)$$

where:

%NDFA—% of nitrogen fixed from the atmosphere,
at % ^{15}N wzbog. fx—^{15}N isotope excess in soybean,
at % ^{15}N wzbog. nfx—^{15}N isotope excess in the control plant—maize;

(b) the percentage of nitrogen derived from fertiliser:

$$\%NDFF = \left[\frac{at\%^{15}\ Nwzbog.fx}{at\%^{15}\ Nwzbog.nawozu}\right] \times 100 \quad (2)$$

where:

%NDFF—% of nitrogen fixed from fertiliser,
at % ^{15}N wzbog. fx—^{15}N isotope excess in soybean,
at % ^{15}N wzbog. nawozu—^{15}N isotope excess in the fertiliser;

(c) the percentage of nitrogen taken up from soil:

$$\%NDFS = 100 - (\%NDFA + \%NDFF) \quad (3)$$

where:

%NDFS—% of nitrogen taken up from the soil,
%NDFA—% of nitrogen fixed from the atmosphere;
%NDFF—% of nitrogen acquired from the fertiliser.

The nitrogen pool (by convention denoted as 'from the soil'—NDFS) comprises all other sources apart from the atmosphere and mineral fertiliser.

2.4. Analysis of Meteorological Conditions

Meteorological observations were conducted in the study years. Air temperature measurements were taken three times per 24 h at 06.00 UTC, 12.00 UTC and 18.00 UTC. Atmospheric precipitation was measured once every 24 h (daily) at 06.00 UTC. Insolation was measured once daily after sunset. The observations were used to characterise the soybean growing season by calculating average monthly air temperatures, monthly atmospheric precipitation sums and monthly insolation. Sielianinov's hydrothermal coefficient is a parameter that combines air temperature and atmospheric precipitation to determine water relationships in the environment [34–36]. In order to determine periods of an occurrence of drought (Table 2), Sielianinov's hydrothermal coefficient was calculated following the formula:

$$K = \frac{P}{0.1\sum t} \quad (4)$$

where:

P—monthly atmospheric precipitation sum,
Σt—monthly sum of air temperature.

Table 2. Classification of the values of Sielianinov's coefficient.

Value	Classification
≤0.4	extremely dry
(0.4; 0.7>	very dry
(0.7;1>	dry
(1; 1.3>	quite dry
(1.3; 1.6>	optimum
(1.6; 2>	moderately wet
(2; 2.5>	wet
(2.5; 3>	very wet
>3	extremely wet

2.5. Statistical Analyses

The effect of meteorological conditions on nitrogen acquisition by soybean plants was estimated in STATISTICA 13.3 using the model of regression trees based on the algorithm C&RT. A regression tree uses the sum of squares and regression analysis to predict values of the outcome (target) variable. The essence of this analysis is to determine a set of 'if–then' logical (split) conditions. During tree building, recursive intervals are formed which lead to the formation of subsets. Construction of the tree is performed so as to ensure the variance is as small as possible. The subsets form a hierarchical structure and are called branches or split nodes (if they undergo further divisions), and leaves or terminal (end) nodes (if there are no further splits beyond them). The process of decision tree construction begins from the main (root) node which is divided into two or more subordinate nodes in order to reduce the sum of squares for the node. For every node to be split, a predictor which contributes to the smallest sum of squares is selected. Variance and the criterion of minimum number of cases in the node being split were the criteria for pruning (for $n \geq 18$).

3. Results and Discussion

3.1. Analysis of Meteorological Conditions

Analysis of precipitation and thermal conditions in the growing seasons of soybean and maize revealed their marked variation in individual study years (Figure 1). The highest precipitation sum was recorded in September (112 mm) and July (96 mm) in 2017 and 2018, respectively. The lowest precipitation sums in both the study years (2017 and 2018) were observed in May (respectively, 46 and 26 mm) and August (respectively, 53 and 29 mm). In 2018, the growing season was considerably warmer compared with 2017. The average monthly air temperature in all the months of the growing season in 2018 was higher compared with 2017. In both the study years, August was the warmest month in the growing season (19.0 °C in 2017 and 19.9 °C in 2018), May and September being the coldest months in 2017 (respectively, 13.1 and 13.9 °C).

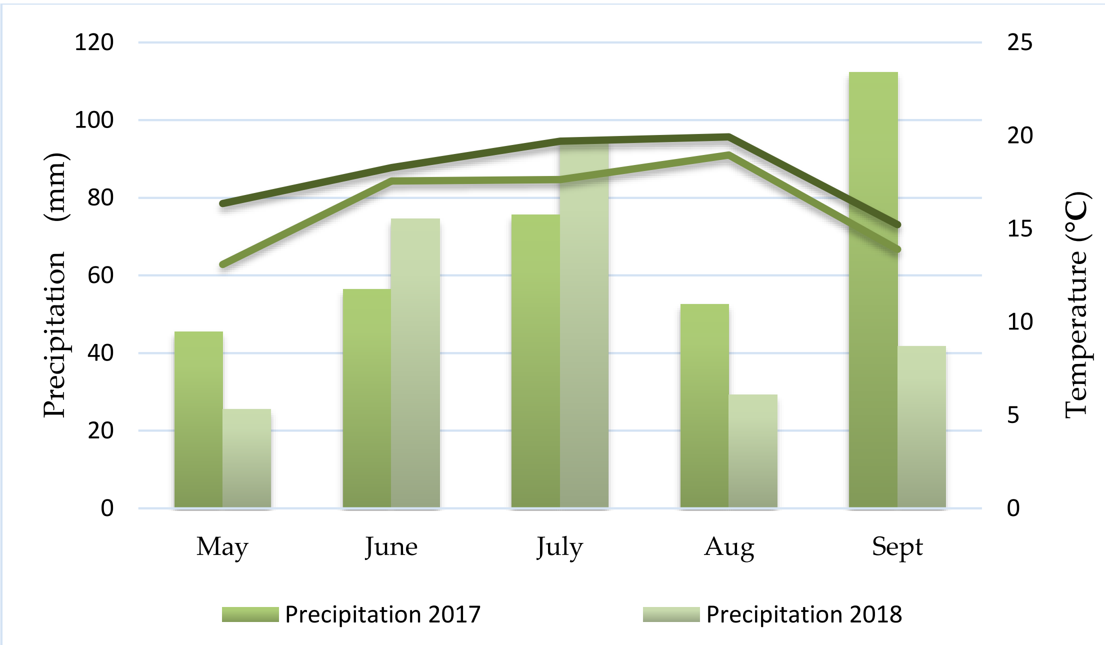

Figure 1. Air temperature and atmospheric precipitation values in 2017 and 2018.

Analysis of insolation, that is, the length of time when direct radiation reaches the earth's surface, demonstrated that the average insolation sum of the growing season was lower in 2017 than 2018 (234 vs. 289 h) (Figure 2). The highest monthly values of this parameter were recorded in May (347 h), June (303 h) and August (306 h) in 2018 whereas the lowest value was obtained for September 2017 (119 h).

Values of Sielianinov's hydrothermal coefficient show that, in 2017, May and June were quite dry, July was optimum and was followed by a dry August and very wet September (Figure 3). In 2018, May and August were very dry, June and July were optimum and September was dry.

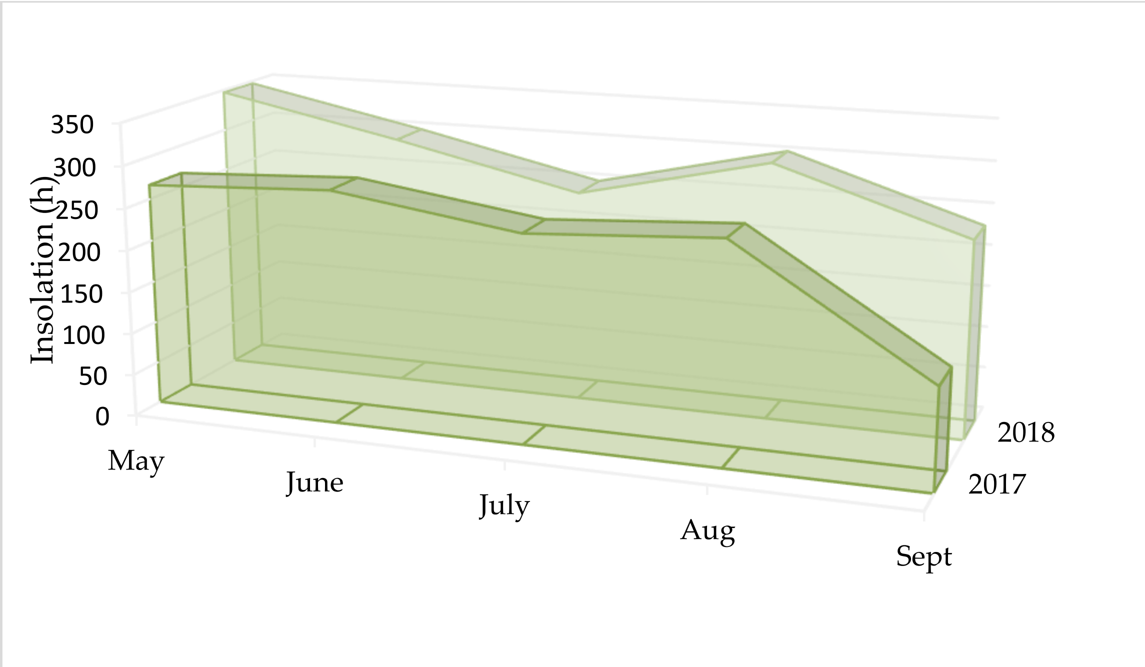

Figure 2. Insolation in 2017 and 2018.

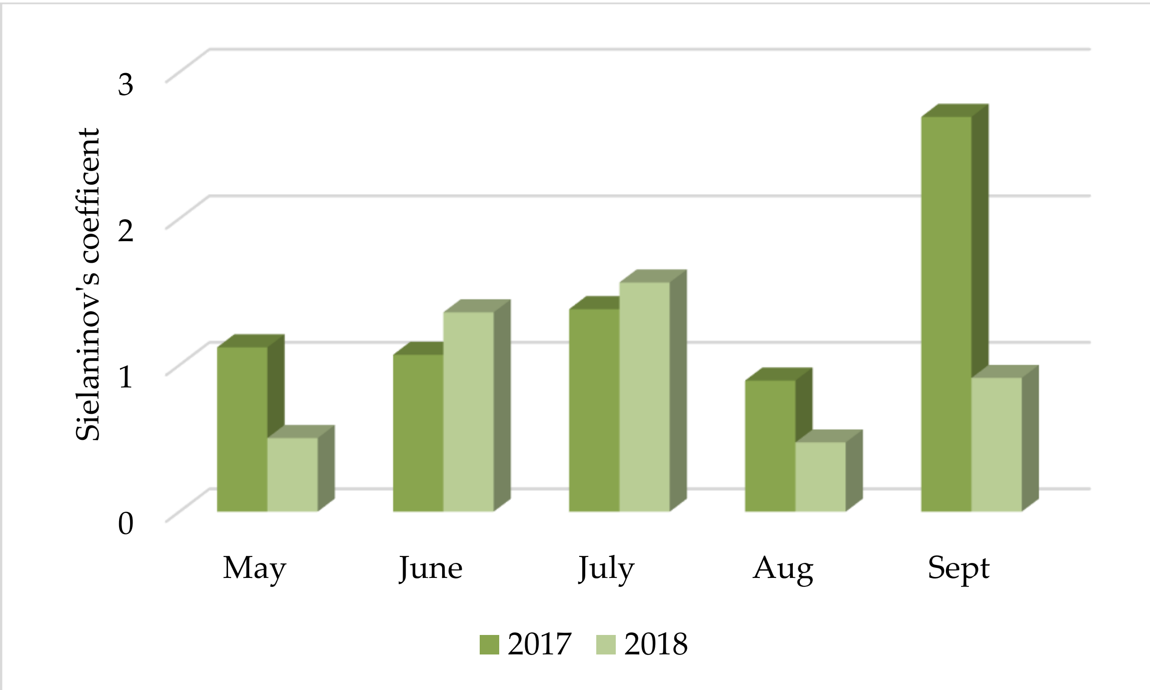

Figure 3. Values of Sielianinov's coefficient in the years 2017–2018.

Table 3 presents variation in meteorological conditions in the study years. Air temperature was the most variable in May 2017 as it ranged from 3.5 to 20.2 °C, the coefficient of variation being 31.9%. In the remaining months of 2017 and 2018, the coefficient values fell within the range of 12.3 to 19.3%. Atmospheric precipitation varied markedly in the study years as, in all the months, the coefficient of variation was higher than 200%. The highest variation in insolation was recorded in September 2017 (V = 105.7%), it being the lowest in August 2018 (V = 38.8%).

Table 3. Minimum and maximum values and coefficient of variation for air temperature, atmospheric precipitation and insolation.

Air Temperature (°C)						
Months		May	June	July	Aug	Sept
2017	Min.	3.5	5.3	14.0	13.6	8.7
	Max.	20.2	22.7	21.8	24.9	18.8
	V	31.9	17.9	12.3	17.6	16.9
2018	Min.	9.2	12.4	12.0	13.5	7.6
	Max.	21.3	22.4	23.5	24.9	21.2
	V	17.6	14.9	13.7	15.6	19.3
Precipitation (mm)						
Months		May	June	July	Aug	Sept
2017	Min.	0.0	0.0	0.0	0.0	0.0
	Max.	22.0	12.0	25.0	20.0	23.0
	V	244.4	206.8	290.4	225.4	275.3
2018	Min.	0.0	0.0	0.0	0.0	0.0
	Max.	10.0	5.0	19.0	13.0	19.0
	V	205.4	267.8	181.9	264.7	183.3
Insolation (h)						
Months		May	June	July	Aug	Sept
2017	Min.	0.2	0.8	1.4	0.0	0.0
	Max.	15.2	15.5	14.7	14.1	12.5
	V	49.0	45.0	43.4	40.9	105.7
2018	Min.	0.0	0.6	0.0	0.0	0.7
	Max.	15.3	15.5	14.8	14.1	11.7
	V	39.0	42.1	51.6	38.8	43.8

Min.—minimum value, Max.—maximum value, V—coefficient of variation (%).

3.2. Analysis of the Effect of Meteorological Conditions on Nitrogen Fixation from the Atmosphere

The constructed regression tree model includes five terminal nodes which indicated that meteorological conditions affected nitrogen fixation from the atmosphere by control soybean plants. When the daily temperature (averaged across 24 h) in July was no more than 20.9 °C and daily insolation in August did not exceed 8.15 h, soybean plants fixed the largest amount of nitrogen from the atmosphere (53.3). The lowest quantity of this nutrient was derived from the atmosphere when the average daily temperature in July went beyond 20.9 °C. The amount of fixed nitrogen was also affected by daily precipitation sums, which was observed when the average daily temperature in July was lower than 20.9 °C, daily insolation in August exceeded 8 h and the average daily temperature in June was not higher than 21 °C (Figure 4). A well-developed deep rooting system, and hairs (which move to be located in a position parallel to solar radiation) present on the leaf surface contribute to soybean being a drought-resistant crop [23]. According to some authors, drought and high temperature negatively affect the symbiosis between soybean and nodulating bacteria (*Bradyrhizobium japonicum*) [37]. Korsak-Adamowicz et al. [38] reported the lowest numbers of nodules in years when the precipitation sum during flowering was lower than 20 mm, and the average air temperature was at least 20 °C. Moreover, Sadeghipour and Abbasi [39] pointed to diminished activity of symbiotic bacteria and a lower number of pods per plant, seeds per pod, seed weight and seed yield resulting from drought-induced stress. Additionally, severe drought causes less vigorous root growth and, as a result, diminishes the possibility of nodule formation. Thus, nitrogen (N_2) fixation by leguminous plants is affected by soil drying. Under conditions of water shortage, losses suffered by soybeans are lower due not only to CO_2 accumulation resulting from poorer development of leaf surfaces, but also to reduced N_2 accumulation [40]. Several factors have been associated with inhibited N_2 fixation including lower oxygen availability, a reduced carbon flux to nodules, declining nodule sucrose synthase activity and an increase in ureides and free amino acids [41–43]. According to Kapusta [44], high air temperature (23–27 °C) and insolation (900–1000 h) during the growing season positively affect the protein content of soybean seeds. The influence of insolation on nodule formation and nitrogen fixation is a derivative of its impact on photosynthesis. Nitrogen fixation (the process of reduction) requires a substantial energy outlay; thus, it is vital that plants grow in conditions that guarantee the appropriate course of photosynthesis. Research has demonstrated that an increase in lighting intensity is followed by a linear increase in the number of nodules and nitrogen fixation efficiency. Cloudy weather is particularly undesirable during the period of root nodule formation.

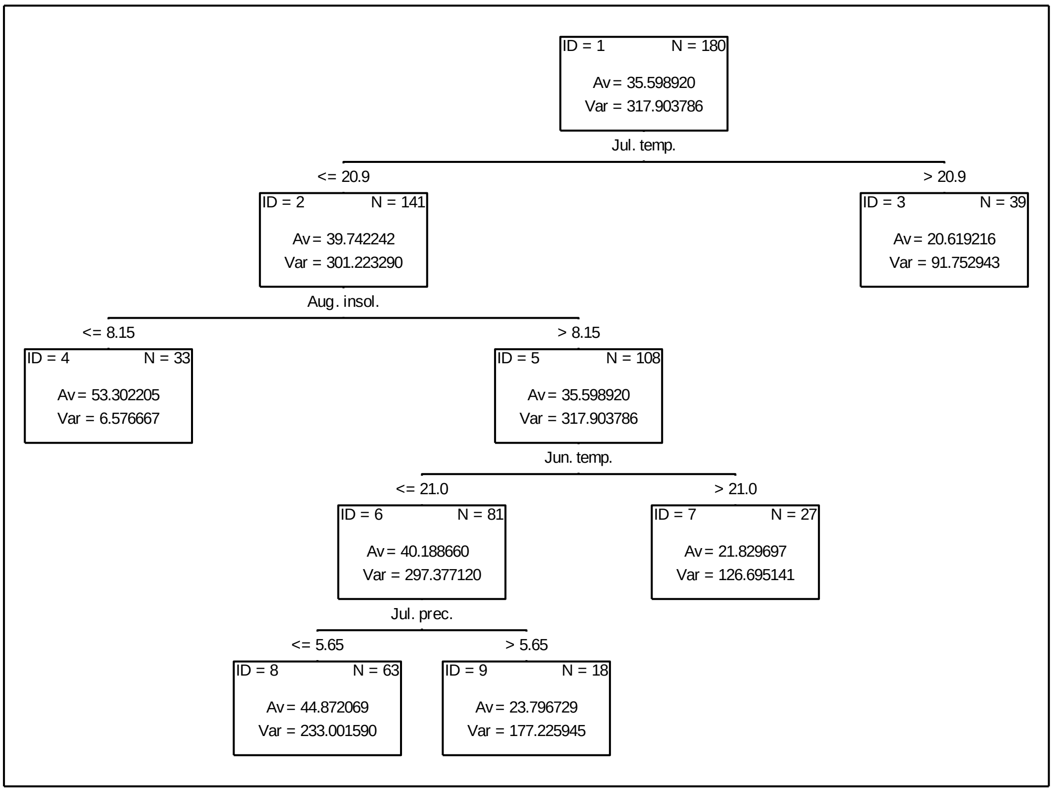

Figure 4. Diagram of regression tree reflecting nitrogen acquisiton from the atmosphere by control soybean plants.

The amount of atmospheric nitrogen acquired by soybean plants treated with biostimulants was higher compared with the control. Similarly to the model for the control unit, in the obtained models (Figures 5 and 6), the first and most important split into branches (marked with the identifiers ID = 2 and ID = 3) was affected by the temperature in July, and the second one (ID = 4 and ID = 5) by insolation in August. Further splits depended on the applied biostimulant and were affected by insolation in July (after treatment with Asahi) or June (after spraying with Improver), and daily precipitation sums in July. If, additionally, daily insolation in July and June did not exceed, respectively, 13.5 and 11.5 h and was accompanied by a daily precipitation sum of up to 5.65 mm, soybean plants tended to fix an average of 61.1 or 59.7 kg nitrogen, respectively (Figures 5 and 6). Szparaga et al. [45] demonstrated that soybean plant growth, yield and biometric parameters of seeds were affected by biostimulant type, concentration and number of applications. Moreover, the authors reported that treatment with synthetic biostimulants affected protein and fat contents in soybean seeds which tended to decline regardless of the number of applications or concentration of the products. There was a strong negative correlation between the total protein content and plant height (r = −0.91), number of seeds (r = 0.72) and seed yield (r = −0.69) following an application of Tytanit. The plants modified their metabolism so that, instead of producing storage proteins, energy was used for stem, leaf and pod growth [46], as well as an accumulation of fat which is the major storage material in soybean seeds. The authors point to the fact that apart from various active compounds in the test biostimulants, their concentration and rate, soybean performance and seed quality were also affected by environmental factors occurring throughout the growing season. Treatments with biostimulants increase the content of 'pest-related' components such as phenols or dietary fibre, both of which usually increase as part of a systemic response of plants to stressors [47,48].

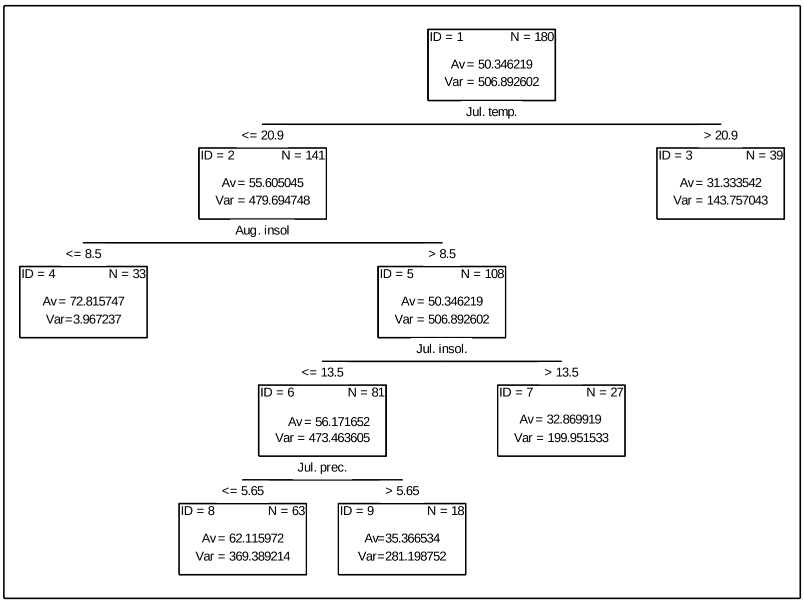

Figure 5. Diagram of regression tree reflecting nitrogen acquisition from the atmosphere by soybean plants treated with Asahi.

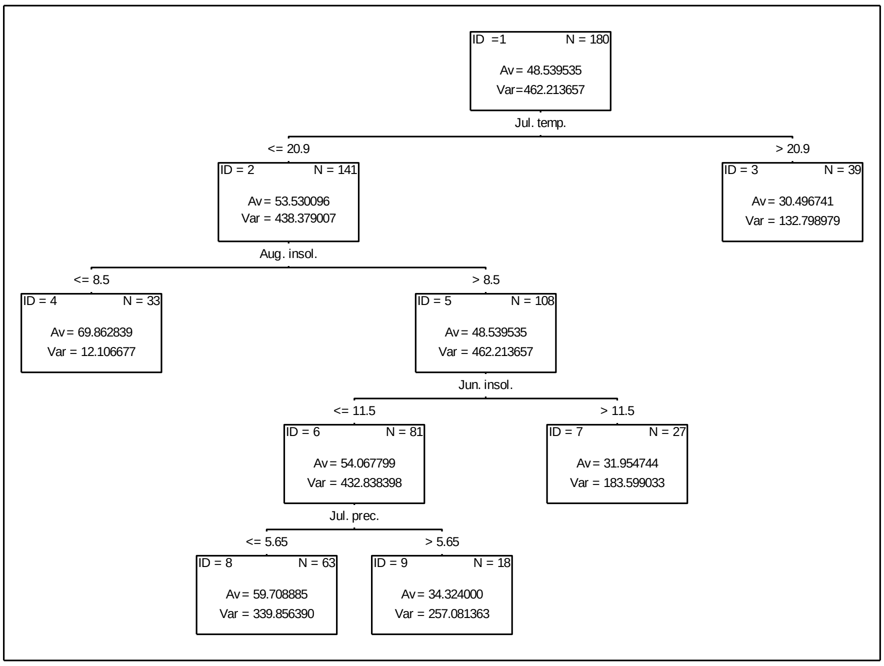

Figure 6. Diagram of regression tree reflecting nitrogen acquisition from the atmosphere by soybean plants treated with Improver.

3.3. Analysis of the Effect of Meteorologial Conditions on Nitrogen Uptake from Fertiliser

Regression trees describing the amount of nitrogen taken up by soybeans from fertiliser consisted of four terminal nodes. Both in the control unit and plots treated with biostimulants, the amount of nitrogen taken up from fertiliser was predominantly affected by temperatures in July and June, and precipitation in July. When the temperature in July exceeded 20.9 °C, fertiliser nitrogen uptake by soybean plants was the lowest and ranged from 7.71 (no biostimulant) to 9.35 kg (treatment with Improver), it being the highest (from 10.64 to 14.98 kg) when the temperatures in July and June did not exceed 20.9 and 13.15 °C, respectively, and daily precipitation sums in July were up to 5.65 mm (Figures 7–9). Research by LeMenza et al. [49] has shown that, when grown under conditions which allow soybean to produce yields higher than 2.5 Mg ha^{-1}, the crop may positively respond to nitrogen fertilisation. Unfortunately, a high concentration of mineral nitrogen forms in soil tends to inhibit elemental nitrogen reduction conducted by nodulating bacteria by hampering the activity of enzymes which are involved in this process, and the development of root nodules. Furthermore, research by Salvagiotti et al. [50] has demonstrated that mineral nitrogen has a negative effect on nodulation and N_2 fixation by soybeans, excluding nitrogen starter fertiliser applied during sowing.

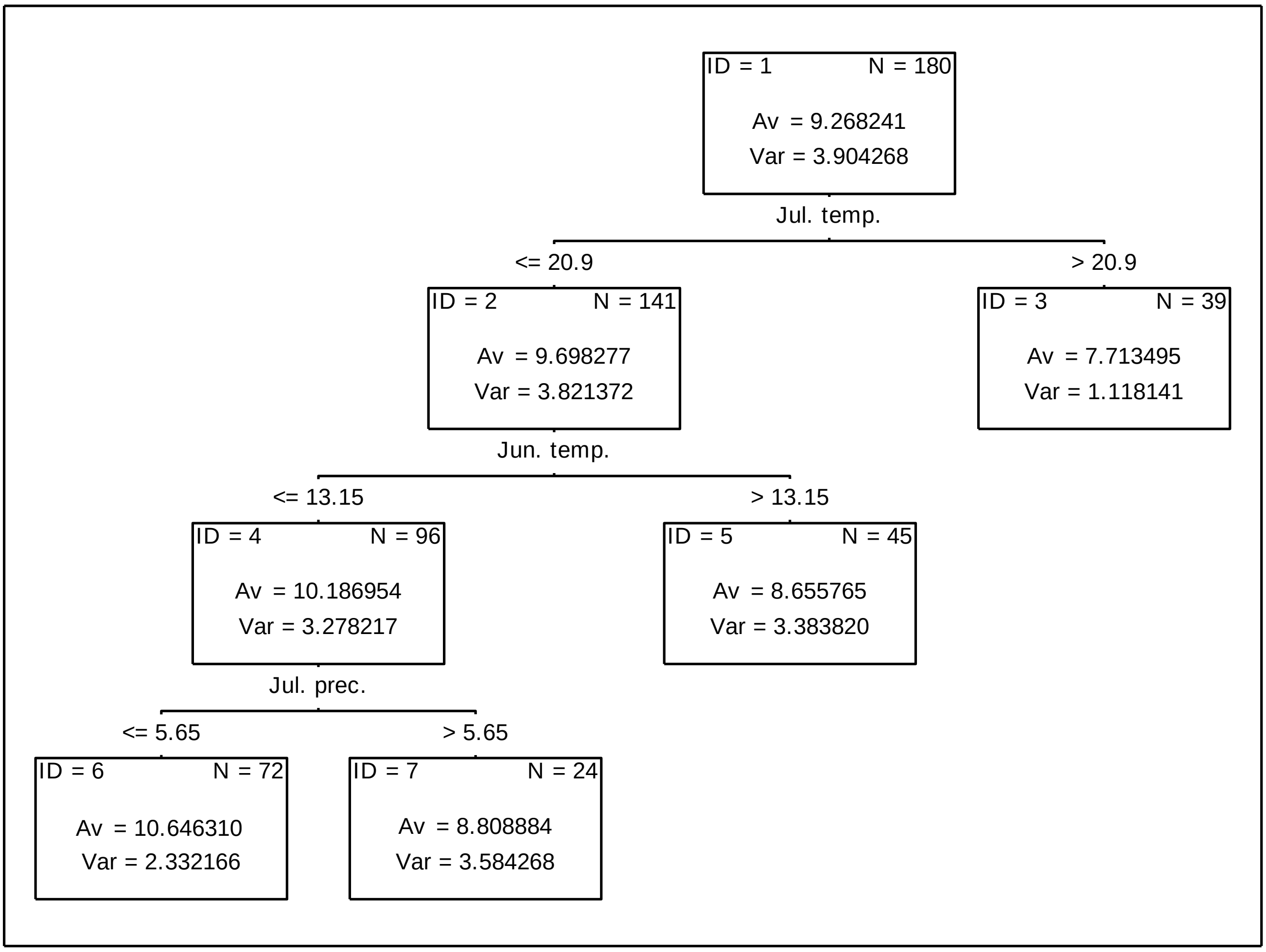

Figure 7. Diagram of regression tree reflecting nitrogen acquisition from fertiliser by control soybean plants.

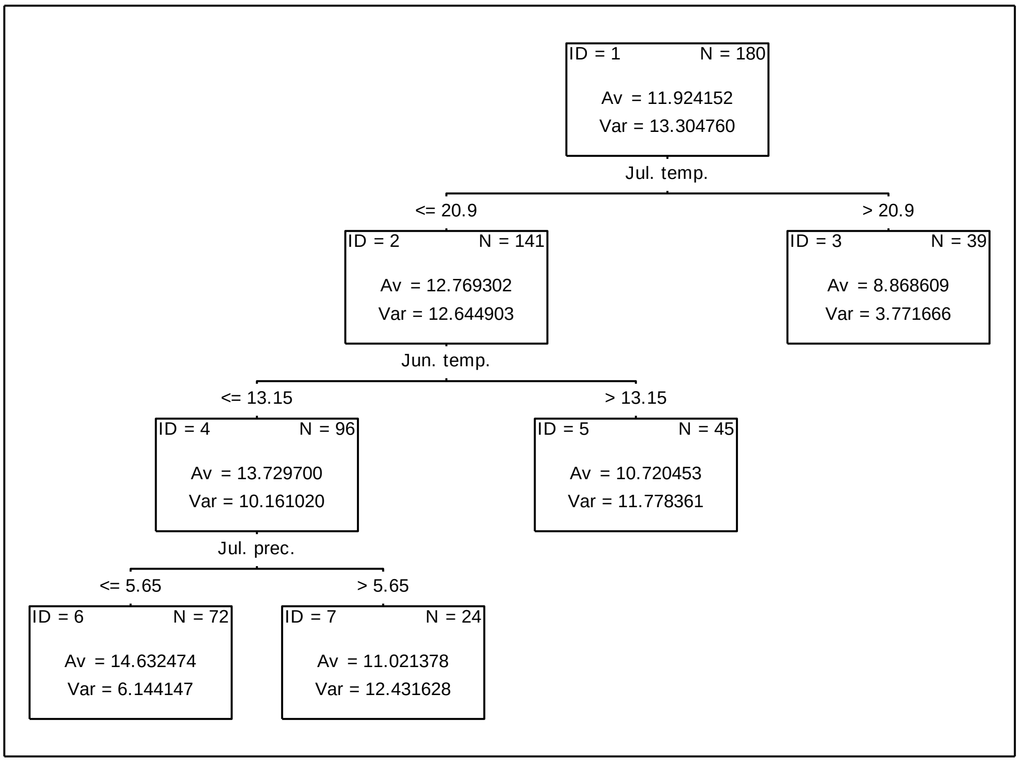

Figure 8. Diagram of regression tree reflecting nitrogen acquisition from fertiliser by soybean plants treated with Asahi.

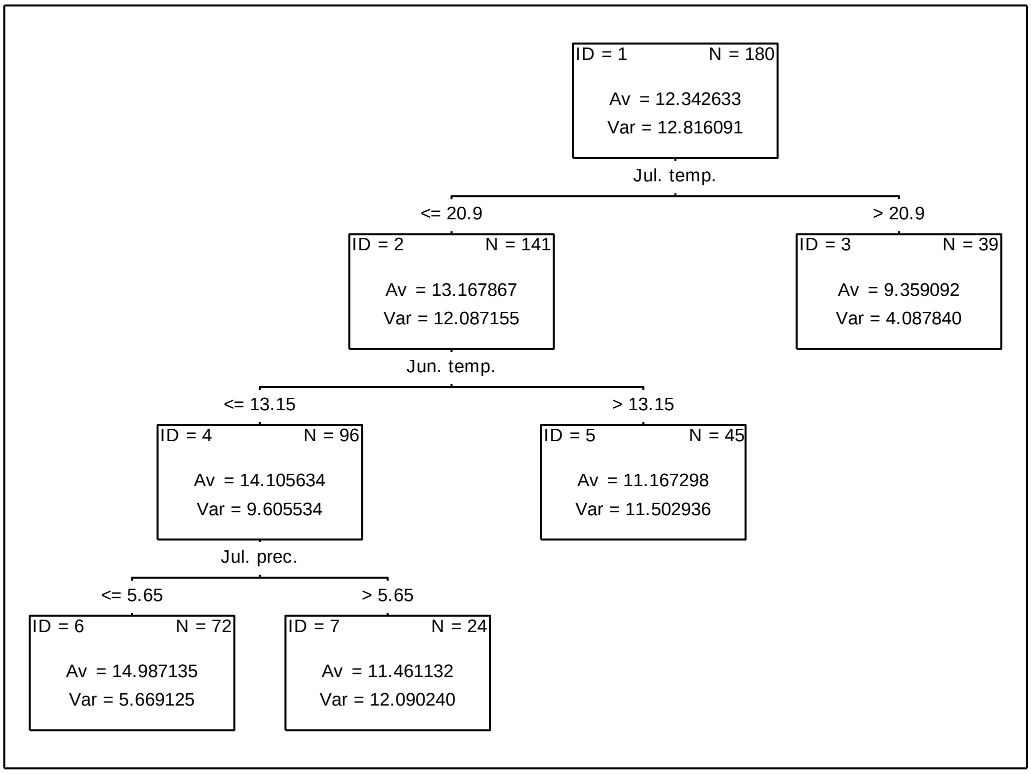

Figure 9. Diagram of regression tree reflecting nitrogen acquisition from fertiliser by soybean plants treated with Improver.

3.4. Analysis of the Effect of Meteorological Conditions on Nitrogen Uptake from Soil

The quantity of nitrogen taken up from the soil by control soybean plants is presented in Figure 10. The crop acquired approximately 56 kg nitrogen from the soil when, in July, the average daily temperature was lower than 20.9 °C, the insolation did not exceed 13.15 h and daily precipitation sums were higher than 1.75 mm. The analysis of non-split nodes revealed that, following treatment with Asahi, the highest nitrogen uptake from soil (77.9 kg) was recorded when the average daily temperature in July was lower than 20.9 °C, and insolation in August did not extend beyond 8.15 h. Nitrogen uptake from soil was the lowest when the average daily temperature in July was higher than 20.9 °C (Figure 11). The regression tree model illustrating the impact of the biostimulant Improver on the amount of nitrogen taken up by soybean plants included four leaves (Figure 12). The diagram demonstrates that nitrogen uptake from soil by soybeans treated with Improver was mostly associated with the average daily temperature in July, insolation in June and daily precipitation sum in July, the highest uptake being recorded when the temperature in July was up to 20.9 °C, the insulation was up to 8.15 h, and the lowest average daily temperature in July of over 20.9 °C. A large quantity of nitrogen taken up from soil reserves in the total soybean biomass may result from a long growing season with weather conditions contributing to soil organic matter mineralisation (the provided mineral nitrogen, high temperature, optimal moisture). A high percentage of nitrogen taken up from soil and present in the total soybean biomass may have also been due to the fact that much more nitrogen present in the seeds of the test cultivars was soil- rather than atmosphere-derived. Differences in nitrogen acquisition between the cultivars cultivated in the same conditions may also be genetically or physiologically conditioned [51,52].

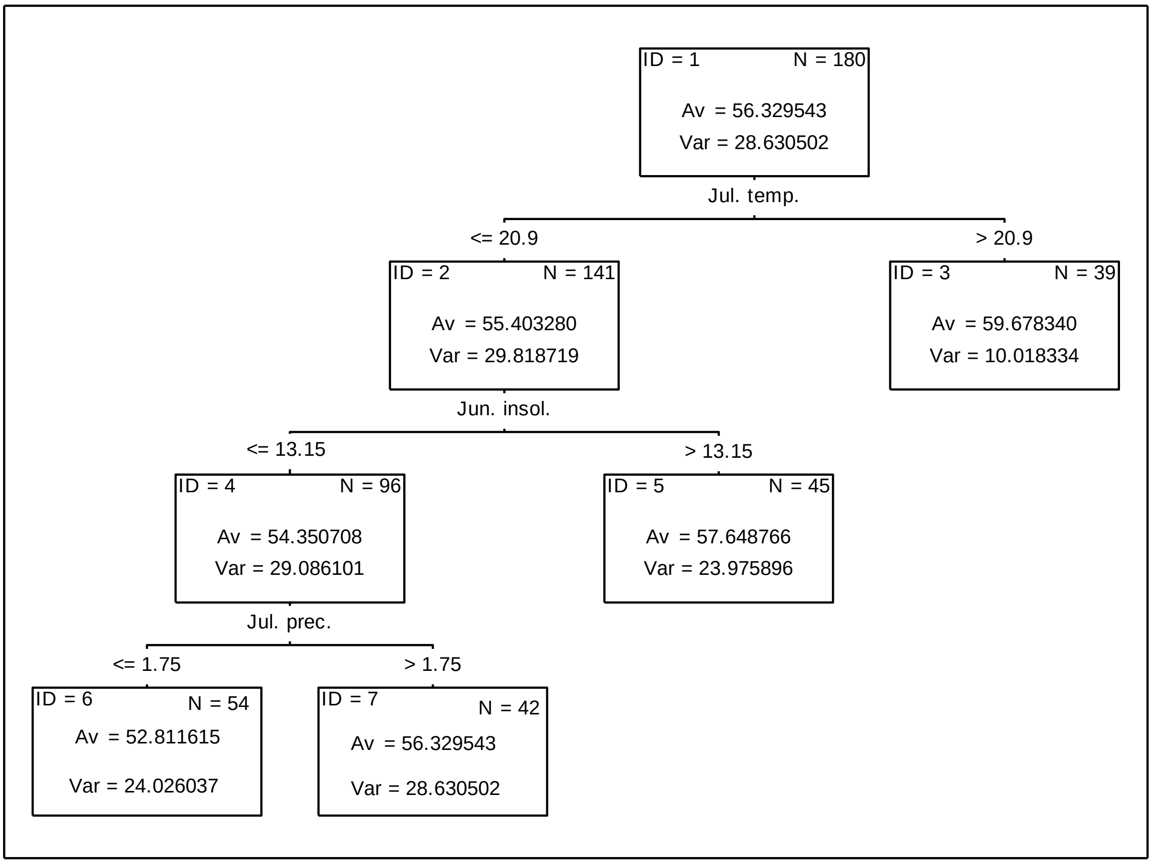

Figure 10. Diagram of regression tree reflecting nitrogen acquisition from soil by control soybean plants.

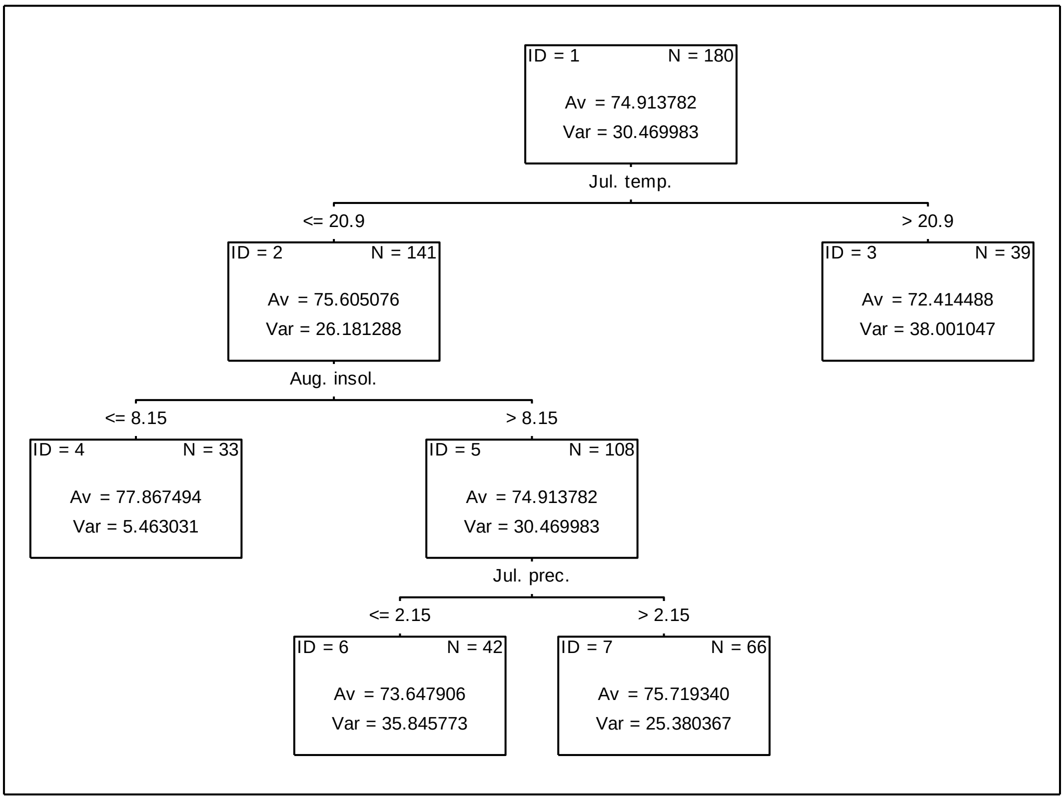

Figure 11. Diagram of regression tree reflecting nitrogen acquisition from soil by soybean plants treated with Asahi.

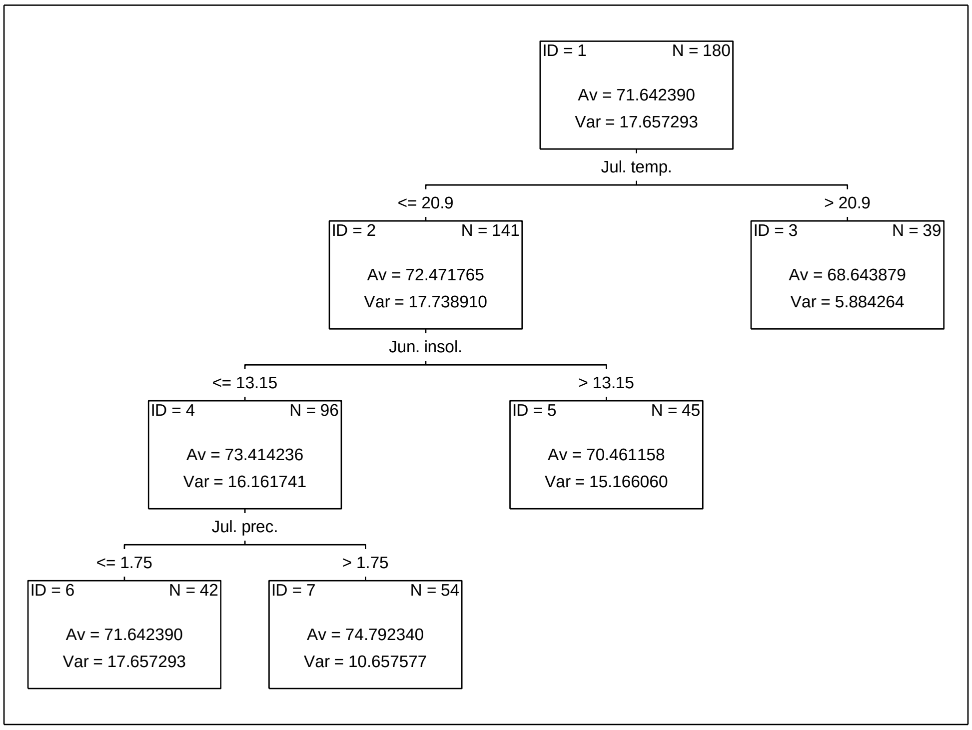

Figure 12. Diagram of regression tree reflecting nitrogen acquisition from soil by soybean plants treated with Improver.

4. Conclusions

Meteorological conditions varied in the study years. Droughts occurred in May, August and September 2017, and only in August 2018. Moreover, the growing season of 2018 was much warmer compared with 2017. Similarly, insolation was higher in 2018 vs. 2017, the respective values being 289 and 234 h.

The amount of nitrogen taken up from the atmosphere by soybean plants was found to be higher following an application of biostimulants compared with the control. However, regardless of the applied biostimulant, atmospheric nitrogen uptake was affected by temperature in July. The soybean uptake of nitrogen from fertiliser was the highest when temperatures in July and June did not exceed 20.9 and 13.15 °C, respectively, and the daily precipitation sum was lower than 5.65 mm. Nitrogen uptake from soil amended with Asahi and Improver was affected by air temperature in July and insolation in August. Hence, it was confirmed that temperature influences the effectiveness of growth regulator activity in plants. The effectiveness of these products is also enhanced by insolation which affects photosynthesis intensity.

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