

## The effect of artificial *vs* natural infection with *Alternaria alternata* on tomatoes, in the climate change context

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### Abstract

Tomatoes (*Solanum lycopersicum* L.) are some of the most popular vegetables in the world. The productivity of crops is determined by a detailed knowledge of the natural environment, the correct application of the knowledge related to the specific technology, and the interaction between species and the environment. The environment itself influences the crops and they may be beneficial to the normal development of the plant or may induce different sensitivities to the cultivated plants. The main objective of this article was to identify the most resistant tomato varieties to *Alternaria alternata* attack, in natural and artificial infection conditions. The biological material used was five varieties of tomatoes with large-fruit: 'San Marzano', 'Marmande', 'Romus' and with cherry-type fruit: 'Perun' and 'Bajaja'. The varieties were chosen due to large scale cultivation, resistance to various stress factors (disease and pest), and different organoleptic qualities. In the present events of climate change, it is necessary to continue testing the genetic material on the resistance and tolerance to the action of pathogens. The aim of the research was to test a group of tomato varieties to *A. alternata* attack. The pathogens can cause plant populations to evolve into an extremely varied set of immune receptors with differing ways of responding. In the Transylvanian Plain area, Romania, 2021 was a favourable year for this pathogen due to suitable infection conditions. Among the varieties of tomatoes, the cherry-type fruit 'Perun' (14.73%), was one of the most resistant to *A. alternata*. At the same time at the opposite end, the 'Bajaja' variety (25.33%) was the most affected cherry-type tomato in case of natural infection in 2022. The differences between the varieties reinforce the fact that climatic conditions significantly impact the dispersion of the pathogen on the field cultivated in both types of infection.

**Keywords:** *Alternaria*; climate change; resistance; tomatoes

### Introduction

Tomatoes (*Solanum lycopersicum* L.) are some of the most popular vegetables in the world. They were originally used as ornamental plants or as pharmaceutical plants because they were thought to be poisonous.

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Today is known that tomatoes have many therapeutic properties such as: detoxification, inhibition of cancer, lowering blood pressure and preventing hypertension and cardiac arrest (Indrea *et al.*, 2012; Collins *et al.*, 2022; Ma *et al.*, 2023).

The productivity of vegetable crops is given by a detailed knowledge of the natural environment, the correct application of the knowledge related to the specific technology and the interaction between species and environment. The environment itself influences the crops and they may be beneficial to the normal development of the plant or may induce different sensitivities to the cultivated plants. In the interaction between the biotic and abiotic factors, the action of each element cannot be accurately established. One of the main biotic factors that can influence the production and the quality of tomatoes are phytopathogens, which can produce different symptoms on all the organs of the plant. One of the main pathogens that can affect tomato crops is the species that originate from the *Alternaria* genus (Indrea *et al.*, 2012; Gannibal, 2016; Lawrence *et al.*, 2016).

The genus *Alternaria* (Fungi, Ascomycota, Pezizomycotina, Dohideomycetes, Pleosporales, Pleosporaceae) includes saprophytic and parasitic species. Most species of this genus are devoid of sexual form, except for a few species, which can have in addition to the anamorphic form, the teleomorphic form given by the presence of the asci and ascospores (Lawrence *et al.*, 2013; Singh *et al.*, 2016). Currently the pathogenic species that can produce a complex of diseases are: *A. alternata* Keissler (Fries)- The fruit rot on tomatoes, *A. linariae* E.G. Simmons- Early Blight of tomatoes, *A. solani* Sorauer- Alternariosis of tomatoes, *A. tomato* Jones (Cooke)- Nail stain shape of the tomatoes and *A. arborescens* E.G. Simmons - Black cancer of the tomato stem (Lawrence *et al.*, 2016; Ramezani *et al.*, 2019; Nishikawa and Nakishima, 2020).

*A. alternata* (Fr.) Keissler belongs to the category of species that have small dimensional spores. Conidiophores can be curved or erect, simple or branched dark brown with a length of up to 50  $\mu\text{m}$ . Conidia are multicellular, ovoid or dark brown clavate and they are usually chained. The length of the conidia is between 20 and 60  $\mu\text{m}$ , and the width is between 8 and 12  $\mu\text{m}$ . On their surface can appear between 3-11 transverse septa and 1-2 longitudinal. The ascogone are formed solitary or grouped and they can have globular or obovoidal shape, the colour is brown, and they are small in size (170-200  $\mu\text{m}$  length, 190-240  $\mu\text{m}$  width). The ascogone shell is between 38-50  $\mu\text{m}$  coloured brown. The asca has a cylindrical form, bitunicate, with dimensions between 170-190  $\mu\text{m}$  length and 23-30  $\mu\text{m}$  width. They can range from 4 to 8 ascospores. The ascospores are ellipsoids, with 3 to 7 transverse septa and 1-2 series of longitudinal septa passing through the centre of the ascospore. Dimensions can vary between 37- 43  $\mu\text{m}$  length and 13-14  $\mu\text{m}$  width (Akhtar *et al.*, 2004; Ariyawansa *et al.*, 2015; Landschoot *et al.*, 2017; Soomro, 2019).

*A. arborescens* E.G. Simmons and *Alternaria alternata* are part of the *Alternaria* section. The difference between these two species is the branching of the conidiophores. The branching is given by the secondary conidiophores which they give a tree like appearance. The conidia have dimensions between 20-60  $\mu\text{m}$  length and 8-12 width. On the surface of the conidia can appear a number of 3 to 7 transverse septa and 1-2 longitudinal. Conidia are formed in simple or branched chains (Hu *et al.*, 2015; Landschoot *et al.*, 2017; Choudhary *et al.*, 2020).

*A. solani* Sorauer has dark brown to brown or yellowish conidia with sizes ranging from 40-143  $\mu\text{m}$  length to 18-26  $\mu\text{m}$  width. Conidia are formed solitary, rarely chained. They have various forms: ovoid, obovates or ecliptics. On the surface of the conidia there are between 9-11 transverse septa and 1-4 longitudinal septa. The conidiophores are short and rarely branched (Marak *et al.*, 2014; Rahmazai *et al.*, 2016; Zhao *et al.*, 2021; Nasr-Esfahani, 2022).

*A. tomato* (Cooke) L.R Jones is devoid of sexual form and is categorized as a species with large conidia. The conidiophores are brown and have sizes between 4 and 130  $\mu\text{m}$ . The conidia have the obovate shape, brown to black colour, chained with dimensions ranging from 35 to 90  $\mu\text{m}$  length and 3 to 14  $\mu\text{m}$  width. On the

surface of the conidia there are several longitudinal septa and 2-7 transverse septa (Patel *et al.*, 2005; Gannibal, 2015; Gannibal, 2016;).

*A. linariae* (Neerg.) E.G. Simmons has the symptomatology and the morphological characteristics of conidia similar to those of *A. solani*. The differences between these species are the dimensions that make it a little smaller in size than *A. solani* and the branching of the beak. The dimension of the conidia is between 20-128  $\mu\text{m}$  length and 16-23  $\mu\text{m}$  width. The length of the cone beak is between 100-306  $\mu\text{m}$  long and the number of branches range between 1 and 4 (Woudenberg *et al.*, 2014; Kokaeva *et al.*, 2022; Schmey *et al.*, 2022; Wang *et al.*, 2022).

This complex of pathogens produces symptoms on all the green organs of tomato plants, affecting their growth and development. The characteristic symptom appeared on the organs of the plants are the circular brown-grey necrotic stain, surrounded by a chlorotic ring. During the periods with high humidity the spots will grow in diameter and deepen into the tissue. The surfaces of the spots become dark brown to black colour with concentric areas where can be found the conidia and the conidiophores. In the case of a strong infection, it could appear a massive defoliation and cause the prematurely falls of the fruit (Marak *et al.*, 2014; Rahmatzai *et al.*, 2016; Rodrigues and Furlong, 2022).

In order for the pathogens to produce infections, the climate needs to be alternately wet and dry. The optimum development temperature is between 25-31 °C and the relative humidity between 70-80%. In the case of favourable climatic conditions, the infection occurs within 1 to 18 hours and the first symptoms appear within 4-6 days. The penetration of the pathogen is also favoured by the presence of lesions that already exist on the plants. In addition to injuries, conidia can penetrate the tomato plants through the stomata onto a healthy tissue. With the infection the pathogen releases toxins that help to weaken the healthy tissues. The mycelium of the pathogen is mainly found on the surface of the affected tissue along with the conidia. Conidia are usually spread by wind, water, animals, insects, etc. Spores that reach the other plants or organs of the tomato plant can cause secondary infections (Hussain *et al.*, 2022; Schmey *et al.*, 2022; Rodrigues and Furlong, 2022; Tian *et al.*, 2023).

The varieties studied show different characteristics related to the resistance to pathogen attack. Some varieties are more resistant ('Marmande', 'Romus', 'Bajaja') (Burpee Homegardens; Biokiskert; Garden seed; 'Podaja' Semena; Fontem, 2003), and others show different sensitivities, or were not created for resistance to the attack of certain diseases ('San Marzano') (Gardeners Basic; Bonnie Plants). The varieties prefer a warm climate with direct exposure to sunlight. Although San Marzano is resistant to the attack of pathogens, it was not designed mainly to resist their attack, but to withstand drought condition, so it becomes susceptible in conditions of abundant precipitation. The Romus variety has a good resistance to the attack of aphids, due to the content of toxins in the stem and leaves; the variety is resistant to the attack of rodents, especially rabbits (The National Gardening Association). Together with the Perun variety, it shows increased resistance to the attack of viruses (Masheva, 2014). From the soil point of view, they prefer a sandy soil, which does not retain a large amount of water. The damage caused by *Alternaria* genus to tomatoes crops is significant causing loses between up to 50-86%, in favourable infection conditions (Severin and Cornea, 2009; Pârnu, 2010; Yigal and Ilaria, 2014).

Because the environment significantly influences, directly or indirectly, plants, pathogens and their antagonists, climate change is strongly associated with different levels of losses caused by a pathogen and is often involved in the emergence of new physiological races. For these reasons, changes associated with global warming (high temperatures, changes in rainfall, increased CO<sub>2</sub> and ozone levels, drought, etc.) may affect the incidence and severity of plant diseases and may influence the coevolution of plants and pathogens (Boland *et al.*, 2004; Beaumont *et al.*, 2011; Pautasso *et al.*, 2012; Yanez *et al.*, 2012; Yigal and Ilaria, 2014; Sadeghi *et al.*, 2022).

Many factors intervene in determining the resistance of plants to the action of pathogens, and one of the most important is the climate. Climatic conditions can affect the development of plants in a negative sense (the moment when the climatic factors are outside the optimal intervals of the plants), but also in a positive

sense. The same principle is equally valid in the case of pathogens. Pathogens being permanently interdependent with climatic conditions, they are affected by any anomaly that appears in the climatic environment, especially that of the climate warming phenomenon. So, changes also occur at the level of pathogens, which evolve due to their own mechanisms. Climate changes can directly affect: the phenophases of plant growth, the normal climatic structure of the area, the distribution of pathogens, the interaction between plants and pathogens, ecosystem management, the genetic diversity of host plants and even migration to other host plants. In addition to these direct influences, previously presented, they are also interdependent in the biological cycle of plants. Many factors determine the health status of plants, whether they act directly or indirectly. The appearance of diseases can result from a permanent coevolution between the plant and the parasitic systems of the pathogens. The gene for gene hypothesis claims that for each pathogen virulence gene, there is a corresponding gene for plant susceptibility (Beaumont *et al.*, 2011, Pautasso *et al.*, 2012; Ramadan and Elkafrawy, 2014; Yigal and Ilaria, 2014).

The aim of the research was to test a group of tomato varieties to *A. alternata* attack. The main objectives of the research were to identify the resistance of tomatoes varieties, in natural and artificial infection and to establish the differences induced by climatic conditions in the relation of plant to the pathogen.

## Materials and Methods

### *Site and climatic parameters*

The thermal regime and rainfall presented in this paper come from the Turda Weather Station. The Turda Weather Station is a subunit of the Northern Transylvania Regional Meteorological Center, part of the Romanian National Meteorological Administration. The weather station records data every hour and data reporting is done at daily values. Subsequently, the data is processed for three monthly decades.

The thermal regime of the Transylvanian Plateau records average annual temperatures of 9 °C. Extreme values can be recorded that constitute climate risks having a negative impact on crop plants. The most extreme temperatures are recorded between the months of July and August, which are between 30-35 °C, imposing restrictions on the development of crop plants due to the lack of breathing and transpiration. The average values of multiannual precipitation are between (500 and 520 mm). Both precipitation and temperatures can register extreme values, so that in a short period of time, the maximum amounts, in 24 hours, can exceed the average monthly amounts (Mărculeț and Mărculeț, 2016).

### *Experimental description*

The preparation of the experimental field was done through an autumn plowing and with a tiller, before planting. The soil was not treated or sterilized. Total area of the experimental field was 75 m<sup>2</sup> and systematized in the split-split-plot arrangement. The field was divided into three replications. The length of each replication was 6.5 m, the width of 3 m, and between the replications was left 3 paths with a distance of 80 cm. The experimental surface contained a total of 300 tomato plants, with each repetition consisting of 100 plants. Out of these, 50 plants underwent artificial infection.

The tomato seedlings were obtained from untreated seeds, purchased from specialty stores. Tomato seedlings were placed randomized. The planting was carried out in an intensive crop system. The planting distance between rows was 40 cm, and between plants at a time was 20 cm. The three repetitions were divided into two equal parts, half of which were artificially infected and the other half was naturally infected. During the experimental year were made 3 observation where was monitored the evolution of the disease (Nichifor and Negulescu, 1983; Prakash *et al.*, 2022). The observations were conducted every 15 days following the artificial infection by repeating the measure each time.

Artificial infections were performed when tomatoes reached phenofase 6 and respectively the phenofase 7 (Meier, 2001). The infection technique used is that of spraying with a pump with a rare sieve. This technique

had the disadvantage since the application of spores was not carried out evenly on the surface of the plant, (Severin and Cornea, 2009).

#### *Biologic material and cultivation*

The biological material used to achieve the main objectives were 5 varieties of tomatoes: with large-fruit: 'San Marzano', 'Marmande', 'Romus' and with cherry-type fruit: 'Perun and 'Bajaja'. The motivation for their use was to diversify and cover a broad spectrum of the genetic variability of tomatoes. This was due to the different morphological characters of the varieties. The plants were grown using an intensive cultivation system, and specific techniques were applied to promote their growth. During months with a high precipitation deficit, 0.67 m<sup>3</sup> of water was supplemented to prevent the plants from wilting due to dehydration.

#### *Fungal isolation, identification, storage and inoculation*

The identification of the pathogens present in the tomato culture was carried out through macro and microscopic examinations of the symptoms present on the plant. To identify the species of *Alternaria* the surface of the attacked organs was examined for characteristic symptoms. Specific stains on the surface of plants are slightly immersed in brown or brown-black tissue with a well delimited edge and concentric rings are highlighted on their surface. Also, on the affected area a brown to black puff is present which was represented by the conidiophore and the conidia fungus (Hulea, 1969; Severin and Cornea, 2009).

After identifying the symptoms of the pathogen, samples were taken from the plant by which we attest and accurately identify the presence of the pathogen with the help of microscopic examinations. To identify the pathogen more accurately the affected biological material was sterilized in alcohol solution with a concentration of 5% and distilled water for 1 minute. The sampled symptomatic tissue was placed on the wet chamber at a temperature of 25 °C for 48 hours to stimulate the fructification of the pathogen (Parvu, 2010; Somappa *et al.*, 2013).

With the help of the microscopic examination, the main morphological characteristics (length, width, number of longitudinal and transverse septa were measured. The presence or absence of tails and the presence or absence of secondary conidiophores on the affected tissue was used to establish the species of the genus *Alternaria*. To study the morphological characteristics and obtain pure cultures, to perform artificial infections, it was necessary to prepare an artificial culture media. The media used was PDA (potato, dextrose and agar) and PCA (potato, carrot and agar) (Alvarenga *et al.*, 2016; Aung *et al.*, 2020).

The culture media was poured in Petri dishes up to 0.2-0.5 mm thickness and then was added the antibiotics to prevent the growth of the bacteria. Antibiotics used were in the form of sulfamethoxazole and trimethoprim. They are part of the class of metabolic inhibitors, which inhibit the synthesis of nucleic acids of bacteria.

To isolate the pathogen, a portion of the affected tissue or parts of the mycelium from the surface of the tissue was taken and placed slightly immersed in the culture medium, inside the Petri dishes. The entire isolation process is carried out under sterile conditions in the biological safety cabinet. At the end, the plates were close tightly to maintain a favourable environment for the development of the pathogen (Severin and Cornea, 2009; Parvu, 2010).

To provide a favourable development environment for species of the *Alternaria*, they were introduced into the thermostat at 25 °C for 7 days considering the circadian cycle, exposing the Petri dishes alternately to 8 hours of light and 16 hours of darkness (Rodrigues *et al.*, 2010; Singh *et al.*, 2014; Kong *et al.*, 2023).

In order to use the isolated pathogen to perform artificial infections, they were stored at temperatures between 4-6 °C until the pathogen was used. To maintain the viability of mycelium and conidia, the whole process explained above will be repeated every 6 to 7 months. If the process is done too often, the pathogen will lose its morphological, biological and physiological characteristics. The *Alternaria* genus is known to have problems maintaining the viability of the culture due to morphological instability. On the surface of the

medium instead of the typical mycelium is found a tufted white-grey sterile mycelium (Severin and Cornea, 2009). *A. alternata* was identified by the characters of the colony on the culture medium and the microscopic identifications. No infectivity tests were performed.

In order to achieve natural infections, the tomatoes were planted and maintained from a technological point of view, and no protection method was applied against the *Alternaria* infections.

The preparation of the spore suspension, used in the artificial infection in the field, was obtained from the pure isolates. On the surface of the Petri plates is added 10 ml of distilled water and with the help of a fine brush the mycelium is removed with the formed spores. To remove impurities the water together with the spores, detached from the media seeps through gauze. From the liquid obtained, a known quantity is taken and the number of spores was determined by counting them with the help of the Thoma chamber (Severin and Cornea, 2009).

The number of spores on a Petri plate was 2,308 X 10<sup>5</sup>. To obtain the suspension for infection, 10 ml of the spore concentrate was taken and diluted in 500 ml of distilled water. 10 ml was applied to each plant by spraying 484, 68 spores on a single plant (Vloutoglou *et al.*, 2000).

The number of spores applied on the plant was approximately 3231. Upon completion of the operation, the plants were covered for 48 hours with plastic film to maintain high humidity. The infection occurred between 19:00-21:00, when the temperature was lower, the risk of water evaporation and the lens effect was also reduced.

#### *Disease monitoring*

To test the resistance of the varieties to the attack of *Alternaria* in the plantation was monitored the:

Attack frequency (F%) is the relative value of the number of plants or plant organs affected by the pathogen (n), reported to the total plants or organs attacked (N) and is calculated by the formula:

$$F\% = (n \times 100) / N \text{ (Puia, 2005).}$$

The Intensity of the attack (I%) it is the index that appreciates the extent of the attack on the plant by referring to the area attacked to the total area observed. The calculation formula for determining the intensity is:

$$I\% = [(i \times f)] / n \text{ (Puia, 2005).}$$

i - note or the percentage of coverage of the attack

f - number of affected plants

n - the total number of plants.

The degree of attack (G.A. %) is the extension of the disease process of the culture or plants under observation. It is calculated by the percentage report between the frequency (F%) and the intensity (I%) of the attack by the formula.

$$G.A.\% = (F \times I) / 100 \text{ (Puia, 2005).}$$

#### *Data analysis*

All data analysis was carried out with R Studio (RStudio, 2019), version 1.4.1106, with packages “psych” (Revelle, 2019) and “agricolae” (De Mendiburu, 2019). Means and standard errors were calculated with the formulas available in the “psych” package. ANOVA was used to analyse the overall impact of each experimental factor (experimental year, type of infection and tomato variety) on the attack parameters (Corcoz *et al.*, 2022). For the detection of differences between the attack parameters, related to the experimental protocol, the Tukey tests were performed (Pop-Moldovan *et al.*, 2022).

## Results

The retrospective isolation of the pathogen on the culture medium determined the presence of *A. alternata* species on the harvested plant material, which was further used to prepare the inoculum required for artificial infection.

### *Climate conditions in the two years of experimentation*

From the data presented in Table 1 it can be seen that the differences between 2021 and 2022, in terms of recorded temperatures (in the five experimental months of 2021, the temperature was higher than in the last 65 years) by approximately 0.86 °C, the warmest month being July with an average of 22.7 °C.

**Table 1.** The temperature regime at Turda 2021-2022

Temperature (°C)	2021					Mean on 5 months
	May	June	July	August	September	
Monthly mean	14.1	19.8	22.7	19.7	15.0	18.26
Mean on 65 years (5 months)	15.0	17.9	19.7	19.3	15.1	17.40
Temperature (°C)	2022					Mean on 5 months
	May	June	July	August	September	
Monthly mean	16.3	21.1	23.1	22.3	14.3	19.42
Mean on 65 years (5 months)	15.0	18.0	19.8	19.5	15.2	17.50

(Source: Meteorological station Turda (longitude: 23° 47'; latitude 46° 35'; altitude 427 m)

In 2022 the temperatures recorded during the experimentation period were higher by approximately 1.92 °C compared to the average for 65 years. In contrast to 2021, in 2022 four of the five months higher temperature values were recorded, the warmest being July (23.10 °C) (Table 1).

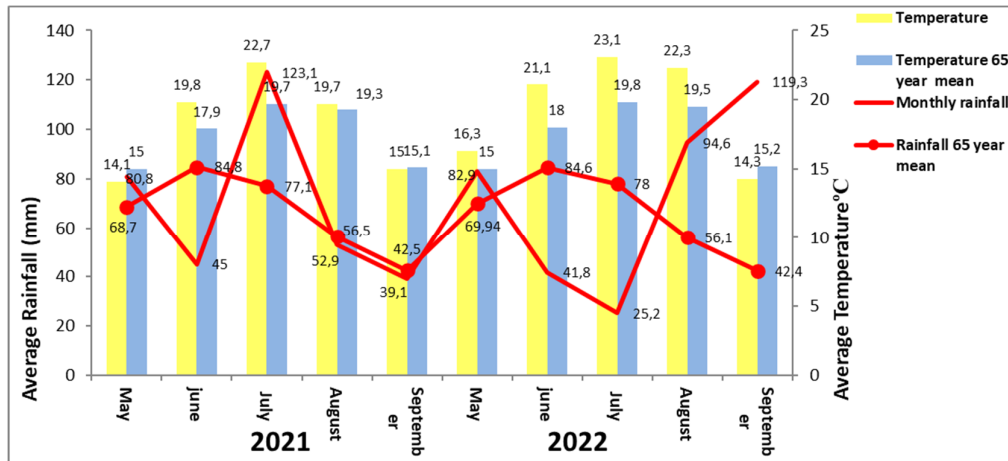
Regarding the rainfall regime from the two experimental years, from the data presented in table 2 the precipitation was different during the experimental period, in both years. In May more than 80 mm of water fell, comparable with the amount recorded exceeding the average for 65 years. In June, the rainfall in the experimental years had close values, but these were lower compared to the monthly amount recorded in June in 65 years, month considered dry from this point of view.

The next three months of the experimentation period differ greatly (Figure 1) in terms of rainfall. July 2021 was an excessively rainy month with 121.1 mm of rainfall during this period. In 2022 this month was excessively dry with only 25.2 mm of rainfall. In this month the water intake required for plant development was provided by additional watering 3l per plant. For August, 2021, the rainfall regime was normal, with values close to the 65-year average. In 2022 in August, the rainfall made the month excessively rainy, the precipitation being about 95 mm. In 2021 a normal month follows, in terms of rainfall recorded and in 2022, with a + 77.5 mm precipitated we have an excessively rainy month (Table 2).

The climatic data recorded in the three experimental years influenced the Early Blight attack differently, an aspect also found in the degree of attack values and the productions obtained.

**Table 2.** The rainfall regime at Turda 2021-2022

Rainfall (mm)	2021					Amount of rainfall on 5 months
	May	June	July	August	September	
Monthly amount	80.8	45.0	123.1	52.9	39.1	340.90
Mean on 65 years (5 months)	68.7	84.8	77.1	56.5	42.5	330.50
Rainfall (mm)	2022					Amount of rainfall on 5 months
	May	June	July	August	September	
Monthly amount	82.9	41.8	25.2	94.6	119.9	364.40
Mean on 65 years (5 months)	69.4	84.6	78.0	56.1	42.4	330.50



**Figure 1.** Interaction of the average temperature and rainfall in 2021 and 2022

*The influence of experimental factors on the attack parameters by Alternaria alternata*

Regarding the influence of experimental factors Table 3 presents the influence of experimental factors and their interaction on the indicators of the Early Blight attack.

Each experimental factor had a very significant positive influence on the frequency of the *A. alternata* attack. According to the values of p ( $p < 0.001$ ) the frequency of the attack in the double interaction between the experimental factors had very significant differences on the attack of *A. alternata*. The significant interactions observed are: in between year x variety ( $p = 0.001$ ), year x observation ( $p = 0.000$ ) and type of infection and the time of observation. (supplementary Table 1).

In case of intensity of the attack by *A. alternata* each experimental factor had a very significant positive influence, according to the values of p ( $p < 0.001$ ). Regarding the double interaction between experimental factors have very significant differences on the attack of *A. alternata* is observed in the case of the interaction year x variety ( $p < 0.001$ ), year x observation ( $p = 0.000$ ), variety x observation ( $p = 0.005$ ) and type of infection interacting with observation time ( $p = 0.000$ ) (supplementary Table 2).

The same trend was maintained in the case of the degree of attack all experimental factors having a very significant positive influence on the degree of attack by *A. alternata*, according to the values of p ( $p < 0.001$ ). Regarding the double interaction between experimental factors, they have very significant differences on the attack of *A. alternata* it was observed in the case of the interaction year x variety ( $p < 0.001$ ), year x observation ( $p = 0.000$ ), variety x observation ( $p = 0.012$ ) and type of infection interacting with observation time ( $p = 0.000$ ) (supplementary Table 3).

**Table 3.** The analysis variance table for frequency, intensity and degree of *Alternaria* attack

Interaction	Frequency (%)		Intensity (%)		Degree of attack (%)	
	Statistics	P. value	Statistics	P. value	Statistics	P. value
Year	95.28	0.000	116.48	0.000	133.24	0.000
Variety	4.45	0.002	5.66	0.000	6.67	0.000
Infection	43.89	0.000	100.34	0.000	109.47	0.000
Observation	363.86	0.000	1381.27	0.000	1498.18	0.000
Year: Variety	4.66	0.001	7.65	0.000	8.39	0.000
Year: Infection	2.71	0.101	2.37	0.126	1.84	0.177
Variety: Infection	0.89	0.471	0.92	0.454	0.67	0.612
Year: Observation	55.10	0.000	24.39	0.000	22.15	0.000
Variety: Observation	1.92	0.110	3.85	0.005	3.36	0.012
Infection: Observation	38.81	0.000	29.24	0.000	26.73	0.000
Year: Variety: Infection	0.54	0.703	0.59	0.670	0.66	0.621
Year: Variety: Observation	2.18	0.074	6.00	0.000	6.33	0.000
Year: Infection: Observation	0.38	0.541	0.58	0.448	0.40	0.526
Variety: Infection: Observation	0.56	0.695	0.27	0.897	0.06	0.993
Year: Variety: Infection: Observation	1.37	0.245	0.50	0.733	0.46	0.766

*The influence of experimental factors on the Frequency of the Alternaria alternata attack.*

According to the data presented in Table 4, in 2021 the values of the attack frequency were higher in artificial infection condition compared to the natural infection, in all varieties studied, at the time of the first observation. The climatic conditions influence, from 2021 favoured the *A. alternata* attack, the values of the frequency of the attack being higher this year, in most varieties, in both conditions of infection. Against the background of climatic conditions in 2021, under natural conditions of infection the variety with the lowest frequency of the attack was ‘Marmande’ (60%) and in 2022 the ‘Perun’ variety was observed with an attack frequency of 43.3% under conditions of natural infection and with the lowest frequency (60%) in high pressure of infection. At the second observation in the most majority of varieties and in both experimental years the frequency of the attack reached 100%. However, the varieties of ‘Romus’, ‘San Marzano’, ‘Marmande’ and ‘Perun’, in which the frequency of the attack was between 70% and 93% in both conditions of infection in 2022.

The differences between frequencies recorded in the 3 observations are an extremely useful tool in assessing the dynamics of the presence of the pathogen in the tested varieties. An important observation is the frequency of 100% in all varieties, regardless the type of infection, at the third observation. Compared to this value, significantly lower levels are found at the ‘Marmande’ and ‘San Marzano’ varieties (2022) natural infection. When comparing the last and first observation, there are significant differences between both varieties in response to the method of infection. The biggest difference is found in the ‘Perun’ variety, which in 2022 and in natural infection has an increased frequency of 56.67%. The ‘San Marzano’ variety has a similar behaviour in conditions of natural infection, with a frequency of the attack more than 50% from the first to the last observation. Overall, there were an increase in the frequency between 16-40% compared to the first observation (natural infection) and 3-30% respectively in case of artificial infection. In most cases, the 100% frequency level is reached at the second observation, which means a rapid dispersion of the pathogen on plants of each variety. There are a number of 4 varieties that showed a frequency below 100% in the second observation, but only in the climatic conditions of 2022. The ‘Marmande’ variety has a pathogen frequency of 70% under conditions of natural infection and increases with 20% in the case of artificial infection. Similarly, the ‘San Marzano’ and ‘Perun’ varieties have a difference of only 10-13% between the two types of infection,

and the ‘Romus’ variety only 7%, which indicates approximately the same resistance of plants to the pathogen attack.

**Table 4.** The early blight Frequency of the attack depending on the climatic conditions and the infection type

Variety	Year	Infection	Frequency % Observation 1	Frequency % Observation 2	Frequency % Observation 3	Difference frequency % 1-2	Difference frequency % 2-3	Difference frequency % 1-3
Marmande	2021	Natural	60.00±0.00c-g	100±0a	100±0a	40.00a	0c	40.00a-e
		Artificial	89.17±0.83a-c	100±0a	100±0a	10.83b-d	0c	10.83e-g
	2022	Natural	53.33±16.67e-g	70±10c	100±0a	16.67a-d	30a	46.67a-c
		Artificial	66.67±8.82a-g	90±0ab	100±0a	23.33a-d	10.00b-c	33.33a-g
Romus	2021	Natural	76.67±3.33a-f	100±0a	100±0a	23.33a-d	0c	23.33b-g
		Artificial	93.33±3.33a-b	100±0a	100±0a	6.67c-d	0c	6.67f-g
	2022	Natural	56.67±3.33d-g	86.67± 67a-c	100±0a	30.00a-c	13.33a-c	43.33a-d
		Artificial	63.33±3.33b-g	93.33±3.33a-b	100±0a	30.00a-c	6.67b-c	36.67a-f
San Marzano	2021	Natural	81.11±5.88a-e	100±0a	100±0a	18.89a-d	0c	18.89c-g
		Artificial	96.67±3.33a	100±0a	100±0a	3.33d	0c	3.33g
	2022	Natural	46.67±6.67f-g	70±5.77c	100±0a	23.33a-d	30a	53.33a-b
		Artificial	70.00±5.77a-g	83.33±3.3a-c	100±0a	13.33b-d	16.67a-c	30.00a-g
Bajaja	2021	Natural	76.67±6.67a-f	100±0a	100±0a	23.33a-c	0c	23.33b-g
		Artificial	86.67±3.33a-d	100±0a	100±0a	13.33b-d	0c	13.33d-g
	2022	Natural	63.33±3.33b-g	100±0a	100±0a	36.67a-b	0c	36.67a-f
		Artificial	86.67±3.33a-d	100±0a	100±0a	13.33b-d	0c	13.33d-g
Perun	2021	Natural	77.33±3.33a-g	100±0a	100±0a	26.67a-d	0c	26.67a-g
		Artificial	90.00±5.77a-c	100±0a	100±0a	10.00c-d	0c	10.00e-g
	2022	Natural	43.33±3.33g	80±5.77b-c	100±0a	36.67a-b	20a-b	56.67a
		Artificial	60.00±5.77c-g	90±0a-b	100±0a	30.00a-c	10.00b-c	40.00a-e

Note: Means±s.e. followed by different letters present significant differences at p<0.05 (Tukey test)

*The influence of experimental factors on the Intensity of the Alternaria alternata attack*

The intensity of the *A. alternata* attack, under artificial infection conditions, throughout the experimental period, had higher percentage values than the natural infection. According to the data presented in Table 5, the intensity of the *A. alternata* attack, at the varieties monitored, at the first observation, did not show statistically significant differences, except for the ‘Romus’ variety which in conditions of artificial infection presented the highest intensity value in 2021 (6.57%). In 2022, at the opposite end is the ‘San Marzano’ variety with the lowest attack intensity value of 0.97% under natural infection conditions.

During the growing season the situation changes, so the intensity of the attack on all varieties increases in both conditions of infection, especially in artificial infection. Thus in 2021 the varieties which had the lowest percentage of intensity, now have the highest values, the highest attack intensity values up to 36.80% in ‘San Marzano’. The ‘Romus’ variety, in 2021, maintains the growth trend throughout the vegetation period, registering one of the highest attack intensity values (36.38%).

In 2022, regarding the ‘San Marzano’ variety, it presents the lowest assessment of the intensity of the attack, maintaining its lowest values throughout the vegetation period. (first observation 0.97%- last observation 14.7%). The ‘Perun’ variety at the first observation, the values, in the case of artificial infection, are slightly lower than those from the natural infection (1.5%), but at the third monitoring, the values rise to 21.67% from the first reading with significant differences compared to the natural infection.

The difference between the intensity of the attack recorded in the 3 observations is an instrument that effectively determines the degradation of the plant, following the presence of the pathogen, in the tested varieties. An important observation is that the intensity of the attack, in all varieties, is significantly influenced by climatic conditions. At the recorded values, there is a significantly higher level of intensity in 2021 than in 2022.

Comparing the two experimental years, in terms of intensity, for the ‘Marmande’ and ‘Romus’ varieties, in both conditions of infection, at the second observation the values of the intensity of the *Alternaria* attack were higher in 2021. So, between the intensity of the attack in 2021 and 2022 at the second observation shows that the ‘Marmande’ variety has the intensity with 4.3% in natural infection and 8.12% in conditions of artificial infection higher in 2021. In the case of ‘Romus’ variety, the difference is 6.5% in natural infection and 11.46% in artificial infection.

When comparing the last and first observation, there are significant differences both between varieties and in response to the method of infection.

The biggest difference is observed in the ‘San Marzano’ variety, which in 2021, in a high-pressure infection, has an increase intensity of the attack (32.99%) compared to the year 2022. ‘Perun’ variety exhibits similar behaviour in conditions of artificial infection with increases in attack intensity of more than 21.67% from the first to the last observation. Overall, there were increases in intensity of 0.5-17.5% comparing the differences between the first observation and the last observation at all varieties in the both experimental years (natural infection) and 14.2-25.5% respectively in the case of artificial infection.

In 2021, in case of high pressure of infection the intensity of the attack was higher in the ‘San Marzano’ varieties (32.99%), ‘Romus’ (29.8%), ‘Marmande’ (29.68%), ‘Bajaja’ (25.23%) and ‘Perun’ (22.97%) This impact can be attributed to the fact that after the time of artificial infections, precipitation favoured the evolution of the attack by *Alternaria alternata* (Table 5).

**Table 5.** The early blight Intensity of the attack depending on the climatic conditions and the infection type

Variety	Year	Infection	Intensity% Observation 1	Intensity% Observation 2	Intensity% Observation 3	Difference intensity% 1-2	Difference intensity% 2-3	Difference intensity% 1-3
Marmande	2021	Natural	1.20±0.6b-c	8.43±1.38d-h	25.43±1.37c-e	7.23a-d	17.00a-c	24.23a-e
		Artificial	4.58±1.58a-c	15.69±0.93a-b	34.26±2.44a-b	11.12a	18.57a-c	29.68a-c
	2022	Natural	1.17±0.48b-c	4.10±1.30h	17.13±1.42e-h	2.93d-e	13.03c	15.97d-g
		Artificial	1.67±0.36b-c	7.57±0.49e-h	25.37±0.77c-e	5.90a-e	17.80a-c	23.70a-e
Romus	2021	Natural	2.07±0.03b-c	11.43±0.55b-e	26.60±0.37c-d	9.37a-c	15.17b-c	24.53a-d
		Artificial	6.57±0.50a	17.63±0.97a	36.38±4.82a	11.07a	18.73a-c	29.80a-c
	2022	Natural	1.37±0.08b-c	4.93±1.33g-h	15.83±1.42f-h	3.57-b-e	10.90c	14.47e-g
		Artificial	1.87±0.24b-c	6.17±0.95f-h	23.83±1.92c-f	4.30b-e	17.67a-c	21.97c-f
San Marzano	2021	Natural	2.81±0.05a-c	10.63±0.35b-f	26.98±0.57b-d	7.82a-d	16.34a-c	24.17a-e
		Artificial	3.81±0.46a-c	11.50±1.04b-e	36.80±0.98a	7.69a-d	25.30a	32.99a
	2022	Natural	0.97±0.08c	3.90±0.70h	14.17±0.44h	2.93d-e	10.27c	13.20f-g
		Artificial	1.80±0.1b-c	5.13±0.43g-h	19.33±0.44d-h	3.33c-e	14.20b-c	17.53d-g
Bajaja	2021	Natural	2.50±0.4a-c	10.6±1.7c-f	25.00±1.89c-e	8.10a-d	14.40b-c	22.50b-f
		Artificial	5.13±0.66a-c	15.00±0.40a-c	30.37±1.69a-c	9.87a-b	15.37b-c	25.23a-d
	2022	Natural	2.13±0.48a-c	7.80±1.05e-h	25.33±0.44c-e	5.67a-e	17.53a-c	23.20a-e
		Artificial	3.27±0.24a-c	11.97±0.71b-e	35.33±1.6a-b	8.70a-d	23.37a-b	32.07a-b
Perun	2021	Natural	2.80±0.43a-c	9.00±0.34d-g	24.50±1.66c-f	6.20a-e	15.50b-c	21.70c-f
		Artificial	5.27±0.76a-b	13.03±0.58a-d	28.23±0.99a-c	7.77a-d	15.20b-c	22.97b-f
	2022	Natural	3.67±2.66a-c	4.17±0.08h	14.73±0.62g-h	0.50e	10.57c	11.07g
		Artificial	1.50±0.2b-c	5.50±0.62g-h	23.17±1.20c-g	4.00b-e	17.67a-c	21.67c-f

Note: Means±s.e. followed by different letters present significant differences at p<0.05 (Tukey test)

*The influence of experimental factors on the degree of attack by Alternaria alternata*

The degree of attack gives us an overview of dynamics of the pathogen presence, because it is calculated based on the values of frequency and intensity of the attack, expressed as a percentage. Given that the frequency value is 100 % for most monitored varieties, regardless of the type of infection, in the second and third reading, the value of the degree of attack, is equal to the intensity of the attack. Under these conditions the differences between the varieties are given by the values of the first observation.

Regarding the degree of attack by *A. alternata* under conditions of artificial infection throughout the experimental period, they had higher percentage values than natural infection. Comparing with the climatic

conditions of experimental years during the growing season, the Marmande variety in natural conditions of infection, showed a slight resistance to the attack of *A. alternata*, but over time, the variety yields to the pressure of infection, reaching a percentage of 25.43% of the degree of attack. The highest values were recorded for the San Marzano and Romus varieties, which in 2021 reached values of 36.80% under conditions of high infection pressure.

According to the data in Table 6, it can be seen that the year 2021 was much more favourable to the *Alternaria* attack. In 2021 the 'Perun' (24.5%) variety, under natural infection conditions, shows the lower degree of attack throughout the growing season than 'Bajaja' (25.0%) and 'Marmande' (25.43%). The 'San Marzano', at the end of the growing season shows the highest level of the attack degree 26.98%, de differences were statistically significant between the two varieties. In 2022, the varieties 'San Marzano', 'Perun' and 'Romus' show the lowest values of the degree of attack compared to the other varieties observed in the condition of natural infection. It retains its characteristic until the end of the vegetation period. The biggest increases in the degree of attack are recorded in the varieties 'Marmande' (30.18%), 'Romus' (30.23%), 'San Marzano' (33.09%) in 2021 and in 2022 in 'Bajaja' (32.51%) which were artificially infected.

This indicator reinforces the fact that climatic conditions have a very significant impact on the dispersion of the pathogen on the surface cultivated in both types of infection.

**Table 6.** The early blight Attack degree depending on the climatic conditions and the infection type

Variety	Year	Infection	Degree of attack % Observation1	Degree of attack % Observation2	Degree of attack % Observation 3	Differenc e degree of attack % 1-2	Differenc e degree of attack % 2-3	Differenc e degree of attack % 1-3
Marmande	2021	Natural	0.72±0.36f	8.43±1.38d-g	25.43±1.37c-e	7.71a-f	17.00a-c	24.71a-e
		Artificial	4.08±1.42a-d	15.69±0.93a-b	34.26±2.44a-b	11.61a	18.57a-c	30.18a-c
	2022	Natural	1.02±0.53e-f	3.13±1.19h-i	17.13±1.42e-h	2.11g	14.00b-c	16.11c-h
		Artificial	1.16±0.38e-f	6.81±0.44f-j	25.37±0.77c-e	5.65b-g	18.56a-c	24.21a-f
Romus	2021	Natural	1.58±0.06e-f	11.43±0.55b-f	26.60±0.37c-d	9.85a-b	15.17b-c	25.02a-e
		Artificial	6.14±0.55a	17.63±0.97a	36.38±4.82a	11.50a	18.73a-c	30.23a-c
	2022	Natural	0.77±0.03f	4.45±1.23g-i	15.83±1.42f-h	3.68d-g	11.38c	15.06f-h
		Artificial	1.17±0.09e-f	5.75±0.86g-i	23.83±1.92c-f	4.58c-g	18.08a-c	22.67c-h
San Marzano	2021	Natural	2.29±0.21b-f	10.63±0.35c-f	26.98±0.57bd	8.35a-d	16.34a-c	24.69a-e
		Artificial	3.71±0.55a-e	11.50±1.04b-f	36.80±0.98a	7.79a-f	25.30a	33.09a
	2022	Natural	0.46±0.10f	2.81±0.68i	14.17±0.44h	2.35g	11.36c	13.71h
		Artificial	1.27±0.17e-f	4.31±0.54g-i	19.33±0.44d-h	3.04e-g	15.03b-c	18.06d-h
Bajaja	2021	Natural	1.94±0.41c-f	10.6±1.70c-f	25.00±1.89c-e	8.66a-d	14.40b-c	23.06b-h
		Artificial	4.43±0.54a-c	15.00±0.40a-c	30.37±1.69a-c	10.57a-b	15.37b-c	25.94a-d
	2022	Natural	1.38±0.39d-f	7.80±1.05e-g	25.33±0.44c-e	6.42a-g	17.53a-c	23.95a-f
		Artificial	2.83±0.21b-f	11.97±0.71b-e	35.33±.16a-b	9.14a-c	23.37a-b	32.51a-b
Perun	2021	Natural	2.08±0.40c-f	9.00±0.34d-g	24.50±1.66c-f	6.92a-g	15.50a-c	22.42c-h
		Artificial	4.82±1.00a-b	13.03±0.58a-d	28.23±0.99a-c	8.22a-e	15.20b-c	23.42b-g
	2022	Natural	0.42±0.4f	3.34±0.30h-i	14.73±0.62g-h	2.92f-g	11.39c	14.31g-h
		Artificial	0.92±0.20f	4.95±0.56g-i	23.17±1.20c-g	4.03c-g	18.22a-c	22.25c-h

Note: Means ± s.e. followed by different letters present significant differences at p<0.05 (Tukey test)

## Discussion

In the event of climate change, it is necessary to continue testing the genetic material on its resistance or tolerance to the action of pathogens, because the environment influences, directly or indirectly, plants, pathogens and the antagonists. Climate change is highly associated with different levels of losses caused by a pathogen, and are often involved in the emergence of new races. For these reasons, changes associated with global warming (high temperatures, changes in rainfall, drought, etc.) may affect the incidence and severity of plant diseases and may influence the coevolution of plants and pathogens (Andersen *et al.*, 2018). Climate influences the incidence as well as the spatial and temporal distribution of plant diseases. The main factors that

promote the evolution and development of diseases are temperature, light and water; similarly, these factors influence the host plant as well. The environment acts positively or negatively on the pathogen, through the following aspects: its survival, vigour, multiplication rate, sporulation, spore dispersion distance, germination speed and tissue penetration. Plant diseases develop under an optimum of well-defined climatic variables such as: temperature, rain, relative humidity, etc.; however, the occurrence and severity of a disease in a culture is defined by the deviation of each climate variable in the optimal range for development of the disease, thus the climate affects all stages of the life of the host plant and pathogen (Brosi *et al.*, 2011; Yanez and Ilaria, 2012).

Early Blight is the major disease symptom caused by the fungus *A. solani* (Ellis & Martin) Sorauer. This disease, which in severe cases can lead to a complete defoliation, is most damaging on to tomatoes (*Solanum lycopersicum* L.) (Chaerani and Voorrips, 2006) in regions with heavy rainfall, high humidity, and high temperatures (24 - 29 °C). Epidemics can also occur in semiarid climates where frequent and prolonged nightly dews occur (Chaerani and Voorrips, 2006). Factors that may contribute to the course of the disease include extensive cultivation of sensitive varieties and hybrids, increasing use of fungicides for controlling late blight or gray mould and which are not effective against Early Blight, but also the tomato monoculture (Vloutoglou and Kalogerakis, 2000).

The varieties taken in this study were chosen due to large scale cultivation ('San Marzano') (Rao *et al.*, 2006), resistance to various stressors ('Marmande') (Alsafari *et al.*, 2019) different organoleptic qualities or resistance to disease and pests. According to data obtained in the two experimental years, the frequency of attack was lower in 2022, in both conditions of infection. Exception when the 'Bajaja' variety which in artificial conditions of infection reached a value of 86.67%. The most significant differences in frequency can be observed at the beginning of monitoring, so that during the growing period the values are uniform. No significant differences between varieties were observed between the two types of infections. In the case of natural infections, the Perun variety has a statistically significantly lower frequency in 2022 than 2021 comparing to the other varieties (supplementary Table 5).

The behaviour of the varieties in the attack by *A. alternata* it was different, the most resistant or tolerant varieties to the Early Blight attack were the 'Perun' varieties (2022) and 'Romus' (2022) in natural condition of infection Even if a low frequency value was recorded in the 'San Marzano' variety, at the first observations, it almost doubles to the second observation, which confirms that this variety has a susceptibility to the attack of *A. alternata* (Bozine-Pullai *et al.*, 2021).

In case of intensity of the attack, differences can be observed only at the beginning of the growing period in 2022 where the varieties 'San Marzano' (0.97%), 'Marmande' (1.17%) and 'Romus' (1.37%) showed the most favourable reaction, in the case of natural infection. At the opposite end the varieties that were most affected by natural infection was the variety 'Bajaja' (2.13%) and 'Perun' (3.67%). During the growing season, in case of attack intensity, the values of the intensity of 'Marmande' variety (Natural: 25.43%/Artificial: 34.26%) in 2021 were almost the same as the highest values in the 'Bajaja' variety (Natural: 25.33%/Artificial: 35.33%) in both condition of infection in 2022 (Table 5).

Recent research (Nekoval *et al.*, 2022) on the influence of climatic conditions on the degree of attack of *A. alternata*, confirms that the degree of attack of alternariosis is influenced by climatic conditions in the vegetation period. In the research carried out, during the experimental period 2018-2020, the attack rate reaches 49% (2018), 67.2% (2019), 58.3% (2020), exceeding the attack rate, and, obtained in our experiences, in some varieties 36.8% (2021) and 35.33% (2022).

The highest value of the degree of attack, calculated after the first observation, was obtained in the 'Romus' variety in 2021, at high pressure of infection (6.14%). In the same year the lowest attack value is recorded in the case of natural infection of the 'Marmande' variety (0.72%).

As expected, the degree of attack differs depending on the type of infection. Under conditions of natural infection, varieties with a degree of attack of less than 1% were 'Marmande', in 2021 and 'Perun' and 'San Marzano' and 'Romus', in 2022.

These varieties have stability in terms of the attack of Early Blight, in natural conditions of infection. Favourable climatic conditions and increased amount of inoculum (artificial infection) lead to variety sensitization, thus, in the climatic conditions of 2021 and in conditions of artificial infection, the variety in 'San Marzano' has the highest value of the attack, after the last observation (36.80%).

The varieties studied behaved differently depending on the experimental year and the inoculation conditions. The evolution of the degree of attack was different (supplementary Table 12) but the identification of the most valuable varieties, with resistance to pathogen attack, can be done after assessing the degree of attack in favourable conditions for the realization of infections and the evolution of the disease. Given that in our experience the favourable conditions for the attack by the *A. alternata* were in 2021, a classification of the resistance of the studied varieties was made according to the values of the degree of attack and of course in the artificial conditions of infection.

The climatic factor may or may not favour the attack of *A. alternata*. Most of the cultivars studied become susceptible to pathogen attack under rainfall conditions, except 'Bajaja', which has some resistance to high humidity. The area of the Transylvanian Plains often exhibits sudden climatic changes, especially the precipitation. Precipitation can be one of the factors increasing the frequency of attack during the growing season, targeting the second observation of most varieties in both experimental years, reaching values of 100%. The rapid dispersion of the pathogen in the experimental field can be caused by several factors such as the sensitivity of the variety, the presence of more resistant physiological races of genus *A. alternata* etc. (Andersen *et al.*, 2018).

Indeed, we cannot say exactly the percentage of action of each biotic and abiotic factor, because they act in a complex way, but this study can raise a series of questions that can be debated and deepened, such as climate change can cause changes in genetics *A. alternata* so that in the current conditions experienced by climatic extremes can lead to the evolution of pathogens thus producing physiological races resistant to climatic variations that can overcome the natural barriers of plant protection. It is in the nature of creation to evolve, perpetuate and defend itself in order to survive. Currently, there is a specific resistance for each variety of tomato to meet the needs of producers and more.

It is necessary that at the moment the continuous retesting of the varieties. Can old varieties with tradition and history cope with new climatic and biological demands?

## Conclusions

Based on our findings, it can be concluded that *Alternaria* complex remains one of the most significant diseases affecting tomato crops, despite the uncertainty surrounding the pathogenicity of species within this genus. Early Blight remains one of the most important diseases in tomato cultivation.

In the Transylvanian Plain area, 2021 was a favourable year for this pathogen due to suitable infection conditions. Among the varieties of tomatoes, the cherry type fruit 'Perun', was one of the most resistant to *Alternaria alternata* with 28.23% in the case of artificial infection and 24.5% in natural infection. At the same time at the opposite end the 'Bajaja' (2021: Natural: 25.0%, Artificial: 30.37%; 2022: Natural: 25.33%, Artificial: 35.33%) variety was the most affected cherry type tomato in both experimental years, in both types of infection. 'San Marzano' variety has the highest susceptibility in the case of artificial infection with 36.8% in the category of tomatoes with large fruits followed by the 'Romus' variety (36.38%) that has a behaviour similar to San Marzano but the degree of attack is slightly elevated from 'Marmande' (34.26%) variety by 2.11%.

In 2022, the order changed as 'San Marzano' (14.17%) had the lowest intensity and degree of attack in the case of natural infection, followed by 'Perun' (14.73%), 'Romus' (15.83%), 'Marmande' (17.13%), and 'Bajaja' (25.33%). In the case of artificial infection 'Bajaja' (35.33%) had the highest value of the degree of attack.

It is evident that the climatic factor is crucial in the emergence and development of pathogens. Therefore, a deeper understanding of the climatic factor is required to have a phytopathological point of view for the future.

### Authors' Contributions

Conceptualization: ALF and LS; methodology: ALF, LS and LMS; software: ALF; validation: VF and LS; formal analysis: ALF; investigation: ALF; resources: ALF, LS and LMS; data curation: ALF; writing—original draft preparation: ALF; writing—review and editing: LS and VF; visualization: ALF and LS; supervision: LS and VF; project administration: LS and VF; funding acquisition: ALF, LS and LMS.

All authors read and approved the final manuscript.

### Ethical approval (for researches involving animals or humans)

Not applicable

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

### References

- Akhtar KP, Saleem MY, Asghar M, Haq MA (2004). New report of *Alternaria Alternata* causing leaf blight of tomato in Pakistan. *Plant Pathology* 53 (6): 816. <https://doi.org/10.1111/j.1365-3059.2004.01099.x>
- Alsafari SA, Galal HK, Bafeel SO (2019). Growth and anatomy of the tomato (*Solanum lycopersicum* Mill.) cultivars Marmande and Oria under salinity stress. *Pakistan Journal of Botany* 51(4):1199-1207. [http://dx.doi.org/10.30848/PJB2019-4\(16\)](http://dx.doi.org/10.30848/PJB2019-4(16))
- Alvarenga TVM, Ribeiro SRR, Souza EA, Pereir F, Pinto CABP (2016). Characterization of *Alternaria* isolates and reaction of potato genotypes to early blight. *Ciencia Rural* 46(10):1783-1789. <https://doi.org/10.1590/0103-8478cr20151447>
- Andersen E, Ali S, Byamukama E, Yen Y, Nepal M (2018). Disease resistance mechanisms in plants. *Genes* 9(7):339. <https://doi.org/10.3390%2Fgenes9070339>
- Aung SL, Liu HF, Pei DF, Lu BB, Oo MM, Deng JX (2020). Morphology and molecular characterization of a fungus from the *Alternaria alternata* species complex causing black spots on *Pyrus sinkiangensis* (Koerle pear). *Mycobiology* 48(3):233-239. <https://doi.org/10.1080/12298093.2020.1745476>
- Beaumont LJ, Pitman A, Perkins S, Zimmermann NE, Yoccoz NG, Thuiller W (2011). Impacts of climate change on the world's most exceptional ecoregions. *Proceedings of the National Academy Sciences of the United States of America* 108(6):2306-2311. <https://doi.org/10.1073/pnas.1007217108>
- Biokiskert (2023). Retrieved 2023 November 30 from: <https://biokiskert.hu/bio/romus/>

- Boland GJ, Melzer MS, Hopkin A, Higgins V, Nassuth A (2004). Climate change and plant diseases in Ontario. Canadian Journal of Plant Pathology 26(3):335-350. <https://doi.org/10.1080/07060660409507151>
- Bonnie Plants (2023). Retrieved 2023 November 30 from: <https://bonnieplants.com/products/san-marzano-tomato>
- Boziné-Pullai K, Csambalik L, Drexler D, Reiter D, Tóth F, Tóthné Bogdányi F, Ladányi M (2021). Tomato landraces are competitive with commercial varieties in terms of tolerance to plant pathogens-A case study of Hungarian gene bank accessions on organic farms. Diversity 13(5):195. <https://doi.org/10.3390/d13050195>
- Brosi GB, McCulley RL, Bush LP, Nelson JA, Classen AT, Norby RJ (2011). Effects of multiple climate change factors on the tall fescue fungal endophyte symbiosis: infection frequency and tissue chemistry. New Phytologist 189(3):797-805. <https://doi.org/10.1111/j.1469-8137.2010.03532.x>
- Burpee Homegardens (2023). Retrieved 2023 November 30 from: <https://www.burpeehomegardens.com/Vegetables/PlantDetails.aspx?plantid=5717>.
- Chaerani R, Voorrips RE (2006). Tomato early blight (*Alternaria Solani*): The pathogen, genetics, and breeding for resistance. Journal General Plant Pathology 72:335-347. <https://doi.org/10.1007/s10327-006-0299-3>
- Choudhary P, Chakdar H, Singh A, Kumar S, Singh SK, Aarthy M, ... Saxena AK (2020). Computational identification and antifungal bioassay reveal phytosterols as potential inhibitor of *Alternaria arborescens*. Journal Biomolecular Structure Dynamics 38(4):1143-1157. <https://doi.org/10.1080/07391102.2019.1597767>
- Collins EJ, Bowyer C, Tsouza A, Chopra M (2022). Tomatoes: an extensive review of the associated health impacts of tomatoes and factors that can affect their cultivation. Biology 11(2):239. <https://doi.org/10.3390/biology11020239>
- Corcoz L, Păcurar F, Pop-Moldovan V, Vaida I, Pleșa A, Stoian V, Vidican R (2022). Long-term fertilization alters mycorrhizal colonization strategy in the roots of *Agrostis capillaris*. Agriculture 12(6):847. <https://doi.org/10.3390/agriculture12060847>
- de Mendiburu F (2019). Agricolae: Statistical procedures for agricultural research. Available at: <https://CRAN.R-project.org/package=agricolae>
- Fontem DA (2003). Quantitative effects of early and late blights on tomato yields in Cameroon. Tropicultura 21(1):36-41.
- Gannibal PB (2015). Distribution of *Alternaria* species among sections. 1. Section *Porri*. Mycotaxon 130(1):207-213. <https://doi.org/10.5248/130.207>
- Gannibal PB (2016). Distribution of *Alternaria* species among sections. 2. Section *Alternaria*. Mycotaxon 130(4):941-949. <https://doi.org/10.5248/130.941>
- Garden Seeds Market Available (2023). Retrieved 2023 November 30 from: <https://gardenseedsmarket.com/tomato-bajaja-low-growing-cherry-type-variety-with-trailing-habit-for-balcony-cultivation.html>
- Gardeners Basics Available (2023). Retrieved 2023 November 30 from: <https://www.gardenersbasics.com/tools/blog/are-san-marzano-tomatoes-harder-to-grow>
- Hu W, Ran Y, Zhuang K, Lama J, Zhang C (2015). *Alternaria arborescens* infection in a healthy individual and literature review of cutaneous alternariosis. Mycopathologia 179(1-2):147-152. <http://dx.doi.org/10.1007/s11046-014-9822-9>
- Hulea A (1969). Guide for micology and bacteriology laboratory. Editura Agrosilvică, Bucharest.
- Hussain R, Gupta V, Arora JK, Sharma SK, Kumar D, Khushboo SS (2022). Weather based prediction of early blight of potato caused by *Alternaria solani*. Agricultural Mechanization in Asia, Africa and Latin America 53(2).
- Indrea D, Apahidean AS, Apahidean M, Mănuțiu DN, Sima R (2012). Vegetable Crops. 3rd. Ceres, Bucharest.
- Kokaeva L, Yu Yarmeeva MM, Kokaeva ZG, Chudinova EM, Balabko PN, Elansky SN (2022). Phylogenetic study of *Alternaria* potato and tomato pathogens in Russia. Diversity 14(8):685. <https://doi.org/10.3390/d14080685>
- Kong H, Fu X, Chang X, Ding Z, Yu Y, Xu H, ... Ding S (2023). The ester derivatives of ferulic acid exhibit strong inhibitory effect on the growth of *Alternaria alternata* *in vitro* and *in vivo*. Postharvest Biology and Technology 196:112158. <https://doi.org/10.1016/j.postharvbio.2022.112158>
- Landschoot S, Carrette J, Vandecasteele M, De Baets B, Höfte M, Audenaert K, Haesaert G (2017). Boscalid resistance in *Alternaria alternata* and *Alternaria solani* populations: an emerging problem in Europe. Crop Protection 92:49-59. <https://doi.org/10.1016/j.cropro.2016.10.011>

- Landschoot S, Vandecasteele M, De Baets B, Höfte M, Audenaert K, Haesaert G (2017). Identification of *A. arborescens*, *A. grandis*, and *A. protenta* as new members of the European *Alternaria* population on potato. Fungal Biology 121(2):172-188. <https://doi.org/10.1016/j.funbio.2016.11.005>
- Lawrence DP, Gannibal PB, Peever TL, Pryor BM (2013). The sections of *Alternaria*: Formalizing species group concepts. Mycologia 105(3):530-546. <https://doi.org/10.3852/12-249>
- Lawrence DP, Rotondo F, Gannibal PB (2016). Biodiversity and taxonomy of the pleomorphic genus *Alternaria*. Mycological Progress 15:3. <https://doi.org/10.1007/s11557-015-1144-x>
- Ma M, Taylor PWJ, Chen D, Vaghefi N, He JZ (2023). Major soilborne pathogens of field processing tomatoes and management strategies. Microorganisms 11(2):263. <https://doi.org/10.3390/microorganisms11020263>
- Marak TR, Ambesh BS, Das S (2014). Cultural, morphological and biochemical variations of *Alternaria solani* causing diseases on *Solanaceous*. The Bioscan 9(3):1295-1300.
- Mărculeț C, Mărculeț I (2016). Favorable and restrictive climatic conditions for culture of plants in Alba Iulia - Turda bazin. Pangeea 16:55-69.
- Masheva S (2014). Recent trends of the breeding programs in main vegetables and potatoes in Bulgaria. Journal of Horticulture 1:2. <https://doi.org/10.4172/2376-0354.1000e102>
- Meier U (2001). Growth stages of mono- and dicotyledonous plants: BBCH monograph. Federal Biological Research Centre for Agriculture and Forestry.
- Nasr-Esfahani M (2022). An IPM plan for early blight disease of potato *Alternaria solani* Sorauer and *A. alternata* (Fries.) Keissler. Archives of Phytopathology and Plant Protection 55(10):1-12. <http://dx.doi.org/10.1080/03235408.2018.1489600>
- Nekoval S, Zakharchenko A, Sadovaya A, Churikova A, Fedoryanskaya I (2022). Assessment of mutant tomato lines as a starting material for breeding varieties resistant to *Alternaria alternata*. Saudi Journal of Biological Sciences 29:1061-1072. <https://doi.org/10.1016/j.sjbs.2021.09.066>
- Nichifor C, Negulescu F (1983). Genetics and the improving resistance to diseases at plants. Academy of Romanian Socialist Republic, Bucharest.
- Nishikawa J, Nakashima C (2020). Japanese species of *Alternaria* and their species boundaries based on host range. Fungal Systematics and Evolution 5:197-282. <https://doi.org/10.3114/fuse.2020.05.13>
- Pârveu M (2010). Practical guide of Phytopathology. University Press of Cluj, Cluj-Napoca.
- Patel NA, Dange SS, Patel SI (2005). Efficacy of chemicals in controlling fruit rot of tomato caused by *Alternaria Tomato*. Indian Journal of Agricultural Research. 39:72-75.
- Pautasso M, Döring TF, Garbelotto M, Pellis L, Jeger MJ (2012). Impacts of climate change on plant diseases opinions and trends. European Journal of Plant Pathology 133:295-313. <http://dx.doi.org/10.1007/s10658-012-9936-1>
- Pop-Moldovan V, Corcoz L, Stoian V, Moldovan C, Pleșa A, Vâtcă S, Vidican R (2022). Models of mycorrhizal colonization patterns and strategies induced by biostimulat or treatments in *Zea mays* roots. Frontiers in Plant Science 13. <https://doi.org/10.3389/fpls.2022.1052066>
- Prodaja semena (2023). Retrieved 2023 November 30 from: <https://www.prodajasemena.com/proizvod/tomato-romuszuti-veoma-rodanukusan-paradajz/>
- Puia EC (2005). Phytopathology - pathology and etiology. Risoprint, Cluj-Napoca.
- Rahmatzai N, Zaitoun AA, Madkour M, Ahmady A, Hazim Z, Mousa MA (2016). Morphological, pathogenic, cultural and physiological variability of the isolates of *Alternaria solani* causing early blight of tomato. International Journal of Advanced Research 4:808-817. <http://dx.doi.org/10.21474/IJAR01/2151>
- Ramadan W, Elkafrawy A (2014). Breeding tomato for early blight resistance under Egyptian conditions. Journal of Plant Production 5(12):1989-2000. <http://dx.doi.org/10.21608/jpp.2014.64762>
- Ramezani Y, Taheri P, Mamarabadi M (2019). Identification of *Alternaria* spp. associated with tomato early blight in Iran and investigating some of their virulence factors. Journal of Plant Pathology 101(3):647-659. <https://doi.org/10.1007/s42161-019-00259-w>
- Rao R, Corrado G, Bianchi M, Di Mauro A (2006). (GATA) 4 DNA Fingerprinting identifies morphologically characterized "San Marzano" tomato plants. Plant Breeding 125(2):173-176. <https://doi.org/10.1111/j.1439-0523.2006.01183.x>

- Revelle W (2019). Psych: Procedures for psychological, psychometric, and personality research. r package version 1.9.12 (Evanston, IL, USA: Northwestern University).
- Rodrigues MHP, Furlong EB (2022). Fungal diseases and natural defense mechanisms of tomatoes (*Solanum lycopersicum*): a review. *Physiological and Molecular Plant Pathology* 122:101906.
- Rodrigues TMS, Maffia LA, Dhingra OD, Mizubuti ESG (2010). *In vitro* production of conidia of *Alternaria solani*. *Tropical Plant Pathology* 35(4):203-212. <http://dx.doi.org/10.1590/S1982-56762010000400001>
- RStudio Team (2019). RStudio: Integrated development environment for r (Boston, MA, USA: RStudio, Inc).
- Sadeghi B, Mirzaei S, Fatehi F (2022). The proteomic analysis of the resistance responses in tomato during interaction with *Alternaria alternata*. *Scientia Horticulturae* 304:111295. <https://doi.org/10.1016/j.scienta.2022.111295>
- Schmey T, Small C, Hoyoz LM, Ali T, Gamboa S, Mamami B, ... Stam R (2022). Small spored *Alternaria* spp. (Section *Alternaria*) are common pathogens on wild tomato species. *Environmental Microbiology* 25(10):1-17. <https://doi.org/10.1111/1462-2920.16394>
- Severin V, Cornea CP (2009). Guide for diagnose of the disease on plants. Ceres, Bucharest.
- Singh D, Prasad R, Yadav SP, Kumar S (2014). Characterization of *Alternaria brassicae* causing *Alternaria* blight of rapeseed mustard (*Brassica juncea* L.) and it's management. *The Bioscan* 9(3):1207-1212.
- Singh SG, Mehta N, Meena PD (2016). *Alternaria* diseases of Crucifers: Biology, ecology and disease management. Springer, Singapore.
- Somappa J, Srivastava K, Sarma BK, Pal C, Kumar R (2013). Studies on growth conditions of the tomato *Alternaria* leaf spot causing *Alternaria solani* L. *The Bioscan* 8(1):101-104.
- Soomro HU (2019). Disease intensity and eco-friendly management of *Alternaria alternata* in chili (*Capsicum annum* L.). *Pure and Applied Biology* 8(4). <http://dx.doi.org/10.19045/bspab.2019.80180>
- The National Gardening Association (2023). Retrieved 2023 November 30 from: <https://garden.org/plants/view/175545/Tomato-Solanum-lycopersicum-Romus/>
- Tian Y, Abdallah MF, De Boevre M, Audenaert K, Wang C, De Saeger S, Wu A (2023). Deciphering *Alternaria* Metabolic responses in microbial confrontation via an integrated mass spectrometric targeted and non-targeted strategy. *Food Chemistry* 404(B):134694. <https://doi.org/10.1016/j.foodchem.2022.134694>
- Vloutoglou I, Kalogerakis SN (2000). Effects of inoculum concentration, wetness duration and plant age on development of early blight (*Alternaria Solani*) and on shedding of leaves in tomato plants. *Plant Pathology* 49(3):339-345. <https://doi.org/10.1046/j.1365-3059.2000.00462.x>
- Wang J, Xiao S, Zheng L, Pan Y, Zhao D, Zhang D, ...Yang Z (2022). Multiomic approaches reveal novel lineage specific effectors in the potato and tomato early blight pathogen *Alternaria Solani*. *Phytopathology Research* 4:29. <https://doi.org/10.1186/s42483-022-00135-z>
- Woudenberg JHC, Truter M, Groenewald JZ, Crous PW (2014). Large spored *Alternaria* pathogens in Section *Porri* disentangled. *Studies in Mycology* 79:1-47. <https://doi.org/10.1016/j.simyco.2014.07.003>
- Yáñez LR, Torres IP, González GRG, Zul HMI, Carranza QJA, García RE (2012). The effect of climate change on plant diseases. *African Journal of Biotechnology* 11(10):2417-2428. <https://doi.org/10.5897/AJB10.2442>
- Yigal E, Ilaria P (2014). Climate change impacts on plant pathogens and plant diseases. *Journal of Crop Improvement* 28(1):99-139. <http://dx.doi.org/10.1080/15427528.2014.865412>
- Zhao D, Fan S, Zhang D, Pan Y, Gu Q, Wang J, Yang Z, Zhu J (2021). Parasexual reproduction in *Alternaria solani*: Simple sequence repeat molecular evidence for haploidization. *Mycologia* 113(5):949-955. <https://doi.org/10.1080/00275514.2021.1922243>



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