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Oat Crown Rust in Jordan: A Comprehensive Survey and Analysis

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Abstract

This research aimed to study the ecological context of *Puccinia coronata* f. sp. *avenae* (Pca), a pathogen affecting oats, in Jordan. The region is identified as a natural habitat for various wild oat species and the Rhamnaceae family, serving as an alternative host for Pca. The prolonged coexistence of the pathogen, wild oats (*Avena* spp.), and *Rhamnus* creates a scenario conducive to natural selection for long-term resistance. Although oats are not a major crop in Jordan, studying this disease is still significant. The survey spans were between 2018 and 2023, focusing on the upper and lower regions of Jordan Valley. The survey involved collecting, coding, preserving, and morphologically identifying Pca-infected leaves, wild oats, and *Rhamnus* specimens. Results indicated the majority of Pca isolates were from wild oats, particularly in the northern region. Wild oat samples mainly belonged to *A. sterilis*. *Rhamnus* spp. specimens, identified primarily as *Rh. lycioides* with subspecies *graecae* and *lycioides* and few *Rh. punctata*showed no roleasan alternative host of Pca infection. The research provides a foundation for future investigations, emphasizing race characterization and evaluating wild oat accessions for potential resistant genes against Pca.

Keywords: *Avenasterilis*,*Avena barbata*, *Rhamus* spp., wild oat, buckthorn.

1. Introduction

Oats (*Avena* spp.), comprising numerous species within the genus *Avena*, have gained prominence as a cultivated crop across various countries globally, assuming a progressively essential role in human and animal diets. Notably, oats stand as the sixth most consequential cereal crop internationally, a distinction underscored by statistical data from 2021 (Statista, 2021). Demonstrating an array of health benefits contributing to its growing popularity (Clemens and Klinken, 2014), oat consumption has risen to prominence.

Among the Mediterranean countries, Jordan emerges as a native habitat for several wild oat species (El-Shatnawi and Ghosheh, 1999), although oat cultivation remains infrequent. However, the prevalence of wild oat species, particularly as a favorable grazing option for diverse livestock classes, holds promise (Carson, 2008).

The cultivation of oats faces a multitude of diseases that significantly impede yield and quality, with crown rust disease being a notable example (Leonard, 2002). Generally, crown rust is a fungal disease affecting several Poaceae plant species, attributed to *Puccinia coronata*. In the context of oats (*Avenae* spp.), the special form of this pathogenic agent (also called *forma specialis,* biological race, or morphotype) is recognized as *P.coronata* f. sp. *avenae* (Pca), a polycyclic heteroecious fungus. The impact of crown rust, Pca, extends to compromising both the quantity and quality of seeds, casting its prevalence across cultivated oat areas (Leonard, 2002; Simons, 1985). Evidencing a successful interaction, symptoms are discernable as pustules full of clusters of uredospore. Subsequently, the uredial phase converts into the overwintering teliospores, distinguished by their black color, and these endure on straw or host plants of the buckthorn genus (Rhamnaceae) until the following spring (Nazareno *et al.*, 2018; Simons, 1985).

Favorable conditions for the proliferation of crown rust predominantly encompass oat plantation regions characterized by moderate to high temperatures (20-25°C), complemented by abundant dew or elevated relative humidity (RH %). The conjunction of temperature and aerial moisture (including rainy or foggy circumstances) exerts the most substantial influence on the incidence and dispersal of crown rust. Notably, cultivated and wild oats thriving in arid environments typically remain unscathed by crown rust infection due to the scarcity of the requisite RH % (Carson, 2011; USDA-ARS, 2017). Instances of heightened incidence and spread correlate with rainy and warm conditions warm droughty and fog-laden nights. Conversely, moderate infection is associated with extreme instances of either factor, such as exceedingly dry warmth,

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adverse hot and dry conditions can delay infection (Soovali and Koppel, 2003).

Genetic resistance via breeding constitutes the most dependable strategy for disease management. The race-specific seedling resistance was characterized by a hypersensitive response, discernible as flecks on leaves devoid of pustules (Murphy, 1935). However, managing disease through resistance genes in cultivated oats confronts challenges engendered by the rapid evolution of Pca, which facilitates the consistent breakdown of resistance genes (Carson, 2011; Leonard, 2002). The pressure of selection hastens this process, consequently limiting the longevity of a resistance (Carson, 2010). For instance, while *Pc38* and *Pc39* conferred resistance in Canada for several years, by 1990, most races had developed virulence against these genes. Furthermore, new races weakened *Pc91*, *Pc94*, and *Pc96* (Chong *et al.*, 2008). Recent endeavors focusing on genetic resistance underscore the significance of resistant genes in wild oats, thus highlighting the pursuit of cultivars capable of withstanding or tolerating crown rust and other consequential diseases.

In addition to *Avena* spp., Jordan has been reported as a native habitat of the Rhamnaceae family, the alternative host of *P. coronata* (Al Eisawi, 1996; Oran, 2014). In general, alternative host has a vital role in regenerating new races and creating virulence diversity of rust diseases due to its role in sexual reproduction (Jin, 2011; Mehmood *et al.*, 2020), and scarcely mutation has a role in the emergence of new races (Chen *et al.*, 2017). Regarding oat crown rust, *Rhamnus* spp. is responsible for the regeneration of *Pca* races (Wahl *et al.*, 1960). Even among the same aecial cluster, genetic diversity from one cup to another exists (Berlin *et al.*, 2017). However, the long-lasting coexistence of the pathogen, wild oat (such as *A. sterilis* in the Middle East) and the alternative host *Rhamnus* creates natural selection toward long-term resistance (Wahl, 1958).

The causal pathogen and its alternative host trace their origins to the Mediterranean, including the Levant region (Clegg and Allard, 1972; Wahl, 1970). Consequently, despite oats not being a staple and cultivated crop in Jordan, studying this disease is important. The Levant has been identified as a prolific source of Pc genes, with over 25 Pc genes predominantly identified in *A. sterilis* (Simons, 1978). The present study endeavors to comprehensively survey diverse locations in Jordan for *P. coronata* f. sp. *avenae* (Pca) infections, and wild oats and buckthorn distributions. This research serves as fundamental for future investigation on race characterization and evaluation of wild oat accessions against Pca races for identifying potential resistant genes.

2. Materials and Methods

2.1. Surveys

2.1.1. Puccinia coronata f.sp. avenae

Collection

The present study aims to comprehensively investigate the distribution of the crown rust pathogen, *P. coronata* f. sp. *avenae* (Pca), across various regions of Jordan, encompassing the North, Mid, and Southern geographical zones. Notably, our surveys emphasized Northern Jordan, following the approach outlined by (Leonard *et al.*, 2004). In addition, differential lines, including susceptible cultivars, recognized for their distinct interactions with the pathogen, were cultivated in three strategically chosen locations with different environmental conditions, namely the Jubiha station (32°00'40.4"N 35°52'19.5" E), Agricultural Research Center, The University of Jordan (32°05'05.0"N 35°35'44.2" E), and Marow station, Bani Kananah (32°36'26.7"N 35°54'04.9" E), in the year 2022, as trap crops. The choice of these locations aimed to capture variations in environmental factors influencing Pca distribution. Furthermore, our investigation also encompassed previously collected isolates spanning the years 2018 to 2020. To ensure accurate tracking of collection sites, precise GPS coordinates, including longitude and latitude, were recorded for each sampling location. During the survey, plant leaves infected with Pca pustules, from oat (*Avena* spp.), were collected and subjected to careful preservation techniques. Specifically, the collected infected leaves were carefully placed in paper bags to facilitate air drying. The desiccation process took place over (3-4) days at room temperature or using a desiccator, ensuring the preservation of the fungal pustules.

Isolate coding

Each Pca isolate collected during our survey was assigned a unique accession code based on the year of collection, thereby facilitating subsequent data management and analysis. As an illustrative example, accession codes may follow the format "Pca2020JOR-01," signifying the pathogen's isolation in Jordan during the year 2020.

Preservation of Pca pustules

Discernible single pustules were selectively excised from the infected plant material and preserved in two distinct manners: (i) living pustules: these pustules were carefully dried and stored in 2 ml test tubes. Subsequently, they were preserved in an ultra-freezer at a temperature of -80°C. This preservation method will allow for the long-term viability of the pathogen, facilitating further race identification through the utilization of differential lines; and (ii) dead pustules in which the selected pustules were immersed in 95% alcohol for one day, followed by thorough drying. This preservation technique ensures the inactivation of the pathogen while retaining its morphological characteristics, thus enabling detailed microscopic examination and future molecular analysis.

Morphological identification and characterization of Pca isolates

Different *Pca* sample isolates were used to describe the morphology for characterization and taxonomy identification purposes. For light macro- and microscopic characterization, isolates were prepared as follows: infected leaves with pustules of both telia and uredia were examined using a stereo microscope (LB-321, Labomed), while spores themselves were scratched and located on a glass slide and then inspected using a light microscope (Microstar 410, Cambridge Instruments). For scanning electron microscope (SEM) micrographing: *Pca* samples of uredia and telia were scratched to obtainuredospore and teliospores (some samples have both). Spores were located

on aluminum stubs and then coated by platin using Emltech K550X sputter coater. Finally, surface micrographs were taken for these processed samples by using SEM at Hamdi Mango Centre (Inspect F50 - FEI Company). Identification of rust species was done using identification key. This key is based initially on the infected host plant which was *Avena* sp. Different details of morphological characteristics of uredium, telium, uredospore, and teliospore were investigated and ensured, relying on those in (Cummins, 2013; Grove, 1913). Additionally, and as observational data, the same keys and characteristics were followed to identify samples of oats with an infection on the stem (which are commonly supposed to be *P graminis* f.sp *avenae* based on the infected part, color and pustule shape), and compared with those findings of *Pca* (Appendix 1).

2.1.2. Wild oat survey (Avena spp.)

Collection and coding of wild oat genotypes

In our investigation, wild oats (*Avena* spp.) were collected from diverse environments, including roadsides and natural habitats, across Jordan mainly in 2021and some in 2022. Each collected specimen was coded to facilitate accurate tracking and documentation based on the collection year, adhering to a standardized nomenclature format, exemplified as "WO2021JOR-01." The comprehensive collection endeavor aimed to encompass unique wild oat genotypes, each representative of distinct ecological niches and geographical locations within the country. A minimum distance of two kilometers was maintained between the different locations where wild oat plants were collected. Seeds from each location were collected randomly from mature parent plants exhibiting typical wild oat characteristics. Location coordinates were collected for each sampling site. This geospatial data will serve as valuable metadata for subsequent analyses, aiding in the elucidation of the genetic and ecological factors shaping the wild oat populations in Jordan.

Seed selection and genotype purity assurance

To ensure the genetic purity of each collected wild oat genotype, a rigorous selection process was implemented. From each collection, a single seed was randomly chosen and subjected to an increase in the greenhouse to guarantee the preservation of its inherent genetic constitution. This approach serves to maintain the integrity of each genotype and minimize genetic variation within collected samples.

Morphological identification and taxonomy

Given the potential presence of multiple oat species within each collection, a comprehensive morphological identification process was undertaken for the entire seed collection. Until species-level identification is achieved, the collected wild oats were designated as "*Avena* sp.". Then, species of samples were identified using the weighted key with “the most important diagnostic characteristics only” by Baum (1977) and, in some cases shifting to another detailed key known as the unweighted key in cases that characteristic is not clear enough to be identified or more comparative characteristics need to be taken in consideration. Also, another key of Loskutov (2007) was used for certainty (Appendix 2).

2.1.3. Buckthorn survey (Rhamnus spp.)

Collection

A survey of buckthorn (*Rhamnus* spp.) populations across diverse regions of Jordan was conducted in 2022-2023. Various regions, including North, South, East, and West, were systematically explored to provide a comprehensive overview of buckthorn distribution and prevalence. Our survey efforts prioritized areas in proximity to wild oat (*Avena* spp.) habitats, as these locations are of particular interest in the context of crown rust epidemiology. GPS coordinates, encompassing longitude and latitude, were recorded for each sampling site. Each collected specimen was coded to facilitate accurate tracking and documentation based on the year of collection, adhering to a standardized nomenclature format, exemplified as "Rh2022JOR-01." Leaf samples from 1-3 different distant plants from the same location were collected.

Morphological identification

The collected *Rhamnus* buckthorn specimens underwent a morphological identification process up to the genus, species, and subspecies level. This process depends on botanical characteristics related to the description of the *Rhamnus* genus, which was described as follows: deciduous (mostly) or evergreen plants, shrubs, or small trees, with grey to brown stem color, branch is alternate or opposite. The leaf's shape is oblong (mostly), oboval or linear, greenish-yellow or green, alternate or opposite. The blade is undivided with serrate or entire margins. Some plants may have two different types of leaf according to leaf apex: one emarginate to obcordate (dimorphic leaf), and the other is obovate. Flowers are yellowish green, single or in-groups, with berry-like fruits and (2-4) drupes. Stems are with or without spines. Habitats are mountainsides, rocky lands, and poor deserts (Nguyen, 2007). A key characteristic was designated to identify collected samples to species level (of the four *Rhamnus* species referred by (Taifour and El-Oqlah, 2016), and to subspecies level (if was of *Rh. lycioides)*. This key originated from those keys and characteristics mentionedby (Davis, 1970; Muschler, 1912; Tutin, 1986) (Appendix 3).

Sample processing for further analysis

In the context of crown rust research, sampled *Rhamnus* plants were examined for the presence of crown rust pustules on leaves. Additionally, leaves were excised and placed immediately in vials containing silica gel. Silica gel serves as a desiccant, effectively drying out the leaves and preventing degradation of DNA. Preserved samples will be used as a genetic source for future genetic diversity studies of buckthorn.

2.2. Statistical analysis

Plants and disease locations were mapped using ArcGIS ArcMap (Version 10.2 for Windows; ESRI, Redlands, CA). Excel from Microsoft Office 2016 was employed to compile summaries detailing the number of surveyed locations and samples collected for *P. coronata*, *Avena* spp., and *Rhamnus* spp.

3. Results

3.1. Surveys

3.1.1. Puccinia coronata f.sp. avenae

A total of 1600single pustule isolates (spi) of *P. coronata* f.sp *avenae* (Pca) were collected from 135 locations over the period spanning 2018 to 2023, except 2019 (Table 1). The majority of these isolates were collected from wild oats, whereas only 196 samples originated from differential oat cultivars, as delineated in Table 1. Notably, the majority of spi were retrieved from the northern region, particularly in Irbid, where the count exceeded one thousand spi (Figure 1A, 1D).

Morphological Identification of Pca

The identification of rust species and determination of biological race were executed employing the taxonomic key outlined by Cummins (2013). In general, all isolates obtained from oat sources were conclusively identified as belonging to the genus *Puccinia*, a classification supported by the distinct characteristics observed in the teliospores. Specifically, these teliospores exhibited pedicellate structures, comprised of two cells, each possessing a single germ pore, and featured echinulate uredospore (Figure 2,3).

Further refinement of the identification process, conducted at the species and special form levels, involved a meticulous examination of teliospores isolated from oat plants. This inspection led to the clear identification of *P. coronata avenae* (Pca) and *P. graminis avenae* (Pga) based on the discernible morphological features described in the taxonomic key. Notably, the teliospores of Pca were distinguished by their coverage of spore cells and digitated tip appearance, as illustrated in Figure (3). In contrast, the teliospores of Pga were characterized by their exposed spore cell configuration, and the tip is without digitations, exemplified in Figure 2. Many morphological features examined exhibited similarities with the descriptions provided by Cummins (2013) and Grove (1913) as cataloged in Appendix 1.

3.1.2. Wild oat survey (Avena spp.)

A comprehensive survey of *Avena* species was conducted in this study, encompassing approximately 264 samples sourced from 184 distinct and documented locations (Figure 1B, 1D). For about 56% of the samples collected from Irbid governorate, the identification of wild oat samples revealed two species, mainly *A. sterilis,* with 90% and 10% *A. barbata* (Table 2, Figure 4, Figure 5). Common characteristics shared by both species and primary characteristics for *A. sterilis* and *A. barbarata* are presented in Appendix 2.

Table 1. Survey summary of *P. coronata*. f.sp *avenae* across governates during 2018-2023.

Source	Governate								Total
	Ajloun	Al-Balqa	Al-Mafraq	Al-Zarqa	Amman	Irbid	Jerash	Madaba	
Differentials	0	96	0	0	0	100	0	0	196
Wild oat	115	47	205	31	9	988	57	30	1482
Total	115	143	205	31	9	1088	57	30	1678
Year									
2018		2020		2021		2022		2023	
Locations	Samples	Locations	Samples	Locations	Samples	Locations	Samples	Locations	Samples
15	60	13	48	14	199	16	292	77	1079
Total locations = 135			Total samples = 1678						

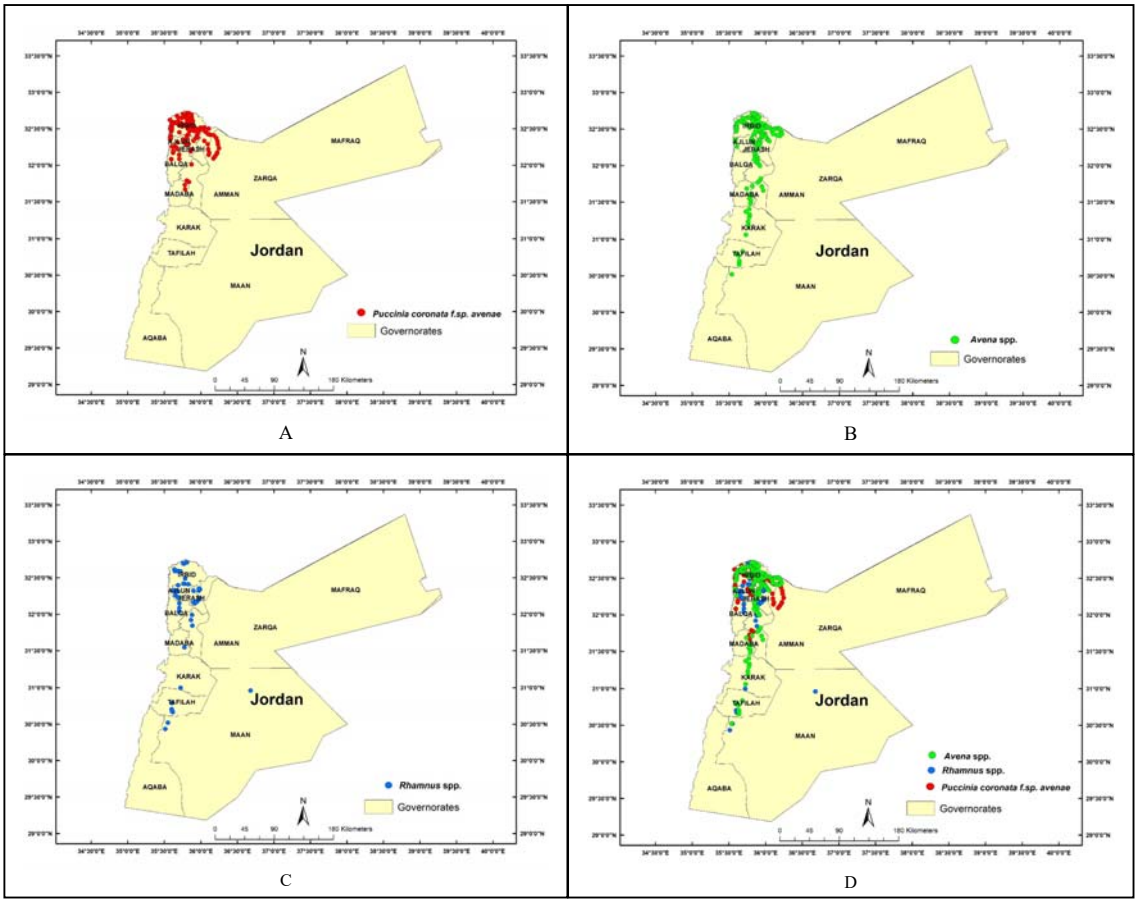

Figure 1. The survey locations conducted for oat crown rust, wild oats, and buckthorn. Sub-figures include: A) A four-year survey overview for oat crown rust (Pca), B) Wild oat (*Avena* spp.) survey conducted in 2021, C) A survey map highlighting locations of buckthorn (*Rhamnus* spp.) in 2022-2023, and D) Combined survey locations for *Rhamnus* spp., *Avena* spp., and oat crown rust (Pca) depicted in a single map.

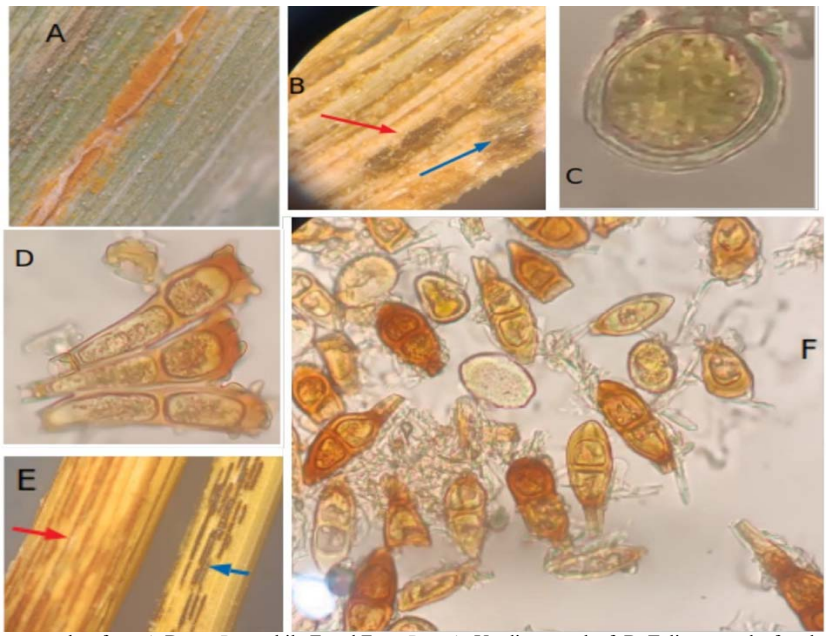

Figure 2. Rust on oat samples, from A-D, are *Pca*, while E and F are *Pga*. A: Urediaon oat leaf, B: Telia on oat leaf, red arrow refers to ruptured pustule while blue arrow refers to unruptured one, C: uredospore, D: teliospores (D is zoomed), E: Uredia (red arrow), telia (blue arrow) both on oat culm, F: both uredospores and teliospores, C, D, F were further inspected under 600x, while A, B and E were inspected under 10x.

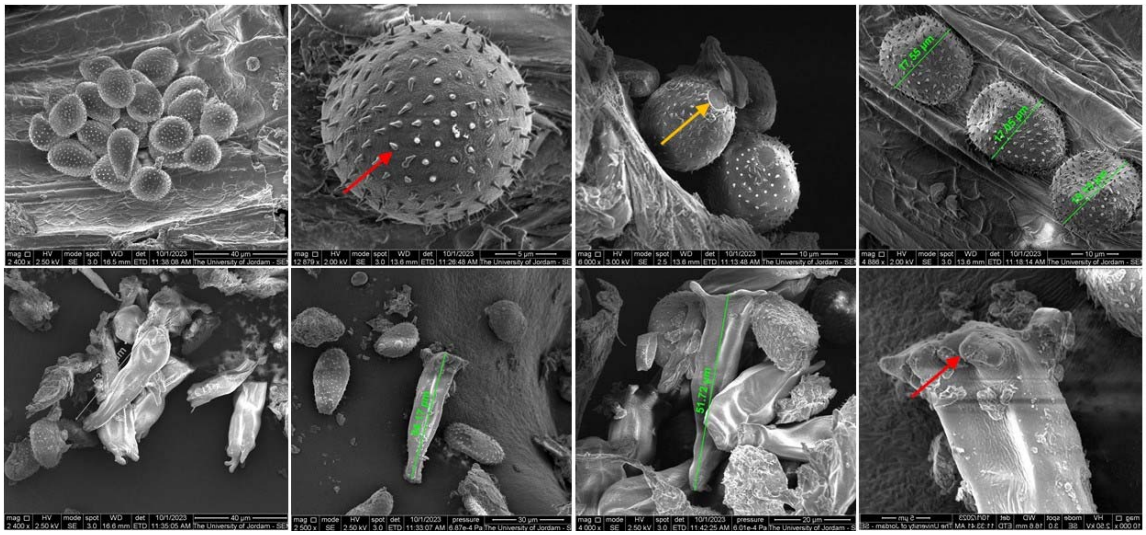

Figure 3. SEM micrographs of *Pca* spores. Upper: uredospores; the red arrow refers to the short spines (echinula), while the yellow arrow refers to the germ pore. Lower part: teliospore, red arrow refers to the digital projections.

Table 2. Survey of wild oat *Avena* spp. collected from different locations in 2021.

Governate	no. of location	no. of samples		
		sterilis	*barbata*	Total
Ajloun	4	7	0	7
Al-Balqa	5	9	0	9
Al-Karak	10	16	4	20
Al-Mafraq	15	13	2	15
Al-Tafilah	8	10	4	14
Amman	11	13	2	15
Irbid	104	139	7	146
Jerash	20	24	2	26
Madaba	7	10	2	12
Total	184	241	23	264

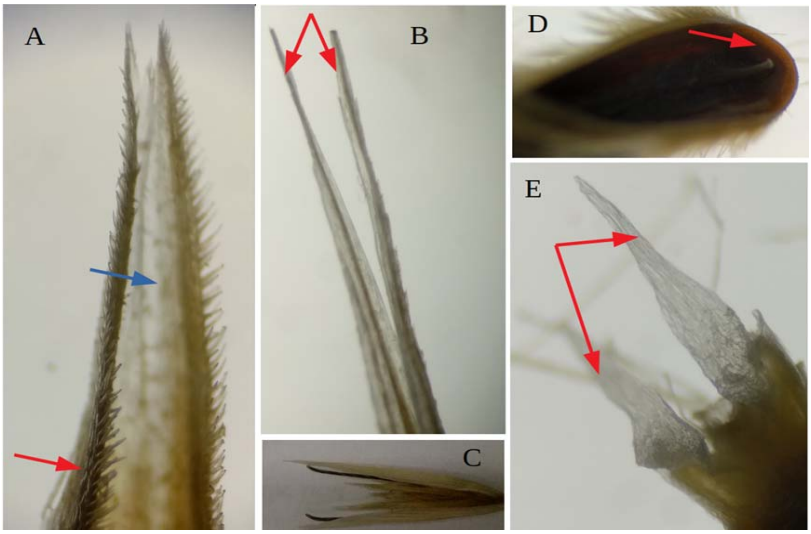

Figure 4. *A. sterilis*, an example of a sample identified according to morphological profile, A: Palea keel with 2 cilia rows (red arrow), palea vestiture with prickles (blue arrow), B: lemma tips are bisubulate, red arrows refer to the subules, C: disarticulation: all florets (here 4 florets) disarticulate as one unit (only the most lower floret disarticulated), spikelet has three florets, glumes are nearly equal, number of glume nerves is 10 nerves, awns inserted in the lower one-third of the lemma (not figured here), D: the shape of primary floret abscission scar is oval, and periphery ring (callus, the shiny part of abscission scar) confined to about (1/3-1/4) of scar, E: lodicule of fatua-type, (no side lobe present).

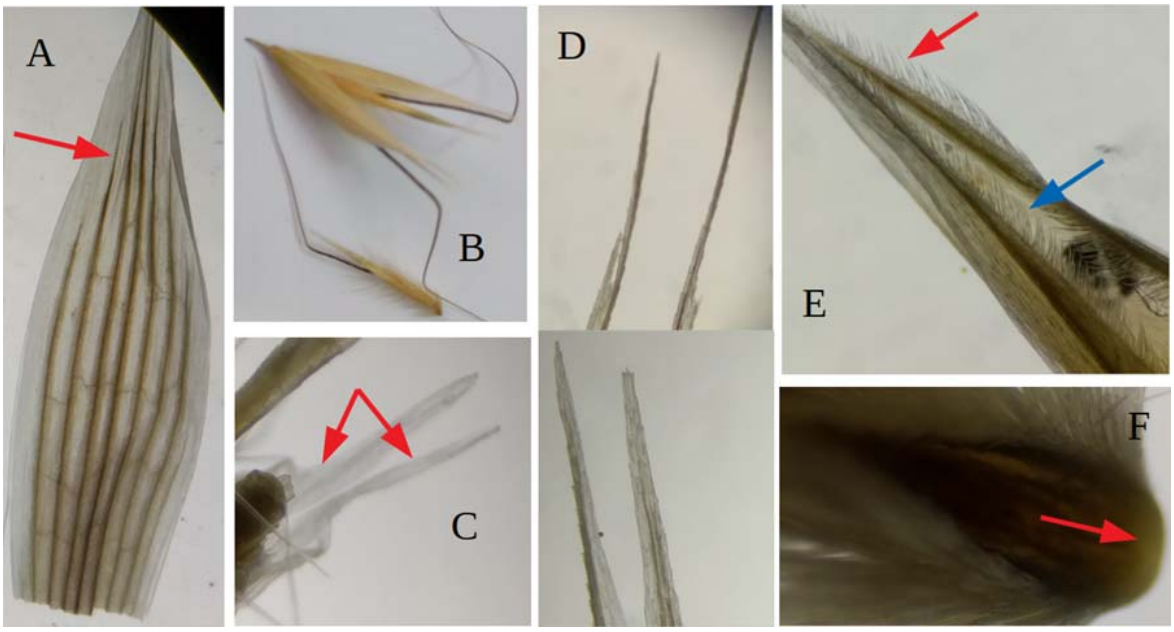

Figure 5. *A. barbata*, A: spikelet glume with 9 nerves (two of them are very short), B: disarticulation: all florets (two florets here) disarticulate, spikelet with nearly equal glumes, awns inserted in the lower (1/3) of lemma, C: two lodicules of fatua-type, red arrows refer to the lodicule arms, D: two types of lemma tips the upper one is biaristulate, the lower one is bisubulate, E: palea keel with one cilia row (red arrow), vestiture of palea back is glabrous (blue arrow), F: the primary floret abscission scar is oval, and ring confined to about (1/3) of scar periphery.

3.1.3. Buckthorn survey (Rhamnus spp.)

This investigation involved the systematic survey of *Rhamnus* plant samples, totaling 102 specimens collected from 46 diverse locations (Table 3). Most of *Rhamnus* plants were concentrated in northern governorates (Figure 1C, 1D). Morphological characteristics were accurately examined to determine their classification within the *Rhamnus* genus. All surveyed plants exhibited spinescent features, characterized by spiny stems, and displayed deciduous leaves (Figure 6). Additionally, a predominant majority of the surveyed plants exhibited an obovate leaf morphology (Appendix 3). The most common *Rhamnus* species was *Rh. lycioides*. Details of the identified species and subspecies are provided in Appendix 3.

In the context of *Rhamnus* potential role as alternative hosts, the surveyed *Rhamnus* plants underwent a thorough screening process. However, none of the examined plants demonstrated any signs of infection by *P. coronata avenae* (Pca). Thus, none exhibited the sexual phase of Pca (aecial stage) throughout the entire collection period. This discerning observation contributes valuable insights into the distribution and host association of Pca, elucidating the absence of its aecial stage on the examined buckthorn plants.

Table 3. Survey of *Rhamnus* spp. from different locations in Jordan during 2022-2023.

Governate	no. of samples	no. of location	Rh. *punctata*	subsp *graecae*	subsp *lycioides*
Ajloun	21	*10*	*0*	10	0
Al-Balqa	8	3	0	3	0
Al-Karak	3	1	0	1	0
Al-Mafraq	2	2	0	2	0
Al-Tafilah	13	4	0	4	0
Amman	7	3	0	3	0
Irbid	22	12	2	8	2
Jerash	9	4	0	4	0
Maan	7	3	0	3	0
Madaba	3	1	0	1	0
Total	102	46	2	42	2

Figure 6.*Rhamnus* in Jordan, at left *R. lycioides* subsp. *lycioides* at right *R. lycioides* subsp. *graeca*. Red arrows refer to the spiny stem; blue arrows refer to the two types of leaf, the obovate and the emarginate-obovate.

4. Discussion

This research represents the first survey in Jordan to investigate the presence of oat crown rust on its primary host, wild oat, as well as its alternative host, buckthorn. In the current investigation, *P. coronata avenae* (Pca) samples, were collected from wild oats over four years from diverse locations and governorates in Jordan. These collected samples underwent thorough processing and preservation for subsequent in-depth analyses.

Conducting a comprehensive Pca survey involved exploring various regions with distinct environmental conditions, even within the same governorate, to ensure a broader spectrum of variability and diversity among Pca isolates (Ali *et al.*, 2021; Lyon and Broders, 2017). Nevertheless, this study showed a concentration of Pca samples in the northern part of Jordan. This region, characterized by prolonged intermediate temperatures and moderated extremes in temperature and humidity, provides optimal conditions for Pca infection. The Northern regions of Jordan exhibit a high prevalence and diversity of host plants, which could be attributed to specific soil characteristics. Additionally, the proximity of the northern region to other Mediterranean countries, which also harbor both Pca and alternative hosts, contributes to the observed distribution patterns.

The assessment of Pca infection revealed a significant variation in disease incidence from one year to another, with most isolates being collected in 2023. This underscores the pronounced influence of environmental conditions on the epidemic status of the disease (Ali *et al.*, 2023). Our geographic regions are characterized by well-documented weather and climatic fluctuations, both seasonally and daily. The persistent presence of this disease has the potential to drive the evolution of Pca genotypes, giving rise to diverse and novel races; such cases were referred to by (Moreau *et al.*, 2023). To investigate deeper into the molecular aspects of this evolutionary process, additional studies are required, specifically focusing on genetic variation analyses among Pca populations in Jordan.

The consistent annual occurrence of natural *P. coronata avenae* (Pca) infection on oats over four years prompted our investigation into the potential involvement of nearby alternative hosts (Zhao *et al.*, 2016). Concurrent surveys of buckthorns, oat, and crown rust indicated their proximity, coupled with the known limited dispersal capability of Pca basidiospores. Despite these observations, screening results revealed no substantiating evidence that the surveyed *Rhamnus* plants function as alternative hosts for Pca in Jordan. While studies often highlight the windborne transmission capacity of uredospore across countries and continents (Berlin *et al.*, 2017; Moreau *et al.*, 2023), our findings did not align with this expectation. Given these results, further investigation, potentially employing more recent methodologies such as multiplex PCR, may be warranted to explore the possible association of Pca with *Rhamnus* in the region.

This study involved the random collection of wild oat plants from various locations, followed by morphological identification at the species level, which is valid and still used (Jabar and Yahya, 2019; Belsariya *et al.*, 2023; Singh *et al.*, 2019). The identification process relied primarily on the keys provided by (Baum, 1977). However, due to the difficult nature of wild oat taxonomy, a combination of keys, sometimes necessitating shifts, was employed to enhance accuracy. The results indicated that the surveyed wild oat samples belonged to two species: *A. sterilis* and *A. barbata;* those two species have been recorded in Jordan by many authors (Loskutov and Rines, 2011; Taifour and Oqlah 2016). *A. sterilis* was more abundant than *A. barbata* by tenfold (Table 2), aligning with previous findings that highlight the superior distribution and multiplication success of *A. sterilis* compared to *A. barbata* (Baum *et al*., 1972).

Taxonomy and classification complexities were evident, with variations among authors regarding species/subspecies designations. The morphological keys, while valuable, were not always straightforward, and differences existed between different keys (Leggett, 1992). *A. barbata* posed more significant identification challenges than *A. sterilis*, with potential confusion arising from similar species such as *A. wiestii*, *A. hirtula*, *A. damacina*, *A. fatua*, and *A. longiglumis* (Loskutov and Rines, 2011). However, the study observed few samples resembling *A. barbata*-like characteristics (all florets disarticulated at maturity) (Table 3).

This study highlighted limitations in morphological identification, showcasing unique cases not covered by existing keys (Kumar *et al.*, 2023). These cases included features such as aristulate-subulate lemma tips, palea backs with long hairs, *A. barbata* with a ring confined to 1/6 of the scar, *A. sterilis* glumes with 12 nerves, and *A. barbata* with fewer than nine nerves. Additionally, the study introduced extra morphological details not previously mentioned in the literature, such as two types of cilia rows (short and long), the fusion of the two subules, and subules with toothed tips. The presence of unique traits not addressed by traditional morphological keys suggests the potential existence of unidentified subspecies or even new species. For that, further studies, including molecular techniques, may be essential in resolving these cases, considering the inheritability of these distinctive traits and the possibility of uncovering previously unrecognized taxa based in the term of phenotype stability (Devi *et al*., 2023).

In this investigation, *P. graminis avenae* (Pga) was incorporated into the study alongside *P. coronata avenae* (Pca) as observational data. This inclusion was motivated by the frequent co-occurrence and potential overlap of both infections, complicating their differentiation (Cabral et al. 2014). Challenges in distinguishing the two species arose from the possibility of late-stage infection on various plant parts, the resemblance in coloration, particularly in the telial stage, and the general similarity in the shape of uredospores as mentioned in (Cummins, 2013). Therefore, a comprehensive characterization of both rust species was conducted at macro- and microscopic levels, including a comparative description. The applied key in this study facilitated differentiation, commencing with the host plant genus. Rust infections within the *Avena* genus were found to be limited to Pga and Pca. Distinguishing between Pga and Pca teliospores was achieved through simple microscopic observation. The later teliospores of Pca exhibited digitate projections, imparting a crown-like appearance to the apical part, while Pga teliospores lacked such digitations. Most characteristics of Pca and Pga aligned with descriptions provided by (Cummins, 2013; Grove, 1913). Additionally, the study introduced supplementary descriptions, including the darker color of Pca apex compared to other teliospore parts, the tapering nature of some Pga apex toward one side in many instances, the notably long pedicel of Pga teliospores, potentially exceeding the combined length of both teliospore and pedicel cells, the relatively shorter echinula of Pca uredospores compared to Pga's (though not quantitatively measured), the generally more globose shape of Pca uredospore s in contrast to the more ellipsoid shape of Pga's, and the relatively thinner cell wall of Pca uredospore's compared to Pga's (with "relatively" indicating the mean cell wall thickness relative to the entire cell).

Irrespective of whether *Rhamnus* species documented in Jordan were previously recognized in the literature as hosts for the sexual stage or not, the current study spanned two years to thoroughly survey this plant. The investigation involved concurrent inspection and random collection of *Rhamnus* plant samples at each visited location for subsequent analyses. Samples were grouped based on morphological similarities, followed by detailed morphological identification. Categorization revealed distinct morphological differences among samples. Consequently, a comprehensive morphological characterization was conducted using keys and traits outlined in the methodology section. Results showed two *Rhamnus* species, one of which has two subspecies; these species had been previously reported in Jordan by Taifour and El-Oqlah (2016).

P. coronata avenae (Pca) presence on *Rhamnus* was assessed through visual observation of leaves. Results showed no discernible evidence supporting their role as alternative hosts for Pca. While further extensive observations and investigations may be necessary, it is noteworthy that both the identified species in this study and those mentioned in prior literature, such as *Rh. diperma* and *Rh. alaternous*, have not been documented as alternative hosts, except for one case reported in Tunisia (Hemmami *et al.*, 2006). It is important to emphasize that the pictures of plants shown in the figures in that research do not align with the morphology of *Rh. lycioides* leaves. In general, such negative interactions of Pca and alternative hosts were found by others (Dietz, 1926).

5. Conclusions

This research represents the first survey in Jordan to investigate the presence of oat crown rust on its primary host, wild oat, as well as its alternative host, buckthorn. In summary, a thorough investigation was conducted on *P. coronata* f.sp. *avenae* (Pca), comprising the collection and morphological identification of 1600 single pustule isolates (spi) throughout 2018 to 2023. All of these isolates were retrieved from wild oats, particularly in the northern region, notably Irbid. Additionally, a comprehensive survey of *Avena* species identified *A. sterilis* as the predominant species in the Irbid governorate. Moreover, the survey of *Rhamnus* species revealed significant concentrations in northern governorates, with 102 specimens collected from 46 locations. Morphological examination facilitated accurate classification within the *Rhamnus* genus, predominantly identifying *Rh. lycioides*. Despite the potential role of *Rhamnus* as alternative hosts, none of the surveyed plants showed signs of infection by Pca, indicating the absence of its aecial stage throughout the collection period. These findings shed light on the distribution and host association of Pca, providing valuable insights into its ecological dynamics.

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References

Al Eisawi D. 1996. Vegetation of Jordan. Regional Office for Science and Technology for the Arab States/UNESCO e Cairo Office, Cairo.

Ali Y, Iqbal S, Aatif HM, Naveed K, Khan AA, Ijaz M, Raza A. 2023. Predicting stripe rust severity in wheat using meteorological data with environmental response modeling. *J King Saud Univ Sci*., **35(4)**: 102591.

Ali Y, Khan AA, Ijaz M, Aatif HM, Rahim A, Ahmad S, Rafiq M. 2021. Characterization of environmental conditions conducive for leaf rust and genetic diversity on wheat crosses based upon physiomorphic traits. *Pakistan J Phytopathol*., **33(1)**: 125-136.

Baum BR, Fleischmann G, Martens JW, Rajhathy T, Thomas H. 1972. Notes on the habitat and distribution of *Avena* species in the Mediterranean and Middle East. *Canad J Bot*, **50 (6):** 1385-1397.

Baum BR. 1977. Oats: wild and cultivated. a monograph of the genus *Avena* L. (Poaceae). Ottawa, Canada: Minister of Supply and Services, 108-114.

Belsariya N, Sahu M, Kak A, Roy S. 2023. Morphological characterization using nonmetric variables in exotic oat (*Avena* sp.) accessions. https://doi.org/10.21203/rs.3.rs-2633376/v1 (Under review).

Berlin A, Samils B, Andersson B. 2017. Multiple genotypes within aecial clusters in *Puccinia graminis* and *Puccinia coronata*: improved understanding of the biology of cereal rust fungi. *Fung Biol Biotechnol*., 4(1): 1-7.

Cabral AL, Gnanesh BN, Mitchell Fetch J, McCartney C, Fetch T, Park RF., Menzies JG, McCallum B., Nanaiah GK, Goyal, A. 2014. Oat fungal diseases and the application of molecular marker technology for their control. In *Future challenges in crop protection against fungal pathogens* (pp. 343-358). New York, NY: Springer New York.

Carson ML. 2008. Virulence frequencies in oat crown rust in the United States from 2001 through 2005. *Plant Dis*., **92(3)**: 379-384.

Carson ML. 2010. Additional sources of broad-spectrum resistance to *Puccinia coronata* f. sp. *avenae* from Canadian accessions of *Avena barbata*. *Plant Dis*., **94(12):** 1405-1410.

Carson ML. 2011. Virulence in oat crown rust (*Puccinia coronata* f. sp. *avenae*) in the United States from 2006 through 2009. *Plant Dis*., **95(12):** 1528-1534.

Chen J, Upadhyaya NM, Ortiz D, Sperschneider J, LiF, Bouton C, Dodds PN. 2017. Loss of AvrSr50 by somatic exchange in stem rust leads to virulence for Sr50 resistance in wheat. *Science*, **358(6370)**: 1607-1610.

Chong J, Gruenke J, Dueck R, Mayert W, Woods S. 2008. Virulence of oat crown rust (*Puccinia coronata* f. sp. *avenae*) in Canada during 2002–2006. *Canad J Plant Pathol*., **30(1):** 115-123.

Clegg MT and Allard RW. 1972. Patterns of genetic differentiation in the slender wild oat species *Avenabarbata*. *Proc Nation Acad Sci USA*, **69(7):** 1820-1824.

Clemens GV and Klinken BJ. 2014. The future of oats in the food and health continuum. *Brit J Nut.*, **112**: S75–S79.

Cummins GB. 2013. The rust fungi of cereals, grasses, and bamboos. Springer Science & Business Media.

Davis PH. 1970. Flora of Turkey and the East Aegean Islands, Volume 2. Edinburgh: Edinburgh University Press, pp. 527.

Devi R, Sood VK, Arora A. 2023. Stability analysis for seed yield and related traits of oat (*Avena sativa* L.) under varied conditions of North-Western Himalayas. *Inter J Env Clim Change*, **13(11):** 2409-2418.

Dietz, SM. 1926. The alternate hosts of crown rust, *Puccinia coronata* Corda*. J Agric Res*., **33:** 953–970.

El-Shatnawi MKJ and Ghosheh HZ. 1999. The influence of sowing rate on the production and growth of wild oat (*Avena fatua*) in the depleted semi-arid rangelands of Jordan. *AfriJ Range For Sci*., **16(2-3):** 96-100. DOI: 10.2989/10220119909485723.

Grove WB. 1913. The British rust fungi (Uredinales): Their Biology and Classification. University Press.

Hemmami I, Allagui MB, Chakroun M, Gazzah ME. 2006. *Rhamnus lycioides* in Tunisia is a new aecial host of oat crown rust. *Europ J Plant Pathol*., **115:** 357-361.

Jabar A and Yahya AA. 2019. Using morphological and chemical characteristics of grains for identification and taxonomy of some oat cultivars (*Avena sativa* L.). *Mesop J Agricul*., **45(1):** 229-242.

Jin Y. 2011. Role of *Berberis* spp. as alternate hosts in generating new races of *Puccinia graminis* and *P. striiformis*. *Euphytica*, **179(1)**: 105-108.

Kumar R, Varghese S, Jayaswal D, Jayaswall K, Yadav K, Mishra G, Kumar S. 2023. Agro-morphological and genetic variability analysis in oat germplasms with special emphasis on food and feed. *Plos One*, **18(2):** e0280450.

Leggett JM. 1992. Classification and speciation in *Avena*. *Oat Sci Technol*., **33**: 29-52.

Leonard KJ, Anikster Y, Manisterski J. 2004. Patterns of virulence in natural populations of *Puccinia coronata* on wild oat in Israel and in agricultural populations on cultivated oat in the United States. *Phytopathology*, **94(5):** 505-514.

Leonard KJ. 2002. Oat lines with effective adult plant resistance to crown rust. *Plant Dis*., **86(6)**: 593-598.

Loskutov IG, and Rines HW. 2011. Avena. Wild crop relatives: genomic and breeding resources: cereals, 109-183.

Loskutov IG. 2007. Oats (*Avena* L.) distribution, taxonomy, evolution and breeding value. Saint-Petersburg: SSC RF VIR Publishing.

Lyon B and Broders K. 2017. Impact of climate change and race evolution on the epidemiology and ecology of stripe rust in central and eastern USA and Canada. *Canad J Plant Pathol*., **39(4)**: 385-392.

Mehmood S, Sajid M, Zhao J, Huang L, Kang Z. 2020. Alternate hosts of *Puccinia striiformis* f. sp. *tritici* and their role. *Pathogens*, **9(6)**: 434.

Moreau ELP, Riddle JM, Nazareno ES, Kianian SF. 2023. Three decades of rust surveys in the United States reveal drastic virulence changes in oat crown rust. *Plant Dis*. DOI: 10.1094/PDIS-09-23-1956-RE.

Murphy HC. 1935. Physiologic specialization in *Puccinia coronata avenae*. Bull. US Depart. *Agriculture*, **433:** 1–48.

Muschler R. 1912. A Manual Flora of Egypt, Volume 1. Berlin: R. Friedlaender & Sohn, Karlstrasse, pp. 618.

Nazareno ES, Li F, Smith M, Park RF, Kianian SF, Figueroa MP. 2018. *Puccinia coronata* f. sp. *avenae*: a threat to global oat production. *Mol. Plant Pathol*., **19(5)**: 1047-1060.

Nguyen, M. L. 2007. Flora of China. Vol. 12, Hippocastanaceae through Theaceae. Edited by Wu Zhengyi, Raven Peter H., Hong Deyuan. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press, 139-162.

Oran SA. 2014. A list of flowering wild plants in Tafila Province, Jordan. *Inter J Biodiv Conserv*., **6(1):**, 28-40.

Simons MD. 1978. Oats: a standardized system of nomenclature for genes and chromosomes and catalog of genes governing characters (No. 509). Department of Agriculture, Science and Education Administration.

Simons MD. 1985. Crown rust. In: The Cereal Rusts, Vol. II, Diseases, Distribution, Epidemiology and Control. A.P. Roelfs and W.R. Bushnell, Eds.; Academic Press: Orlando, FL, pp. 131-172.

Singh A, Chaudhary M, Chaudhary NK, Chiranjeev N. 2019. Stability analysis for morphological characters in oats (*Avena sativa* L.). *Int J Chem*., **7(5)**: 3172-3178.

Soovali P and Koppel M. 2003. Genetic control of oat rust diseases. *Agron Res*., **1(2):** 245-251.

Statista. 2021. Grain production worldwide by type, 2020/21. Available at: https://www.statista.com/statistics/263977/world-grain-production-by-type/

Taifour H and El-Oqlah, A. 2016. The plants of Jordan, an annotated checklist. Kew Publishing Royal Botanic Gardens, U.K., Kew.

Tutin TG. 1968. Flora Europaea: Rosaceae to Umbelliferae, Ed.; Cambridge Univ. Press, Volume 2, pp. 244.

USDA-ARS CDL. 2017. Oat crown rust. USDA-ARS Cereal Disease Laboratory. Available at: https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/cereal-rusts/oat-crown-rust/

Wahl I. 1958. Studies on crown rust and stem rust on oats in Israel. *Bull Res Counc Isr Sect. D*, **6(3)**.

Wahl I. 1970. Prevalence and geographic distribution of resistance to crown rust in *Avena sterilis*. *Phytopathology*, **60(5):** 746-749.

Wahl L, Dinoor A, Halperin J, Schreiter S. 1960. The effect of *Rhamnus palaestina* on the origin and persistence of oat crown rust races. *Phytopathology*, **50(7)**: 562-567.

Zhao J, Wang M, Chen X, Kang Z. 2016. Role of alternate hosts in epidemiology and pathogen variation of cereal rusts. *Ann RevPhytopathology*, **54:** 207-228.

Appendix 1. Characterization of *Pca* in comparison to *Pga.*

Characteristic	*Pca*	*Pga*	Main possible similarity
Macroscopic characterization			
Uredium and telium location	Mostly the infected part of host is the leaf, uredia are on both sides of leaf (amphigenous) but mainly on upper side (epiphylous), telia are hypophyllous	The infected part of the host is mainly the culm and sheath, beginning on parts near soil. Both types of pustules are amphigenous	In a few cases, both may infect most plant parts especially in advanced infection and late in the season
Uredium and telium color	Uredium with orange color	Uredium with brown to brick color	Both have black telium
Uredium shape, size, and arrangement	In general, smaller, oblong, and less elongated, arranged irregularly or in rows	In general, bigger, linear, and more elongated, arranged in rows	Both pustules are pulverate (have a powdery nature)
Telia shape, size, and arrangement	In general, smaller, (appear as tiny dots), oblong to a linear pustule, arranged irregularly, teliaremain with epidermis for long time (rigid)	In general, bigger, elongated pustule, telia became naked soonly so consider as pulverate, arranged in rows,	
Microscopic characterization			
Apical of the teliospore upper cell	Nearly curved to flat apix, with crown-shaped digital projections, apical and projections are darker in colors than other parts	Apix tapered toward one side, curved to ovate (candle flame-shape) or nearly circular and always without digital projections	
Number of projections on the upper cell tip of teliospore and its length	(2-7) but five is the most frequent, projections are darker than other parts of teliospore diam= (2.5-7) µm	No projections	
Teliospore shape	Wedge-shaped (width decrease gradually toward the pedicel)	Shape is the same (not decreased) along the two cells and there is clear difference in width with pedicel	
Teliospore cells	Covered cells, both cells are with nearly equal length	In exposed cells, the lower cell in most cases, is longer than the upper one	Both are 2-celled spore
Teliospore pedicel	Pedicel is shorter	Pedicel is longer and, in some spores, may has length more than the sum of both spore cells length	Both are pedicelated
Teliospore colour	In general, darker	In general, lighter	Both may have colourless teliospore
Uredospore ornamentations	Echinula are relatively shorter = (0.7-1) µm	Echinula are longer (not measured)	Both are echinulated
Uredospore general shape	Most of spores are nearly globose, (some ovate or irregular)	Most of spores are ellipsoid (some are ovate or oblong)	Both are unicellular
Uredospore cell wall thickness	Relatively thinner	Relatively thicker	
Uredospore dimensions as an average	(17.5-19.15) x (19.5-22) µm	(22-43) x (16-22) µm	
Teliospore dimensions as an average	(34-54) x (10-14) µm	(35-60) x (12-22)µm	
Dimensions of uredospore germ pore	3-4 µm	Not measured	
Presence of paraphyses			No paraphyses found

Appendix 2

Common and specific characteristics for *Avena* spp. collected in this study.

a. Common characteristics shared by both species include:

- Annual lifespan, with none of the samples identified as perennial.
- Erect or erect-geniculate culms.
- Green coloration in early stages, with no glaucous samples.
- Plant height ranges from 70 to 150 cm.
- Mostly equilateral or slightly flagged panicles.
- Glumes are either equal or nearly so in length.
- Awn insertion is approximately at the lower third of the lemma back.
- Lemma are distinct from glumes and possess a tough texture.
- The lemma back, under awn insertion, is densely covered with microhairs, and none were found to be
- glabrous.
- Scar shapes mostly oval with some being elliptic or nearly round.
- Lodicules of fatua-type and consistently devoid of hairs.
- Periphery ring confined to 1/3-1/4 of the scar in *A. sterilis* or 1/5-1/2 in *A. barbata.*
-

b. Primary characteristics for *Avena sterilis*:

- Disarticulation at maturity occurs solely on the lowermost floret.
- Most of samples have bisubulate lemma tips, few have bidenticulate or
- bimucronate lemma tips.
- Mostly, palea keels bears more than one cilia rows (2-3).
- Palea back with prickles.
- The number of florets per spikelet ranges from 2 to 5, with 9 to 11 glume nerves.

a. Primary characteristics for *Avena barbata*:

- All spikelet florets disarticulated at maturity.
- Most lemma tips are biaristulate and the less exhibit bisubulate lemma tips, some samples have
- Bothtypes.
- Mostly, palea keels bears 1 cilia row and palea back is glabrous.
- Arista in biaristulated lemma tips is never longer than 5 mm.
- Lodicule length rarely falls below 8 mm.
- The number of florets per spikelet ranges from 2 to 3, with 9 to 11 glume nerves, some are with 7 to
- 9nerves.

Appendix 3. Key to identify *Rhamnus* species in Jordan (*Rh. lycioides, Rh. disperma, Rh. punctata* and *Rh. alaternus*):

0….. Plants are with soft stipules, stipules are not persistent (mostly absent) winter buds covered with scales, stipules are subulate.

Rhamnus. spp.

1….. Plants leaves are evergreen:

2….. Plants without spines

Rh. alaternus (leaves are entire, shiny, and leathery).

2….. Plants with spiny stems.

Rh. lycioides.

1….. Plants leaves are deciduous:

3….. Plant leaf margins with small teeth (serrate).

Rh. lycioides (leaf is flat, branch is direct).

3….. Plant leaf entire, with smooth margins:

4….. Plant leaf margins are revolute, leaf densely pubescent below.

Rh. punctata (branch is direct, lateral veins are conspicuous, glabrous fruits).

4….. Plants branches are tortuous, bark always with ash-colour.

Rh. disperma (2-seeded drupes,much spiny plant, plant habitat of rocky-desert type).

Key to identify the *Rh. lycioides* subspecies:

1….. leaf is linear or linear-spatulate, lateral veins are invisible on upper side, mature drupe is black.

Rh. lycioides subsp. *lycioides*

1….. leaf is obovate or obovate-elliptical:

2….. Plant is evergreen leaves; lateral veins are conspicuous.

Rh. lycioides subsp. *oleoides*

2….. Plant is decidiuos leaves, lateral veins are inconspicuous.

Rh. lycioides subsp. *graecus*.