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## Wheat productivity in different tillage systems after using biostimulants and their mixtures

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### Abstract

Policies to reduce the use of mineral fertilisers and chemical pesticides have spurred research into biological approaches to soil and plant protection and biostimulation. The two-factorial field experiment was carried out on the crops of spring and winter wheat at the Experimental Station of Vytautas Magnus University Agriculture Academy, Lithuania, from 2018 to 2020. The aim of the experiment was to evaluate the influence of biostimulants and their mixtures in different soil tillage systems on the productivity of spring and winter wheat (*Triticum aestivum* L.). Biostimulants were more effective to stimulate plant development at the no-till + catch crop (NT + CC) treatment. The effect became more evident in the third year of the experiment. The biostimulant with *Trichoderma* sp. and the biostimulant with *Azotobacter* sp. and mixtures with them increased grain yield when NT + CC technology was used. Only mixtures of biostimulants with *Trichoderma* sp. and with *Azotobacter* sp. increased yield when applying PT technology. Although biostimulants do not always increase the yield of agricultural crops, their application is more sustainable agricultural practice.

Keywords: elements of productivity, *Triticum aestivum*, grain yield.

### Introduction

In recent decades, climate change and human activities have particularly accelerated global soil degradation (DeLong et al., 2015). Scientists suggest that around 45% of Europe's land is at potential risk of degradation, and urgent solutions are needed to protect soil, preserve soil functions, restore degraded soils, and provide the necessary nutrients for growing crops (EUR-Lex, 2006; Strafella et al., 2021). The European Green Deal presented by the European Commission aims to reduce nutrient losses by at least 50%, while preventing the deterioration of soil fertility, and to reduce the use of fertilisers by at least 20% by 2030 (FAO..., 2019; Zhang, Peng, 2021). Policies to reduce the use of mineral fertilisers and chemical pesticides have spurred research into biological approaches to soil and plant protection and biostimulation. Over the past years of research, many different types of products including biostimulants, bioproducts, and microbial inoculants have been invented to improve the health, vitality, growth, and yield of plants or to protect them from abiotic and biotic stresses including plant pathogens (Pylak et al., 2019). This can enable high levels of agricultural production to be sustained, as the world will need about 840 million tons of wheat by 2050 up from the current 642 million tons, and this must be achieved with less land and resources

through innovative soil health and, especially, resource-conserving technologies (FAO, 2009).

Biostimulants are defined as substances supplied to plants primarily to improve the efficiency of nutrient uptake as well as to improve tolerance to abiotic stress and the qualitative characteristics of the products grown, regardless of the nutrient content in the soil (Du Jardin, 2015). Thus, one of the benefits of using biostimulants is improving nutrient uptake and assimilation. Biostimulants are often attributed to at least one of the following: can increase soil activity both microbiologically and enzymatically, affect the root system, and alter the solubility and transport of micronutrients (Colla et al., 2015; Lucini et al., 2015). Artyszak and Gozdowski (2020) point out that the effectiveness of biostimulants is greatly enhanced under unfavourable agroecological conditions: lack and/or excess of heat and moisture, heavy metal contamination of the soil, and lack of mineral nitrogen. Asseng et al. (2015) suggest that warming is already slowing down yield increases in most wheat-growing regions. Global wheat production is estimated to decrease by 6% with each further increase in temperature by one degree and becomes more variable in space and time. Numerous papers are published about the effect of biostimulants but there are no clear conclusions about

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the effect on crop yield. Major findings of meta-analysis based on 171 peer-reviewed publications were that the effectiveness of biostimulants was higher in dry climate, at higher soil phosphorus levels, and low organic matter content (Schütz et al., 2018). After summarising the data of 180 field experiments, Li et al. (2022) concluded that biostimulants can increase the yield of agricultural plants. Biostimulants improve crop yield by reducing yield reductions under stress conditions.

Maintaining soil fertility without compromising crop productivity and soil biodiversity loss requires an increasingly sustainable agricultural and productivity system. The main aspects are the reduction of tillage operations, the increase of organic residues in the topsoil layer by the mass of catch crops, the use of biological tools to maintain soil fertility, the improvement of plant growth, and the promotion of the mineralisation of plant residues (Wanic et al., 2013; Piotrowska-Długosz, Wilczewski, 2014a; b). The cultivation and incorporation of catch crops into the soil have been reported by researchers to increase agricultural production in all climates, soils, and farming systems (Wanic et al., 2019; Zhang, Peng, 2021). The intensification of agricultural production is responsible for emerging threats to human well-being, rates of biodiversity loss, soil degradation, and environmental pollution (Gomiero, 2016; Lanz et al., 2018; Evans et al., 2019).

In view of the emerging issues in agriculture, the aim of the study was set to evaluate biostimulants and their mixtures that enhance plant growth and promote the mineralisation of crop residues in different soil tillage systems for the productivity of spring and winter wheat (*Triticum aestivum* L.). In the experiment, biostimulants were used alone and in mixtures in different tillage systems with and without catch crops. The wider use of biobased products requires an accurate selection of beneficial micro-organisms and their mixtures and readiness for future agricultural challenges.

**Table 1.** Biostimulants and their mixtures used in the experiment

| Title of treatment | Explanation of treatment   |
|--------------------|--|
| C                  | Control, no biostimulants  |
| N <sub>8</sub>     | Compensatory nitrogen (N), 8 kg per ton of straw, no biostimulants   |
| T                  | Contains fungus <i>Trichoderma</i> sp., which has an antibacterial effect, releases antibiotic substances that protect plants from pathogens, and reduces their activity. Intended to activate the decomposition and mineralisation of plant residues. Suitable for all types of soils, restores their natural properties. Enriched with phytohormones, which regulate cell processes and stimulate plant growth and development when they enter the plant. Due to the mentioned properties, the productivity of outdoor plants increases. Can be used in mixtures with other biological products and herbicides; 1.0 L ha <sup>-1</sup> |
| B                  | Produced from Bentonite clay under electric and magnetic fields. Improves mineralisation and humification of plant residues and maintains natural soil fertility. To accelerate straw mineralisation, it can be used in combination with azo-bacterial products. This combination promotes the destruction and transformation of straw into more complex compounds and improves the activity of organic matter-degrading micro-organisms in the soil. Its detailed composition is not disclosed by the manufacturer (Artyszak, Gozdowski, 2020); 0.2 L ha <sup>-1</sup>  |
| A                  | <i>Azotobacter</i> sp. bacteria in the preparation fix atmospheric N making it available to plants, increasing the N content in the soil, and improving its structure; 1.0 L ha <sup>-1</sup>  |
| T+B                | 0.0 L ha <sup>-1</sup> + 0.2 L ha <sup>-1</sup>  |
| T+A                | 1.0 L ha <sup>-1</sup> + 1.0 L ha <sup>-1</sup>  |
| B+A                | 1.0 L ha <sup>-1</sup> + 1.0 L ha <sup>-1</sup>  |
| T+B+A              | 1.0 L ha <sup>-1</sup> + 0.2 L ha <sup>-1</sup> + 0.5 L ha <sup>-1</sup>   |

rate of 14.0 kg ha<sup>-1</sup>. For wheat sowing, a seed drill Rapid 300 (Väderstad, Sweden) was used. Catch crop was left in field during the winter period and spring wheat was sown into it in the spring. The average yield of catch crop biomass was evaluated: 9.83–13.45 t ha<sup>-1</sup> green biomass and 1.32–1.86 t ha<sup>-1</sup> dry biomass. In the experiment, fungicides were not used.

Spring wheat: sowing rate 320 kg ha<sup>-1</sup>, locally fertilised with complex fertiliser N<sub>43</sub>P<sub>43</sub>K<sub>43</sub>, and fertilised

## Material and methods

**Experimental site.** The field experiment was carried out at the Experimental Station of Vytautas Magnus University Agriculture Academy, Lithuania, from 2018 to 2020. The soil of the experiment was carbonate shallow clayey loam (*Calc(ar)i-Epihypogleyic Luvisol*) according to WRB (2015). The soil has a granulometric composition of 40% sand and 38% clay. The soil pH is close to neutral of 6.0–6.7 with high levels of mobile phosphorus (P<sub>2</sub>O<sub>5</sub>) averaging 285.8 mg kg<sup>-1</sup> and mobile potassium (K<sub>2</sub>O) averaging 240.0 mg kg<sup>-1</sup>. In the experimental fields, the organic carbon (C) content varied from 1.28% to 1.11% and the total nitrogen (N<sub>tot</sub>) from 0.110% to 0.009%.

A two-factorial stationary field experiment was carried out on the crops of wheat (*Triticum aestivum* L.): winter 'Seilor' and spring 'Wicki'. Treatments of the experiment were as follows: 1) tillage systems: no-tillage + catch crop (NT + CC) and ploughless tillage (PT) with the use of a disc tillage cultivator (factor A); the use of biostimulants (factor B). The experimental plots were of the same size as gross plot – 72 m<sup>2</sup> and net plot – 60 m<sup>2</sup>. The treatments were arranged randomly in four replication blocks.

In the site selected for the experiment, winter wheat was grown in 2017 and 2019, spring wheat in 2018 and 2020. The NT was used from 2017. In 2016, winter oilseed rape was grown using conventional tillage (CT). After harvesting, the straw was chopped and spread on the stubble over the whole experimental area. The stubble was treated with N<sub>8</sub> and sprayed with different biological agents and mixtures thereof (Table 1).

Halfway through the experiment (in cloudy weather), the crop residues were immediately (within 1 h) incorporated 5–7 cm deep into the soil with a disc cultivator (PT). The rest was left uncultivated (NT + CC). The spring wheat was preceded by catch crop of white mustard and root radish at a ratio of 50:50 at a total seed

additionally with N<sub>41</sub> at wheat tillering stage, herbicides Elegant 0.4 L ha<sup>-1</sup> (florasulam 6.25 g L<sup>-1</sup> + 2.4-D 300 g L<sup>-1</sup>) and Trimmer 10 g ha<sup>-1</sup> (tribenuron-methyl 500 g kg<sup>-1</sup>).

Winter wheat: sowing rate 170 kg ha<sup>-1</sup>, locally fertilised with complex fertiliser N<sub>8</sub>P<sub>20</sub>K<sub>30</sub>, 350 kg ha<sup>-1</sup>, in autumn sprayed with herbicide Komplet SC 560 0.4 L ha<sup>-1</sup> (flufenacet 280 g L<sup>-1</sup> + diflufenican 280 g L<sup>-1</sup>). In spring, the crop was treated with N at a rate of 200 kg ha<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (BBCH 23–25) and with

an additional fertiliser  $\text{NH}_4\text{NO}_3$  at a rate of  $100 \text{ kg ha}^{-1}$  (BBCH 29).

**Methods of analysis.** Wheat productive stems were counted in each experimental plot at six locations using a longitudinal meter count and converted to the number of seedlings per  $\text{m}^2$ . Grain yield was assessed by harvesting with a combine harvester Wintersteiger Delta (Wintersteiger, Austria) equipped with a weighing and moisture determination system. The cleanliness of the grains was determined, and the yield was converted into the standard yield of 14% moisture content and 100% cleanliness of wheat  $\text{t ha}^{-1}$ .

**Determination of plant biometric and grain yield structure parameters.** Before harvesting,  $1 \text{ m}^{-2}$  reference plots were cut in each experimental field. The samples of each field were brought to the laboratory. For analysis, 30 plants were randomly selected from each plot. The biometric and yield structure parameters (number and weight of grains per ear) of each plant were determined. The 1000-grain weight was evaluated using an accurate seed counter Elmor C1 from the samples after grain harvesting with a plot harvester Wintersteiger.

**Meteorological conditions.** Lithuania's meteorological conditions have a major impact on the microbiological processes in the soil, which determine the nutrient uptake by plants. These processes in the environment determine the yield and quality of agricultural crops. In 2018, September was relatively warm, but the rainfall was insufficient for good wheat germination. In October, the air temperature was higher at  $2.3^\circ\text{C}$  and the rainfall was similar to normal for this period. November was also warmer at  $2.19^\circ\text{C}$ , but the rainfall was 30.5 mm below the long-term average. December, January, and February were characterised by positive temperatures, liquid precipitation, and no snow cover. The spring of 2019 was exceptionally dry. The wheat sown was very difficult to germinate with the first seedlings appearing only after a month. May was cool and rainy, favourable for the formation of the plant productivity elements. At the end of July, conditions

were favourable for harvesting. The spring of 2020 was exceptionally dry. The wheat sown was very difficult to germinate with the first sprouts appearing only a month later. May was cool and wet when the elements of cereal productivity were forming. In July, conditions were favourable for harvesting.

**Statistical analysis** of the experimental data was carried out by two-factor analysis of variance (ANOVA), the significance of the differences between the means of the variants was determined according to the Fisher's criterion, and the least significant difference was found to be  $\text{LSD}_{0.05}$  at the 95% probability level ( $P < 0.05$ ). To assess the relationships between traits, correlation-regression analysis was used.

## Results and discussion

**Productivity of spring wheat in 2018.** Seed germination is the first critical step in the life cycle of a plant and the basis of agricultural production (Nonogaki et al., 2018). The meteorological conditions in 2018 were particularly unfavourable for the germination and subsequent tillering of spring wheat with a low precipitation since the beginning of the year and 42 mm at the sowing time in early April. However, dry weather returned later. The conditions were also unfavourable for the action of soil biostimulants containing microorganisms. Higher temperatures accelerated the growth and development of the plant but shortened the duration of the development stages and reduced productivity. Water deficit and high temperatures can promote the formation of free radicals and reactive oxygen compounds that impair plant metabolism (Long, Ort, 2010; Liu et al., 2014; Driedonks et al., 2015). The climatic conditions in 2018 confirmed the scientists' claims that dry and hot weather affects the productivity of spring wheat and the harvest starts particularly early in the country. Due to the lack of moisture at the beginning of the growing season, germinated spring wheat was weakly tillered resulting in a low formation of productive stems (Table 2).

**Table 2.** Spring wheat productivity indicators in different tillage systems with biostimulants and their mixtures in 2018

| Biostimulants used and their mixtures | No-till + catch crop                              |                                 |                            |                      |
|---------------------------------------|---|---------------------------------|----------------------------|----------------------|
|                                       | number of productive stems, units $\text{m}^{-2}$ | number of grains per ear, units | weight of grains in ear, g | 1000-grain weight, g |
| 1. Control, no biostimulants          | 577 a   | 2.50 bc                         | 0.50 c                     | 43.60 a              |
| 2. $\text{N}_8$ , no biostimulants    | 579 a   | 28.4 a                          | 0.67 a                     | 43.96 a              |
| 3. T                                  | 564 bc  | 24.4 c                          | 0.56 bc                    | 43.56 a              |
| 4. B                                  | 545 bc  | 26.19 b                         | 0.55 bc                    | 43.56 a              |
| 5. A                                  | 534 c   | 24.9 bc                         | 0.55 bc                    | 42.08 c              |
| 6. T+B                                | 566 ab  | 27.1 a                          | 0.52 c                     | 42.00 c              |
| 7. T+A                                | 558 b   | 27.1 a                          | 0.58 b                     | 42.32 bc             |
| 8. B+A                                | 585 a   | 25.6 b                          | 0.61 ab                    | 43.10 ab             |
| 9. T+B+A                              | 583 a   | 27.9 a                          | 0.63 a                     | 42.54 bc             |
| Ploughless tillage                    |   |                                 |                            |                      |
| 1. Control, no biostimulants          | 614 a*  | 25.9 b                          | 0.52 c                     | 45.00 b*             |
| 2. $\text{N}_8$ , no biostimulants    | 619 a*  | 26.3 ab                         | 0.65 a                     | 45.90 b*             |
| 3. T                                  | 585 bc  | 26.1 ab                         | 0.56 bc                    | 47.96 a*             |
| 4. B                                  | 574 bc*   | 25.6 bc                         | 0.57 bc                    | 45.38 b*             |
| 5. A                                  | 572 c*  | 22.7 d                          | 0.56 bc                    | 42.18 d*             |
| 6. T+B                                | 585 b   | 27.5 a                          | 0.56 b*                    | 43.36 c*             |
| 7. T+A                                | 600 a*  | 28.4 a                          | 0.63 ab*                   | 42.96 cd             |
| 8. B+A                                | 591 b   | 26.3 ab                         | 0.59 b                     | 45.84 b*             |
| 9. T+B+A                              | 606 a   | 24.1 cd                         | 0.56 bc*                   | 42.88 cd             |

*Note.* Explanation of treatments in Table 1; means marked with different letters (a, b, c...) indicate a significant effect of the biostimulants and their mixtures and those marked with an asterisk (\*) indicate a significant effect of the tillage system,  $P < 0.05$ .

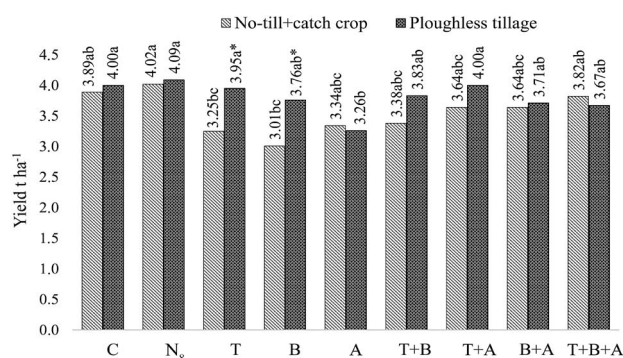
When biological products were applied to the stubble and incorporated with the use of a disc tillage cultivator (PT), spring wheat formed on average 11.2% more productive stems than in a field where the biostimulants were applied but not incorporated (NT +

CC); in most cases, the difference was significant. The number of productive stems was more influenced by the mixtures of biostimulants at the PT crop with an average of 13.0% more stems compared to the crop with no soil biostimulant mixtures (control). At the NT + CC, the

difference in these results was two times lower (6.7%). The use of the mixtures B+A and T+B+A resulted in more productive stems than the NT crop with N<sub>8</sub> fertilisers, but the difference was not significant. When the tested biostimulants and their mixtures were incorporated into the soil (PT), spring wheat produced the highest number of productive stems in the crop where N<sub>8</sub> was applied, while the number of productive stems was lower but not significantly different when the mixtures T+A, B+A, and T+B+A were applied. Spring wheat produced more productive stems in the crops where mixtures of biostimulants and N<sub>8</sub> were applied to stimulate straw mineralisation compared to the use of a single product. Lestingi et al. (2007) found that biostimulants with mycorrhizal fungi and N-fixing bacteria have a positive effect on the yield and quality of triticale and are most effective in the early stages of plant growth.

The number and weight of grains in the ear were not significantly affected by a tillage system (Table 2). The biostimulants and their mixtures increased the average number and weight of grains per ear, but only in a small number of cases significantly compared to the crop without biostimulants and with N<sub>8</sub> application at the NT + CC only. The tillage system had a significant effect on 1000-grain weight with an average increase of 4.5% at the application of PT compared to NT + CC. At the NT + CC, 1000-grain weight was significantly higher at the N<sub>8</sub> application and in the control crop and was comparable to the use of biostimulants B and T in the NT crop and the mixture B+A. At the PT, biostimulants did not have a significant effect with a significantly higher grain weight only after the application of a non-mixed biostimulant with *Trichoderma* sp. (T). At the N<sub>8</sub> and in the control crop, 1000-grain weight was significantly higher compared to the biostimulant mixtures.

**Spring wheat grain yield in 2018.** The grain yield was particularly low, ranging from 3.0 to 4.09 t ha<sup>-1</sup>, given the growing conditions and the drought that year (Figure 1).



Explanations of treatments in Table 1, of statistical analysis under Table 2

**Figure 1.** Spring wheat grain yield in different tillage systems with biostimulants and their mixtures in 2018

Spring wheat was most productive with N<sub>8</sub> application, both by incorporation (PT) and by leaving it on the stubble (NT+CC). Spraying with the mixtures T+A, B+A, and T+B+A resulted in a lower wheat productivity, but the difference was not significant. The application of biostimulants B and A alone, not in mixtures, resulted in a significantly lower grain yield compared to their application in mixtures with other products and, also, to the application of N<sub>8</sub>. The application of the tested mixtures of biostimulants to the soil resulted in a higher spring wheat grain yield, but the difference was not

significant in all treatments. High temperatures during the grain filling stage are one of the main environmental factors limiting grain yield in temperate climate wheat crops (Liu et al., 2014).

In the temperate zone, wheat is often subjected to high temperature stress during the grain filling and maturation stages (Sadras, Dreccer, 2015; Hu et al., 2018). The likelihood of heat stress increases with climate change, which negatively affects wheat productivity (Long, Ort, 2010). Soluble starch synthase in the endosperm of wheat crops is known to be highly sensitive to heat stress. Heat stress leads to the inactivation of many heat-stable proteins, the accumulation of harmful reactive oxygen species, and even the programmed cell death (Xue, McIntyre, 2011; Liu et al., 2014; Driedonks et al., 2015). The climatic conditions in Lithuania in 2018, especially the dry and hot start of the growing season, were uncharacteristic of this zone. This explains the researchers' claims that heat stress has a negative impact on the formation of wheat productivity elements (Long, Ort, 2010; Liu et al., 2014).

Scientists have carried out extensive research on biological tools to improve plant health, vitality, growth, and yield, and to protect plants from abiotic and biotic stresses. They highlight that the effectiveness of these tools varies and is highly dependent on a number of factors such as soil moisture, air temperature, and precipitation, especially if they contain micro-organisms (Du Jardin, 2015; Pačuta et al., 2018).

**Productivity of winter wheat in 2019.** The influence of tillage systems on the number of productive stems in winter wheat was limited with only a few trends observed (Table 3). The application and incorporation of biostimulants into the soil (PT) significantly increased the number of productive stems in the crop sprayed with biostimulant T by 13.4% compared to its application in the crop at the NT + CC. The number of productive stems of winter wheat was not significantly affected by the tillage system when using other products and their mixtures.

The mixture B+A increased the number of productive stems of winter wheat under both tillage systems. At the NT + CC, the application of this mixture resulted in a significant increase of 15.9% in the number of productive stems compared to the application of biostimulant B alone. The other tested products resulted in a lower number of productive stems of winter wheat, but the decrease was not significant. At the PT, in which the tested products were incorporated after application, the highest number of productive stems was produced by winter wheat sprayed with mixture B+A. The application of mixture B+A increased winter wheat tillering by 6.0% compared to the application of N<sub>8</sub>, but the difference was not significant. The above-mentioned mixture significantly increased (on average by 16.4%) the number of productive stems compared to the mono-component biostimulants T, B, and A, and the mixture T+B. At the NT + CC, winter wheat produced the most grains and a high grain weight in the ears after application of T, T+A, and N<sub>8</sub> (Table 3). The number and weight of grains were lower with the mixture B+A, but not significantly different compared to the N<sub>8</sub> application. Winter wheat produced significantly less grains and had the lowest grain weight with the application of biostimulant A and mixtures T+B and T+B+A. At the PT, winter wheat produced the highest number of grain spikes and a high grain weight with the application of mixture T+A and biostimulant B. The number of grains and weight

**Table 3.** Winter wheat productivity indicators in different tillage systems with biostimulants and their mixtures in 2019

| Biostimulants used and their mixtures | No-till + catch crop                              |                                 |                                |                      |
|---------------------------------------|---|---------------------------------|--------------------------------|----------------------|
|                                       | number of productive stems, units m <sup>-2</sup> | number of grains per ear, units | weight of grains in the ear, g | 1000-grain weight, g |
| 1. Control, no biostimulants          | 488 a   | 34.2 a                          | 1.47 a                         | 43.81 c              |
| 2. N <sub>8</sub> , no biostimulants  | 478 ab  | 33.8 ab                         | 1.41 ab                        | 43.78 c              |
| 3. T                                  | 491 a   | 32.4 ab                         | 1.31 ab                        | 38.82 e              |
| 4. B                                  | 433 b   | 31.2 b                          | 1.37 ab                        | 43.77 c              |
| 5. A                                  | 465 ab  | 28.2 c                          | 1.07 c                         | 42.73 d              |
| 6. T+B                                | 467 ab  | 31.2 b                          | 1.18 c                         | 45.53 b              |
| 7. R+A                                | 478 ab  | 31.8 ab                         | 1.31 ab                        | 45.46 b              |
| 8. B+A                                | 502 a   | 30.7 bc                         | 1.32 ab                        | 46.34 a              |
| 9. T+B+A                              | 470 ab  | 31.2 b                          | 1.25 bc                        | 45.79 b              |
| Ploughless tillage                    |   |                                 |                                |                      |
| 1. Control, no biostimulants          | 539 a*  | 31.3 abc*                       | 1.30 a                         | 44.22 cd             |
| 2. N <sub>8</sub> , no biostimulants  | 484 bc  | 29.2 c*                         | 1.11 b*                        | 37.88 f*             |
| 3. T                                  | 433 d*  | 30.0 bc                         | 1.17 b*                        | 38.75 e*             |
| 4. B                                  | 466 cd  | 32.9 a                          | 1.42 a                         | 38.43 e*             |
| 5. A                                  | 468 cd  | 29.1 c                          | 1.29 a*                        | 44.85 b*             |
| 6. T+B                                | 457 cd  | 31.0 abc                        | 1.20 b                         | 46.28 a*             |
| 7. T+A                                | 474 bc  | 32.9 a                          | 1.32 a                         | 44.01 d*             |
| 8. B+A                                | 513 ab  | 31.2 abc                        | 1.26 a                         | 44.56 bc*            |
| 9. T+B+A                              | 485 bc  | 32.2 ab                         | 1.34 a                         | 45.09 b              |

Explanations of treatments in Table 1, of statistical analysis under Table 2

per ear were significantly lower when the stubble was sprayed with N<sub>8</sub> and biostimulant T was applied, whereas the number of grains was lower, but the weight was not significantly different when biostimulant A was applied. The high grain weight in the ears was obtained with mixtures T+A, B+A, and T+B+A.

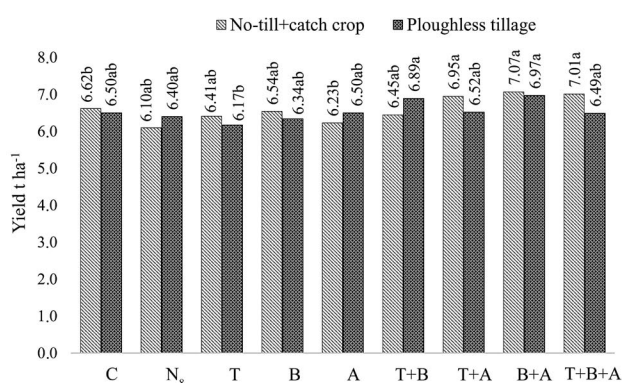
At the NT + CC, N<sub>8</sub> increased the number of grains per ear by 9.2% compared to the PT. Grain weight was also higher when N<sub>8</sub> and biostimulant T were applied at the NT + CC, while biostimulant A increased grain weight significantly at the PT. The effect of tillage system on 1000-grain weight after the application of the products in the stubble of the pre-crop was in most cases significant. The application of mono-component biostimulants at the PT resulted in a significant increase in 1000-grain weight compared to the NT + CC with an average increase of 6.5%. The use of biostimulant mixtures resulted in an overall average increase of 17.2% in 1000-grain weight at the NT + CC. The use of N<sub>8</sub> to stimulate straw mineralisation did not have a significant effect on 1000-grain weight in both tillage systems.

At the NT + CC, the use of soil mixture B+A significantly increased 1000-grain weight by an average of 7.8% compared to the other biostimulants and their mixtures and by 7.5% compared to the N<sub>8</sub>. At the PT, the application of mixture T+B resulted in a significantly higher 1000-grain weight by 6.0% compared to the N<sub>8</sub> application and by an average of 8.7% compared to the application of other biostimulants and their mixtures.

**Winter wheat grain yield in 2019.** The use of biostimulants and their mixtures increased winter wheat grain yield compared to the use of N<sub>8</sub> to stimulate straw mineralisation (Figure 2).

At the PT, the application of biostimulants A, T, and B in the stubble winter wheat grain yield increased from 2.0% to 8.0%, while the application of mixture T+B by 6.2%, but the difference was not significant. A significant grain yield increase was obtained with the following mixtures: T+A – 13.9%, B+A – 15.9%, and T+B+A – 14.9%. The NT + CC also increased the grain yield of winter wheat compared to the N<sub>8</sub> to stimulate straw mineralisation but only with the following mixtures: T+B – 7.6%, B+A – 8.9%, and T+B+A – 7.6%. The grain yield of winter wheat was independent of tillage with a non-significant difference between NT + CC and PT.

**Productivity of spring wheat in 2020.** The number of productive stems of spring wheat was not



Explanations of treatments in Table 1, of statistical analysis under Table 2

**Figure 2.** Winter wheat grain yield in different tillage systems with biostimulants and their mixtures in 2019

significantly affected by a tillage system, but more stems were produced at the NT + CC (Table 4). The effect of mono-component biostimulants on the number of productive stems was insignificant when compared to the use of N<sub>8</sub>, but better tillering was observed (2.5%) when biostimulants B and A were applied. The use of all biostimulant mixtures increased the number of productive stems of spring wheat better than N<sub>8</sub>. The number of productive stems increased by 6.8% with the mixture T+B and by 8.1% with the mixture T+B+A. At the PT, all biostimulants used increased the number of productive stems of spring wheat significantly better than N<sub>8</sub>: on average, mono-component ones increased by 21.7% and mixtures by 20.8%.

The number of grains per ear was significantly, on average by 29.0%, higher at the NT + CC compared to the PT (Table 4). The NT + CC had a positive effect on grain formation in the ear. The number of grains in the ear was directly related to the spring wheat grain yield ( $y = 1.22 + 0.14x$ ;  $r = 0.68$ ;  $P < 0.05$ ); there was a strong direct positive correlation between these variables. No such correlation was found at the PT. In both tillage systems, the wheat crops, where biostimulant mixtures were used, formed more grain in the ears with significant differences compared to the use of N<sub>8</sub>.

The weight of grains per ear was also significantly, on average by 28.8%, higher at the NT +

**Table 4.** Spring wheat productivity indicators in different tillage systems with biostimulants and their mixtures in 2020

| Biostimulants used and their mixtures | No-till + catch crop                              |                                 |                                |                      |
|---------------------------------------|---|---------------------------------|--------------------------------|----------------------|
|                                       | number of productive stems, units m <sup>-2</sup> | number of grains per ear, units | weight of grains in the ear, g | 1000-grain weight, g |
| 1. Control, no biostimulants          | 521 c   | 32.1 ab*                        | 1.49 ab*                       | 42.56 b              |
| 2. N <sub>8</sub> , no biostimulants  | 555 ab*   | 30.1 b*                         | 1.37 b*                        | 41.48 b              |
| 3. T                                  | 534 bc  | 33.1 ab*                        | 1.51 ab*                       | 41.31 b              |
| 4. B                                  | 569 ab  | 30.4 b*                         | 1.48 ab*                       | 46.27 a*             |
| 5. A                                  | 569 ab  | 33.7 ab*                        | 1.48 ab*                       | 43.96 ab             |
| 6. T+B                                | 593 a   | 35.3 a*                         | 1.44 b*                        | 42.35 b              |
| 7. T+A                                | 569 a   | 34.9 a                          | 1.53 ab*                       | 46.27 a              |
| 8. B+A                                | 588 a   | 34.9 a*                         | 1.64 a*                        | 45.24 a              |
| 9. T+B+A                              | 600 a   | 35.8 a*                         | 1.69 a                         | 46.16 a              |
| Ploughless tillage                    |   |                                 |                                |                      |
| 1. Control, no biostimulants          | 542 b   | 21.2 d                          | 1.10 bc                        | 41.80 ab             |
| 2. N <sub>8</sub> , no biostimulants  | 467 c   | 22.5 cd                         | 1.02 c                         | 40.75 b              |
| 3. T                                  | 589 a   | 23.7 cd                         | 1.00 c                         | 41.59 ab             |
| 4. B                                  | 554 ab  | 26.1 bc                         | 1.03 c                         | 42.66 ab             |
| 5. A                                  | 583 ab  | 25.7 bc                         | 1.18 b                         | 42.78 ab             |
| 6. T+B                                | 557 ab  | 28.2 ab                         | 1.24 b                         | 42.48 ab             |
| 7. T+A                                | 558 ab  | 31.7 a                          | 1.21 b                         | 43.92 ab             |
| 8. B+A                                | 543 b   | 27.7 b                          | 1.24 b                         | 43.36 ab             |
| 9. T+B+A                              | 595 a   | 27.7 b                          | 1.57 a                         | 44.53 a              |

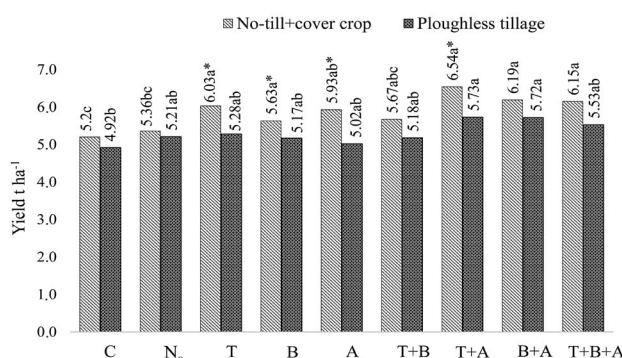
Explanations of treatments in Table 1, of statistical analysis under Table 2

CC (Table 4). The higher grain weight per ear increased the grain yield of spring wheat. In this crop, there was a strong significant correlation between the grain weight per ear and the wheat grain yield ( $y = -72.25 + 101.58x - 32.9x^2$ ;  $r = 0.79$ ;  $P < 0.05$ ). The effect of all biostimulants used was better compared to the use of N<sub>8</sub>. At the NT + CC, the grain weight in the ear was significantly increased by the mixtures B+A and T+B+A, while at the PL, the application of all biostimulant mixtures resulted in a higher grain weight in the ear compared to the application of N<sub>8</sub> and mono-component biostimulants, but a significant increase was observed with the application of mixtures B+A and T+B+A only.

The 1000-grain weight was not significantly affected by tillage systems, but the spring wheat was larger at the NT + CC (Table 4). In both crops, 1000-grain weight was higher with the biostimulant mixtures compared to the mono-component biostimulants. The effect of mixture T+B was similar to that of the stubble application of N<sub>8</sub> and/or biostimulants outside the mixture.

In 2020, the spring wheat grain yield was 10.4% higher at the NT + CC compared to the PT (Figure 3). A significant effect of tillage systems on the grain yield was only found in a few cases: the use of biostimulants T, B, and A and also a mixture T+A. At the NT + CC, biostimulants and their mixtures increased spring wheat grain yield in most cases compared to the use of N<sub>8</sub>. The use of catch crops had positive implications for improving further land use efficiency. Immobilisation of plant residues in the soil resolves the C to N ratio over time and solves the problem of non-decreasing biomass of micro-organisms in the soil by increasing soil organic N stocks (Mooshammer et al., 2014; Mateus et al., 2020). At the NT + CC, the effect of biostimulant mixtures on the spring wheat grain yield was particularly pronounced with an average of 14.5% higher yield compared to the application of N<sub>8</sub> and 7.9% compared to the application of mono-component biostimulants. The use of biostimulants at the PT increased the spring wheat grain yield but did not have a stronger effect compared to the NT + CC. The effect of mono-component products on the spring wheat grain yield was similar to that of N<sub>8</sub>, but the mixtures T+A, B+A, and T+B+A tended to increase the spring wheat grain yield.

The results of the three-year experiment showed that the biostimulants that improve plant development



Explanations of treatments in Table 1, of statistical analysis under Table 2

**Figure 3.** Spring wheat grain yield in different tillage systems with biostimulants and their mixtures in 2020

and promote residue mineralisation increased the wheat grain yield but not after the first year of application. Biostimulants were more effective in stimulating plant development at the NT + CC. Catch crop is reported to induce changes in the abundance and structure of microbial communities and in soil enzyme activity. They stimulate the growth and enzymatic activity of soil-dwelling micro-organism groups while inhibiting the growth of other microbial groups (Elfstrand et al., 2007). These differences are due to changes in the physical soil properties and the ready availability of C and N. They are a source of nutrients for micro-organisms both during and after the growing season (Thorup-Kristensen et al., 2003), thus catch crops are particularly important for the use of biostimulants containing live micro-organisms.

Scientists point out that the application of NT increases biomass yield of soybeans, corn, and sorghum as well as general and soil inorganic N stocks, but has a little effect on grain yield, especially if the growing season is dry. Therefore, they are recommended for crop rotation with beans and bell crops that should be grown using a NT with intermediate crops producing a high biomass (Silva et al., 2020). Buah et al. (2017) agree that NT technology allows the cultivation of cereal crops in such an environment, where there is a lack of moisture, and the weather is extremely hot. Other researchers who investigated crop losses due to water shortages and high



temperatures report that NT technology resulted in 57% and 33% higher corn and soybean yield, respectively, compared to the CT. The authors explain this yield increase by crop diversity and the adapted tillage system affecting soil N availability (Chu et al., 2017; Ginakes et al., 2018; Rocha et al., 2020). In this context, legumes such as soybean contribute to increasing soil N content through biological N fixation (Chu et al., 2017; Wu et al., 2017). In addition, grasses with a high aboveground and belowground biomass increase soil C sequestration and soil organic matter content improving soil N stocks (Raphael et al., 2016). The cultivation of catch crops and the application of NT are particularly important when incorporating biological measures into the cultivation of agricultural production, in order to preserve soil fertility without reducing the productivity of agricultural plants and soil biodiversity.

## Conclusions

1. Biostimulants were more effective to stimulate plant development at the no-till + catch crop (NT + CC) treatment. The effect became more evident in the third year of the experiment.

2. The biostimulant with *Trichoderma* sp. (T) and the biostimulant with *Azotobacter* sp. (A) and mixtures with them increased wheat grain yield when no-till + catch crop (NT + CC) technology was used. Only mixtures of biostimulants with *Trichoderma* sp. and with *Azotobacter* sp. increased yield when applying ploughless tillage (PT) technology.

3. Though the effect of biostimulant on crop yield is often inconsistent, the application of biostimulants is more sustainable agricultural practice.

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## Kviečių produktyvumas skirtingose žemės dirbimo sistemose naudojant biostimuliantus ir jų mišinius

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### Santrauka

Mineralinių trąšų ir cheminių pesticidų naudojimo mažinimo politika paskatino taikyti biologinius dirvožemio ir augalų apsaugos biostimuliacijos metodus. Dviejų veiksnių lauko eksperimentas su vasarinių kviečių ‘Wicki’ ir žieminių kviečių ‘Seilor’ pasėliais buvo vykdytas 2018–2020 m. Vytauto Didžiojo universiteto Žemės ūkio akademijos Bandymų stotyje. Tyrimo tikslas – įvertinti augalų augimą gerinančių ir augalinių liekanų mineralizaciją skatinančių biologinių produktų įtaką žieminių bei vasarinių javų produktyvumui taikant skirtingas žemės dirbimo sistemas. Trejų metų eksperimento rezultatai parodė, kad augalų vystymąsi gerinantys ir liekanų mineralizaciją skatinantys biostimuliantai didino javų grūdų derlingumą, tačiau ne pirmaisiais naudojimo metais. Trečiųjų eksperimento metų rezultatai parodė, kad biologiniai produktai augalų vystymuisi ir šiaudų skaidymui paskatinti buvo efektyvesni taikant tiesioginę sėją ir auginant tarpinį pasėlį. Vasariniai kviečiai pirmaisiais eksperimento metais geriau krūmijosi taikant bearimą žemės dirbimą, bet vėlesniais (2019 ir 2020) metais daugiau produktyvių stiebų suformavo taikant tiesioginę sėją ir auginant tarpinį pasėlį. Taikant bearimą žemės dirbimą ir auginant tarpinį pasėlį kviečiai užmezgė daugiau grūdų varpoje ir jų masė buvo didesnė, palyginus su bearimio žemės dirbimo pasėliais. Pirmaisiais eksperimento metais biostimuliantų ir jų mišinių naudojimas reikšmingesnės įtakos javų krūmijimuisi neturėjo. Esminiai skirtumai, palyginus su kompensacinio azoto naudojimu ir pasėliu be tirtų priemonių, išryškėjo tik trečiaisiais eksperimento vykdymo metais. Daugiausia produktyvių stiebų suformavo kviečiai, auginami naudojant biostimuliantų mišinius. Produktus naudojant ne mišinyje, jų efektyvumo javų derlingumui nenustatyta bearimio žemės dirbimo pasėliuose, palyginus su pasėliais, kuriuose biostimuliantai nenaudoti. Vasarinių kviečių grūdų derlingumą esmingai didino visi biostimuliantai ir biostimuliantų mišinys, sudarytas iš preparato, skirto aktyvinti augalinių liekanų skaidymą ir mineralizaciją, ir preparato su *Azotobacter* sp. bakterijomis taikant tiesioginę sėją ir auginant tarpinį pasėlį, lyginant su bearimio žemės dirbimo pasėlių derlingumu.

Reikšminiai žodžiai: biologiniai produktai, produktyvumo elementai, *Triticum aestivum*, grūdų derlingumas.