Research objective is to determine the role of chemical compounds, among others, nitric oxide (NO), hydrogen sulfide (H_2S) and volatile sulfur-containing compounds, in strengthening the natural protection of tomato plants against gray mold disease, through proecological biocontrol.

The main reasons for undertaking this research is the fact that gray mold is one of the most common fungal diseases on vegetables, fruits, and ornamental plants. *Botrytis cinerea* fungi, causing gray mold, is one of the pathogens that cause significant losses in plant cultivation, transport and storage. Tomato is one of the vegetables most frequently infected by *B. cinerea*. Apart from a few transgenic tomato varieties, there are no commercially acceptable varieties resistant to *B. cinerea*, and the use of chemical plant disease agents in the form of fungicides, which according to the latest EU restrictions should be limited to a minimum, is not effective enough in protecting tomatoes against this pathogen. In times of intensive development of organic farming and integrated plant production, combating gray mold is quite a challenge. Therefore, even more than in the last decade, the scientific and economic community emphasizes the need to look for new, alternative methods of plant protection, including biocontrol of pathogens. Biocontrol, by combating pathogens with natural microbiological preparations, is a complex phenomenon that scientists are investigating all over the world. *Trichoderma* fungi are one of the groups of microorganisms intensively studied as biocontrol agents. The results of studies conducted on the Polish strain *T. virens* TRS106 showed that it limits the development of the disease caused by *B. cinerea*, partly by directly inhibiting the pathogen, and primarily by strengthening the defense mechanisms in tomato plants. These responses were strongly associated with NO accumulation and potentially enhanced emission of sulfur-containing secondary metabolites. Due to the fact that the role of NO in plant defense responses against *B. cinerea* is little known, and the role of H_2S and volatile sulfur-containing compounds has not been taken into account so far in the defense responses enhanced by *Trichoderma* and TISR (*Trichoderma*-Induced Systemic Resistance), in this project such a research problem is addressed.

Description of the research The research will be carried out on two tomato varieties, i.e. *S. lycopersicum* L. "Perkoz", less susceptible, and *S. lycopersicum* L. "Remiz", more susceptible to the infection with *B. cinerea*. The plants will be grown under controlled conditions established in preliminary tests. Spore suspension of *T. virens* TRS106 will be applied to the soil, while the *B. cinerea* suspension will be applied to the leaves. The tested plant groups will include (I) plants grown in the soil without *T. virens* TRS 106 spores (Control), (II) plants grown in the soil with *T. virens* TRS 106 spores (TRS 106), (III) plants infected with *B. cinerea*, grown in the soil without *T. virens* TRS 106 spores (Bc) and (IV) plants grown in the soil with *T. virens TRS* 106 spores, infected with *B. cinerea* (TRS 106+Bc). Plants will be tested at four time points: 0, 24, 48 and 72 h after *B. cinerea* infection, in order to determine the time at which the strongest inhibitory effect on the development of gray mold occurs. Then, the plants will be subjected to a series of biochemical and molecular analyses, using modern measurement equipment, including: a non-invasive system for imaging the fluorescence of photosynthetic pigments, FluorCam. The parameters tested will include, among others (i) determination of the dynamics of NO and H_2S accumulation in cell organelles, among others, cell nuclei and chloroplasts, and in apoplast, in parallel with the analysis of the changes in their ultrastructure and the state that they are in, (ii) analysis of the expression of genes related to defense responses (PR proteins) and NO and H_2S synthesis in tomato leaves, as well as (iii) detection of volatile sulfur-containing compounds as signaling molecules of defense responses of plants against *B. cinerea*.

The understanding and description of new potential signaling pathways that involve NO, H_2S and volatile sulfur-containing compounds, in the strengthening of the natural defense responses of tomato plants against the pathogen *B. cinerea*, is **the main expected effect of the project**. In the context of international and multidisciplinary scientific research, the project results will be another important step in elucidating the mechanisms of biocontrol of fungal pathogens through natural, indirect plant protection. The project results may be important for the development of various fields of science, from plant physiology and biochemistry, through microbiology, to agronomy. Understanding the molecular mechanisms of action of NO, H_2S, and sulfur-containing volatile compounds in plant defense reactions may open new perspectives for their practical application. In the longer term, the obtained results may contribute to the introduction of alternative, ecological plant protection against gray mold, allowing the protection of tomato varieties with special food and taste values, the withdrawal of which from agriculture is related to, among others, their greater sensitivity to attack by fungal pathogens. Indirectly, the obtained results may indicate the direction of research conducted on tomato and other plant fruits in order to find ecological methods of protecting them against *B. cinerea* during transport and storage.