Thesis for doctoral (*Ph.D.*) dissertation

EFFECT OF BIOTIC STRESSORS ON THE ANTIOXIDANT ENZYMES ACTIVITIES, PROTEIN CONTENT, AND PHYSIOLOGICAL PARAMETERS OF MAIZE

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1. Introduction and objectives of the doctoral research

Agriculture is one of the most important sectors both domestically and globally. Among climate factors, global warming is the biggest problem for agriculture because it affects crop production in indirect and direct ways. Directly it causes abiotic stresses such as drought (Tuberosa et al., 2003), salinity stress (Flowers and Yao, 1990) and extreme weather conditions (Naglaa et al., 2014), as well as flooding (Ashraf and Mehmood, 1990). Indirectly, it reduces crop resistance and creates a suitable environment for the spread of pests. (Gregory et al., 2004).

The goal of this research was to investigate the impacts of the corn smut (*Ustilago maydis* DC. Corda) infection, which is an increasingly damaging pathogen with global warming, on the plant physiological, morphological, and quality parameters of two fodder and two sweet corn hybrids. The experiments were set up in a greenhouse and field conditions. In the greenhouse conditions, the effects of the infection on the relative chlorophyll content, the amount of photosynthetic pigments, the malondialdehyde content (MDA content), proline concentration, plant height, stem diameter, dry weight, as well as the activities of ascorbate peroxidase, guaiacol peroxidase and superoxide dismutase of maize hybrids were investigated. During the field experiment, the effects of different concentrations of the corn smut infection (2,500, 5,000, and 10,000 sporidia/mL) on the relative chlorophyll content, the amount of photosynthetic pigments, the malondialdehyde content (MDA content), the proline concentration, on plant height, stem diameter, dry weight, as well as ascorbate peroxidase, guaiacol peroxidase, and superoxide dismutase activity, and in the generative stage on tube length, tube diameter, kernel weight, fresh and dry weight of 100 kernels, quality parameters (dry matter, crude fiber, crude fat, crude ash, crude protein, and nitrogen) and the element contents of corn grains (Al, Br, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P, Pb, S, and Zn). The goal of the greenhouse experiment was to find a new, effective method of protection against corn smut. Therefore, the effects of different plant hormones (cytokinin, gibberellin, auxin, and ethylene) on the corn smut-infected plants infected were studied. In the field experiment, the effects of the different concentrations of the corn smut infections were investigated. These tests will allow us to get a more comprehensive view of the plant physiological changes that take place during the disease process, especially the plant immune responses that occur after the formation of reactive oxygen species (ROS), which can be measured by enzyme activities (ascorbate peroxidase, guaiacol peroxidase, and superoxide dismutase), malondialdehyde content and proline concentration.

2. Materials and Methods

The inoculum for infection was prepared according to the method of Szőke and Tóth (2020) in the laboratory of the University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Plant Protection. The infection took place in the vegetative stage (4-5 leaf stage in both experiments) and the generative stage (beginning of cob development in the field experiment). In the greenhouse experiment, sampling was done 7 and 11 days after infection with the pathogen (DAPI), in the field experiment, 7 and 14 DAPI in the vegetative stage, and 21 DAPI in the generative stage. Five to five plants were used for the measurements and statistical analyses. The greenhouse experiment was done at the University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Horticulture. The field experiment was done in the garden of the Institute of Plant Protection.

The fodder corn hybrids Armagnac and P9025 and the sweet corn hybrids Desszert 73 and Noa were used in the experiment. In the greenhouse experiment, the effects of cytokinin (2 x 10^{-4} M), gibberellin (10^{-3} M), auxin (2 x 10^{-3} M), and ethylene (1%) were also investigated. The cytokinin kinetin, the gibberellin gibberellic acid (GA_3), the auxin α-naphthalene acetic acid, and ethylene in the form of ethephon were injected into the plants at the same time with the infection. The corn smut infection concentration was 10,000 sporidia/mL in the greenhouse experiment. In the field trial, corn smut infection concentrations were 2,500, 5,000, and 10,000 sporidia/mL. Two comparative methods were used in the greenhouse experiment. First, I investigated the effects of corn smut infection on the physiological and morphological characteristics of the plants by comparing untreated infected plants with the control. Secondly, I investigated the influence of plant hormones on the intensity of corn smut infection by comparing hormone-treated infected plants with untreated infected plants. In the field experiment, I studied the effects of different corn smut concentrations on the physiological and morphological characteristics of the plants.

The statistical analyses were carried out with the software IBM SPSS Statistics 25 (Armonk, NY, USA).

2.1. Determination of plant physiological parameters

2.1.1. Determination of the relative chlorophyll content

The relative chlorophyll content was measured by Chlorophyll Meter SPAD-502 Plus (Minolta, Japan) on the fourth and fifth well-developed leaves. The SPAD value was measured in five repetitions per plant and then took the average of them to obtain the value of the relative chlorophyll content.

2.1.2. Determination of the content of photosynthetic pigments (chlorophyll-a, b, and carotenoids)

The contents of photosynthetic pigments (chlorophyll-a, b, and carotenoid) were measured by the methods of Moran and Porath (1980) and Wellburn (1994) from the fifth leaf.

2.1.3. Determination of the activity of antioxidant enzymes

For the determination of ascorbate peroxidase (APX) and guaiacol peroxidase (POD) activities, the plant samples were prepared according to the method of Pukacka and Ratahczak (2005), and for the determination of superoxide dismutase (SOD) activity, the plant samples were prepared according to the method of Giannopolities and Rice (1977). APX activity was measured by the method of Mishra et al. (1993), POD activity was measured according to the method of Zeislin and Ben-Zaken (1991), and SOD activity according to the method of Beyer and Fridovich (1987) from the fifth leaf.

2.1.4. Determination of proline concentration

The proline concentration was determined by the method of Carillo and Gibon (2011) from the fifth leaf.

2.1.5. Determination of the level of lipid peroxidation based on the malondialdehyde (MDA) content

The formed MDA content was measured from the fifth leaf according to the method of Heath and Packer (1968).

2.1.6. Determination of element content with an ICP-OES device

The contents of Aluminum (Al), boron (Br), calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), lead (Pb), sulfur (S), and zinc (Zn) was determined from the corn kernels. The ICP measurement was performed by the method of Tóth et al. (2020).

2.1.7. Determination of the quality parameters of grains

The crude protein, crude fibre, crude fat, crude ash, nitrogen, and dry matter content of the corn kernels were measured based on the method of Csapó et al. (2020).

2.2. Measurement of plant morphological parameters

2.2.1. Measurement of the stem diameter

The stem diameter was measured with a sliding calliper on the stems which were located between the second and third nodes.

2.2.2. Measurement of the plant height

The plant height was measured with a measuring tape from the soil surface to the emergence of the most developed leaf.

2.2.3. Measurement of the dry weight

For the dry weight measurement, after sampling, the entire plant was cut off from the soil surface, placed in a paper bag, and then dried at 65 °C for 3 days in a drying chamber. After that, the weight of the dried samples was measured by Ohaus AX223 (OHAUS Corporation, Parsippany, NJ, USA) analytical balance.

2.2.4. Measurement of morphological parameters in the generative stage

The corn cob length was measured with a measuring tape from the beginning of the cob to the end of the cob. The cob diameter was measured with a calliper. After counting 100 grains the fresh weight was weighed and after drying the dry weight was weighed. Drying took place at 65 °C for 3 days in an oven. After sampling, the mass of the corn cob was weighed on an analytical balance, in the case of infected plants, the entire cob was weighed first, and then after removing the tumours, the cob weight was weighed, then subtracted the cob weight from the cob mass.

3. Results

3.1. Results of the greenhouse experiments

3.1.1. Effects of corn smut infection on the chlorophyll content

In the greenhouse experiment, infection with the two corn smut strains (1x7 and 7x12) had different effects on the relative chlorophyll content and the amount of photosynthetic pigments per leaf, hybrid, and sampling time. On the fourth leaf, infection with the 1x7 strain reduced SPAD-unit by 23% in the Armagnac hybrid at 7 DAPI, by 17% in the Desszert 73 hybrid at 11 DAPI, and by 36% and 60% in the Noa hybrid at both sampling dates. Infection with the 1x7 corns smut strain significantly reduced the relative chlorophyll content of each hybrid's fifth leaf at both sampling dates. The relative chlorophyll content decreased by 16% and 21% in the Armagnac hybrid, 20% and 34% in the P9025 hybrid, 15% and 27% in the Dessert 73 hybrid, and 38% and 38% in the Noa hybrid. Infection with the 1x7 corn smut strain altered the amount of photosynthetic pigments. Armagnac and Dessert 73 hybrids at 11 DAPI (with 40%, 43%, and 89% for Armagnac hybrids and, 32%, 77%, and 53% for Dessert 73 hybrids). In P9025 and Noa hybrids, chlorophyll-a, chlorophyll-b, and carotenoids diminished in Noa hybrids at both sample dates after infection with the 1x7 corn smut strain. In the P9025 hybrid, chlorophyll-a was reduced by 38% and 25%, chlorophyll-b by 31% and 18%, and carotenoids by 45% and 23%, in the Noa hybrid, they were reduced by 78%, 44%, and 84%, respectively 90%, 181% and 185% at 7 and 11 DAPI.

Infection of the fourth leaf with the 7x12 corn smut strain decreased relative chlorophyll content by 25% in the Armagnac hybrid at 7 DAPI, by 27% in the Dessert 73 hybrid at 11 DAPI, and by 49% and 34% in the Noa hybrid at both sample dates, while it was not affected in the P9025 hybrid. Infection with the 7x12 corn smut strain reduced the relative chlorophyll content of the fifth leaf by 31% and 28% in the Armagnac hybrid, 25% and 45% in the P9025 hybrid, and 11% and 15% in the Desszert 73 hybrid, 21% and 11% in the Noa hybrid, at 7 and 11 DAPI. In both sampling times, infection with the 7x12 corn smut strain diminished the amount of photosynthetic pigments (chlorophyll-a, chlorophyll-b, and carotenoids) in the P9025, Desszert 73 and Noa hybrids. In the Armagnac hybrid, chlorophyll-a was reduced by 38% at 7 DAPI, the amount of carotenoids by 34% and 108% at 7 and 11 DAPI, in the P9025 hybrid chlorophyll-a by 50% and 24%, chlorophyll-b by 35% and 24%, the amount of carotenoids by 76% and 85% at 7 and 11 DAPI, in the Desszert 73 hybrid, chlorophyll-a by 146% and 189%, chlorophyll-b by 76% and 184%, the amount of carotenoids by 79% and 119% at 7 and 11 DAPI, in the Noa hybrid, chlorophyll-a by 105% and 152%, chlorophyll-b by 176%

and 349%, the amount of carotenoids by 176% and 119% compared to the control at 7 and 11 DAPI. Regarding the effects of the plant hormones, it is concluded that the cytokinin, gibberellin, and auxin treatments increased the relative chlorophyll content and the amount of photosynthetic pigments differently for each hybrid and leaf at the two sampling times. In the fourth leaf of infected Armagnac hybrids with the 1x7 corn smut strain, the cytokinin treatment increased relative chlorophyll content by 18% at 7 DAPI, while the auxin treatment increased relative chlorophyll content by 22% at 11 DAPI. In both sample intervals, ethylene treatment decreased the relative chlorophyll content of the fourth leaf of infected plants by 30% and 55%, respectively. Compared to untreated infected plants, treatments with cytokinin, gibberellin, and auxin increased SPAD levels in the fifth leaf by 22%, 28%, and 21% at the first sampling time and by 18%, 28%, and 28% at the second sampling time. The relative chlorophyll content in the fifth leaf of the infected plants was reduced by 28% at 7 DAPI as a result of the ethylene treatment. The cytokinin and gibberellin treatments increased the relative chlorophyll content in the fourth leaf of the P9025 hybrids by 22% and 19% at 7 and 11 DAPI, respectively, while the ethylene treatment decreased it by 15% at 7 DAPI. On the fifth leaf of infected plants 7 and 11 days after infection, the treatments with cytokinin, gibberellin, and auxin increased the SPAD unit by 24%, 25%, and 30%; and by 30%, 21%, and 40%, respectively. In the Desszert 73 hybrid, I measured 41%, 25%, and 27% higher SPAD Units at 7 DAPI and 44%, 42%, and 50% higher SPAD unit at 11 DAPI in the fourth leaf as a result of the cytokinin, gibberellin, and auxin treatments compared to hormone untreated infected plants. In the fifth leaf, cytokinin treatment increased the relative chlorophyll content of infected plants by 44% and 58%, auxin treatment by 21% and 48% at both sampling dates and gibberellin treatment by 33% at 11 DAPI. In the Noa hybrid, the cytokinin, gibberellin, and auxin treatments increased the relative chlorophyll content in the fourth leaf of infected plants by 39%, 37%, and 29%, respectively, to 71% and 88% at the first sampling date, but the ethylene treatment decreased the relative chlorophyll content by 89% at the second sampling date. The relative chlorophyll content of the fifth leaf increased by 59%, 55%, and 65% by the cytokinin, gibberellin, and auxin treatments, and by 65%, 76%, and 73% in the infected plants not treated with hormones az 7 and 11 DAPI.

Cytokinin, gibberellin, and auxin treatments increased SPAD unit after infection with the 7x12 corn smut strain in the fourth leaf of Armagnac hybrid at both sampling times (by 15%, 27%, and 25%; and by 13%, 13%, and 25%, respectively); ethylene treatment reduced SPAD unit by 14% at 11 DAPI. The gibberellin and auxin treatments increased the relative chlorophyll content of the fifth leaf by 41% and 34%; and by 44% and 37%, respectively. The cytokinin treatments increased the relative chlorophyll content of the infected plants by 35% at 7 DAPI. In the P9025 hybrid, auxin treatment in the fourth leaf increased SPAD unit by 15% and 21% (at 7 and 11 DAPI), and ethylene treatment decreased it by 29% (at 7 DAPI). In the fifth leaf, the SPAD Unit increased as a result of the cytokinin and auxin treatments at both sampling dates (19% and 31% in the first and 34% and 44% in the second), and by 27% in response to gibberellin treated infected plants at 11 DAPI. In Dessert 73 hybrids, the SPAD unit increased in the fourth leaf at both sampling dates as a result of treatment with cytokinin, gibberellin, and auxin by 31%, 27%, and 21% in the first sampling date, and by 24%, 28%, and 15% in the second sampling date. The relative chlorophyll content decreased by 105% and 53% compared to untreated, infected plants. In the fifth leaf, as a result of the cytokinin, gibberellin, and auxin hormone treatments, I measured 44%, 32%, and 41% higher relative chlorophyll content at 7 DAPI. At the second sampling time, cytokinin and gibberellin treatments increased SPAD units by 41% and 32%, respectively, compared to the non-hormone-treated infected plants. In Noa hybrid, cytokinin, gibberellin, and auxin treatments significantly increased SPAD units in the fourth leaf of infected plants by 41%, 52%, and 74%; and by 67%, 16%, and 64% at 7 and 11 DAPI, respectively. Ethylene treatment significantly reduced relative chlorophyll content in the fourth leaf of infected plants by 15% and 23% at 7 and 11 DAPI, respectively. At both sampling times, the relative chlorophyll content of the fifth leaf of infected plants treated with ethylene was 22% to 30% lower and 50% to 74% lower than that of hormone-free infected plants. Cytokinin treatment increased the amount of chlorophyll-a by 20% at 7 DAPI and the amount of chlorophyll-b and carotenoids (by 25% and 78%, respectively) in the 1x7 strain infected plants at 11 DAPI in the Armagnac hybrid compared to the hormone-free infected plants. The infected plants treated with gibberellin had statistically detectable lower levels of chlorophyll-a (by 76%) and chlorophyll-b (by 168%) and lower levels of carotenoids (by 21%) at 7 DAPI. As a result of auxin treatment, the amount of chlorophyll-a and carotenoids decreased by 21% and 29% at 7 DAPI, and the amount of chlorophyll-b decreased (by 61% and 86%, respectively) at both sampling dates. compared to untreated infected plants. Ethylene treatment diminished the amount of chlorophyll-b (by 21% and 28%) at 7 and 11 DAPI and the amount of carotenoids by 36% at 7 DAPI in the infected plants. In the P9025 hybrid, chlorophyll-a content increased by 87% at 11 DAPI due to cytokinin treatment, by 44% and 31% at the first sampling time, and by 25% and 37% at the second sampling time due to gibberellin and auxin treatment, respectively, compared to the non-hormone treated infected plants. Chlorophyll-b content was significantly increased by cytokinin treatment at 7 and 11 DAPI by 87% and 13%, respectively, and by gibberellin and auxin treatment at 7 DAPI by 63% and 74%, respectively, compared to the non-hormone-treated infected plants. The amount of carotenoids were significantly increased in the infected plants by cytokinin and gibberellin treatment at both sampling times (by 19% and 36%, respectively, and by auxin treatment by 13%) at 7 DAPI. As a result of the ethylene treatment, I measured significantly lower chlorophyll-b content in 7 DAPI, by 18% and 13%, compared to the non-hormone-treated infected plants. In the Desszert 73 hybrid, chlorophyll-a content was significantly grown by cytokinin treatment by 29% and 30% in 7 and 11 DAPI, and by gibberellin and auxin treatment by 36% and 21% in 11 DAPI in the infected plants. As a result of the cytokinin treatment, 48% higher chlorophyll-b content was measured in the infected plants at both sampling times, and 97% and 62%; 69%, and 64% higher chlorophyll-b content at 7 and 11 DAPI by the gibberellin and auxin treatments, respectively. The amount of carotenoids was reduced by 70% by the auxin treatment at the first sampling time, while the cytokinin, gibberellin, and auxin treatments increased it by 43%, 51%, and 29%, respectively, at the second sampling time. As a result of ethylene treatment, I measured significantly lower chlorophyll-a and chlorophyll-b content and 104%, 221%, and 65% lower carotenoid content at 7 DAPI. At both sampling times, cytokinin, gibberellin, and auxin treatments significantly increased chlorophyll-a content in Noa hybrids (by 129%, 117%, 115%; and 99%, 100%, 80%, respectively), chlorophyll-b content (by 61%, 31%, 74%; and 188%, 142%, 192%, respectively), and carotenoid content (by 137%, 76%, 127%; and 170%, 133%, 146%, respectively) at both sampling times. Ethylene treatment significantly reduced chlorophyll-a and carotenoid content by 188% and 122%, respectively, at 11 DAPI and chlorophyll-b content by 75% at 7 DAPI.

In the Armagnac hybrid infected with the 7x12 corn smut strain, cytokinin treatment increased the amount of chlorophyll-a at both sampling times (by 27% and 48%) and gibberellin treatment at 7 DAPI by 42% compared to non-hormone treated infected plants. As a result of gibberellin and auxin treatment, the amount of chlorophyll-b in the infected plants was 51% and 75% lower than in the non-hormone-treated infected plants at 7 DAPI. The amount of carotenoids increased by 139% and 49% with cytokinin (7 and 11 DAPI) treatment, by 78% with gibberellin, and by 67% with auxin (7 DAPI for both plant hormones). Ethylene treatment significantly reduced the amount of chlorophyll-a and carotenoids by 60% and 144% at 11 DAPI, and the amount of chlorophyll-b by 29% and 54% at 7 and 11 DAPI, respectively, compared to the non-hormone-treated infected plants. The P9025 hybrid had significantly higher levels of chlorophyll-a (by 45%, 50%, 27%; and 52%, 46%, 44%, respectively) and chlorophyll-b (100%, 67%, 32%; and 147%, 125%, 344%, respectively) as a result of the treatments with cytokinin, gibberellin, and auxin at both sampling dates. The amount of carotenoids was increased by cytokinin and gibberellin treatments at 7 and 11 DAPI (by 55% for both hormones in the first and by 47% and 35% in the second sampling time) and by auxin treatment to 117% at 11 DAPI in the infected plants. As a result of the ethylene treatment, the amount of chlorophyll-a and carotenoids was significantly reduced (by 23% and 57%, respectively) in 7 DAPI, and the amount of chlorophyll-b was decreased (by 79%) in 11 DAPI. At Desszert 73, treatments with cytokinin, gibberellin, and auxin significantly increased the amount of chlorophyll-a by 59%, 135%, and 132% at the first sampling time and by 200%, 203%, and 141% at the second sampling time compared to non-hormone treated infected plants. The amount of chlorophyll-b was increased by 29%, 33%, and 103%; and by 187%, 232%, and 140% by the above plant hormones at 7 and 11 DAPI, respectively. The content of carotenoids was significant growth by the cytokinin, gibberellin, and auxin plant hormones by 128%, 120%, and 115% at 7 DAPI, and by 186%, 211%, and 140% at 11 DAPI in the infected plants. Ethylene treatment decreased the amount of chlorophyll-a by 35%, the amount of chlorophyll-b by 42%, and the amount of carotenoids by 323% compared to the hormone-free infected plants at 7 DAPI. In the case of the Noa hybrids, higher levels of chlorophyll-a, chlorophyll-b, and carotenoids were measured in the infected plants compared to the hormone-free infected plants at both sampling times. The ethylene treatment reduced the amount of chlorophyll-a by 35%, the amount of chlorophyll-b by 42%, and the amount of carotenoids by 323% compared to the hormone-free infected plants at 7 DAPI. Cytokinin, gibberellin, and auxin treatments significantly increased the amount of chlorophyll-a by 99%, 122%, and 182% at the first and 111%, 117%, and 87% at the second sampling time, respectively, compared to hormone-free infected plants. The amount of chlorophyll-b was grown by 321%, 229%, and 265%; and by 442%, 270%, and 162% by the above plant hormones at 7 and 11 DAPI, respectively. Carotenoid content was significantly increased by the plant hormones cytokinin, gibberellin, and auxin by 232%, 187%, and 210% at 7 DAPI and by 248%, 182%, and 96% at 11 DAPI in the infected plants. At any sampling time, the ethylene treatment did not affect the amount of photosynthetic pigments.

3.1.2. Effects of corn smut infection on the activity of the antioxidant enzyme

3.1.2.1. Effects of corn smut infection on the ascorbate peroxidase (APX) activity

As a result of infection with the 1x7 corn smut strain, APX activity increased at both sampling times, by 91% and 181% for Armagnac, by 139% and 157% for Desszert 73, and by 127% and 125% for Noa, but for P9025, APX activity was significantly increased by 97% at 7

DAPI. The effects of plant hormones were demonstrated as the activity of APX decreased by cytokinin, gibberellin, and auxin treatment in the hybrids, in Armagnac (by 106%, 73%, and 76%), Desszert 73 (121%, 52%, and 43%), and Noa (60%, 139%, and 107%) at 7 DAPI. At the second sampling time, the same results were obtained, as the activity of APX was significantly reduced under the influence of the above hormones (cytokinin, gibberellin, and auxin), 146%, 74%, and 100% for the Armagnac hybrid, 118%, 76%, and 74% for the P9025 hybrid, 157%, 135%, and 50% for the Desszert 73 hybrid, and 66%, 123%, and 146% for the Noa hybrid. The ethylene treatment induced a higher APX activity in the plants infected with the 1x7 corn smut strain, in the Armagnac hybrid (by 20%) at 7 DAPI; in the P9025 hybrid (by 60% and 53%); in the Dessert 73 hybrid (by 19% and 16%); and the Noa hybrid (by 31% and 39%), at 7 and 11 DAPI.

As a result of infection with the 7x12 corn smut strain, APX activity increased in the infected plants in all four hybrids at both sampling times (by 148% and 181% in Armagnac, by 133% and 98% in P9025, by 141% and 141% in Desszert 73, by 102% and 119% in Noa). In plants treated with cytokinin, gibberellin, and auxin infected with corn smut, APX activity was significantly reduced at 7 and 11 DAPI in all four hybrids. At the first sampling date, the activity of APX under the influence of the above hormones was reduced by 112%, 67%, and 152% in the P9025 hybrid, 109%, 415%, and 195% in the Dessert 73 hybrid, 54%, 40% and 54% in the Noa hybrid. In the Armagnac hybrid, the treatments with cytokinin and gibberellin reduced the APX activity of the infected plants by 27% and 24% at the first sampling time. At the second sampling time, the hormone-treated infected plants showed lower APX activity by 38%, 105%, and 89% in the Armagnac hybrid, by 89%, 49%, and 22% in the P9025 hybrid, 85%, 144% and 191% in the Desszert 73 hybrid, and by 67%, 43% and 66% in the Noa hybrid due to cytokinin, gibberellin and auxin treatments, respectively. Ethylene treatment induced higher APX activity in the infected plants, in the Armagnac, Desszert 73, and Noa hybrids with 72%, 19%, and 34% at 7 DAPI, with 22%, 16%, and 22% at 11 DAPI, in the P9025 hybrid with 41% at 11 DAPI.

3.1.2.2. Effects of corn smut on the guaiacol peroxidase (POD) activity

Infection 1x7 corn smut strain induced higher activity of POD, the rate of increase was 55% and 93% for the Armagnac hybrid, 71% and 137% for the P9025 hybrid, 247% and 208% for Desszert 73, 113% and 116% for the Noa hybrid, compared to control at 7 and 11 DAPI. The cytokinin, gibberellin, and auxin treatments reduced the negative effect of infection of the corn smut strain, and the POD activity was reduced in the case of the Armagnac hybrids by 50%, 19%, and 101%; and 93%, 101%, and 82%, in the case of the P9025 hybrids, and by 47%, 26%, and 66%; and 47%, 52%, and 43%, in the case of the Dessert 73 hybrid, by 123%, 72% and 118%; and by 92%, 96%, and 164%, in the case of the Noa hybrid by 75%, 135%, and 85%; and by 68%, 106%, and 40% in the case of 7 and 11 DAPI, respectively. The ethylene treatment resulted in higher POD activity in the infected hybrids (Armagnac, P9025, Dessert 73, and Noa) by 24% and 14% in the case of the Armagnac hybrid, by 43% and 65% in the case of the P9025 hybrid, by 15% and 12% in the case of the Dessert 73 hybrid, and by 20% and 22% in the case of the Noa hybrid at the first and second sampling dates, respectively.

Plants infected with 7x12 corn smut strain showed higher POD activity compared to the control in the hybrids. Armagnac POD activity was increased by 101% and 102%, P9025 by 123% and 290%, Desszert 73 by 242% and 218% and Noa by 110% and 101% compared to control plants at 7 and 11 DAPI. The treatments with cytokinin, gibberellin, and auxin reduced the activity of POD in the plants infected with the 7x12 corn smut strain, in the Armagnac hybrid by 75%, 53%, 95% and 84%, 57%, 59%; in the P9025 hybrid by 99%, 98%, 60%; and 132%, 66%, 96%; in the Dessert 73 hybrid by 61%, 86%, 102% and 49%, 103% 107%; in the Noa hybrid by 86%, 52%, and 45% and by 81%, 30%, and 38% POD activity was reduced at 7 and 11 DAPI, respectively. Ethylene-treated infected plants showed higher POD activity by 14% in the Armagnac hybrid at 11 DAPI, by 13% and 17% in Desszert 73 hybrid, and by 18% and 27% in the Noa hybrid, while POD activity was not significantly affected in P9025 hybrid compared to hormone-free infected plants.

3.1.2.3. Effects of corn smut infection on the superoxide dismutase (SOD) activity

The SOD activity was significantly increased in all hybrids as a result of infection with the 1x7 corn smut strain (by 36% and 42% in the Armagnac hybrid, 37% and 82% in the P9025 hybrid, 49% and 68% in the Desszert 73 hybrid, and by 68% and 29% in the Noa hybrid), compared with the control at both sampling times. As a result of the cytokinin, gibberellin, and auxin treatments, infected plants showed lower SOD activity, in Armagnac (by 24%, 94%, and 29% in 11 DAPI), P9025 (by 43%, 31%, and 19%; 74%, 41%, and 34%, respectively), Dessert 73 (by 24%, 57%, and 51%; 68%, 46%, and 43%, respectively), and Noa (by 39%, 96%, and 99%; 13%, 81%, and 48%, respectively) compared to the hormone-free infected plants at 7 and 11 DAPI. Ethylene treatment increased the activity of SOD in the Armagnac hybrid by 20% at 7 DAPI, in the P9025 hybrid by 22%, and 17% at 7 and 11 DAPI, but decreased the activity of SOD in the Noa hybrid by 18% at 7 DAPI.

Infection with the 7x12 corn smut strain also resulted in higher SOD activity in the corn hybrids, by 12% and 44% (at 7 and 11 DAPI) in Armagnac; by 84% and 136% (7 and 11 NFU) in P9025; by 25% and 86% (7 and 11 NFU) in Desszert 73; and by 38% and 39% (7 and 11 NFU) in Noa. The effect of the hormones cytokinin, gibberellin, and auxin was also demonstrated, as they increased the activity of SOD in the Armagnac hybrid by 23%, 23%, 100%, and 22%, 34%, and 98%; in the P9025 hybrid by 136%, 155%, and 145%, and 194%, 242% and 266%; and in the Dessert 73 hybrid by 55%, 26% and 43%, and 61%, 60%, and 93%, in the Noa hybrid by 54%, 73%, 31%, and 69%, 58% and 45% compared to the non-hormone treated infected plants, at the first and second sampling dates. Ethylene treatment increased SOD activity by 10% (at 11 DAPI) in the Armagnac hybrid but had no significant effect on the other hybrids.

3.1.3. Effects of the corn smut infection on the proline concentration

As a result of infection with the 1x7 corn smut strain, the proline concentration in the P9025 hybrid decreased by 14% at 11 DAPI and in the Noa hybrid by 49% at 7 DAPI compared to the control. The proline concentration in the Armagnac and Desszert 73 hybrids was not affected by the infection. In the Armagnac hybrid, the cytokinin treatment increased proline concentration by 34% at 7 DAPI, and the cytokinin and auxin treatments diminished the proline concentration in the infected plants by 21% and 34% at 11 DAPI, respectively. In the P9025 hybrids, gibberellin and ethylene treatments reduced proline concentration by 27% and 26% at 7 DAPI, respectively, and ethylene treatment reduced proline concentration by 20% at 11 DAPI. In the Desszert 73 hybrid, the auxin-treated infected plants had 36% and 23% lower proline concentrations than the hormone-untreated infected plants at both sampling times. In the Noa hybrid, cytokinin and ethylene treatments increased proline concentration in infected plants by 40% and 39% at 7 DAPI, and the cytokinin, gibberellin, and auxin treatments increased proline concentration by 19%, 20%, and 20% at 11 DAPI, respectively.

Infection with the 7x12 corn smut strain diminished proline concentration by 16% and 17% at 7 DAPI in the P9025 and Noa hybrids, 9% at 11 DAPI in the Desszert 73 hybrid, and did not affect proline concentration in the Armagnac hybrid. In the Armagnac hybrid, gibberellin treatment significantly reduced proline concentration in infected plants by 8% at 11 DAPI. In the P9025 hybrid, higher proline concentrations were measured in auxin and ethylene-treated infected plants at 7 DAPI (by 19% and 28%, respectively). Gibberellin treatment significantly diminished proline concentration by 11%, and ethylene treatment significantly increased it by 12% at 11 DAPI. In the Desszert 73 hybrid, cytokinin treatment reduced proline concentration by 11% at 7 DAPI, ethylene treatment reduced proline concentration by 11% at

11 DAPI, and auxin treatment reduced proline concentration by 13% and 10% at both sampling times.

3.1.4. Effect of corn smut infection on the MDA content

MDA content was 238% higher in Armagnac at 11 DAPI, 138% and 132% higher in P9025, 139% and 843% higher in Desszert 73, and 65% and 158% higher in Noa hybrids at 7 and 11 DAPI, respectively, compared to the control plants, due to the effect of the 1x7 corn smut strain. In the Armagnac hybrid, cytokinin, gibberellin, and auxin treatments decreased the MDA content by 138%, 120%, and 154% at 11 DAPI in the infected plants, respectively. Lower MDA content (by 93%, 76%, 99%, and 82%, 43%, 168%; 262%, 276%, 241%, and 111%, 139%, 208%; 82%, 269%, 187%, and 128%, 69%, 128%) was measured in P9025, Dessert 73, and Noa hybrid as a result of the cytokinin, gibberellin, and auxin treatments compared to the non-hormone treated infected plants at both sampling times. The ethylene treatment resulted in higher MDA content in the infected plants of Armagnac by 343% at 7 DAPI, in P9025 by 37% at 11 DAPI, in Desszert 73 by 31% and 51%, and in Noa by 128% and 96% at 7 and 11 DAPI, respectively.

As a result of the infection with the 7x12 corn smut strain, higher MDA content was measured compared to the control, in the Armagnac hybrid by 114% at 7 DAPI, in the P9025 hybrid by 182% and 215% at 7 and 11 DAPI, in the Desszert 73 hybrid by 262% and 117% at 7 and 11 DAPI, and in the Noa hybrid by 254% and 151% at 7 and 11 DAPI. In the Armagnac hybrid, cytokinin treatment reduced MDA content in infected plants by 84% at 7 DAPI, and gibberellin treatment also reduced it by 46% and 38% at 7 and 11 DAPI, respectively. Cytokinin, gibberellin, and auxin treatments significantly reduced MDA content in infected plants by 93%, 76%, and 99%; and 82%, 43%, and 168% for P9025 hybrids, 262%, 276%, and 241%; and 111%, 139%, and 208% for Desszert 73 hybrids, and 82%, 269%, and 187%; and 128%, 69%, and 128% for Noa hybrids at both sampling dates, respectively. As a result of the ethylene treatment, 25% and 173% higher MDA content were measured in the Armagnac hybrid than in the non-hormone treated infected plants, 60% and 26% in the P9025 hybrid at 7 and 11 DAPI, and 57% and 134% in the Dessert 73 hybrid at 11 DAPI.

3.1.5. Effect of corn smut infection on the morphological parameters

3.1.5.1. Effect of corn smut infection on the stem diameter

As a result of infection with the 1x7 corn smut strain, a significantly thicker stem diameter (by 22%) was measured in the Desszert 73 hybrid at 7 DAPI compared to the control, the other hybrids were not significantly affected by the corn smut infection. The gibberellin treatment increased the stem diameter of the infected plant by 26% in the Armagnac hybrid and decreased it by 12% in the Desszert 73 hybrid at 11 DAPI. As a result of the ethylene treatment, I measured a thicker stem diameter in the Armagnac hybrid by 18% and 18%, in the P9025 hybrid by 16% and 21%, in the Dessert 73 hybrid by 29% and 37%, and in the Noa hybrid by 21% and 33% at 7 and 11 DAPI.

Infection with the 7x12 corn smut strain resulted in a significant increase in stem diameter compared to the control in Armagnac by 17% at 7 DAPI and in Desszert 73 by 43% and 25% at 7 and 11 DAPI. In the Armagnac hybrid, cytokine treatment significantly reduced the stem diameter of infected plants by 12% at the second sampling time. In the P9025 hybrid, as a result of the ethylene treatment, a larger stem diameter was observed in the infected plants at both sampling times by 29% and 24%. In the Desszert 73 hybrid, stem diameter was significantly reduced by cytokinin, gibberellin, and auxin treatment at both sampling times (by 36%, 41%, and 36% at the first sampling time and 33%, 47%, and 29% at the second sampling time). In the Noa hybrid, ethylene treatment increased stem diameter by 36% at 11 DAPI.

3.1.5.2. Effect of the corn smut infection on the plant height

Infection with the 1x7 corn smut strain significantly reduced plant height by 19 % at 7 DAPI compared to the control in the Desszert 73 hybrid. In the Armagnac hybrid, plant height was significantly increased by 29%, 80%, and 60% by treatment with cytokinin, gibberellin, and auxin at 7 DAPI. In 11 DAPI, plant height was reduced by 29% and 41% by the cytokinin and ethylene treatments, respectively. In the P9025 hybrid, gibberellin treatment increased plant height by 56% and 24%, and ethylene treatment decreased plant height by 36% and 35%, respectively, at both sampling dates. In the Desszert 73 hybrid, gibberellin treatment increased plant height by 24% at 7 DAPI. Lower plant height (by 27% and 77%, respectively) was measured due to ethylene treatment at both sampling dates. In the Noa hybrid, the infected plant treated with cytokinin had a 26% higher plant height at the first sampling time, and the infected plants treated with gibberellin had a 54% and 70% higher plant height at both sampling times. The ethylene treatment significantly reduced plant height by 59% and 64% at 7 and 11 DAPI.

Infection with the 7x12 corn smut strain significantly diminished plant height compared to control plants by 17% in the Armagnac hybrid at 11 DAPI, by 20% and 15% in the P9025, and by 20% and 17% in the Noa hybrid at both sampling times. In the Armagnac hybrid, the cytokinin treatment increased plant height by 33% the first time, and the gibberellin and auxin treatments significantly increased plant height by 80% and 70%; 36% and 22%, respectively, at both sampling dates, compared to the non-hormone treated infected plants. In the P9025 hybrid, auxin-treated infected plants were 36% and 23% taller than non-hormone-treated infected plants at 7 and 11 DAPI, respectively. In Desszert 73, the gibberellin-treated infected plants had a higher plant height (by 15%) at 11 DAPI. The ethylene treatment reduced plant height at 7 and 11 DAPI by 77% and 99%, respectively. In the Noa hybrid, the gibberellin (65% and 96%) and auxin (25% and 14%) treatments increased plant height at both sampling times, and the cytokinin treatment increased plant height by 16% at the second sampling date. Due to the effect of the ethylene treatment, the infected plants had a lower plant height at both sampling dates, 36%, and 27% for the Armagnac hybrid, 31% and 48% for the P9025 hybrid, 77% and 99% for the Dessert 73 hybrid, and 59% and 42% for the Noa hybrid.

3.1.5.3. Effect of the corn smut infection on the biomass production

Infection with the 1x7 corn smut strain reduced the dry weight of hybrids Armagnac and P9025 by 21% and 34%, respectively, compared to control plants at 11 DAPI. Auxin treatment increased dry weight by 50% in the first, and gibberellin treatment by 85% and 22% in both sampling dates. Cytokinin and ethylene treatments diminished the dry weight of infected plants by 45% and 46% at 11 DAPI, respectively. In the P9025 hybrid, gibberellin-treated infected plants had higher (by 33% and 64%) and ethylene-treated infected plants had lower (85% and 41%) dry weights compared to non-hormone-treated infected plants at both sampling dates. Auxin treatment significantly increased biomass production by 31% at 11 DAPI. In the Desszert 73 hybrid, a higher dry weight (by 48% and 69%) was measured due to the effects of gibberellin and auxin treatments, and a lower dry weight at 7 and 11 DAPI due to the effect of ethylene treatment. In the Noa hybrid, gibberellin treatment induced (by 35% and 28%), and ethylene treatment reduced (by 59% and 64%) biomass production at both sampling times.

Infection with the 7x12 corn smut strain significantly diminished the dry weight of infected plants compared to control plants by 34% at 11 DAPI for the P9025 hybrid and by 41% at 7 DAPI for the Desszert 73 hybrid. The dry weight of infected plants was significantly increased by 47% and 61% by treatment with gibberellin and auxin in the Armagnac hybrid at 7 DAPI. The ethylene-treated infected plants had lower biomass production at both time points (by 85% and 79%, respectively). The P9025 hybrid had a higher dry weight due to the effects of gibberellin and auxin treatments (by 53% and 36% and 75% and 23%, respectively) and a lower dry weight (38% and 56%) due to the effects of ethylene treatment at 7 and 11 DAPI. In the Desszert 73 hybrid, the gibberellin treatment increased the biomass production of the infected plants by 62% and 59% at both sampling dates, and the ethylene treatment decreased it by 60% and 156%.

3.2. The results of the field experiment

3.2.1. Effects of the corn smut infection on the chlorophyll content

The relative chlorophyll content of the Armagnac hybrid was significantly reduced by 30%, 48%, and 49% by the concentrations of 2,500, 5,000, and 10,000 sporidia/milliliter (mL) at 7 DAPI. Different concentrations (2,500, 5,000, and 10,000 sporidia/mL) decreased SPAD units in infected plants by 41%, 56%, and 67%, respectively, compared to the control at 14 DAPI. Infection with a concentration of 10,000 sporidia/mL statistically reduced the SPAD unit by 31% and 25% in the P9025 hybrid at 7 and 14 DAPI, respectively. The 2,500 and 5,000 sporidia/mL treatments had no significant effect on relative chlorophyll content. In the Desszert 73 hybrid, significantly lower relative chlorophyll content was measured in plants infected with a cell count of 2,500 by 40%, in plants infected with a cell count of 5,000 by 52%, and in plants infected with a 10,000 sporidia/mL by 57% compared to the control at 7 DAPI. At the second sampling time, SPAD units were significantly higher by 58%, 70%, and 64% in plants infected with concentrations of 2,500, 5,000, and 10,000 sporidia/mL, respectively. In the Noa hybrid, a strong relative reduction in chlorophyll content was observed as a result of treatments with different concentrations of sporidia numbers. As a result of treatments with concentrations of 2,500, 5,000, and 10,000 sporidia/mL, the SPAD unit was reduced by 37%, 53%, and 42% at 7 DAPI and by 105%, 120%, and 101% at 14 DAPI, compared to the control.

Treatments with different concentrations had a statistically detectable effect on the amount of photosynthetic pigments under field conditions in all hybrids. In the Armagnac hybrid, chlorophyll-a, chlorophyll-b, and carotenoid content were significantly reduced by each treatment at both sampling times. The treatments with concentrations of 2,500, 5,000, and 10,000 sporidia/mL reduced chlorophyll-a content by 66%, 97%, and 101%, chlorophyll-b content by 55%, 76%, and 71%, and the amount of carotenoids by 32%, 74%, and 97%, respectively, compared to the control at 7 DAPI. In the second sampling, the treatments diminished the amount of photosynthetic pigments by 64%, 74%, and 81% for chlorophyll-a, 56%, 62%, and 64% for chlorophyll-b, and 83%, 89%, and 99% for carotenoids in response to treatments with 2,500, 5,000, and 10,000 sporidia/mL, respectively. In hybrid P9025, the amount of chlorophyll-a was 16%, 86%, and 81%, the amount of chlorophyll-b was 67%, 85%, and 95%; and the amount of carotenoids was 53%, 65%, and 73% lower than in the control when treated with 2,500, 5,000, and 10,000 sporidia/mL corn smut infection. At the second sampling time, chlorophyll-b was decreased by 68%, 79%, and 97%, and carotenoids by 58%, 85%, and 102% by the different concentrations of infection. Plants infected with 5,000 and 10,000 sporidia/mL had significantly 127% and 131% less chlorophyll-b compared to the control at 14 DAPI. In hybrid Dessert 73, plants infected with concentrations of 2,500, 5,000, and 10,000 sporidia/ml had significantly 87%, 127%, and 147% less chlorophyll-a, 46%, 89%, and 116% less chlorophyll-b, and 40%, 48%, and 60% fewer carotenoids compared to the control at 7 DAPI. At the second sampling time, the treatments (2,500, 5,000, and 10,000 sporidia/mL) reduced the concentration of chlorophyll-a by 255%, 286%, and 315%, and the concentration of chlorophyll-b by 76%, 107%, and 127%, while the concentration of carotenoids was reduced by 53%, 217%, and 203% compared to the control. In the Noa hybrid, the concentrations of infection (2,500, 5,000, and 10,000 sporidia/mL) reduced the amount of photosynthetic pigments. At the first sampling time, statistically detectable, chlorophyll-a was decreased by 79%, 75%, and 106%, chlorophyll-b by 65%, 119%, and 121%, and carotenoids by 123%, 185%, and 319% compared to the control as a result of the 2,500, 5,000, and 10,000 sporidia/mL treatments. Fourteen days after infection, this ratio was 129%, 162%, and 169% for chlorophyll-a, 170%, 214%, and 244% for chlorophyll-b, and 221%, 380%, and 447% for carotenoids.

3.2.2. Effects of corn smut infection on the activities of antioxidant enzymes

3.2.2.1. Effects of corn smut infection on the activity of ascorbate peroxidase (APX)

Infection with the concentrations of the 7x12 corn smut strain (2,500, 5,000, and 10,000 sporidia/mL) statistically increased the APX activity of all hybrids at both sampling times. For the infected plants of the Armagnac hybrid', the ratio was 54%, 128%, and 144% at 7 DAPI, 81%, 117%, and 178% at 14 DAPI compared to the control. In P9025 hybrid APX, the activity of infected plants with different concentrations also grew by 72%, 63%, and 83% at the first sampling time and by 60%, 96%, and 88% at the second sampling time. In Desszert 73 hybrids, APX activity increased by 99%, 129%, and 145%, and by 126%, 159%, and 190% in plants infected with concentrations of 2,500, 5,000, and 10,000 sporidia/mL of corn smut at 7 and 14 DAPI. In the Noa hybrid, APX activity increased by 66%, 96%, and 119% and by 114%, 184%, and 186%, respectively, in response to concentrations of 2,500, 5,000, and 10,000 sporidia/mL, compared to the control.

3.2.2.2. Effects of corn smut infection on the activity of guaiacol peroxidase (POD)

The effect of infection with the concentrations of the 7x12 corn smut strain (2,500, 5,000, and 10,000 sporidia/mL) was established as higher POD activity was measured in the infected plants at 7 and 14 DAPI in all hybrids compared to the control. In the Armagnac hybrid, POD activity was increased by 45%, 53%, and 57%, and by 65%, 111%, and 164%, respectively; in the P9025 hybrid by 43%, 74%, and 65%, and by 81%, 94%, and 91%; in the Dessert 73 hybrid, by 149%, 197%, and 259%, and by 163%, 230%, and 274%, respectively; and in the Noa hybrid by 78%, 82%, and 112%, and by 84%, 179%, and 248% compared to the control at 7 and 14 DAPI.

3.2.2.3. Effects of corn smut infection on the activity of superoxide dismutase (SOD)

Concentrations of the 7x12 corn smut strain (2,500, 5,000, and 10,000 sporidia/mL) induced higher SOD activity in the infected plants compared to the control in all hybrids at both sampling times. Concentrations of corn smut infection induced higher SOD activity by 33%, 30%, and 49% and 55%, 55%, and 53% for the Armagnac; by 38%, 63%, 57%, and 73%, 79%, and 75% for the P9025; by 24%, 29%, and 46% and 41%, 60% and 74%, respectively, for the Dessert 73; and by 82%, 93%, and 107% and 81%, 88%, and 119% for the Noa hybrids, respectively, compared with the control at 7 and 11 DAPI.

3.2.3. Effects of corn smut infection on the proline concentration

The concentrations (2,500, 5,000, and 10,000 sporidia/mL) of the 7x12 corn smut strain affected the proline concentration differently in the individual hybrids. In the Armagnac hybrid, all concentrations (2,500, 5,000, and 10,000 sporidia/mL) of infection grew proline concentration by 16%, 24%, and 46%, respectively, at the first sampling time; the 5,000 sporidia/mL concentration of infection increased proline concentration by 12% in infected plants at the second sampling time. For P9025, infection with 10,000 sporidia/mL significantly increased proline concentration by 40% at 7 DAPI. In the Desszert 73 hybrid, proline concentration at 7 and 14 DAPI increased significantly by 14% and 13%, respectively, as a result of treatment with 5,000 sporidia/mL. In the Noa hybrid, infected plants had lower proline concentrations (16% and 23% at 7 DAPI and 18% and 19% at 14 DAPI, respectively) as a result of treatment with 5,000 and 10,000 sporidia/mL.

3.2.4. Effects of corn smut infection on the MDA-content

The MDA content was significantly increased by the concentrations of the 7x12 corn smut strain (2,500, 5,000, and 10,000 sporidia/mL) in all hybrids compared to the control. As a result of infection with the different corn smut concentrations, MDA content was higher by 53%, 88%, and 67%, and by 113%, 164%, and 183% in the Armagnac hybrid, respectively, and by 77%, 85%, and 86% and 63%, 93%, and 83% in the P9025 hybrid, by 153%, 204%, and 272%, and 147%, 193%, and 241% in Desszert 73 hybrid and by 162%, 188%, and 235% and 196%, 213% and 251% in Noa hybrid compared to the control at 7 and 14 DAPI.

3.2.5. Effects of corn smut infection on the morphological parameters

3.2.5.1. Effects of corn smut infection on the stem diameter

The hybrids reacted differently to the infection with the concentrations of the 7x12 corn smut strain (2,500, 5,000, and 10,000 sporidia/mL). In the Armagnac hybrid, infection with a concentration of 10,000 sporidia/mL increased stem diameter thickness by 15% at both sampling times and 16% at the first, and infection with a concentration of 5,000 sporidia/mL increased stem diameter thickness at the second sampling time compared to the control. In the P9025 hybrid, the plants infected with a concentration of 10,000 sporidia/mL had significantly thicker stems at 7 DAPI, by 22%. In the Desszert 73 hybrid, all concentrations of infected plants at 7 DAPI showed 16%, 24%, and 24% higher stem diameter, and 16%, 17%, and 24% at 14 DAPI. In the Noa hybrid, infection with 10,000 sporidia/mL increased the stem diameter of infected plants by 17% and 24% at both sampling dates and infection with 2,500 and 5,000 sporidia/mL increased the stem diameter of the infected plants by 17% and 21% at the second sampling date.

3.2.5.2. Effects of the corn smut infection on the plant height

Infection with the 7x12 corn smut strain also had an impact on plant height. In the Armagnac and P9025 hybrids, infection with a concentration of 10,000 sporidia/mL resulted in a significant reduction in plant height of 14% and 16% in the Armagnac hybrid and 24% and 25% in the P9025 hybrid, respectively, compared to the control at 7 and 14 DAPI. In the Desszert 73 hybrid, concentrations of 2,500 and 10,000 sporidia/mL significantly diminished plant height by 31% and 24% at the first and by 36% and 42% at the second sampling time, respectively. In the Noa hybrid, lower plant heights of 21%, 23%, 29% and 28%, 35%, and 41% were measured by the treatments of 2,500, 5,000, and 10,000 sporidia/mL at 7 and 14 DAPI, respectively.

3.2.5.3. Effects of corn smut infection on the cob parameters of Desszert 73 and Noa hybrids

A concentration of 10,000 sporidia/mL corn smut infection significantly reduced cob length in hybrids Desszert 73 and Noa (by 19 % and 30%, respectively) at 21 DAPI. There was no significant effect on cob diameter.

The highest concentration of infection significantly diminished the total grain weight of hybrids Desszert 73 and Noa at 21 DAPI by 41% and 18%, respectively.

The corn smut infection reduced the fresh weight of 100 grains in hybrids Desszert 73 and Noa by 21% and 17%, respectively, compared to the control at 21 DAPI. The effect of infection on the dry weight of 100 grains in both hybrids (Desszert 73 and Noa) was also observed, grain weight of infected plants was decreased by 51% and 59% compared to the control at 21 DAPI.

3.2.6. Effects of corn smut infection on the quality parameters

The effect of the corn smut infection on some grain quality parameters was stated. The infected plants of Desszert 73 and Noa hybrids had lower contents of dry matter (by 10% and 5%), crude fat (by 9% and 11%), and protein (by 13% and 15%) compared to control plants. The infection did not affect the content of crude fiber, crude ash, and nitrogen.

3.2.7. Effects of corn smut infection on the nutrient contents

Corn smut infection reduced Mg and Mn content in the kernels of both hybrids compared to the control by 15% and 25% in Desszert 73 and by 8% and 15% in the Noa hybrid. Infection significantly increased the concentrations of Al, Ca, and S by 182%, 12%, and 7% in Desszert 73 and by 105%, 8%, and 14% in the Noa hybrid, respectively. The infected grain of Desszert 73 contained 11%, 12%, and 6% less B, P, and Zn, respectively, than the uninfected control. Infection reduced K concentration by 5% but increased Na concentration by 13% in the kernel of Noa hybrids at 21 DAPI.

4. New results of the thesis

1. **All four hybrids in the experiment (Armagnac, P9025, Noa, and Desszert 73) showed symptoms of corn smut infection after the artificial infection in the vegetative phase. From this, it can be concluded that these maize hybrids are not resistant to corn smut infection.**

2. **Higher MDA content in the infected plants was measured as a result of the corn smut infection, compared to the control, non-infected plants, both in the greenhouse and in the field experiments. The MDA content was increased by 50-200% in the infected plants after infection with the 1x7 corn smut strain infection, and by 100-300% after infection with the 7x12 corn smut strain. The rate of increase was different for each hybrid and for each sampling time.**

3. **The antioxidant enzyme activities (APX, POX, SOD) were increased after the corn smut infection. As a result of infection with the 1x7 corn smut strain, APX activity was increased by 90-100%, the POD activity by 50-140%, the SOD activity by 30-90%, after infection with the 7x12 corn smut strain this ratio was increased by 90-180% for the APX activity, by 100-300% for the POD activity and 10-140% for SOD activity, compared to control. The increase in enzyme activity was different for each hybrid and for each sampling time.**

4. **Artificial tuber infection in the generative stage infected the sweet corn hybrids, the fodder corn hybrids were resistant to the infection. As a result of the 7x12 corn smut strain infection, the cob length decreased by 19 % in the Desszert 73 hybrid and by 30 % in the Noa hybrid. Kernel weight also decreased, by 41 % for the Desszert 73 hybrid and by 18 % for the Noa hybrid, 100-grains fresh weight by 21 % and 17 %, 100 grains dry weight by 51 % and 59 % for the Desszert 73 and Noa hybrids, respectively.**

5. **Cytokinin, gibberellin, and auxin treatments had a favorable effect on the reduction of the intensity of the corn smut infection, as they had a positive effect**

on the responses of the cultivated plant to the corn smut infection. Among the plant physiological parameters, the chlorophyll content was higher, the activity of the antioxidant enzymes (ascorbate peroxidase, guaiacol peroxidase, and superoxide dismutase), and the MDA content were decreased compared to the values which were measured in the hormone-free treated infected plants. In plants infected with the 1x7 corn smut strain, the aforementioned plant hormones increased the relative chlorophyll content by 10-100%, the amount of photosynthetic pigments by 15-200%, APX activity by 43-150%, POD activity by 19-164%, SOD activity was reduced by 5-81% and MDA content by 48-337% compared to the hormone-free infected plants. Cytokinin, gibberellin and auxin hormones increased the relative chlorophyll content in the 7x12 corn smut strain infected plants by 13-74%, the amount of photosynthetic pigments by 27-321%, APX activity by 24-415%, POD activity by 45- 132%, SOD activity was reduced by 22-266% and MDA content by 43-266% compared to the hormone-free infected plants. The increase and decrease varied depending on the hybrid, sampling time, corn smut strain and plant hormone.

6. **On the other hand, ethylene treatment increased the effect of the infection. Among the plant physiological parameters, the chlorophyll content decreased, the activity of antioxidant enzymes (ascorbate peroxidase, guaiacol peroxidase, superoxide dismutase), and the MDA content were increased compared to the hormone-free infected plants. In plants infected with the 1x7 corn smut strain, the ethylene hormone reduced the relative chlorophyll content by 15-39%, the amount of photosynthetic pigments by 13-192%, the APX activity by 16-53%, the POD activity by 12-65%, it increased SOD activity by 17-22% and MDA content by 31-343% compared to hormone-free infected plants. In plants infected with the 7x12 corn smut strain, the ethylene hormone reduced the relative chlorophyll content by 14-105%, the amount of photosynthetic pigments by 23-323%, the APX activity by 16-72%, the POD activity by 13-27%. The MDA content was increased by 25-173% due to the effect of ethylene teatment. The rate of increase and decrease was different for each hybrid and for each sampling time.**

5. Practical use of the results

1. Both the two corn smut strains (1x7 and 7x12) successfully infected all four maize hybrids in both the greenhouse and field experiments. With this, it is provided that the **cultivated plant is not resistant to infection in the vegetative stage**, which is why prevention is a very important element of protection.

2. In the field experiment, lower concentration (2,500 and 5,000 sporidia number/ml) infections were well tolerated by fodder corn (Armagnac and P9025) and Desszert 73 hybrids. No significant changes in the morphological parameters (plant height and stem diameter) were caused by the corn smut infection. It was stated that in the generative phase, the hybrids also tolerated lower concentration (2,500 and 5,000 sporidia number/ml) infections as corn smut was formed after the artificial infection. With appropriate indirect protection, i.e. by avoiding mechanical damage, using insecticide treatments, and proper care of the crop area, the amount of pathogen inoculum (infectious material) concentration can be reduced, because, with appropriate indirect protection, the optimal environmental conditions for the pathogen can be reduced, as well as the optimal conditions for the development conditions for the cultivated plant can be created. The experiment proved that lower concentrations of corn smut infection (2,500 and 5,000 sporidia number/ml) can be tolerated by resistant and less susceptible hybrids.

3. In the Noa hybrid, the corn smut infection with all sporidia numbers was associated with a high rate of plant death in the field experiment. **Among sweet corn hybrids and varieties, some varieties are very susceptible to corn smut infection, the experiment proved that plant death occurs when the highly susceptible variety (Noa) is infected.**

4. **As a result of the cytokinin, gibberellin, and auxin treatments, positive changes occurred when examining the biochemical parameters in the greenhouse experiment** (Chlorophyll content was increased, the activity of antioxidant enzymes (APX, POD, and SOD) and MDA content was decreased. Using hormones could promote the use of bio fungicides, about which few publications are currently available. Hormone-acting herbicides are widespread in practice, as they are very effective against

dicotyledonous weeds. However, hormone-acting preparations are not currently used in practice to mitigate the effects of gorse thorn infection. **The experiment proved that with plant hormones, it is possible to successfully protect against the corn smut fungus, against which there is currently no effective fungicide preparation.**

5. Ethylene is widely used in horticultural and field crops, horticulture to accelerate maturation, and in field crop production for stem strengthening, as it reduces plant height. Based on the experimental results, **it can be stated that the use of ethylene is not recommended in the case of corns smut pathogen**, as it increases the effect of the infection, and caused plant death several times after the second sampling, especially in the case of sweet corn hybrids.

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7. Publications of the issue of thesis

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Candidate: Lóránt Szőke
Doctoral School: Kálmán Kerpely Doctoral School
MTMT ID: 10072250

List of publications related to the dissertation

Hungarian book chapters (2)

1. Rácz, D., **Szőke, L.**, Széles, A.: A nitrapyrin hatása a kukorica (Zea maysL.) biomassza gyarapodására és egészségügyi állapotára. Utánközlés másodközlés, In: Kutatás-fejlesztés-innováció az agrárium szolgálatában II. kötet. Szerk.: Szabó Péter, Simon Brigitta, Soós Adrienn, Faludi Gergely, Fitos Gábor, Doktoranduszok Országos Szövetsége, Budapest, 155-166, 2021. ISBN: 9786155586736

2. **Szőke, L.**, Kovács, G. E., Radócz, L., Takácsné Hájos, M., Kovács, B., Tóth, B.: Gibberellin és etilén kezelések hatása a golyvásüszöggel (Ustilago maydis /DC./ Corda) fertőzött kukorica (Zea mays L.) néhány fiziológiai és morfológiai paraméterére. In: Kutatás-fejlesztés-innováció az agrárium szolgálatában II. kötet. Szerk.: Szabó Péter, Simon Brigitta, Soós Adrienn, Faludi Gergely, Fitos Gábor, Doktoranduszok Országos Szövetsége, Budapest, 112-118, 2021. ISBN: 9786155586736

Hungarian scientific articles in Hungarian journals (2)

3. **Szőke, L.**, Radványi, C., Szilágyi, A.: Az ázsiai gyapjúfű (Eriochloa villosa (Thunb.) Kunth) kompetíciós hatása a kukorica morfológiai paramétereire és a fotoszintetikus pigmentek mennyiségére. *Magy. Gyomkut. Technol. 22* (2), 45-53, 2021. ISSN: 1586-894X.

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5. **Szőke, L.**, Moloi, M. J., Kaczur, D., Radócz, L., Tóth, B.: Examination of Different Sporidium Numbers of Ustilago maydis Infection on Two Hungarian Sweet Corn Hybrids' Characteristics at Vegetative and Generative Stages. *Life (Basel). 13* (2), 1-26, 2023. EISSN: 2075-1729. DOI: https://doi.org/10.3390/life13020433 IF: 3.251 (2021)

6. Rácz, D., Gila, B., **Szőke, L.**, Széles, A.: N-Stabilizer and Foliar Fertilizer Treatments Enhance Tolerance to Specific Pathogens in Maize (Zea mays L.).
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7. **Szőke, L.**, Moloi, M. J., Kovács, G. E., Biró, G., Radócz, L., Takácsné Hájos, M., Kovács, B., Rácz, D., Danter, M., Tóth, B.: The Application of Phytohormones as Biostimulants in Corn Smut Infected Hungarian Sweet and Fodder Corn Hybrids.
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21. **Szőke, L.**, Kovács, G. E., Radócz, L., Takácsné Hájos, M., Kovács, B., Tóth, B.: Investigation of Ustilago maydis on maize chlorophyll content, rate of lipid peroxidation and phenotypic traits.
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22. Makleit, P., Veres, S., **Szőke, L.**: Hazai rozs (Secale cereale L.) fajták ciklikus hidroxámsav-tartalma és kiválasztása.
Növénytermelés. 66 (2), 47-68, 2017. ISSN: 0546-8191.

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23. Jagunić, M., Diaz-Lara, A., **Szőke, L.**, Al Rwahnih, M., Stevens, K., Zdunić, G., Vončina, D.: Incidence and Genetic Diversity of Grapevine Virus G in Croatian Vineyards.
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26. Rácz, D., **Szőke, L.**, Tóth, B., Kovács, B., Horváth, É., Zagyi, P., Duzs, L., Széles, A.: Examination of the Productivity and Physiological Responses of Maize (Zea mays L.) to Nitrapyrin and Foliar Fertilizer Treatments.
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31. Makleit, P., **Szőke, L.**: Közönséges búza (Triticum aestivum L.) ciklikus hidroxámsav-tartalmának és lisztharmat (Blumeria graminis f. sp. tritici) fertőzöttségének vizsgálata szántóföldi körülmények között.
In: XIII. Növényorvosi Nap, Magyar Növényvédő Mérnöki és Növényorvosi Kamara, Budapest, 77-79, 2018.

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Foreign language abstracts (4)

33. Tóth, B., Grusak, M. A., Maryke, L., Guzmán, C., **Szőke, L.**, Kaczur, D., Harangi, R., Kovács, B., Radócz, L., Makhsatova, S., Danter, M., Nagy, J., Moloi, M. J.: Evaluation of the Impacts of Stressors on Crops in the Context of Climate Change.
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37. **Szőke, L.**, Tóth, B.: A golyvásüszög-fertőzés hatásának vizsgálata a csemegekukorica klorofilltartalmára.
Mezőhír. 27 (2), 36-38, 2023. ISSN: 1587-060X.

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39. Tóth, B., **Szőke, L.**: Az őszi búza termésbiztonságát megalapozó agrotechnológiai tényezők a bokrosodás és szárbaindulás időszakában.
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41. **Szőke, L.**, Kovács, G. E., Tóth, B.: Őszi kalászosok kórtani problémái- a prevenció és az időzített védekezés jelentősége.
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42. Szilágyi, A., **Szőke, L.**, Radócz, L.: Újonnan megjelenő, inváziós gyomnövények a magyar mezőgazdaságban.
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46. Kovács, G. E., **Szőke, L.**, Tóth, B., Radócz, L.: A szelídgesztenye károsítói, illetve a kéregrákos megbetegedést okozó Cryphonectria parasitica jelentősége a hazai termesztésben.
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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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