SUMMARY

Fungi of the genus Epichloë is optional symbionts of many species of grasses, also pasture. The sexual stage of the fungus is a parasite; it forms specific structures so called stromata on all or some of the blades of the plant, thereby preventing grass flowering and seed production. It may also significantly decrease photosynthesis intensity by reducing the surface area of vegetative shoots. In the asexual stage Epichloë is an endophyte and its presence does not manifest in any visible way. However, it is associated with the production of alkaloids which have a toxic effect on livestock. Intensification of research on endophytes has begun in the late 80's and 90's and by knowing the details of the biology of these organisms we are more aware of their properties.

There is still, however, a lot of unsolved issues concerning the interaction of the fungus-plant-environment. Understanding of which would allow to eliminate the negative (from a practical point of view) effects of endophyte infection. One of the vital questions is: whether there are any specific, physically, chemically or biologically measurable parameters of the environment (habitat), which either promote or reduce the likelihood of endophytic infection? There are only a few experimental studies on this subject. These reports, however, do not provide clear and consistent answers to frequently asked questions. The factors that can initiate infection and affect "switching" fungus cycle from asexual to sexual stage remain unknown. Details of the relationship between the endophyte, grass and the environment are still unclear, due to their complexity and the difficulties they cause when researchers try to study them in the field or reproduce in controlled experimental conditions. Finding clear relationship between habitat factors and the level of infection could also have practical application, since the farmer to some extent can modify habitat conditions, e.g. by fertilization, liming, irrigation/drainage.

The aim of the study was to clarify whether soil factors (weight moisture – WW, electrical conductivity – SEC, which is a measure of salinity and reaction) favor infection at the level of habitat and what is the impact of infection on the size of the biomass and characteristics associated with reproduction of plants.

Two populations of weeping alkaligrass (*Puccinellia distans*), which was diagnosed in earlier studies with *Epichloë typhina* infection were selected for this research. Both populations grow in Kujawy, near Inowrocław, in anthropogenically strongly salinated habitats. The study began with collecting of the plant material and soil samples from the same randomly selected points. Coordinates of their location were precisely determined by RTK GPS. In addition low-height remote sensing of the research areas was made. The use of

remote sensing, among others, has allowed creating a high-resolution orthophotomaps, through which it was possible to mark out microhabitats within the study plots. It was crucial for the performed at a later stage, spatial analysis. In addition, in order to accurately characterize the soil profile, drilling was made. In the laboratory the infection status of harvested plants was determined (E - – no endophyte, E + – endophytic infection, P + – stromata present). The condition of infected and non-infected grasses were evaluated by taking into account the size of the biomass (total and green), the biomass of seeds and the number of vegetative shoots (V) and generative (G). In the case of soil samples the pH, electrical conductivity and weight moisture were measured. For some of the samples particles size and organic matter content was determined.

In order to verify the significance of differences in mean values of the soil parameters between defined groups Student's t-test was used. To verify the hypothesis that the analyzed parameters in the compared groups (in the samples collected from the points with grass and with no clumps, with infected and noninfected plants) have similar variances Levene, Brown-Forsyth and F tests were performed. Nonparametric tests Mann-Whitney U, Wald-Wolfowitz and Kolmogorov-Smirnov was used to verify the hypothesis of compliance of whole distributions in the compared samples. Also Kruskal-Wallis, median, Chi-square, and Tukey tests were used.

Geostatistical methods were used to identify the accuracy of the spatial distribution of analyzed soil parameters, the occurrence of *Puccinellia* specimens, their infection status and condition. This included the calculation and mathematical modeling of spatial autocorrelation parameters. The resulting models of spatial autocorrelation and measurement data were used to perform the kriging estimation. It allows to estimate value of analyzed traits at any point in the test area, using the available measurement data from other locations. This allowed to create maps showing the spatial variability of soil parameters and maps of the probability of *Puccinellia* occurrence and the status of plant infection. In addition, the use of simple kriging with local means (SKlm) allowed to prepare maps which take into consideration the potentially abrupt changes in habitat conditions.

Geostatistical approach in the ecological studies is rarely used. Attempts such analyzes have already been performed in the past 20 years, but the number of publications on the subject is limited, due to the barrier which is complicated methodology. Besides using of them in this

study to map the occurrence of Puccinellia and its infection in the context of specific values of the analyzed soil factors represents a significant novelty.

The research revealed: (1) a significantly higher total biomass of infected plants (E+/P+) in relation to the plant without endophyte (E-, but only one of the stations), (2) the lack of statistical difference in the case of seed biomass and (3) number of generative and vegetative shoots produced by E and E+/P + (however it is interesting that infected plants have higher average number of G and V tillers). In addition it was observed: (4) higher average number of V and lower of G shoots of plants with stromata in comparison to E+ plants, although the differences were not statistically significant, (5) on one of the research areas (Kościelec) relation between alkaligrass occurrence and the specified mean value of the conductivity (lack of grass was associated with higher values of this parameter) and the connection between SEC and the status of grass infection (presence of plants P+ was associated with a higher average value of SEC, while in the case of groups E- and E + values of this parameter were quite similar), (6) at the second area (Janikowo) weight moisture (WW) was the major factor influencing presence/absence of *Puccinellia* (the higher moisture, the more frequent occurrence of grass).

The result proving that in Kościelec comparisons between E+ and P+ does not always mean that the average number of shoots is higher in the group with endophyte infection is interesting; the individuals with stromata had on average more V tillers. This may be connected with the fact that in the situation of the presence of sexual stage infection, plants are a kind of "compelled" to produce higher number of green shoots, to balance the loss associated with reduced surface of assimilation.

Comparing the average values of the soil parameters in groups of samples collected from locations with clumps of alkaligrass and from places where it did not grow in Kościelec it was found significant differences in the SEC (mean higher for points without grass). Such a relationship is not surprising, because although *Puccinellia* is a facultative halophyte, however, salinity in the study area is generally very high and can be a limiting factor. SEC in Kościelec Kujawski plot is the only soil parameter which spatial variation shows the dependence on the distribution of microhabitats.

The spatial distribution of *Puccinellia* specimens and its infection status shows spatial autocorrelation, which means that their diversity are not random. This autocorrelation has characteristics similar to those which characterize the spatial distribution of analyzed soil parameters. This shows the cause and effect relationship between them.

Alkaligrass is unusual halophyte, which does not belong to any of the four basic types of halophytes, distinguished because of the occurrence within the spectrum of salinity. This species is one of the few, the presence of which is associated with all the possible range of this parameter - from low to very high. At research areas SEC oscillate in a relatively wide range, given the small area of the experiment. The results presented in this study are therefore consistent with previous reports. One explanation for this might be a close relation of plants with endophyte, which helps it to survive in extreme conditions.

The application of presented in this work geostatistical analyzes must be considered only as a starting point – the possibilities are much wider than it has been presented at this stage. Even with only the data collected so far it is possible to perform more advanced analysis (e.g. quantify spatial crosscorelation).