

NITROGEN FEEDING OF WINTER PEAS AT THE SPRING VEGETATION RECOVERY STAGE

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ABSTRACT. In a field experiment from 2020–2023 on the southern chernozems of the Odessa region of Ukraine, we studied the effect of the doses and timing of mineral nitrogen application when growing winter peas on the tuberization process and crop productivity. Mineral nitrogen in doses of 30, 45 and 60 kg/ha in the form of ammonium nitrate was applied in different development phases of winter peas under seedbed cultivation: foliar top dressing with urea solutions during the resumption of spring vegetation, the budding phase and the beginning of grain filling. Winter pea of the Enduro variety was sown annually on October 20, with a row spacing of 15 cm and a rate of 1.2 million germinating seeds per hectare. The previous crop was winter wheat. The conditions of the Southern Steppe of Ukraine for winter sown peas were shown for the first time. The introduction of mineral nitrogen at a rate of 30–60 kg/ha

into the soil during sowing inhibited the formation of nodules from the active spring growth phase. The mass of nodules on the roots of pea plants decreased by 30–50% compared to the control variant without nitrogen application. The same norms of mineral nitrogen introduced in early spring during the resumption of vegetation on the leaf in the form of aqueous solutions did not have a negative impact on tuberization. The correlation coefficient between the dose of mineral nitrogen and the number and weight of nodules did not exceed 0.37, indicating a weak relationship between these indicators. The share of influence of mineral nitrogen norms did not exceed 13.7%. With this method of application, the nitrogen content in the vegetative mass of winter peas exceeded the control variant by 18–27%.

Keywords: calcic chernozem doses; mineral nitrogen; nodules; peas; terms; winter sowing.



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INTRODUCTION

The most accessible factor for regulating the growth and development of plants is mineral fertiliser. The results regarding the effect of mineral fertilisers, especially nitrogen fertilisers, on the productivity of leguminous crops have ambiguous conclusions with a wide range of fluctuations, from the complete denial of their use to the assumption of a so-called starter fertiliser or a half norm, based on the needs of the culture. At the same time, no unanimous position has been formed regarding the nutrition system of leguminous crops, particularly peas.

Rosso (2023), director of Terres Univia, believes that rhizobia can supply up to 80% of nitrogen to peas, with the rest of the nitrogen coming from the soil. Abbady *et al.* (2016) showed the effectiveness of nitrogen fertilisers for prolonged solubility. Experiments by Faligowska *et al.* (2022) using a nitrogen isotope demonstrated the possibility of using small doses of mineral nitrogen, as nitrogen accumulated in pea seeds from three sources: the atmosphere (average value 35.2%), fertilisers (6.8%) and soil (57.9%). Hu *et al.* (2018) showed that low nitrogen application increased the nitrogen concentration in sorghum and that nitrogen improved the development of plant leaf area, affecting leaf size, longevity and higher grain yield. However, Achakzai (2007) concluded that the formation of nodules was inhibited by all of the studied nitrogen doses (from 25 to 125 kg/ha) and that only the application of phosphorus-potassium fertilisers slightly improved the situation; therefore, they did not recommend using mineral nitrogen when

growing peas, and other scientists agreed (Ghodsi *et al.*, 2022).

According to Vlasova (2023), application of mineral nitrogen at a dose of 20–30 kg/ha is necessary if, at the time of sowing peas, nitrate nitrogen reserves in the arable layer are less than 30 mg/kg of soil, and doses of up to 60 kg/ha are applied to poorly cultivated soils with humus below 2%. The results of our long-term research (16 years) in a stationary experiment showed both the effectiveness of mineral fertilisers and the dependence of their action on the amount and quality (gradation) of precipitation during the spring vegetation period. The payback of mineral nitrogen in the composition of complete mineral fertiliser ($N_{30}P_{30}K_{30}$) was 18.1 kg of pea grain per kg of active substance when precipitation during the spring vegetation period was more than 140 mm, and the share of non-productive plants was not more than 58%, decreasing to 7.4 kg/kg of active substance with precipitation from 50 to 70 mm, and the share of unproductive precipitation was 75–80% (Burykina *et al.*, 2020). Didur and Shevchuk (2020) established that $N_{30}P_{30}K_{30}$ fertiliser in a complex with an inoculant and a plant growth regulator increased the symbiotic activity of pea plants, while the nodules were mostly concentrated on the main root and had a pink colour.

These results refer to common peas sown in spring, but regarding the winter forms of this culture with which we conducted experiments, there are almost no scientific studies. There are only testimonies of practicing agronomists who apply $N_{45-100}P_{15-45}K_{15-45}$ depending on the culture of the soil and conduct top dressing with nitrogen fertilisers at a rate

of 45–60 kg/ha on frozen soil or at the beginning of vegetation recovery (Pavlyuk, 2023; TGWP, 2023). In the conditions of the Southern Steppe of Ukraine in the fields of the "Tavria-Skif" farm in 2016, sub-winter pea fertilisation was carried out on frozen soil (end of February - beginning of March) at a rate of 70 kg/ha of nitrogen and received more than 3.0 t/ha, and in the Zhitomir and Vinnitsa regions (forest-steppe zone), 150 kg of mineral fertiliser nitroammophoska (15:15:15) was applied at sowing in the fall, at the beginning of the recovery of spring vegetation - N_{70} , and two foliar top dressings during the vegetation period - the crop was formed at the level of 3.7 to 4.7 t/ha (Avramchuk, 2023; Kozak, 2019).

Some scientists in Ukraine note the importance of the optimal combination of biological nitrogen and nitrogen from mineral fertilisers, as well as the correct combination of main and pre-sowing fertilisation and top dressing in the winter pea fertilisation system (Gospodarenko, 2019).

There have already been scientific attempts to develop the technology of growing peas under winter sowing for the conditions in southern Ukraine, considering climatic changes, in particular, in the issue of finding optimal sowing dates and sowing rates (Rudenko *et al.*, 2022; Solomonov *et al.*, 2022). In our research, we are trying to develop a pea fertilisation system for sub-winter sowing for the specified region and have already received preliminary results regarding the impact of this element of technology on crop yield (Burykina *et al.*, 2022).

The purpose of the present work was to investigate the influence of early spring nitrogen fertilisation and the post-sowing application of nitrogen fertilisers on the process of nodule formation and the productivity of winter-sown pea crops.

MATERIALS AND METHODS

The experiments were carried out from the fall of 2020 in the experimental field of the Odesa State Agricultural Research Station of the Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine, which is territorially located in the Odesa district of the Odesa region. The surface of the experimental field is a plain with a poorly developed microrelief and a slope of 0–1° with southern exposure. The soil of the experimental site is chernozem southern low humus (Calcic chernozems (WRB, 2022)), with heavy loam on the loess (Polupan *et al.*, 2005). The thickness of the humus horizon is 50–55 cm. The density of assembly is 1.12–1.30 g/cm³, and porosity is 56.5% (WRBSR, 2022).

The experiments were replicated 4 times in a randomised design. The area of the elementary plot was 20 m², and the accounting plot was 14 m². Experiments are laid out in accordance with the recognised methods of conducting field experiments (Dospikhov, 1971; Mazur *et al.*, 2020).

Mineral nitrogen was used in the form of ammonium nitrate (34%) for sowing and urea (46%) for foliar fertilisation during the growing season. Variants of the experiment: 1) control without nitrogen introduction; 2) N_{30} ; 3)

N₄₅; 4) N₆₀ (in options 2–4, mineral nitrogen was introduced into the soil at sowing); in options 5–7, N₃₀; N₄₅: N₆₀ were applied in the form of solutions on the leaves of plants during vegetation restoration in the spring, during budding and the pea grain pouring phase.

Winter pea variety Enduro was sown every year on October 20; the sowing rate was 1.2 million seeds per hectare. The sowing method was continuous (width between rows was 15 cm). The previous crop was winter wheat.

Since we considered only the effectiveness of the post-sowing application of mineral nitrogen in comparison with the first fertilisation in this work, pea plants were selected after vegetation recovery in the spring before fertilising and two weeks in a row after. For this, on the days of observation, plant samples were taken in three places in each of the plots and placed in special bags with a plot number. The samples were divided into vegetative mass and roots. The mass of the dry matter was determined after drying to a completely dry state. During the first hour, fixation was carried out at 115–120°C, and then complete drying occurred at 105°C. The nitrogen content was determined in the obtained samples: in the vegetative mass on a Spectran-119M infrared analyser, and in the roots, according to Kjeldahl (DSTU 4117, 2007; DSTU 7169, 2011).

The working principle of the Spectran-119M analyser is based on the measurement of the diffuse reflectance coefficients of the ground samples in the near-infrared region of the spectrum. The calibration of the device was carried out by specialists according to the results of the standard Kjeldahl method.

The nitrogen content was determined according to Kjeldahl, based on the digestion of organic matter with sulphuric acid in the presence of a catalyst. All nitrogen released at the same time turns into ammonium sulphate ((NH₄)₂SO₄). The latter emits ammonia in an alkaline environment, which, as a result of steam distillation, is distilled into a receiving flask with boric acid and mixed indicator additives. The final measurement procedure was titration using a standard acid solution, changing the colour of the indicator. The nitrogen content was determined by the amount of ammonia.

Combustion of the sample was carried out on a digester with a built-in thermostat and a central display. The process took place at 420 ± 10°C for 2–3 hours until a pure green-blue colour appeared, which disappeared after cooling. The catalyst included metallic selenium (2 g), copper sulphate (10 g), and sodium sulphate (100 g). Approximately 0.7 g of the catalyst was added to the test tube for combustion, with a sample weight of 1 g and 12 ml of concentrated sulphuric acid. The analysis was carried out using the "Kjeltec Auto 1030 Analyzer" system device to sequentially carry out three main stages: digestion, distillation and titration.

For a more accurate count of nodules on the roots, soil monoliths measuring 55 × 45 × 15 cm were selected. The roots were then washed and the nodules were counted and weighed.

Statistical processing of the obtained results was carried out by the methods of dispersion, correlation, regression and graphic analyses using

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Excel and Statistika applications (Tsybulnyk and Lysikova, 2017; Ushkarenko *et al.*, 2008).

Weather conditions during the growing season

The weather conditions of the research years differed greatly in terms of temperature, amount of precipitation and their distribution by growth periods of winter pea plants. The amount of precipitation from October to May was 336 mm in 2021, 208.6 mm in 2022 and 356 mm in 2023.

In all the years of observation, the pre-sowing period and the period from sowing to germination were provided with insufficient precipitation. The pre-sowing time was the worst in 2021 when only 11.5% of the total fell (*Figure 1*).

Pea seedlings suffered from a lack of moisture in all years. In the winter period, the largest share of precipitation occurred in 2022 and the least (20.9%) in 2023. During the spring growing season, pea plants felt the worst in 2022;

in three months, they received only 46.1 mm of precipitation, which was 22.1% of the total. Spring moisture availability was the best in 2023, from March to May; the month received 176 mm of precipitation (49.5% of the total).

The air temperature in all years during the growing season of the plants was higher than the climatic norm (*Figure 2*).

RESULTS

During the period of vegetation restoration, the nitrogen content in the dry above-ground mass of pea plants of the control variant was 3.92% on average over three years (*Table 1*). With an increase in the dose of post-sowing application of mineral nitrogen from N₃₀ to N₆₀, it increased from 4.38% to 5.14%, and the nitrogen content in the roots exceeded the option without fertiliser application by 6.1% at N₃₀ and by 17.1% at N₆₀.

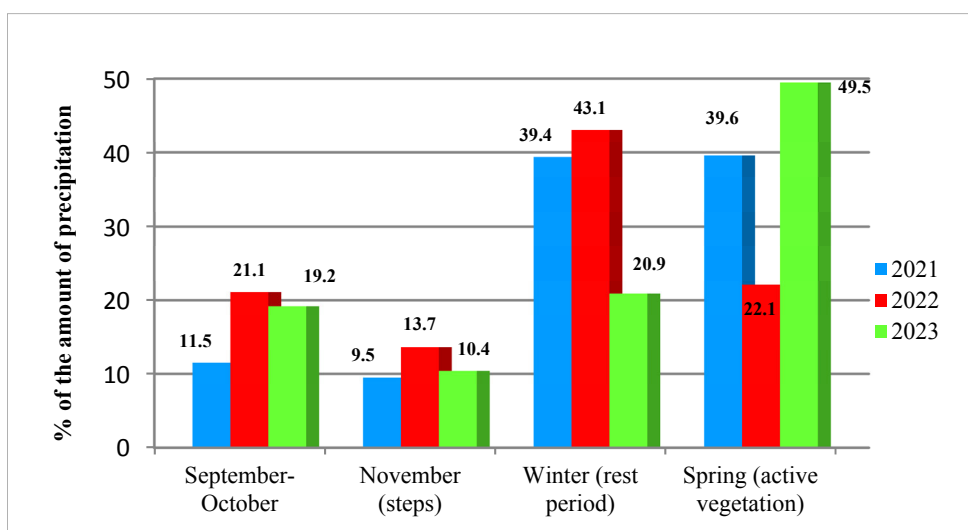


Figure 1 – Distribution of precipitation according to the periods of pea development under winter sowing in the years of research

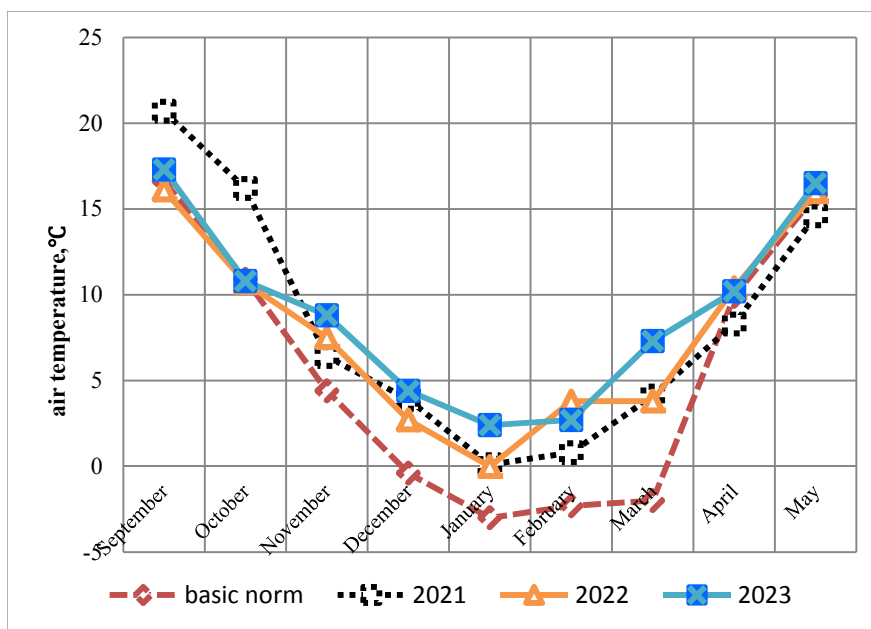


Figure 2 – Average monthly air temperature during the years of research

During this period of development, in the variants of the experiment, where fertilisation was planned but not yet carried out, the vegetative mass and roots of the plants differed from the control variant in terms of nitrogen content by an insignificant amount: from 1.8% to 2.8%.

Two weeks after fertilising, the nitrogen content in the vegetative mass of winter peas in these options increased by an amount proportional to the dose of mineral nitrogen from 38.3% to 47%, and in the post-sowing options – from 18.9% to 4.2%. In the latter case, there was a tendency to decrease the growth rate of nitrogen with an increase in the dose of nitrogen fertilisers (*Figure 3*).

In the option without fertiliser application, the nitrogen content in the vegetative mass increased by 19.8% over a two-week period, and if we assume that it is due to symbiotic

activity, then in the options of foliar feeding, the increase in nitrogen concentration can be explained by the synergistic effect of nitrogen fixation and mineral nitrogen, due to which the nitrogen content increased from 18.3% to 27.2%. However, in the variants in which mineral nitrogen was introduced into the soil, antagonism was observed between nitrogen fixation and mineral nutrition since the growth of the nitrogen content in the vegetative mass was less than the zero variant.

Indirectly, this assumption was confirmed by the change in nitrogen content in the roots of winter peas during the period indicated above (*Figure 4*). In the variant without fertilisers, the growth was 15.8%; in areas with nitrogen application during sowing in the soil, there was a drop in nitrogen content from 0.2% to 9.1%, apparently due to its entry into the aboveground mass.

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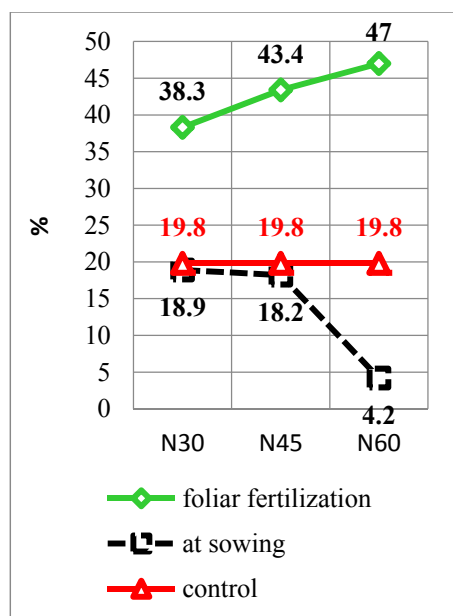
In the variants with foliar fertilisers, the nitrogen content in the roots increased from 19.0% to 24.0%. It is logical to assume that the nitrogen needs of the plants of these variants were satisfied by their entry through the

leaves. The element came in smaller quantities from the roots, and therefore its concentration exceeded the control variant by 3.2%, 8.2% and 6.2% according to the nitrogen dose.

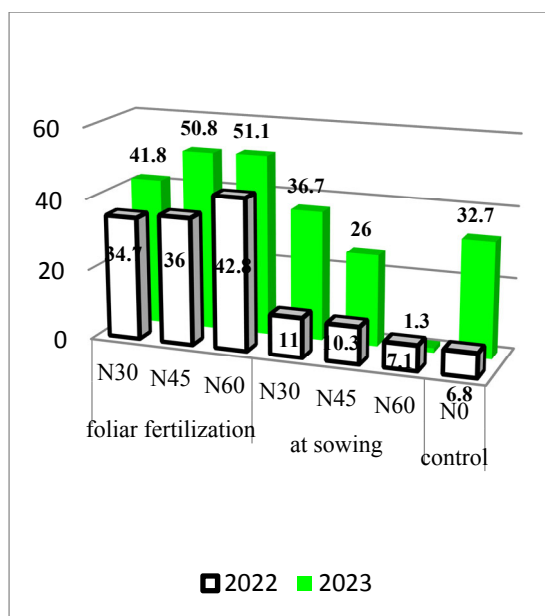
Table 1 – Nitrogen content in the vegetative mass of winter peas during the spring restoration of vegetation on different backgrounds of nitrogen nutrition

Years	N ₀		N ₃₀		N ₄₅		N ₆₀		HCP _{0.05}
	M±m	V,%	M±m	V,%	M±m	V,%	M±m	V,%	
Main application									
2021	3.86±0.08	2.1	4.14±0.08	2.0	4.16±0.03	1.0	4.50±0.02	4.6	0.43
2022	3.42±0.19	5.1	4.07±0.07	1.8	4.46±0.15	3.3	4.83±0.16	3.2	0.40
2023	4.28±0.13	3.1	4.93±0.07	1.5	5.08±0.09	1.7	6.08±0.25	4.1	0.33
average	3.92±0.34	8.7	4.38±0.48	10.9	4.57±0.47	10.3	5.14±0.83	16.2	1.63
2 weeks after feeding at SRV									
2022	3.62±0.19	5.1	4.59±0.13	2.7	4.83±0.05	1.0	4.97±0.16	3.2	0.51
2023	5.72±0.16	2.8	6.19±0.13	2.1	6.45±0.11	1.7	6.05±0.10	1.4	0.31
average	4.67±1.48	31.8	5.39±1.13	21.0	5.64±1.14	20.3	5.51±0.76	13.9	-

SRV - spring resumption of vegetation



a) average over two years



(2022 pik LCD_{0.95} = 5.2%; 2023 pik LCD_{0.95} = 4.1)
b) by years of research

Figure 3 – Increase in the nitrogen content in the vegetative mass of winter peas 2 weeks after the first feeding, % of the content during vegetation restoration

The process of symbiotic nitrogen fixation of peas was characterised by the number and mass of nodules formed on plant roots. Nodule formation on the roots of winter peas began in the fall at the time of germination; at the time of the spring resumption of vegetation, their mass per plant fluctuated significantly, both by year and by research options (*Table 2*).

Within one year, the variability of the mass of nodules on the roots of one plant ranged between 3.5 and 8.7%, except for the option of at-sowing N_{60} application, where the variability of indicators according to experiment repetitions was greater than the ten percent level (10.8–15.4%). However, the absolute indicators differed several times between years, which may be explained by the very different moisture availability at the time of vegetation recovery. Therefore, if the reserves of productive moisture in the metre layer of the soil in 2023 were taken as 100%, then they amounted to 70.5% in 2022 and 88.6% in 2021. There was an even greater difference between these indicators at the time of sowing (October 20 of the previous year) – 45.1% and 12.0%, respectively.

During the recovery of winter pea vegetation, post-sowing application dose of mineral nitrogen did not have a significant effect on nodulation in any of the years of study (the difference with the control variant was less than $LSD_{0.05}$), the same trend was observed after 2 weeks of active vegetation, except for the variant with application of N_{60} , where the mass of formed nodules was significantly smaller than the option without fertiliser application. In 2023,

with good moisture availability of the root layer of the soil, all doses of the at-sowing application of mineral nitrogen had a negative effect on the indicator. On average, over three years, the mass of nodules on the roots of one plant decreased by 29.4% (N_{30}), 31.9% (N_{45}) and 51.9% (N_{60}) compared to the null variant. Nitrogen doses of foliar fertilisation during vegetation restoration did not have any effect on the process of symbiotic fixation since the mass of nodules was not significantly different from the control variant.

Correlation dependencies

Correlation analysis makes it possible to reveal the presence of a relationship and its extent between the characteristics that change during the growth and development of plants. The correlation between the parameters is considered close when $r \geq 0.70$, weak when $r = 0.2-0.3$, and moderate – $r = 0.3-0.5$. The correlation between traits was considered significant if the actual correlation coefficient was higher than the critical one.

Calculation of the correlation coefficients (*Table 3*) showed that the presence of nitrogen fertilisers had almost no effect on the initial stage of spring nodulation ($r = 0.37$); its intensity was determined by 60.8% ($r = 0.78$) of productive moisture reserves at the time of resumption of winter pea vegetation.

Furthermore, the concentration of nitrogen in the vegetative mass of winter peas at the stage of early spring vegetation did not depend on the number of nodules on its roots ($r = 0.31$ with fluctuations over the years from 0.25 to 0.37), but it was limited by nitrogen from mineral fertilisers ($r = 0.98$).

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Correlation coefficients of 0.25–0.41 indicate a weak and moderate relationship between the signs, but they are not mathematically reliable, since they are less than the critical value, which is 0.58.

The level of productivity of winter-sown peas was mainly a function of the reserves of productive moisture ($r = 0.90\text{--}0.98$) and the level of nitrogen nutrition at the time of vegetation recovery ($r = 0.88$; the range of fluctuations according to the years of research – 0.77 ... 0.98), which can be regulated, as our results showed, by the rates of mineral nitrogen applied after sowing or early spring fertilisation. The relatively lower intensity of dependence of any parameter with doses of mineral fertilisers occurred in 2022, the driest of the research years. The productivity of winter pea crops depended on the dose of mineral nitrogen, but little depended on the method of application of this dose: at-sowing application or early spring fertilisation. *Figure 5* shows the three-year average increases in grain yield by nitrogen doses without considering the application timing.

The coefficient of determination of the regression equation for yield increases (1) was 0.81, indicating a high degree of probability of proceeding in this direction (Tsybulnyk and Lysikova, 2017).

$$Y = 23.437 \cdot X^{0.2088} \quad (1)$$

where Y - yield increase, %; X - nitrogen content in the vegetative mass of peas during spring vegetation renewal, % on dry matter.

DISCUSSION

In the presented results, we considered the impact of mineral

nitrogen at application doses of 30–60 kg per hectare on the processes of nitrogen fixation only in the initial stage of the spring vegetation of winter peas and established that the at-sowing application of nitrogen fertiliser (N_{30} and N_{45}) had a negative effect on the processes of nitrogen fixation, and application of N_{60} inhibited bubble formation regardless of the weather conditions. The nitrogen rate of the first foliar feeding at the stage of vegetation recovery had no effect on the ability of winter pea plants to form nodules. Our results partially coincide with the conclusions of some researchers (Hu *et al.*, 2018; Rosso, 2023) and partially contradict those of others (Achakzai, 2007; Ghodsi *et al.*, 2022) but are confirmed by the results of agrarian producers regarding the positive effect of mineral nitrogen on the productivity of winter-sown peas (Avramchuk, 2023; Kozak, 2019; Pavlyuk, 2023).

Some researchers believe that the dose of the starting application of nitrogen fertiliser depends on the nutrient content in the soil (Gospodarek *et al.*, 2020) in general and, specifically, on nitrate nitrogen reserves. Thus, Huang *et al.* (2017) claimed that the starting dose of N_{30} with nitrate nitrogen reserves of 10 kg/ha increased pea yield by 19.00%, and with reserves of 44.0 kg/ha, it reduced it. In the experiments of Hu *et al.* (2018), basic application of 90 kg N/ha during sowing and topdressing of 45 kg N/ha during flowering of peas increased the nitrogen content in plants, the distribution by structural elements of peas and nitrogen availability to the grain. Janusauskaite (2023) revealed the absence of a

negative effect of nitrogen fertilisation on root nodules at doses of N₁₅₋₄₅ against the background of P₄₀K₈₀. Some believe that peas can withstand only 10 kg N/ha (Dona et al., 2020). This indicates the inconsistency of data on fertiliser doses; the fluctuation is from 10 to 90 kg N/ha.

The bulk of research results relate to spring peas. We conducted research with peas of the Enduro variety, which is sown in winter, and showed the dependence of the effect of mineral nitrogen not only on the application dose but also on the method of application.

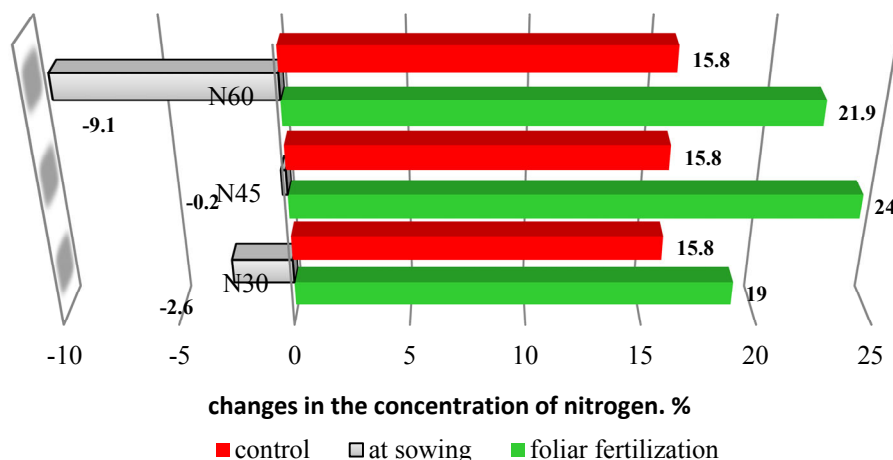


Figure 4 – Changes in the concentration of nitrogen in the roots of winter pea plants two weeks after vegetation restoration and fertilising (average over two years)

Table 2 – Mass of nodules on the roots of one plant (mg)

Year	control	N ₃₀	N ₄₅	N ₆₀	LSD _{0.05}
At-sowing application at the time of vegetation recovery					
2021	33.7	30.9	31.4	30.8	11.7
2022	23.3	25.0	24.3	20.8	8.8
2023	77.8	67.2	61.2	64.7	17.6
average	44.9	41.0	39.0	38.7	-
At-sowing application in two weeks					
2021	50.2	44.6	39.9	32.2*	10.7
2022	35.6	30.8	28.4	20.4*	11.2
2023	160.0	98.0*	99.0*	66.0*	17.5
average	81.9	57.8	55.8	39.5	-
Options with foliar fertilisation at the time of vegetation recovery before fertilising					
2022	30.9	30.0	30.8	28.1	6.5
2023	86.2	85.1	86.8	85.0	11.4
average	58.6	57.6	58.8	56.6	-
Options with foliar fertilisation at the time of vegetation recovery two weeks after fertilisation					
2022	42.4	44.0	41.0	39.8	8.2
2023	166.6	169.0	167.0	165.0	15.4
average	104.5	104.5	104.0	102.4	-

*- a mathematically significant difference with a confidence level $\geq 95.0\%$

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Table 3 – Pairwise correlation coefficients

	Signs	Average value	Oscillation interval
nitrogen of mineral fertilisers	harvest	0.88	0.77–0.98
	plant height	0.97	0.96–0.98
	nitrogen content in the vegetative mass of winter peas in the SVR phase	0.98	0.97–0.99
	the number of nodules on the roots of winter peas at SVR	0.37	0.33–0.41
nitrogen content in the vegetative mass of winter peas in the SVR phase	grain harvest	0.81	0.78–0.85
	the number of nodules	0.31	0.25–0.37
reserves of productive moisture in the 0–20 and 0–100 cm layer at SVR	harvest	0.99	0.98–0.99
	nodulation	0.78	0.76–0.80
* $r_{critical}$			0.58

* $r_{critical}$ – the minimum absolute value of the correlation coefficient at which the relationship is significant at the 5% level of significance (reliability equal to 95%)

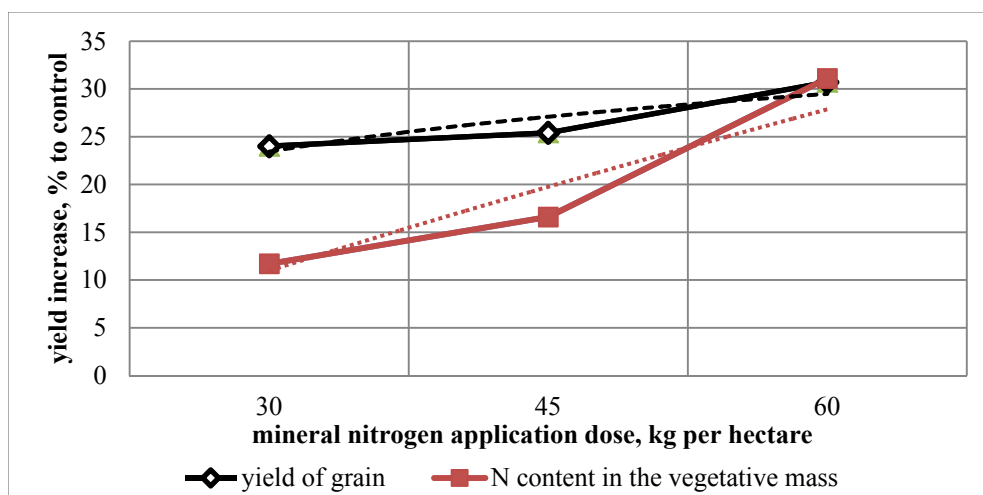


Figure 5 – Effect of doses of mineral nitrogen on the growth of winter pea grain yield

Application during sowing at the rate of 30–60 kg N /ha reduced the mass of pea root nodules by up to 50% compared to the control. The same doses of nitrogen, when used for foliar feeding in the phase of spring vegetation recovery and budding, did not significantly affect the number and weight of nodules but increased the nitrogen content in the vegetative mass

of peas up to 27% relative to unfertilised areas.

In the future, we will continue to study the effect of the distribution of 30, 45 and 60 kg/ha nitrogen for two and three periods of application according to the phases of spring vegetation recovery, budding and filling of pea grain sown in winter.

CONCLUSIONS

Foliar fertilisation of winter pea crops with mineral nitrogen at a dose of 30 to 60 kg/ha (urea) did not have a negative effect on the nodulation process ($r = 0.37$). With this method of application, winter pea plants received a combined type of nitrogen nutrition: mineral - through the leaf apparatus (18–27%) and the rest due to symbiotic fixation and soil.

At-sowing application of mineral nitrogen N_{30-60} (ammonium nitrate) suppressed the process of nitrogen fixation from the active spring growth phase (BBCH 12-15). The mass of nodules on the roots of winter pea plants decreased by 30–50%. Therefore, it could be presumed that plants switch to the consumption of mineral nitrogen and limit nitrogen fixation from the air.

Author Contributions: S. Burykina – conducting research, writing - manuscript preparation; L. Serhieiev – project administration, conceptualisation, software; I. Kohut – methodology, statistical analysis; V. Sharii – viewing and editing. All authors declare that they have read and approved the publication of the manuscript in the present form.

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Conflicts of Interest: The authors declare that the submitted work was carried out in the absence of any personal, professional or financial relationships that could potentially be interpreted as a conflict of interest.

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