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EFFECT OF NITROGEN FERTILIZATION ON THE YIELDING AND HEALTH STATUS OF SELECTED NONPAPILONACEOUS PLANT SPECIES GROWN IN STUBBLE INTERCROP

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ABSTRACT

Field experiments were carried out over 2002-2004 at the Experiment Station of the University of Technology and Agriculture at Mochelek, in the vicinity of Bydgoszcz, on a very good rye complex soil. High biomass yields were recorded for all the nonpapilionaceous plants researched, grown in stubble intercrop. Upon no nitrogen fertilization, sunflower showed to be most productive. The treatments which involved a high nitrogen dose (90 kg ha⁻¹) oil radish yielded significantly higher that phacelia. The plant produced also significantly more post-harvest residue mass than sunflower and phacelia, and also used nitrogen applied prior-to-sowing most considerably. An increase in the dose from 0 to 90 kg of nitrogen resulted in an increase in its accumulation in the plant biomass by 74.4 kg (82.7% of the nitrogen applied), as compared with the non-fertilized treatments. Sunflower used only 49.0 kg (54.4%), while phacelia - 48.2 kg (53.6%). The nitrogen fertilization applied in the experiments significantly increased the overground plant biomass yield of the crops researched. The greatest reaction to N fertilization was demonstrated by oil radish. The dry matter yield of this plant increased by 26.5% due to the application of 45 kg ha⁻¹, as compared with the yield collected from unfertilized objects. Increasing the nitrogen dose from 45 to 90 kg ha⁻¹ resulted in an 18.4% increase in the dry matter yield of radish. The effectiveness of nitrogen fertilization of sunflower was definitely lower and was, respectively, 14.6 and 7.0%. There was observed a relatively low infection with fungal pathogens in sunflower and phacelia, much higher in radish. A significant effect of the fertilization dose on the health status of the plant roots was found. Sunflower roots were most healthy when 45 kg·ha⁻¹ was applied, and the most heavily infected when treated with 90 kg N·ha⁻¹. Radish fertilization with nitrogen, irrespective of the dose, increased the root infection with fungi. There was found no clear effect of the nitrogen fertilization dose on the health status of phacelia roots. The pathogenic fungi isolated from infected roots of plants grown as intercrops were dominated by Alternaria alternata, as well as Fusarium spp., especially F. solani, F. avenaceum and F. culmorum.

Key words: stubble intercrop, sunflower, radish, tansy phacelia, plant health status, nitrogen fertilization.

INTRODUCTION

In the 70s in Poland intercrops were very common cheap crops, which considerably met the animal requirements for bulky feeds. In the 80s and 90s, the decrease in the cattle and sheep stock was accompanied by a decreasing interest of farmers in these crops [16,17,18,20,21]. Now there is a more and more clear need for growing green intercrops in order to enrich the soil with humus and to limit leaching of fertilizer nutrients unused during the vegetation [19,25]. As early as in 1990 there was noted a decreasing role of the biomass of crops grown in stubble intercrops and intercrop companion crops as animal feed and its growing importance as fertilizer [18,21]. According to Songin [19], growing intercrops and using the biomass produced as green manure brings about a number of positive ecological effects. It can be most important to enhance the biological equilibrium of soil, helping the inactivation of the residue of pesticides and other substances of industrial origin. Besides intercrops counteract water and air erosion of soil and can alleviate the consequences of mechanization in agriculture.

Because of a different management of plant biomass, it seems justifiable to develop new technologies of crop cultivation as intercrops to be ploughed-in, which mostly applies to fertilization which, when intercrops were used for fodder, factored in the risk of the accumulation of excessive amounts of free nitrogen and potassium in green forage. According to Michałowski [12], one should apply fertilization exceeding 100 kg·ha⁻¹ P and K under stubble intercrops, while nonpapilionaceous plants should be also fertilized with nitrogen doses of at least 50 kg·ha⁻¹ N. Minerals and the doses can also affect a changing resistance of plants to pathogens. Excessively high doses of nitrogen fertilizer can intensify the occurrence of plant diseases [6,14].

The working hypothesis assumed the existence of a varied potential of the plants researched for managing the nitrogen remaining in soil after the forecrop harvest. Introducing additional nitrogen into soil in a form of ammonium nitrate facilitates the potential for uptaking and accumulating large amounts of this nutrient in the plant biomass, during a short vegetation period of plants grown in stubble intercrop.

The aim of the present research was to determine the effect of the nitrogen dose on yielding and heath status of roots of oil radish, tansy phacelia and sunflower, grown in stubble intercrop and the evaluation of potential for accumulating nitrogen unused by the plant grown as a forecrop as well as applied in a form of ammonium nitrate prior to intercrop sowing.

MATERIALS AND METHODS

Over 2002-2004 at the Mochelek Experiment Station of the Bydgoszcz University of Technology and Agriculture (17°51' longitude and 53°13' northern latitude), a strict two-factor field experiment was carried out following the randomized split-plot method, in four replications. The research was established on lessive soil, formed from heavy loamy sand, representing very good rye complex, very rich in available phosphorus (95.5 mg P in dm³) and potassium (330 mg K in dm³) and an average content of magnesium (59.8 mg Mg in dm³). The soil reaction in 1M KCl was 5.7.

The experimental scheme included: 1) nitrogen fertilization [kg·ha⁻¹]: I = 0, II = 45, III = 90; 2) crops grown in stubble intercrop:

- 'Adagio' oil radish,
- 'Wielkopolski' sunflower,
- 'Stala' tansy phacelia.

The area of harvest plots was 27 m^2 . Seeds were sown after spring barley harvest between August 5 and 9 each year, and collected 71-77 days after sowing.

Straight after barley harvest 60 kg·ha⁻¹ P_2O_5 and 80 kg·ha⁻¹ K_2O were applied. Once mineral fertilizers were used, disc-harrowing was performed, followed by plough about 12 cm deep. Prior to seed sowing, nitrogen fertilizers were applied and the soil seedbed prepared with the cultivation unit.

The sowing rates were as follows $[kg \cdot ha^{-1}]$: radish – 12; sunflower – 30; phacelia – 10, at 12.5cm row-spacing.

Once the plants were harvested, there were determined the yield of fresh matter and dry matter of underground parts and post-harvest residue. The yield of post-harvest residue was defined based on the $25 \times 25 \times 25$ cm soil monoliths sampled. The content of nitrogen determined in the samples of overground biomass and post-harvest residue with the Kjeldahl method and the dry matter yield were used to calculate a total accumulation of N in the biomass of intercrops.

The root infection with a pathogen complex was evaluated during the harvest using a 5-degree scale $(0-4^\circ)$, where: 0° – healthy roots – no symptoms, 1° – to 10% of the root surface infection, 2° – 10-30% of the root surface infection, 3° – 30-60% of the root surface infection, 4° – over 60% of the root surface infection. Each time the health status of 25 plants randomly selected from each plot was analyzed.

The quality classification data expressed as infection degrees were converted into the infection index following the Townsend and Heuberger's formula [24].

The macroscopic root health status evaluation was supplemented with the analysis of the species composition of fungi infecting roots with disease changes. The mycological material was randomly sampled from the plants analyzed. Each time from 100 selected plants, separately for each species, 1 root fragment, about 5 mm, was cut out, rinsed under running water for 45 minutes, disinfected for 5 seconds in 75% solution of C_2H_5OH and for 15 seconds in 0.01% solution of $HgCl_2$, and then rinsed three times in distilled sterile water and placed onto the PDA medium acidified up to pH 5.5.

The variance analysis was made with the AWAR software, developed by IUNG in Puławy. The analysis was made for the split-plot model. The significance of the differences was verified with the Tukey's test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Weather conditions over the research period were very favorable for the growth and development of plants grown in stubble intercrop (Table 1). In 2002 and in 2004 high total rainfall in July and August provided conditions enhancing seed germination and an initial plant development. According to Sypniewski et al. [23], intercrops require even rainfall August through September of a minimum of 90 mm. In 2003, which recorded definitely low rainfall over that period, the density of phacelia and radish after emergence was 34% lower, while that of the sunflower – similar, as compared with the other vegetation periods. Nevertheless the growth and development of the crops researched was adequate and fresh and dry matter yields were high due to high July rainfall, much higher than the mean multi-year total for that month. Besides, the year 2003 recorded very favorable temperature conditions in September, thanks to which the growth and development of the plants in that month was very intensive and the plants could make up for worse conditions in August.

Month	2002	2003	2004	Mean for 1949-1999				
Total precipitation, mm								
July	77.9	106.2	53.5	69.5				
August	58.0	17.7	138.7	47.9				
September	70.5	16.7	40.0	39.9				
October	111.8	34.0	63.8	31.4				
Total July through October	318.2	174.4	296	188.7				
Mean air temperature, °C								
July	18.9	19.2	16.4	17.8				
August	19.9	18.5	17.9	17.4				
September	12.9	13.6	12.7	13.2				
October	6.2	4.8	8.8	8.3				
Mean July through October	14.5	14.0	14.0	14.2				

Table 1. Weather conditions in the region of the research

According to Demidowicz and Gonet [1], temperature is a factor which limits the cultivation of intercrops, mostly because it determines the potential vegetation period length. According to Gromadziński [4], any temperature below $+5^{\circ}$ C very strongly inhibits the increase in the plant biomass. Reports by Gonet [2] indicate that already a longer decrease in temperature below $+10^{\circ}$ C inhibits an increase in the dry matter of spring crops. Mean air temperature below 12° C considerably limits the cultivation of intercrops or makes the cultivation impossible [3]. In the present research mean temperature August through October ranged from 12.3 to 13.1°C and created good conditions for nonpapilionaceous plants. The analysis of the effect of the weather course on the growth, development and yielding of stubble intercrop, as well as earlier observations indicate that rainfall July 20 through August 20 is most critical for the intercrop success. If it exceeds 60 mm, good nonpapilionaceous plants yielding can be expected.

The present observations confirm the intercrop requirements reported on earlier concerning air temperature in September. In the years with mean temperature over 13°C satisfactory yields are obtained even if rainfall is

insufficient. In 2004 the total rainfall July through October was 56.9% higher than the multi-year mean for that period. The course of the growth and development of plants was deteriorated by hailstorm which occurred on August 29 and which resulted in leaf damage and a few-day development inhibition.

The yields of the plant species compared, both the overground fresh and dry matter did not differ significantly (Figs 1 and 2). There was found a varied reaction of plants to the fertilization applied. In objects non-fertilized with nitrogen, the dry matter yield of sunflower was significantly higher than that of radish and phacelia. The greater the fertilization nitrogen dose, the more visible the high potential of oil radish which as a result of the application of 45 kg and 90 kg N yielded significantly higher than phacelia, while the productivity of fresh matter was also higher than that of sunflower.





a, b, c – means followed by the same lower-case letters for N doses within plant species and the same capital letters across nitrogen doses for species were not significantly different x, y, z – means followed by the same lower-case letters for N doses and the same capital letters for species were not significantly different





Nitrogen fertilization showed a significant effect on the yielding of the crops researched. An increasing dose of N fertilization was observed to be accompanied by a significant increase in the yield of fresh and dry matter. There was found a varied effect of increasing doses of nitrogen on the yielding of the plant species researched. The application of 45 kg·ha⁻¹ N resulted in an increase in the yield of dry matter, as compared with the non-fertilized objects, from 14.6% (sunflower) to 26.5% (radish). Increasing the dose from 45 to 90 kg·ha⁻¹ N was definitely less effective and facilitated the yield increase from 7.0% (sunflower) to 18.4% (radish). A lower effectiveness of the increased nitrogen fertilization of sunflower can be due to high thermal requirements of this plant and a very strong reaction to the sowing date. The reports by Gromadziński [5] and Sypniewski and Skinder [22] show a highly significant decrease in the fresh and dry matter yield of sunflower when the sowing date was postponed from the third decade of July to the first decade of August. Radish, as a plant of lower thermal requirements, probably can grow better at lower temperatures of the second half of September and October.

The mean yield of dry matter of post-harvest residue was $1.87 \text{ t}\cdot\text{ha}^{-1}$ and accounted for 37.5% of the total biomass produced. Out of all the species researched, significantly highest mass of post-harvest residue was produced by oil radish, while the lowest – by tansy phacelia. Nitrogen fertilization significantly increased the fresh matter yield of oil radish, while the dry matter was not affected by this factor (Figs 3 and 4).





Fig. 4. Dry matter yield of post-harvest residue - means for 2002-2004



The mean content of nitrogen in the overground biomass ranged from 2.33% (sunflower) to 2.57% (radish) and from 2.21% in non-fertilized objects to 2.78% after the application of 90 kg N·ha⁻¹ (Fig. 5). Irrespective of the nitrogen fertilization dose, the significantly lowest concentration of this nutrient in green forage was noted in sunflower. The higher the nitrogen dose, the higher the nitrogen content in the overground plant parts; a significant increase in the concentration of this nutrient was found in all the crops researched only after the use of 90 kg N·ha⁻¹.



Fig. 5. Content of nitrogen in the dry matter of overground parts of stubble intercrops – means for 2002-2004 $\,$

Underground parts of the plants researched contained almost half less nitrogen than the overground biomass (Fig. 6). The highest concentration of nitrogen was found in post-harvest residue of oil radish, significantly less in phacelia, while the least – in sunflower. Increasing the nitrogen dose resulted in a significant increase in its concentration, although in the case of radish and phacelia, a significant increase in its content was recorded when not less than 90 kg·ha⁻¹ N was applied.



Fig. 6. Content of nitrogen in the dry matter of post-harvest residue of stubble intercrops – means for 2002-2004

A high concentration of nitrogen in the radish biomass must have been the cause of a low yielding of this plant in the objects non-fertilized with this mineral. A full production potential of radish could be seen only when the limiting factor, that is nitrogen deficit, was eliminated. Sunflower plants, containing much lower amounts of this macroelement, developed very well in the objects which have not been fertilized with nitrogen, however as a plant of high thermal requirements, it did not manage, when exposed to decreasing temperature, to use additional amounts of this nutrient, introduced into soil prior to sowing.

The nonpapilionaceous plants researched accumulated in the biomass, on average, from 76.2 kg·ha⁻¹ of nitrogen in the objects non-fertilized with this macroelement, to 133.4 kg·ha⁻¹, following the application of 90 kg N·ha⁻¹ (Fig. 7). Oil radish plant accumulates in its biomass the highest amount of nitrogen and uses the fertilization most effectively. Increasing the N dose from 0 to 90 kg increased its accumulation in the biomass of this plant by 74.4 kg (82.7% of the nitrogen applied), as compared with the non-treated plants. Sunflower managed only 49.0 kg (54.4%), while phacelia – 48.2 kg (53.6%).





The literature reports demonstrate that the effectiveness of intercrops fertilization with nitrogen depends on weather conditions. Gromadziński [5] in his research carried out under rainfall deficit observed no significant effect of increasing the nitrogen dose from 60 to 120 kg·ha⁻¹ on the yield of nonpapilionaceous plants, while Zieliński and Zielińska [26], under favorable conditions, showed a significant increase in the fresh and dry matter yield of white mustard, fodder radish, spring rye, cow cabbage and winter rape already when the N dose was increased from 70 to 90 kg·ha⁻¹. The weather conditions discussed earlier over the research period were rather favorable for the growth, development and yielding of nonpapilionaceous plants grown in stubble intercrop, which allowed for a good effectiveness of nitrogen fertilization, especially for oil radish, containing significantly more nitrogen in the biomass than phacelia and sunflower [25], which, for good yielding, needs a high fertilization dose of 45 kg·ha⁻¹ N was more adequate.

Three-year observations of plants grown in stubble intercrop indicated relatively low infection in common sunflower and tansy phacelia roots, and much higher – in oil radish (Figs 8-10). The mean value of the sunflower root infection index was the lowest of all the plants researched and accounted for 7.3%. Despite such a low infection, there was found a significant effect of the nitrogen fertilization dose on the health status of sunflower roots. The lowest infection was recorded when sunflower was fertilized with nitrogen at the dose of 45 kg·ha⁻¹, the highest – at the dose of 90 kg·ha⁻¹.



Fig. 8. Effect of nitrogen fertilization on sunflower root health status - infection index

means followed by the same lower-case letters across the research years were not significantly different

The mean value of the phacelia root infection index was 9.3% (Fig. 9). Based on the three-year research means, there was found, however, no significant effect of the nitrogen fertilization dose on the health status. A significant variation was found only in 2002 and 2003. In 2002 the healthiest roots were observed for the objects fertilized with $45 \text{ kg} \cdot \text{ha}^{-1}$ N, most infected after the application of 90 kg·ha⁻¹. In 2003 the roots were most healthy when no nitrogen fertilization was applied, and at the dose of 45 kg N, the highest infection index value was recorded.



Fig. 9. Effect of nitrogen fertilization on tansy phacelia root health status - infection index

Out of all the plants researched, the highest infection was recorded for oil radish roots (19.8%). The root health status was considerably affected by the nitrogen fertilization dose. The healthiest roots were noted on the plots where no N was applied, while the use of 45 kg of this macroelement resulted in a significant infection increase. Further increasing of the nitrogen dose did not differentiate the radish health status (Fig. 10).



Fig. 10. Effect of nitrogen fertilization on oil radish root health status - infection index

Majchrzak et al. [10] report on spring *Brassicaceae* plants, including oil radish, being among those plants which are scarcely infected with fungal pathogens. In earlier research reported by Lemańczyk et al. [9] on the effect of different organic fertilizers on the health status of plants grown in stubble intercrop, there were also found low values of the sunflower (8.5%) and phacelia (7.2%), as well as radish (10.4%) infection index. However there were found no clear differences in the health status caused by the dose of the fertilizer applied. In the present research, however, there was recorded a significant effect of the mineral nitrogen dose on the root health status. According to Idziak and Michalski [6] and Plaskowska et al. [14], mineral nitrogen fertilization increases the infection with pathogens. Martyniuk [11] claims otherwise by stating that well-nourished plants grown in intercrops show a greater resistance to pathogens. Orlikowski [13] and Kurowski et al. [8] report on the adequate dose of nitrogen fertilizer being the only one which can create optimal conditions in the soil environment, thus making it possible for the microorganisms capable of inhibiting pathogen development to develop. Soil, a natural environment of microorganisms, has a specific composition of these organisms remaining in equilibrium, which depends on many factors; nitrogen fertilization can be one of them [6,13].

A higher root infection of plants fertilized at the dose of 90 kg N did not result in a significant effect on the yielding, which could have been due to a relatively low degree of infection, even following the application of 90 kg ha⁻¹ N and, first of all, due to a strong reaction of nonpapilionaceous plants grown in stubble intercrop (especially oil radish) to nitrogen fertilization.

As a result of the mycological analysis of fungi infesting the infected roots of plants grown in stubble intercrop (<u>Table 2</u>), the highest number of isolates was obtained from the roots of phacelia (202 isolates), then from radish (148), and finally the lowest number – from sunflower (102). Out of all the pathogenic fungi, *Alternaria alternata* and *Fusarium* spp. dominated, *F. solani, F. avenaceum* and *F. culmorum*, especially.

Chasies	Sunflower		Tansy phacelia		Oil radish	
Species		%**	Σ	%	Σ	%
Alternaria alternata (Fries.) Keiss	50	49.0	82	40.6	3	2.0
Aspergillus niger van Tieghen	1	1.0	—	—	—	-
Aspergillus niger van Tieghen	-	—	_	-	1	0.7
Aureobasidium bolleyi (Sprague) von Arx	-	—	19	9.4	_	-
Botrytis cinerea Pers.	4	3.9	-	-	-	-
Epicoccum nigrum Link	-	—	6	3.0	3	2.0
Fusarium avenaceum (Fr.) Sacc.	8	7.8	26	12.9	12	8.1
Fusarium culmorum (W.G. Smith) Sacc.	-	—	19	9.4	8	5.4
Fusarium equiseti Sacc.	4	3.9	-	-	1	0.7
Fusarium graminearum Schwabe	1	1.0	2	1.0	1	0.7
Fusarium oxysporum Schlecht.	1	1.0	2	1.0	5	3.4
Fusarium poae (Peck.) Wollenw.	1	1.0	-	-	-	-
Fusarium solani (Mart.) Sacc.	5	4.9	25	12.4	47	31.8
Mucor spp.	7	6.9	6	3.0	11	7.4
Penicillium spp.	3	2.9	-	-	4	2.7
Phoma spp	1	1.0	2	1.0	_	-
Pythium sp.	2	2.0	-	-	_	-
Rhizoctonia solani Kühn	-	—	3	1.5	-	-
Trichoderma harzianum Rifai	-	—	-	-	2	1.4
Trichoderma koningii Oud.	9	8.8	4	2.0	20	13.5
Trichoderma polysporum (Link ex Pers.) Rifai	-	—	-	-	3	2.0
Trichoderma viride Pers. ex Gray	5	4.9	6	3.0	21	14.2
Zygorhynchus sp.		_	_	_	4	2.7
Non-sporulating colony	_	_	_	_	2	1.4
Total	102	100.0	202	100.0	148	100.0

Tabla 2	Funai i	colotod	from infact	d roots of	nlante	cultivated in	n stubblo	intereron	(2002	2004)
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* total number of isolates

**percentage share of respective species of fungi

From the infected sunflower roots *A. alternate* was isolated most frequently; it accounted for an average of 49.0% of all the fungi isolated. The share of *Fusarium* genus was 19.6%; *F. avenaceum* (7.8%), *F. solani* (4.9%) and *F. equiseti* (3.9%) were most frequently isolated.

Similarly, A. alternata was most often observed on the infected phacelia roots (40.6%). Of all the fungi *Fusarium* genus (36.6%), *F. avenaceum* (12.9%), *F. solani* (12.4%) and *F. culmorum* (9.4%) dominated. The pathogenic fungi isolated were also represented by *R. solani* (1.5%).

Radish roots were most often infested by *Fusarium* genus fungi, which accounted for an average of 50.0%; mostly *F. solani* (31.8%), whereas *F. avenaceum* (8.1%), *F. culmorum* (5.4%) and *F. oxysporum* (3.4%) were isolated much less frequently. *Trichoderma* spp. (31.1%) was also important, especially *T. viride* (14.2%) and *T. koningii* (13.5%).

From infected roots of radish cultivated in stubble intercrop, Lemańczyk et al. [9] isolated, most of all, *F. oxysporum, F. avenaceum*, whereas from the roots of sunflower and phacelia – mainly *A. alternate.* As for *Fusarium* genus, sunflower was dominated by *F. oxysporum* and *F. avenaceum*, phacelia – by *F. avenaceum* and *F. culmorum*. According to Majchrzak et al. [10], root rot can be caused by e.g. *Fusarium* spp. and *A. alternata*, often isolated in the present research. Besides the fungus often occurs as a saprotroph on plants which can undergo a secondary infestation.

A considerable group was made up by *Trichoderma* fungi which show a definite antagonism towards pathogens, including *Fusarium* spp. The enzymes they produce are of special importance, e.g. gluconases, which facilitate the cell wall decomposition in *R. solani* and other fungi [7]. *Trichoderma* genus fungi can inhibit the development of fungi on seeds considerably, and help decreasing the root infection with pathogens [15].

CONCLUSIONS

- 1. The nitrogen doses applied significantly increased the yield of dry matter of the nonpapilionaceous plants species researched. Oil radish showed the strongest reaction to the fertilization and was the only one of all the species researched which enhanced yielding both when 45 kg·ha⁻¹ was introduced and when the dose was increased to 90 kg·ha⁻¹ N. Sunflower yielded significantly higher than the other species in the objects non-fertilized with nitrogen.
- 2. Common sunflower and tansy phacelia sown in stubble intercrop in the first decade of August do not use the nitrogen doses exceeding 45 kg·ha⁻¹ rationally.
- 3. Oil radish has the greatest, of all the crops researched, potential for managing nitrogen which has not been used by the forecrop and applied in a form of mineral fertilizers prior to sowing, which is due to both a high yielding potential and a significantly higher, than in the sunflower and phacelia, content of nitrogen in the overground biomass and post-harvest residue of that crop.
- 4. Nitrogen fertilization can have a significant effect on the root health status of plants cultivated in stubble intercrop. Excessively high doses resulted in an increase in root infection which, however, did not mean a significant decrease in plant yielding.
- 5. Disease symptoms which occur on the roots of plants cultivated in stubble intercrop were mainly caused by *Fusarium* genus fungi, especially *F. solani*, *F. avenaceum* and *F. culmorum*, as well as *Alternaria alternata*, *Aureobasidium bolleyi* and *Rhizoctonia solani*.

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