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Research Article

ASSESSMENT AND SUSTAINABLE CONTROL OF WHITE RUST (*PUCCINIA HORIANA* HENN.) IN THE *CHRYSANTHEMUM* CULTIVARS COLLECTION OF THE IAȘI BOTANICAL GARDEN (ROMANIA)

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Abstract: Chrysanthemum white rust (CWR), caused by *Puccinia horiana* Henn., is a quarantine disease that poses a serious threat to chrysanthemum production under protected cultivation. This study aimed to evaluate the incidence, severity, and cultivar-specific responses to CWR in a greenhouse collection of *Chrysanthemum* × *grandiflorum* Ramat. at the "Anastasie Fătu" Botanical Garden of Iași, Romania. A total of 35 cultivars (1,120 plants) were monitored under uniform greenhouse conditions between 2020 and 2023 growing seasons. Disease assessment was based on systematic visual inspections, microscopic confirmation of the pathogen, and quantitative evaluation using Disease Incidence, Disease Severity Index, and standardized Combined Score integrating both parameters. Cultivars were classified into susceptibility categories ranging from tolerant to highly susceptible. In 2020, disease incidence ranged from 0% in resistant cultivars to over 90% in highly susceptible ones, with corresponding variations in disease severity. The implementation of integrated management strategies, including cultural practices, curative fungicide applications at 13 and 15 weeks after planting, and preventive treatments at 5 weeks after planting, resulted in a significant reduction in disease incidence and severity in 2021. Continuous monitoring combined with preventive treatments in 2022 and 2023 led to the complete suppression of CWR, with no visible symptoms observed across all cultivars. Several cultivars consistently exhibited resistance throughout the study, while others showed high susceptibility under initial disease pressure. The findings demonstrate that the integration of resistant cultivars with early preventive measures and continuous monitoring is essential for the sustainable management of *P. horiana* in greenhouse chrysanthemum production. This study provides a practical framework for disease assessment and supports informed cultivar selection and integrated disease control strategies for ornamental crops.

Keywords: chrysanthemum horticultural varieties, disease management, *Puccinia horiana*, resistance, white rust.

Introduction

Chrysanthemum is an important traditional ornamental crop, ranking among the four most widely produced cut flowers globally, ranking second globally in the cut flower trade, after rose [MEKAPOGU & al. 2022, SUN & al. 2011]. The crop is highly susceptible to a range of diseases, including *Alternaria* leaf blight, brown rust, white rust, wilt, bacterial blight, and various viral and viroid infections [TROLINGER & al. 2018]. Among these, *Chrysanthemum* white rust (CWR), caused by *Puccinia horiana* Henn., is particularly destructive, posing a major threat to commercial cultivation. This disease has a significant impact on chrysanthemum production worldwide [DICKENS, 1990] and is recognized as a quarantine pest in many

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countries, often triggering strict eradication programs upon detection. Characterized by its rapid spread and severe effects on both ornamental quality and market value, *P. horiana* represents a major challenge for growers and botanical collections [ZENG & al. 2013].

Chrysanthemum white rust (CWR), caused by *P. horiana* Henn., is one of the most destructive fungal diseases affecting chrysanthemum crops worldwide. The pathogen is indigenous to Japan, where it was first reported in 1895, and remained confined to China and Japan until 1963. Since 1964, it has spread rapidly through the international trade of infected cuttings and is now established across Europe, Africa, Australia, Central and South America, and the Far East. The global dissemination of *P. horiana* is facilitated by the high susceptibility of chrysanthemum cultivars and favorable microclimatic conditions that promote epidemic development [BONDE & al. 2015; EPPO, 2025; USDA-APHIS, 2020, 2025].

The disease significantly impacts both ornamental quality and commercial value, leading to major economic losses in the floriculture industry. As a quarantine pathogen in many countries, its detection triggers strict regulatory measures and eradication programs to prevent further spread. The obligate biotrophic nature of *P. horiana* makes early detection and accurate diagnosis critical for effective management. Botanical gardens, nurseries, and commercial cultivations are particularly vulnerable due to dense plantings and the presence of susceptible cultivars. Understanding the pathogen's biology, epidemiology, and host interactions is therefore essential to develop sustainable control strategies and to safeguard genetic diversity in chrysanthemum collections [ZENG & al. 2013; KUMAR & al. 2021].

In Romania, the growing popularity of chrysanthemum cultivars in horticulture and public gardens increases the necessity for rigorous disease monitoring and management - especially within conservation-oriented institutions. The *Chrysanthemum* collection of the "Anastasie Fătu" Botanical Garden of Iași represents a valuable scientific, educational, and ornamental resource, a reservoir of genetic diversity and a national reference for horticultural practices. Safeguarding the health of this collection demands precise detection, thorough characterization, and the development of sustainable control strategies against pathogens such as *P. horiana*.

According to the EPPO Global Database (2025), *P. horiana* is reported in Romania with the status "present, restricted distribution." The first documented occurrence was recorded in 1995 in Vidra, Romania [COSTACHE & COSTACHE, 1995], with subsequent reports appearing in later studies, including NEGREAN (2003), and NEGREAN & ANASTASIU (2009). No recent peer-reviewed scientific articles from Romania reporting experimental data on *P. horiana* were found in accessible databases, and most sources consist of internal reports, popular articles, control guides, or phytosanitary observation records. Overall, the situation in Romania appears to reflect mainly presence and risk assessments rather than systematic research [BUTA & al. 2011], indicating a lack of recent experimental studies or limited publication in international journals.

Puccinia horiana Henn. is a rust fungus belonging to the order Pucciniales (formerly Uredinales), family Pucciniaceae. Unlike many rusts that complete their life cycle on two unrelated hosts, *P. horiana* is an autoecious microcyclic rust fungus, completing all developmental stages on chrysanthemum hosts [BONDE & al. 2015]. The pathogen forms characteristic buff-white telia that differentiates it from other rust fungi, notably *Puccinia chrysanthemi* Roze, which produces orange-brown uredinia [DEMERS & al. 2015; CUMMINS & HIRATSUKA, 2003].

The pathogen is an obligate parasite and cannot be cultured on artificial media. Infection begins when basidiospores, produced from germinating teliospores, penetrate leaf tissues through stomata. High humidity (>95%) and temperatures between 10-20°C are key factors supporting infection, sporulation and secondary spread [RHS, 2025]. After penetration, the fungus develops an intercellular mycelium that forms haustoria in host cells, enabling nutrient uptake and systemic spread within plant tissues.

Typical symptoms include pale yellow chlorotic spots on the upper leaf surface; white to cream-coloured pustules (telia) on the underside of leaves; premature defoliation and chlorosis; stunted growth, deformed shoots and compromised flowering.

Under favorable conditions of high humidity and persistent leaf wetness, the disease can spread rapidly, leading to severe damage: defoliation, stunted growth, and loss of ornamental and commercial value. In advanced infections, systemic colonization may occur, leading to severe plant decline or death [BONDE & al. 2015].

Given these risks, accurate and early detection of *P. horiana*, especially before visible symptoms appear, is essential. Effective and quick detection methodologies are required to mitigate yield loss and time constraints associated with monitoring and management of CWR.

The scientific literature demonstrates that *P. horiana* remains a major phytosanitary threat to chrysanthemum collections globally. Modern diagnostics, especially molecular techniques, are essential for early detection and effective management. Sustainable control strategies integrate cultural, ecological and minimal chemical methods, aligning with the mission of botanical gardens to preserve biodiversity while ensuring environmental safety.

Ensuring phytosanitary stability within such collections requires rigorous monitoring of plant pathogens, with *P. horiana* representing a major threat due to its rapid spread and capacity to remain latent in infected tissues [ALAEI & al. 2009; PEDLEY, 2009]. This study aims to assess the presence and progression of white rust within *Chrysanthemum* collection, to characterize the pathogen and to identify environmentally responsible methods for its long-term management. Through these objectives, the research contributes to improved plant protection protocols and the preservation of chrysanthemum genetic resources in Romania.

In this context, the present work aims to: (1) detect the presence of *P. horiana* in the *Chrysanthemum* cultivars collection of the Iași Botanical Garden, using traditional mycological methods; (2) characterize the pathogen (morphology, infection patterns, possibly cultivar susceptibility); (3) propose and evaluate sustainable control strategies, combining good horticultural practices, early detection, sanitation, and minimal targeted intervention, to safeguard the collection's health and preserve its ornamental and genetic value. By achieving these aims, this study will contribute to improved phytosanitary management of ornamental chrysanthemum germplasm in Romania and support broader efforts to monitor and control white rust in botanical gardens and horticultural settings.

Material and methods

Study site and plant material

The study was conducted at the „Anastasie Fătu" Botanical Garden of Iași - Romania, under protected greenhouse conditions (47°11'17.69" N, 27°33'22.8" E - DMS system, 150 m a.s.l.). The observation took place in a single greenhouse with a total area of 50 m², designed to ensure uniform growing conditions and to minimize the influence of external climatic factors.

The plant material consisted of 35 *Chrysanthemum × grandiflorum* cultivars, selected for their horticultural relevance and phenotypic diversity: cv. *Alec Bedser* (cv.1), *Astro* (cv.2), *Axillia* (cv.3), *Blanche* (cv.4), *Cassandra* (cv.5), *Crimson Robe* (cv.6), *Cristal* (cv.7), *Diplomate Orange* (cv.8), *Escort Gelb* (cv.9), *Escort Orange* (cv.10), *Escort Roth* (cv.11), *Evelyn Busch* (cv.12), *Flame Blaier* (cv.13), *Good Bust* (cv.14), *Hannenburg* (cv.15), *Hagoromo* (cv.16), *Homaro* (cv.17), *Inga* (cv.18), *Jonson* (cv.19), *Margaret* (cv.20), *Marielle Purple* (cv.21), *Marielle Red* (cv.22), *Nob Hill* (cv.23), *Nyll Zwager* (cv.24), *Pink Always* (cv.25), *Prince de Monaco* (cv.26), *Princess Armgard* (cv.27), *Promenade* (cv.28), *Sheer Purple* (cv.29), *Sterling* (cv.30), *Stramer* (cv.31), *Taylor* (cv.32), *Tom Pierce* (cv.33), *Vienna Cooper* (cv.34), *Vienna White* (cv.35).

For each cultivar, 32 individual plants were included in the observation, resulting in a total of 1,120 plants analyzed throughout the study. All plants were cultivated in greenhouse compartments under identical horticultural practices, including substrate composition, irrigation regime, fertilization, and plant protection measures. Environmental parameters such as temperature, relative humidity, and light conditions were maintained at optimal levels for chrysanthemum growth and development. The experimental design allowed for comparative evaluation among cultivars under uniform protected conditions, ensuring the reliability of the obtained results.

The cultivars used in this study originate from the living chrysanthemum collection of the Botanical Garden of Iași, which comprises over 450 cultivars of *Chrysanthemum* × *grandiflorum* Ramat. and *Chrysanthemum indicum* L., representing the most extensive and important chrysanthemum collection in Romania and an essential resource for research, conservation, and ornamental plant breeding [COJOCARIU, 2016; PETRE & al. 2017; COJOCARIU & al. 2018].

CWR monitoring and visual detection

Monitoring of Chrysanthemum white rust (CWR), caused by *P. horiana*, was performed between 2020-2023, through systematic visual inspections of chrysanthemum plants cultivated under protected greenhouse conditions. The monitoring aimed to identify the onset, spread, and severity of disease symptoms, as recommended for quarantine pathogens affecting ornamental crops [EPPO, 2023].

Visual detection focused on the identification of typical CWR symptoms, including chlorotic or necrotic spots on the adaxial leaf surface and the development of white to pinkish pustules (telia) on the abaxial surface [O'KEEFE & DAVIS, 2015; PEDLEY, 2009]. Individual plants were inspected at regular intervals (one week, in August-October) to ensure detection of infection and accurate assessment of disease progression.

Assessment of CWR on *Chrysanthemum*

The incidence and severity of Chrysanthemum white rust (*Puccinia horiana* Henn.) on chrysanthemum cultivars were evaluated using a combination of quantitative and semi-quantitative parameters. For each plant, control strategies against Chrysanthemum white rust (CWR) were implemented using an integrated approach, combining cultural and chemical measures, in accordance with good horticultural practices for protected ornamental crops.

Cultural control strategies were applied to reduce disease pressure and limit the spread of *P. horiana* within the greenhouse. These measures included: plant spacing (*Chrysanthemum* plants were arranged at appropriate distances to prevent canopy overlap and to reduce leaf-to-leaf contact, thereby limiting the conditions favorable for pathogen transmission and sporulation), sanitation practices (regular sanitation measures were strictly applied, infected leaves and plant debris showing visible CWR symptoms were promptly removed and destroyed to reduce the inoculum source, tools and equipment used during plant handling were disinfected to prevent mechanical dissemination of the pathogen), ventilation management (natural ventilation was used to maintain air circulation and to create unfavorable conditions for fungal growth, to reduce relative humidity and leaf wetness duration, both of which are critical factors for CWR infection and disease development).

Chemical Control Measures for CWR was carried out using fungicide treatments based on azoxystrobin, a systemic fungicide belonging to the strobilurin (Q_oI) group, known for its preventive and curative activity against rust pathogens [BARTLETT & al. 2002].

Fungicide applications were performed according to the manufacturer's recommendations, considering the appropriate dosage, application interval, safety regulations for greenhouse use, and to ensure complete foliar coverage. Treatments were applied at the early stages of symptom appearance to ensure maximum efficacy. Treatments were applied twice per growing season (2020, 14 and 28 September, and 2021, 13 and 27 September, representing a latent period of 13 and 15 weeks after planting, WAP) at a 0.1% recommended concentration, and also as preventive treatment in 2021 and 2022 at 5 WAP (2021, 19 July and 2022, 18 July).

Data collection

The collected data on CWR were analyzed to evaluate disease incidence, severity, and cultivar responses under greenhouse conditions. The following procedures were applied for calculation of disease parameters:

Disease Incidence (DI, %)

Disease incidence was calculated as the proportion of infected plants in each cultivar, expressed as a percentage:

$$DI(\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Disease Severity Index (DSI, %)

Disease severity was assessed using a standardized visual scoring scale (1-5) and expressed as a disease severity index (DSI, %), calculated as follows [CHIANG & al. 2017]:

$$DSI(\%) = \frac{\sum(n_i \times v_i)}{N \times V} \times 100$$

where: n_i = number of plants in each severity category; v_i = numerical value of each category (1-5); N = total number of plants assessed; V = maximum disease score (5).

All 1,120 plants, representing 35 chrysanthemum cultivars, were individually evaluated using a standardized visual scoring scale (1-5), *VSS*, based on symptom severity, adapted from commonly used disease assessment protocols [EPPO, 1990], where:
1 = no visible symptoms; 2 = very mild infection (few isolated lesions); 3 = moderate infection (multiple lesions on several leaves); 4 = severe infection (numerous lesions, chlorosis, leaf deformation); 5 = very severe infection (extensive pustule coverage, necrosis, premature senescence).

This index provides a quantitative measure of disease intensity, facilitating comparison between cultivars. Disease incidence (%) and severity scores were calculated for each cultivar. This combined approach allowed for an objective evaluation of the pathogen's impact while providing a standardized framework for comparing susceptibility among chrysanthemum cultivars. This monitoring approach enabled the identification of tolerant and susceptible cultivars and supported the evaluation of host–pathogen interactions involving *P. horiana* under greenhouse conditions.

To classify chrysanthemum cultivars according to their susceptibility to CWR, both Disease Incidence (DI) and Disease Severity Index (DSI) were integrated into a *standardized*

Combined Score (CS). First, the individual values of DI and DSI were normalized by dividing each percentage by 100, yielding values between 0 and 1: $DI_{norm} = DI/100$, $DSI_{norm} = DSI/100$

Normalization ensures that both variables contribute equally to the combined evaluation. The Combined Score (CS) was then calculated as the average of the normalized values: $CS = (DI_{norm}+DSI_{norm})/2$

The CS integrates both the proportion of infected plants and the intensity of symptoms, providing a comprehensive measure of cultivar response. Based on the CS value, cultivars were assigned to susceptibility categories as shown in Table 1:

Table 1. Susceptibility categories of *Chrysanthemum* cultivars based on Combined Score (CS) of Disease Incidence and Severity

CS range values (0-1)	Susceptibility Category
0 – 0.20	Tolerant
0.21 – 0.40	Moderately Tolerant
0.41 – 0.60	Moderately Susceptible
0.61 – 0.80	Susceptible
0.81 – 1.00	Highly Susceptible

This method allows for a quantitative and integrative assessment of all 35 cultivars included in the study, facilitating comparisons and identification of tolerant, moderately susceptible, and highly susceptible genotypes.

Results and discussions

Detection and Identification of Chrysanthemum White Rust (CWR)

Chrysanthemum white rust (CWR), caused by *P. horiana*, was first detected in September 2020 in the protected greenhouse environment of the "Anastasie Fătu" Botanical Garden of Iași and subsequently monitored throughout four consecutive growing seasons (2020-2023). The occurrence of the disease was identified through regular visual inspections, which revealed the characteristic symptoms of CWR, including chlorotic to necrotic lesions on the adaxial (upper) leaf surface and the development of white to pinkish spore-producing pustules (telia) on the abaxial (lower) surface (Figures 1, 2A-D).

Symptom appearance and progression were systematically recorded during the critical period from August to October, when environmental conditions within the greenhouse were favorable for pathogen development. Weekly monitoring allowed for the detailed tracking of disease dynamics, including the timing of initial symptom expression, the rate of disease spread, and the progression of symptom severity across cultivars. This intensive surveillance enabled early detection of infection foci, which proved essential for the prompt implementation of integrated control measures.

The early identification of CWR symptoms, combined with consistent monitoring, facilitated an accurate assessment of cultivar-specific responses to *P. horiana* and supported timely disease management decisions. This approach not only limited further disease development within the greenhouse but also provided a reliable framework for evaluating host susceptibility and the effectiveness of applied control strategies over multiple growing seasons.

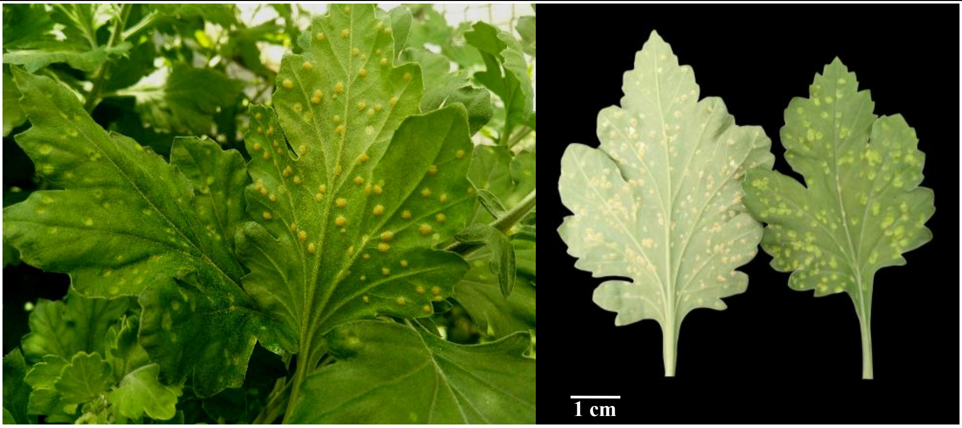

Figure 1. Typical symptoms of Chrysanthemum white rust (*Puccinia horiana*): chlorotic spots on the adaxial leaf surface and white to pinkish pustules (telia) on the abaxial surface (original).

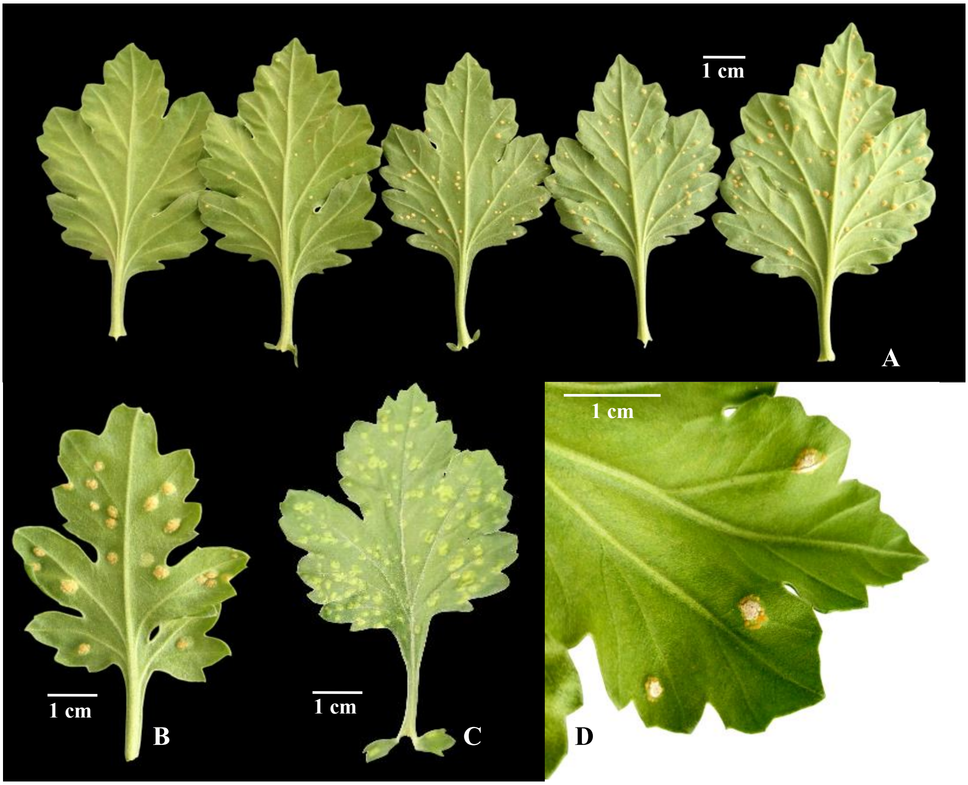

Figure 2. Different stages of Chrysanthemum white rust (*Puccinia horiana*) infection on chrysanthemum leaves (A); abaxial (lower) leaf surface with white to pinkish telial pustules (B); adaxial (upper) leaf surface showing chlorotic to necrotic lesions (C); detailed view of pustules illustrating infection and spore-producing structures (D) (original).

Disease symptoms were observed on different plant organs, with the leaves being predominantly affected. Initial symptoms appeared as pale green to yellow chlorotic spots, up to 5 mm in diameter, on the adaxial surface of infected leaves. As the disease progressed, these lesions darkened, becoming brown and necrotic. On the abaxial leaf surface, spore-producing pustules developed, initially buff to pink in color, later turning white upon maturation. Microscopic examination of these pustules revealed the presence of teliospores borne on pedicels up to 52 µm in length (Figure 3). Teliospores were pale yellow, two-celled, oblong to oblong-clavate, slightly constricted, measuring 30-52 x 11-18 µm, with thin walls (1-2 µm) that were distinctly thickened at the apex (4-9 µm). These morphological features are characteristic of *P. horiana*, confirming the identity of the causal agent.

Figure 3. Characteristic teliospores of *Puccinia horiana* observed microscopically (200x, 400x) (original)

Leaf samples of *Chrysanthemum* × *grandiflorum* showing characteristic symptoms of Chrysanthemum white rust were collected and deposited as voucher material in the Herbarium of the Faculty of Biology, "Alexandru Ioan Cuza" University of Iași (Index Herbariorum acronym: I), under voucher number **I207021**.

Disease Incidence and Severity

Quantitative evaluation of Chrysanthemum white rust revealed a wide spectrum of responses among the 35 *Chrysanthemum* × *grandiflorum* cultivars evaluated under protected greenhouse conditions. Disease Incidence (DI, %) varied considerably, ranging from 0% in resistant cultivars to over 90% in highly susceptible genotypes, while the Disease Severity Index (DSI, %) showed comparable variability across cultivars and growing seasons (Table 2). These results highlight pronounced differences in host response to *P. horiana* infection.

The application of a standardized visual scoring system (1-5) enabled an objective and reproducible assessment of symptom intensity, facilitating reliable comparisons among cultivars and between growing seasons. Cultivars exhibiting complete resistance showed no visible symptoms throughout the evaluation period, resulting in DI and DSI values of 0%. In contrast, susceptible cultivars developed extensive chlorosis, necrosis, and abundant telial pustules, leading to high DI and DSI values.

During the 2020 growing season, several cultivars, including *Axillia*, *Diplomate Orange*, *Marielle Purple*, *Marielle Red*, *Nob Hill*, *Vienna Cooper*, *Vienna White*, and *Taylor*, displayed very high disease incidence (≥ 75%), accompanied by increased severity levels (DSI > 55%). Conversely, cultivars such as *Blanche*, *Escort Gelb*, *Escort Roth*, *Evelyn Busch*, *Hannenburg*, *Jonson*, *Princess Armgard*, and *Stramer* remained symptomless, indicating strong resistance under the experimental conditions.

In the 2021 growing season, a general reduction in both DI and DSI values was observed across most cultivars. This decrease may be attributed to the implementation of preventive fungicide treatments at 5 weeks after planting (WAP), combined with curative applications at 13 and 15 WAP, as well as the consistent application of cultural control measures. Despite this overall reduction, cultivar-specific differences in susceptibility were maintained, suggesting that genetic factors played a dominant role in determining disease response.

Normalized values of disease incidence (DI_{norm}) and disease severity (DSI_{norm}), expressed on a 0-1 scale, and enabled direct integration of both parameters into a standardized Combined Score (CS). This approach allowed for a more comprehensive evaluation of cultivar susceptibility by accounting for both the proportion of infected plants and the intensity of symptoms. As shown in Table 2, CS values ranged from 0.00 in resistant cultivars to values exceeding 0.80 in highly susceptible ones.

Overall, the combined analysis of DI, DSI, and CS demonstrates substantial variability in cultivar response to *P. horiana*. These findings emphasize the importance of integrating multiple disease parameters when evaluating resistance and provide a basis for the classification of chrysanthemum cultivars into susceptibility categories. The results also underscore the potential of tolerant and resistant cultivars for use in breeding programs and sustainable chrysanthemum production under protected cultivation systems.

Sustainable control and integrated management

Across cultivars, a consistent downward shift in DI, DSI, and CS values was observed in 2021 compared with 2020, indicating a general reduction in disease pressure between growing seasons. This trend was particularly pronounced in cultivars that exhibited high disease levels in 2020, where large absolute and relative decreases were recorded. Cultivars such as *Axillia*, *Diplomate Orange*, *Marielle Purple*, *Marielle Red*, *Nob Hill*, *Taylor*, *Vienna Cooper*, and *Vienna White* showed substantial reductions in DI and DSI, with corresponding decreases in normalized indices and combined scores, suggesting a strong seasonal effect on disease expression.

The distribution of normalized disease indices in 2021 was narrower and shifted toward lower values compared with 2020, reflecting reduced variability and lower overall disease intensity across the cultivar set. This pattern is further supported by the decrease in CS values for most cultivars, indicating an overall improvement in health status under the applied management regime. In contrast, cultivars that remained symptom-free in both seasons (*Blanche*, *Escort Gelb*, *Escort Roth*, *Evelyn Busch*, *Hannenburg*, *Jonson*, *Princess Armgard*, *Stramer*) consistently exhibited zero variance for all disease parameters, highlighting stable resistance under the experimental conditions.

The observed seasonal differences are attributed to the disease control strategy implemented prior to and during the assessment period. The two treatments applied in 2020 likely resulted in a statistically meaningful reduction of the initial inoculum load, which was translated into lower baseline DI and DSI values at the beginning of 2021 growing season. Moreover, the preventive treatment program applied in 2021 appears to have further constrained early disease development, as indicated by systematically lower normalized values and reduced combined scores across susceptible and moderately susceptible cultivars.

Taken together, the data indicates a strong treatment effect, with disease parameters in 2021 consistently lower than those recorded in 2020. Although no formal inferential statistics are presented, the magnitude, consistency, and directionality of the changes across multiple cultivars and disease metrics support the conclusion that the treatment programs applied in 2020 and preventively in 2021 had a significant impact on reducing initial disease incidence and severity in *Chrysanthemum* × *grandiflorum*.

Several cultivars (*Blanche*, *Escort Gelb*, *Escort Roth*, *Evelyn Busch*, *Hannenburg*, *Jonson*, *Princess Armgard*, and *Stramer*) showed no disease symptoms in either year, indicating a high level of tolerance or resistance under the applied management conditions. Conversely, cultivars with intermediate susceptibility (e.g., *Alec Bedser*, *Cassandra*, *Homaro*, *Inga*, *Pink Always*, *Prince de Monaco*) also benefited from the treatment programs, displaying moderate but consistent reductions in disease parameters in 2021.

The comparative analysis between the two growing seasons highlights the cumulative effect of disease control measures, demonstrating that the curative treatments applied in 2020, together with the preventive strategy adopted in 2021, played a decisive role in reducing initial disease incidence and severity across *Chrysanthemum* × *grandiflorum* cultivars. Importantly, the monitoring program was continued in 2022 and 2023, during which preventive fungicide treatment was systematically applied at 5 weeks after planting (WAP) each year. Under these conditions, no visible symptoms of CWR were recorded during the subsequent growing seasons, indicating the effective suppression and eventual eradication of *P. horiana* from the chrysanthemum crop within the Botanical Garden of Iași. This outcome underscores the critical importance of early preventive interventions, continuous monitoring, and the integration of cultural and chemical control measures in managing quarantine pathogens under protected cultivation. The sustained absence of disease symptoms in 2022 and 2023 further confirms that the implemented management strategy was not only effective in reducing disease pressure but also successful in breaking the pathogen's infection cycle, thereby ensuring long-term phytosanitary safety of the chrysanthemum collection.

The results obtained in the present study are consistent with previous research highlighting the high variability in host response to *P. horiana* among chrysanthemum cultivars and related taxa. Similar patterns of differential susceptibility were reported by ZENG & al. (2013), who evaluated resistance to chrysanthemum white rust in 19 accessions of *Ajania* and *Chrysanthemum* species using artificial inoculation. Their comprehensive assessment, based on latent period, infection type, disease incidence, and severity, demonstrated substantial genetic variation in resistance, supporting the cultivar-dependent responses observed in our study under natural greenhouse infection conditions.

More recently, KUMAR & al. (2021) screened multiple *Dendranthema grandiflora* genotypes for resistance to *P. horiana* and confirmed infections using microscopic diagnostic techniques. Their findings emphasized the rapid spread of white rust in greenhouse and nursery environments, particularly under cool and humid conditions, and documented symptom development remarkably like those recorded in the present investigation. The progression from chlorotic spots on the adaxial leaf surface to necrotic lesions and the formation of buff to pinkish pustules on the abaxial surface, which later turn white upon maturation, closely matches the symptomatology observed in our monitored cultivars. These parallels further validate the accuracy of visual and microscopic diagnosis applied in the current study.

The wide range of Disease Incidence (DI) and Disease Severity Index (DSI) values recorded among cultivars also aligns with findings by SRIRAM & al. (2020), who quantified the effectiveness of fungicides against chrysanthemum white rust under controlled conditions.

Their results demonstrated that although chemical control can significantly reduce disease incidence and severity, cultivar susceptibility remains a critical factor influencing overall disease expression. In the present study, the marked reduction in disease parameters following the application of azoxystrobin-based treatments, particularly when combined with preventive applications at early growth stages, supports the effectiveness of integrated disease management strategies, as also recommended by SRIRAM & al. (2020).

Morphological confirmation of *P. horiana* based on teliospore characteristics observed in this study agrees with recent micromorphological investigations conducted on naturally infected chrysanthemum plants. Detailed descriptions of teliospore size, shape, wall thickness, and pedicel length reported in these studies closely correspond to our observations, reinforcing the reliability of classical morphological criteria for pathogen identification. Similarly, O'KEEFE & DAVIS (2015) provided an in-depth characterization of *P. horiana* teliospores from naturally infected hosts, emphasizing the importance of combining field symptomatology with microscopic examination to ensure accurate diagnosis.

Importantly, the findings of BONDE & al. (2015) regarding the systemic nature of *P. horiana* infections have significant implications for disease monitoring and management. Their demonstration that the pathogen can colonize vascular tissues and persist in asymptomatic stems and crowns highlights the potential for latent infections and disease re-emergence. This systemic behavior underscores the necessity of long-term monitoring, even in the absence of visible symptoms, and supports the preventive strategy adopted in the later years of the present study. Continued monitoring during 2022 and 2023, combined with early preventive fungicide applications, likely contributed to the successful suppression and eventual eradication of the disease within the chrysanthemum collection of the Botanical Garden of Iași.

The findings of RAHARDJO & al. (2019), who evaluated both biological and chemical fungicides for controlling white rust in chrysanthemums grown under open-field conditions, highlight the effectiveness of combining chemical and biocontrol measures. Their results reinforce the concept that integrated management strategies, tailored to environmental conditions and host susceptibility, can substantially reduce disease incidence and severity, complementing observations from greenhouse-based studies.

The results of this study on the application of azoxystrobin-based fungicide treatments align with earlier research demonstrating the effectiveness of Q_oI (quinone outside inhibitor) fungicides in managing *P. horiana*, the causal agent of chrysanthemum white rust. Previous work has shown that various strobilurin compounds, including formulations like azoxystrobin, significantly reduced pustule formation and disease development when applied preventively or curatively on chrysanthemum leaves, resulting in visibly lower pathogen activity compared to untreated controls. In these experiments, chrysanthemums protected with strobilurin fungicides exhibited substantially fewer active pustules and symptoms than non-treated plants, indicating that the mode of action of azoxystrobin effectively disrupts the infection cycle of *P. horiana* at key stages of spore germination and early colonization [WOJDYŁA, 2007]. These findings support the use of azoxystrobin as an important tool within integrated chemical control programs for chrysanthemum white rust under greenhouse conditions. However, reports have also identified strains of *P. horiana* with reduced sensitivity to Q_oI (quinone outside inhibitor) fungicides, suggesting that reliance on a single mode of action can lead to tolerance development in pathogen populations, and highlighting the need for careful fungicide rotation and integration with cultural practices to preserve long-term efficacy.

Overall, the present study corroborates existing evidence that chrysanthemum white rust is a highly aggressive disease under favorable environmental conditions, particularly in protected cultivation systems. While fungicides remain an effective component of disease management, their repeated use raises concerns related to environmental impact and production costs, as previously noted by WAARD & al. (1993). Therefore, the identification and utilization of resistant or tolerant cultivars, as demonstrated in this study, represent a sustainable and economically viable approach for long-term management of chrysanthemum white rust. The integration of host resistance, preventive chemical treatments, and strict cultural practices emerges as a strategy for controlling *P. horiana* in greenhouse-grown chrysanthemums.

Conclusions

The present study provides a comprehensive evaluation of Chrysanthemum white rust (*Puccinia horiana* Henn.) under protected greenhouse conditions, based on systematic monitoring, quantitative disease assessment, and integrated disease management strategies. The investigation, conducted on 35 *Chrysanthemum* × *grandiflorum* cultivars from the living collection of the "Anastasie Fătu" Botanical Garden of Iași, revealed pronounced variability in cultivar response to CWR, highlighting the importance of host genotype in determining disease incidence and severity.

Visual detection, supported by microscopic examination of teliospores, allowed for accurate identification of *P. horiana* and confirmed the presence of the pathogen during the 2020 growing season. The use of standardized disease parameters such as Disease Incidence (DI), Disease Severity Index (DSI), and a normalized Combined Score (CS), proved effective in integrating both the proportion of infected plants and symptom intensity, enabling classification of cultivars into susceptibility categories ranging from tolerant to highly susceptible.

Results demonstrated that several cultivars exhibited complete resistance, remaining symptom-free throughout the monitoring period, while others showed high susceptibility, with DI values exceeding 90% and severe symptom expression. The comparative analysis between growing seasons highlighted a consistent reduction in disease incidence and severity in 2021, attributable to the combined effect of cultural practices, curative fungicide applications at 13 and 15 weeks after planting (WAP), and the introduction of a preventive treatment at 5 WAP.

Continued monitoring in 2022 and 2023, combined with the systematic application of preventive treatments at early growth stages, resulted in the absence of visible CWR symptoms, indicating the successful suppression and eradication of *P. horiana* from the chrysanthemum crop within the Botanical Garden of Iași. This outcome emphasizes the critical role of early detection, continuous monitoring, and integrated disease management in controlling quarantine pathogens under protected cultivation.

Overall, the study underscores the value of resistant and tolerant cultivars for sustainable chrysanthemum production and provides a practical framework for disease assessment and management. The findings contribute valuable information for breeding programs, greenhouse production systems, and phytosanitary management, supporting long-term biosecurity and the preservation of ornamental plant collections.

Table 2. Disease Incidence (DI, %) and Disease Severity Index (DSI, %) of *Chrysanthemum* × *grandiflorum* cultivars evaluated at 13 Weeks After Planting (WAP) each growing season (2020, 2021), under two treatments (azoxystrobin) applied at 13 and 15 WAP in 2020 and a preventive treatment at 5 WAP in 2021, including Normalized Values (DI_{norm}, DSI_{norm}) and Combined Score (CS)

Legend: DI (%) = *Disease Incidence*; DSI (%) = *Disease Severity Index*; DI_{norm} = *Normalized Disease Incidence*; DSI_{norm} = *Normalized Disease Severity Index*; CS = standardized Combined Score.

Chrysanthemum x *grandiflorum cv.*	Growing season/2020					Growing season/2021*				
	DI (%)	DSI (%)	DI_{norm}	DSI_{norm}	CS	DI (%)	DSI (%)	DI_{norm}	DSI_{norm}	CS
cv.1 Alec Bedser	25.00	35.62	0.25	0.36	**0.30**	15.63	28.12	0.16	0.28	**0.22**
cv.2 Astro	28.13	32.50	0.28	0.33	**0.30**	28.13	31.87	0.28	0.32	**0.30**
cv.3 Axillia	93.75	64.37	0.94	0.64	**0.79**	37.50	36.87	0.38	0.37	**0.37**
cv.4 Blanche	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.5 Cassandra	34.38	35.63	0.34	0.36	**0.35**	18.75	33.75	0.19	0.34	**0.26**
cv.6 Crimson Robe	37.50	37.50	0.38	0.38	**0.38**	31.25	31.87	0.31	0.32	**0.32**
cv.7 Cristal	31.25	36.88	0.31	0.37	**0.34**	31.25	33.75	0.31	0.34	**0.33**
cv.8 Diplomate Orange	93.75	70.63	0.94	0.71	**0.82**	50.00	45.63	0.50	0.46	**0.48**
cv.9 Escort Gelb	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.10 Escort Orange	28.13	33.12	0.28	0.33	**0.31**	28.13	32.50	0.28	0.33	**0.30**
cv.11 Escort Roth	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.12 Evelyn Busch	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.13 Flame Blaier	6.25	23.13	0.06	0.23	**0.15**	0.00	0.00	0.00	0.00	**0.00**
cv.14 Good Bust	25.00	28.12	0.25	0.28	**0.27**	25.00	28.13	0.25	0.28	**0.27**
cv.15 Hannenburg	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.16 Hagoromo	18.75	28.75	0.19	0.29	**0.24**	15.63	26.25	0.16	0.26	**0.21**
cv.17 Homaro	37.50	36.25	0.38	0.36	**0.37**	25.00	30.63	0.25	0.31	**0.28**
cv.18 Inga	56.25	47.50	0.56	0.48	**0.52**	28.13	30.63	0.28	0.31	**0.29**
cv.19 Jonson	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.20 Margaret	37.50	41.62	0.38	0.42	**0.40**	0.00	0.00	0.00	0.00	**0.00**
cv.21 Marielle Purple	81.25	60.62	0.81	0.61	**0.71**	37.50	34.38	0.38	0.34	**0.36**
cv.22 Marielle Red	87.50	67.50	0.88	0.68	**0.78**	43.75	38.13	0.44	0.38	**0.41**
cv.23 Nob Hill	81.25	65.62	0.81	0.66	**0.73**	40.63	37.50	0.41	0.38	**0.39**
cv.24 Nyll Zwager	21.88	33.75	0.22	0.34	**0.28**	0.00	0.00	0.00	0.00	**0.00**
cv.25 Pink Always	68.75	55.62	0.69	0.56	**0.62**	34.38	33.75	0.34	0.34	**0.34**
cv.26 Prince de Monaco	75.00	53.75	0.75	0.54	**0.64**	31.25	31.88	0.31	0.32	**0.32**
cv.27 Princess Armgard	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.28 Promenade	25.00	31.87	0.25	0.32	**0.28**	21.88	29.38	0.22	0.29	**0.26**
cv.29 Sheer Purple	6.25	23.75	0.06	0.24	**0.15**	6.25	21.88	0.06	0.22	**0.14**
cv.30 Sterling	18.75	28.75	0.19	0.29	**0.24**	9.38	23.13	0.09	0.23	**0.16**
cv.31 Stramer	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
cv.32 Taylor	81.25	65.00	0.81	0.65	**0.73**	37.50	36.25	0.38	0.36	**0.37**
cv.33 Tom Pierce	50.00	43.75	0.50	0.44	**0.47**	37.50	37.50	0.38	0.38	**0.38**
cv.34 Vienna Cooper	81.25	56.88	0.81	0.57	**0.69**	40.63	40.00	0.41	0.40	**0.40**
cv.35 Vienna White	87.50	67.50	0.88	0.68	**0.78**	46.88	41.25	0.47	0.41	**0.44**

Legend: DI (%) = *Disease Incidence*; DSI (%) = *Disease Severity Index*; DI_{norm} = *Normalized Disease Incidence*; DSI_{norm} = *Normalized Disease Severity Index*; CS = standardized Combined Score (interval 0-1).

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