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ABOUT THE PHYTOSANITARY STATE OF THE SOIL AND THE EFFECT OF THE CEREAL SEED TREATMENT WITH THE DAROSTIM® BOSTAR ARRAY AGAINST PHYTOPATHOGENIC BACTERIA AND FUNGI

В 2015 году было начато систематическое исследование фитосанитарного состояния опытных площадей в Германии как одна из составных частей международной программы Tandem[12/21](2012-2021). До сегодняшнего дня на наличие фитопатогенных бактерий и грибов проанализированы и классифицированы 172 площадей. Предварительная оценка результатов показывает, что доля фитопатогенных бактерий и грибов охватывает диапазон от 0 до 95 %. CFD-измерения показывают, что на 9-11 календарной неделе активность процесса фотосинтеза PHS озимых зерновых на опытных полях с возрастанием доли фитопатогенов снижается на 10,1 %. На полях, где семенной материал не обрабатывался препаратом daRostim®BOSTAR, падение PHS ещё больше – до 17 %; на полях, где обработка проводилась, наблюдалось повышение PHS на 7,9 %.

As part of the international program Tandem[12/21] (2012-2021), the systematic screening of the phytosanitary state of the German trial areas was started in 2015. To date, 172 trial sites have been screened and classified for the presence of phytopathogenic bacteria and fungi. An initial evaluation shows that the proportion of phytopathogenic bacteria and fungi covers a range of 0 to 95 %. CFD measurements show that in the 9-11 calendar week, the photosynthetic power PHS of winter crops on these trial areas decreases by almost 10.1 % as the proportion of phytopathogenic increases. On unprepared areas, the loss of PHS is even greater at -17 %; on areas treated with daRostim®BOSTAR, a 7.9 % increase of PHS was observed.

Ключевые слова: Tandem[12/21]*;* почва; фитопатогенные бактерии и гриы; фотосинтез.

Keywords: Tandem[12/21]*;* soil; phytopathogenic bacteria and fungi; photosynthesis.

Introduction

The international long-term program Tandem[12/21] (2012-2021) and the two previous research projects Radostim A*B (2005-2008) and future[9/12] (2009-2012) have been investigating the potential of phytohormone-humic acid combinations (PHC compounds) [1; 2] since 2005 to increase soil biological fertility and to create a biological nutrient reserve in the soil. As part of this program, in the spring an application of the plants with the PHC preparation daRostim TANDEM F and in the autumn an application of the soil with the preparation daRostim TANDEM H. As a result of the additional activation of soil biology by the PHC preparations under the conditions of an intensive farming on the 172 German trial plots, we found an average increase in yield of 13.7 CU

in 2016 with a simultaneous reduction in the use of nitrogen fertilizer of 26.2 kgN/ha [3]. The mean concentration of air nitrogen-binding bacteria increased from 13.7 million CFU/g (2006-2012) to 20.9 million CFU/g (2012-2018), while that of the phosphor-mobilizing bacteria increased from 3.2 million CFU/g to 7.6 million CFU/g. So far, little is known about the phytosanitary initial state of the 172 German trial areas and the possible influence of phytopathogenic bacteria and fungi on the efficacy of the tandem preparations. For this reason, we started a systematic screening of the experimental areas in the years 2015 to 2018 and today we can report the first results.

It is known that many plant diseases that are caused by soil borne pathogens can be difficult to predict, detect and diagnose. The soil environment has a very complex composition and structure. This reduces the effectiveness of pathogen research. Many soil pathogens are able to survive outside the host organism for a long time due to the ability to form reliable resting structures (for example, cysts, spores).

To reduce the risk of developing plant diseases and the spread of soil phytopathogenic microorganisms, it is necessary to carry out a number of biosecurity measures. For example, it is necessary to estimate the amount of phytopathogenic microorganisms per unit volume or mass of soil. Destroy infectious plants to reduce the spread of the disease. Understand the mechanisms of survival of pathogens in the soil. In order to prevent the transfer of phytopathogens to neighboring agricultural land, to introduce the practice of sanitary cultivation of land.

Thus, knowledge of the peculiarities of the phytosanitary situation of the soil is a prerequisite for obtaining a quality, healthy and rich harvest.

The treatment of seeds with fungicides and insecticides is an effective instrument for ensuring a stable and healthy growth of the plant in the initial stage. In recent years many synthetic fungicides and all synthetic insecticides have been banned because of their harmful effect on health and the environment. The search for alternative preparations is has become a current day job. The Tandem BOSTAR array is a successful attempt to solve this problem. BOSTAR (Bio Organic Seed Treatment Array) is the result of international tandem cooperation, based on the combinatorial effect of phytohormones and humic acids (PHC) [4]. It is free of chemically synthesized fungicides and insecticides (Tab.1).

The width of the multifunctional activity spectrum of BOSTAR can be adapted by the modular selection of the additives used in the various systems of the treatment of seeds. Currently, there are 6 systems in the Tandem array of BOSTAR: Basic, Standard, +1, +2, +3, +4. Each system can successfully work as an additive, but also be can used alone to treat seeds. Also electron beam treated seeds can be treated with the systems. BOSTAR is very effective even in problematic or older seeds [5].

Table 1. The daRostim®Tandem BOSTAR Array and its components

daRostim® Tandem BOSTAR Array for cereals (6 systems)						
BOSTAR-Array	**Basic**	**Standard**	**BOSTAR+1**	**BOSTAR+2**	**BOSTAR+3**	**BOSTAR+4**
phytohormones	X	X	X	X	X	X
humic acids	X	X	X	X	X	X
biosurfactants	X	X	X	X	X	X
endophytes		X	X	X	X	X
adhesive		X	X	X	X	X
Bio-insecticides			X	X	X	XX
Bio-nematicides			X	X	X	XX
Bio-fungicides				X	X	XX
micronutrients					X	XX

Methodology

The determination and evaluation of the phytosanitary status of the test areas was carried out according to a method of Zheldakova and Myamin described by Feklistova [6]. With the help of 5 test methods, all bacteria and fungi isolated from the soil samples were examined and classified for phytopathogenic behavior.

Isolation of microorganisms from soil samples was carried out according to generally accepted procedures. A sample of soil weighing 1-3 g was placed in a sterile flask 100 ml with saline solution and the contents were shaken for 30 minutes. Then it were plated on the surface of a number of full nutrients medium and incubated at 28 ° C for 3 days.

Since the range of factors involved in the development of plant diseases is quite wide to determine the belonging of a particular species of bacteria to a group of phytopathogenic microorganisms, it was necessary to evaluate the totality of the results of various tests. The ability to macerate plant tissue, the ability to degrade pectic substances; cellulolytic activity and the ability to induce necrosis of plant tissue were determined.

To determine the ability of bacterial strains to macerate the plant tissue, the tubers of potatoes are washed, sterilized with ethanol, and a disc with a diameter of 1 cm and a thickness of 3-5 mm is cut with a sterile cork drill. Disks are placed on the surface of a 1.5 % agarized potato medium. On each disc, 50 μl of a 24-hour culture of the test bacteria is placed and incubated for 24-72 hours, and presence/absence of maceration is determined.

To determine the capacity of pectin substances degradation the bacterial cultures are inoculated with medallions onto the surface of the polypeptal gel in Petri dishes. Next, Petri dishes are placed in a thermostat at a temperature that is optimal for the growth of bacteria. When the production of pectolytic enzymes occure, holes/alveolus on the surface of the polypeptide gel are formed.

The most reliable test for determining whether a strain belongs to phytopathogens is to determine the ability of bacteria to cause necrosis of plant tissue not typical for this pathogenic microorganism. In this case, the plant cells at the place of hit of the pathogen rapidly die, forming a zone of necrosis and preventing the spread of the phytopathogen across the plant. As a test plant, the tobacco (Nicotiana tabacum) was used to determine the necrotic ability. The test bacterial strains are incubated for 24 hours. The cells are washed off from Petri dishes with saline solution and injected into the pulp of the leaf using a sterile syringe. As a "positive" control phytopathogenic strain *Erwinia carotovora atroseptica* is used, and "negative" - saprotrophic strain *Escherihia coli*. The hypersensitivity reaction manifests as the darkening area of the leaf blade at the site of injection of the bacterial suspension for 24-72 hours after inoculation.

In addition to defining the phytopathogenic properties, the economically useful characteristics of each isolated strain of microorganisms were studied.

Definition of the ability to fix nitrogen. To study the ability of the bacteria to fix nitrogen, the Ashby's non-nitrogen medium was used. The investigated bacteria were incubated in a thermostat at a temperature of 28-30 °C for 5-7 days. The ability of bacteria to fix nitrogen is determined by the presence of bacterial growth on the plates.

Definition of the ability to mobilize phosphates. Muromtsev's medium was used in experiments to assess the bacteria's ability to dissolve poorly soluble inorganic phosphorus compounds. Strains of microorganisms were incubated in a thermostat at a temperature of 28 °C for 5-7 days. The ability of bacteria to mobilize phosphorus is determined by the appearance of the transparent zones on the plates.

In autumn, about 30 % of winter cereals (wheat, barley) were treated with daRostim®BOSTAR Basic or particular by BOSTAR+1, +2 and drilled on the trial areas. The CFD photosynthesis power PHS was determined at the end of the winter of the following year (9-11 calendar week) by a method developed by Nowick [5]. The PHS was compared with the response of the nontreated areas.

Results

The tandem trial areas are very different in terms of the present concentration of microorganisms and the proportion of phytopathogenic bacteria and fungi. Depending on the proportion of phytopathogens, the phytosanitary state (Figure 1, 2) can roughly divided into 5 zones (A-E) (Tab.2).

Table 2. Zoning of the phytosanitary state

State zone	A	B	C	D	E	
Phytopathogens (%)	<20%	20-40%	40-60%	60-80%	80-100%	total
Number of areas	87	38	26	15	6	172
Proportion of areas (%)	50,6	22,1	15,1	8,7	3,5	100,0

In autum of 2015 a total quantity of 55 program areas was seeded with winter cereals A typical array of measured CFD-curves in the 9/11th calendar week in the year 2016 is plotted. (Figure 3). From these 55 cereals 33 was winter wheat, 13 from these seed treated by BOSTAR. From average CFD-curves, we estimated a higher photosynthetic efficiency (PHS) for the treated wheat, which continued to the 16/18 calendar week and later (Tab.3).

Table 3. Photosynthetic efficiency at different times with and without BOSTAR

	Photosynthetic efficiency (PHS) 9/11 calendar week	Photosynthetic efficiency (PHS) 16/18 calendar week
without BOSTAR	52,4 %	58,5 %
with BOSTAR	53,8 %	61,8 %

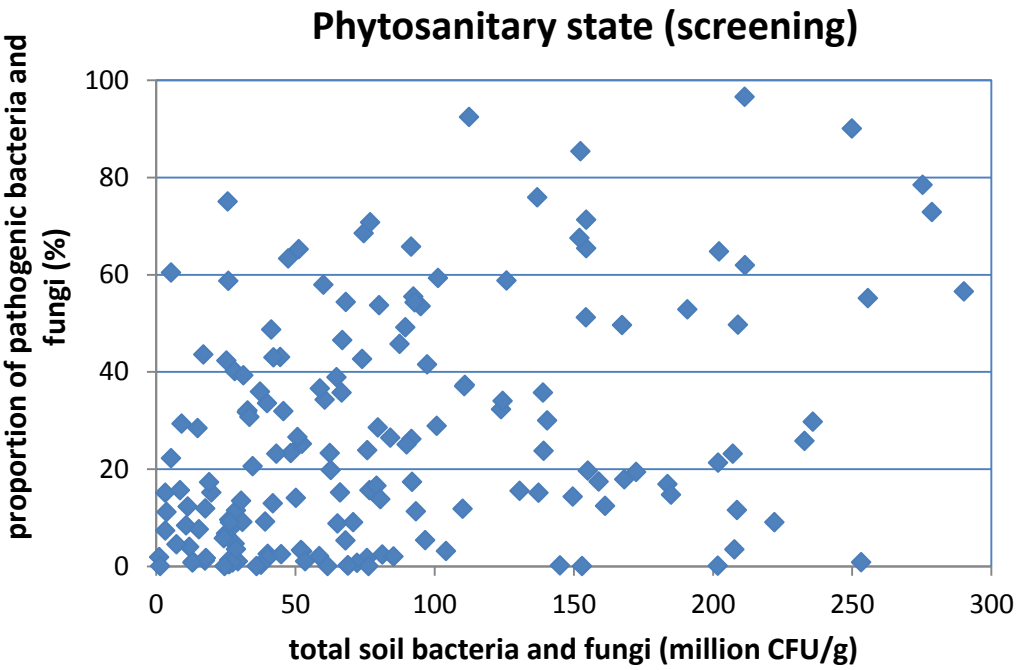

Figure 1. The phytosanitary state of 172 tandem program areas in Germany (2015-2019)

A similar situation we fund in the Years 2016, 2017, 2018 [7] and 2019. In addition to many other factors, the apparent cause a high dispersion of the photosynthesis, the average photosynthetic

efficiency PHS from total 172 cereal areas of 53.2 % decrease in the tendency to 47.8 % with increasing concentration of phytogenic bacteria and fungi in the soil, i.e. absolutely by almost 10.1 %. (Figure 4). A more detailed analysis shows, that on unprepared areas, the loss of PHS is even greater at -17.2 %, while by BOSTAR-Treatment against phytopathogenic in soil the photosynthetic efficiency clearly increase. We observe a 7.9 % increase of PHS (Figure 5).

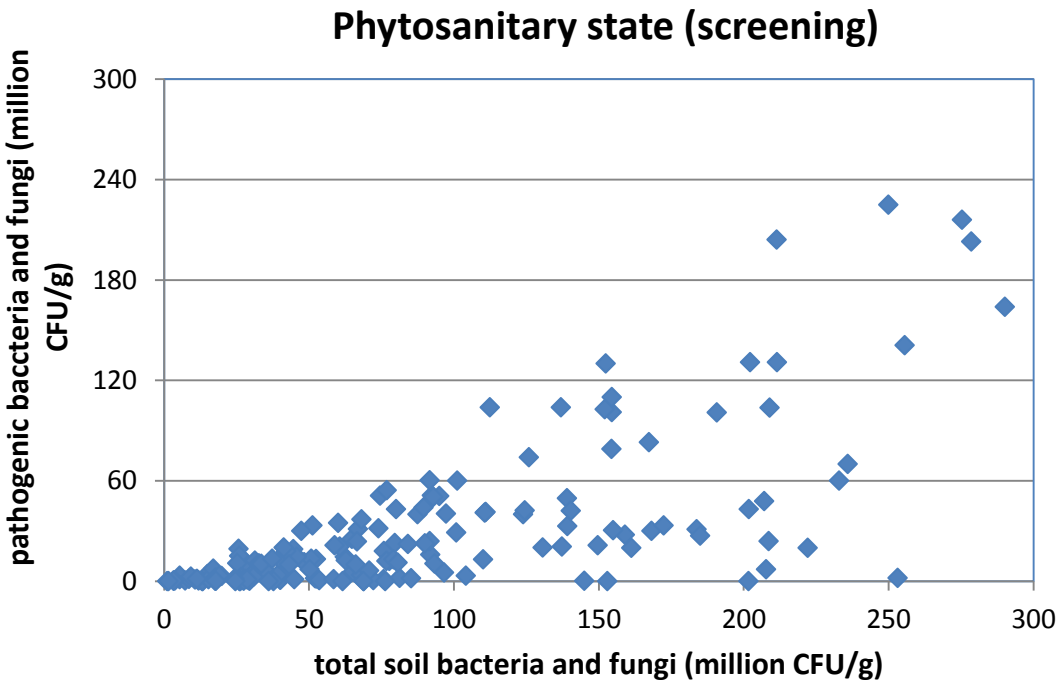

Figure 2. The phytosanitary state of 172 tandem program areas in Germany (2015-2019)

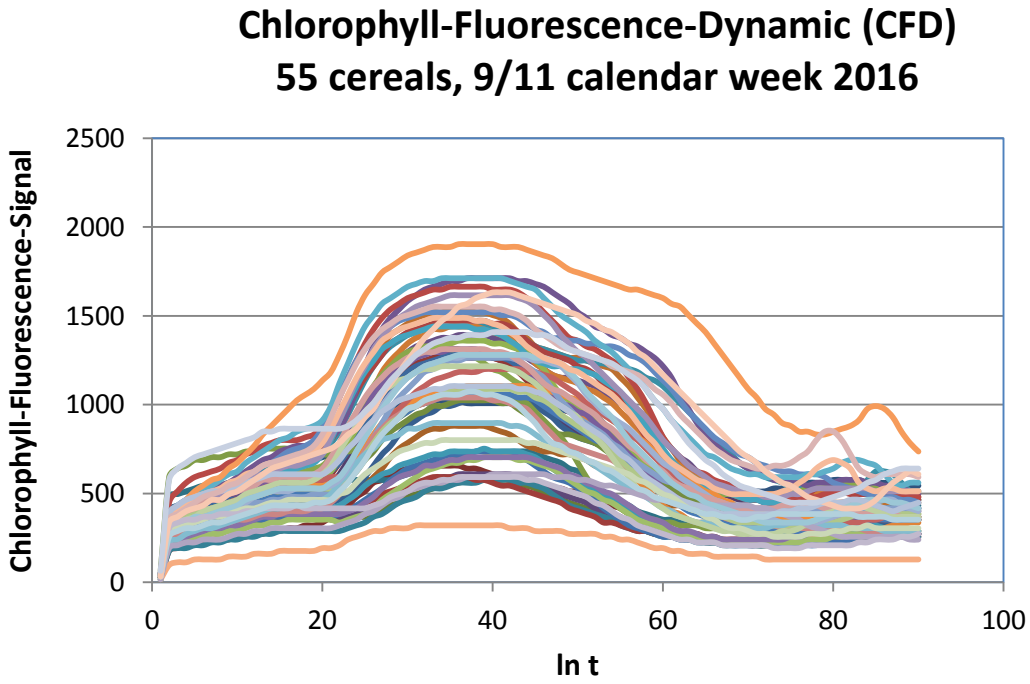

Figure 3. Chlorophyll-Fluorescence-Dynamic (CFD) from 55 cereal areas, 9/11 calendar week 2016

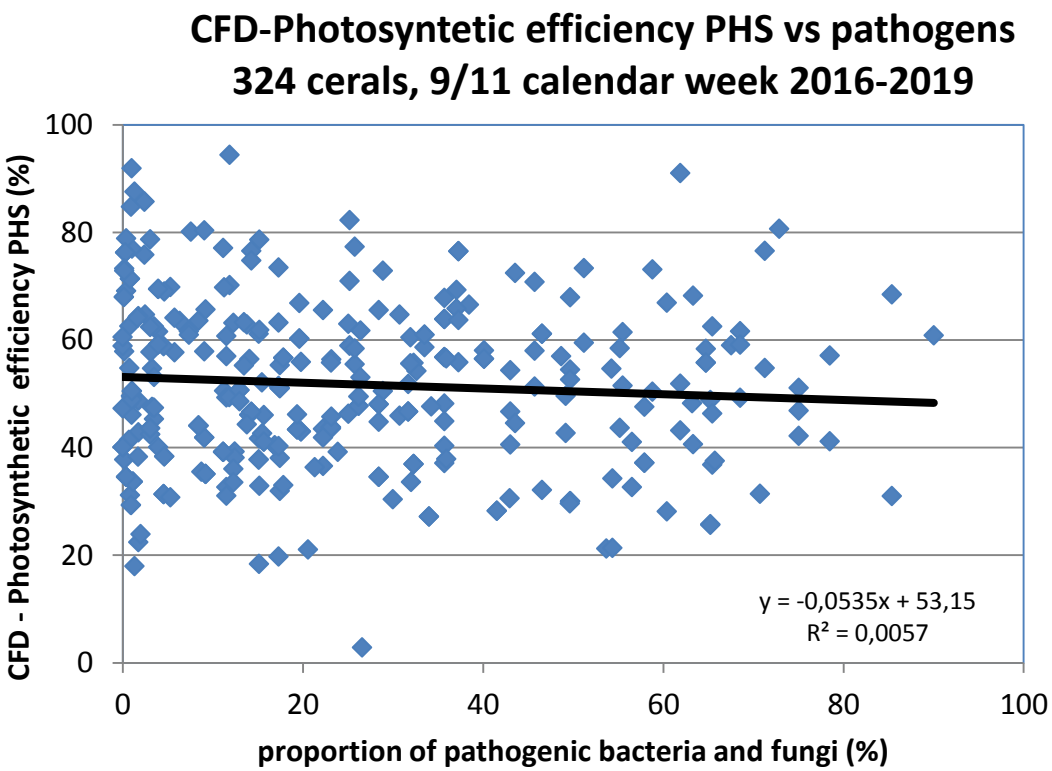

Figure 4. CFD-Photosynthetic efficiency PHS vs pathogens for all 324 cereals areas

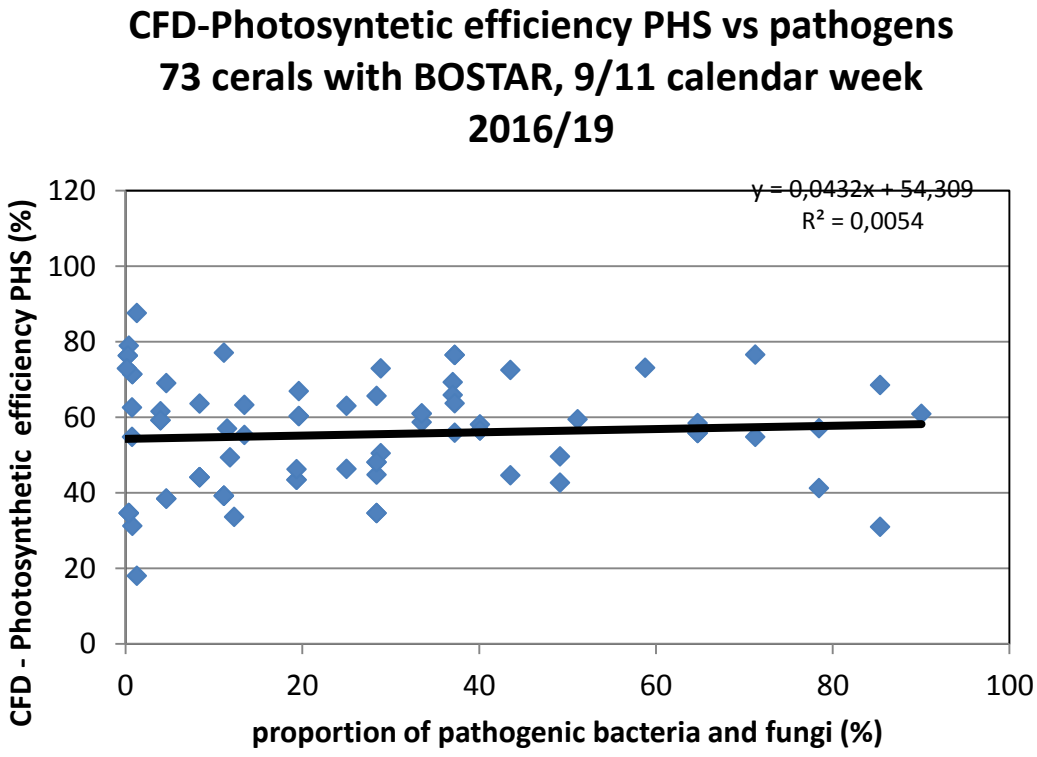

Figure 5. CFD-Photosynthetic efficiency PHS vs pathogens for all 324 cereals areas

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