Life history of Dactylopius opuntiae (Hemiptera: Dactylopiidae) on Moroccan resistant cactus germplasm

SHORT TITLE: Characterization of resistance of cactus genotypes to Dactylopius opuntiae

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Abstract

The important damages caused by *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) to cactus crops around the world require an integrated pest management (IPM) approach, based on the combination of several techniques (varietal resistance, biological, chemical methods, etc). In this sense, this study evaluated the resistance of 10 Moroccan cactus genotypes to *D. opuntiae* in order to characterize the expression of antixenosis and/or antibiosis. Antixenosis was accessed in the greenhouse and in the laboratory (26± 2ºC) using choice and non-choice tests with 1st instar nymphs. Aakria and Cherratia showed a strong antixenosis effect towards *D. opuntiae* (0-0.3 *D. opuntiae* alive 30 after infestation). For antibiosis assessment, 30 1st instar nymphs were confined on cladodes of the 10 selected genotypes under the same laboratory conditions to allow their development, as well as the life cycle performance and behavior of *D. opuntiae* on the 10 selected cactus genotypes, were evaluated under greenhouse conditions. No influence of genotypes on insect oviposition was observed, indicating that the mealybug does not prefer any genotypes over the others for oviposition. The mealybug failed to develop on genotypes Aakria and Cherratia and did not grow beyond the young female stage on all other resistant genotypes tested. Similarly, first instar nymphs fed on genotypes Marjana, Melk Zhar, and A200 died without reaching the second instar nymph stage. In addition, all genotypes tested had a negative effect on nymph viability (<24%), indicating resistance (antibiosis and/or antixenosis) to the cactus scale. These cactus genotypes may all be useful in breeding programs focused on cactus resistance to mealybugs.

Keywords: Cactus, *Dactylopius opuntiae*, Resistance, Antixenosis, Antibiosis

Introduction

Worldwide, approximately 130 genera and 2,000 species belonging to the family Cactaceae (order Caryophyllales) have been recorded, almost all of which are native to the Americas. Mexico is where the center of diversity for cacti in the world is based [1, 2]. Cacti occur in a wide variety of sizes and shapes, from the smallest species measuring 9 mm in diameter to the largest measuring nearly 20 m [3]. Cactaceae generally have a diploid chromosome system of 2n = 22, but many species in the family Opuntioideae are known to be polyploid [3].

The cactus is well known as a multipurpose crop with an important ecological and economic role. Indeed, cacti can be used as fodder, medicinal plants, for human consumption (vegetables and fruits), and as ornamental plants [4]. It can also be used as a food additive, nutritional supplement, and for cosmetic and pharmaceutical purposes [4]. The cactus is grown commercially as a fruit plant in only five countries: Chile, Mexico, Italy, the United States, and South Africa [5]. Cactus fruits are very nutritious. They are rich in vitamins, minerals, proteins, phenolic compounds, and other elements with high nutritional value [6, 7]. This crop is known as a good indicator of harmfulness (Nobel, 1994) and as a living fence to protect agricultural areas [8]. However, this crop is subject to a number of biotic constraints such as fungal diseases, lepidopterans, gastropods, cactus mealybugs (*Diaspis echinocacti* and *Dactylopius opuntiae*) that have very dangerous impacts on the yield and the sustainability of cactus cultivation even around the world. Indeed, *D. opuntiae* (Cockerell) is one of the eleven species belonging to the monogenic family Dactylopiidae [9], considered among the main pests of *Opuntia ficus-indica* (L.) Miller (Caryophyllales: Cactaceae) and other cultivated and wild Opuntia species in many countries in the world [10, 11, 12]. The mealybug (*D. opuntiae*) has also been known as a biological agent for the control of Opuntia in countries where they behave as invasive plants [13]. *Dactylopius opuntiae* is a sap-sucking insect that can have a strong negative impact on both prickly pear production for fresh consumption and cladodes used as fodder for livestock [14]. The mealybug tends to form colonies of variable size on cladodes, which in some

cases are completely covered by the insect [15, 16], which triggers the desiccation and loss of cladodes, premature drop of fruits and total death of the cactus plant [17]. Thus, the severe damage caused by this insect requires an integrated pest management (IPM) approach, based on the combination of several techniques, including genetic, mechanical, physical, biological, chemical, etc. methods [18, 19, 20], in order to obtain better results in the control of this pest.

For Morocco, the prickly pear has been introduced since the 16th century [21]. The cactus is very common in most regions of the country where the crop has become important. But unfortunately with the appearance for the first time in Morocco of the wild mealybug of cactus "*Dactylopius opuntiae*" in 2014, the cactus sector found itself in front of a very big scourge. The rapid and unpredictable spread of this mealybug from the first outbreak to other cactus production areas of the country has led to the destruction of several hedgerows and cactus plantations where the mealybug has devastated thousands of hectares and kilometers of cactus plantations, causing huge socio-economic and environmental losses. Similar cases were reported by Lopes et al. in 2009 where *D. opuntiae* attacks on a cactus forage species, *Opuntiae ficus indica*, in Brazil, resulted in the loss of 100,000 ha, valued at 25 million dollars [22].

Considering the urgency of the mealybug, and to avoid the spread of this epidemic, the Ministry of Agriculture, Maritime Fishing, Rural Development, and Water and Forests-Morocco, put in place a major emergency plan for the control of this mealybug in 2016. This plan also included a research program covering the most important management elements such as host plant resistance [23], pesticides, beneficial insects [24, 25, 26, 27, 28], and biopesticides [29]. Of all the investigated research pathways, the identification of ten mealybug-resistant genotypes is the part that offers great hope for the revival of the cactus industry at the present time [23]. Resistant genotypes are showing positive results in Brazil as well. Matos et al. (2021) reported that the best alternative for the cultivation of cactus in regions attacked by this insect is to plant cultivars resistant to the carmine scale [30]. Thus, to provide effectively and simultaneously environmentally and human-friendly

95 alternatives, the use of resistant genotypes can be a valuable strategy for integrated pest management
96 (IPM) [31].
97 Identifying and characterizing the defense mechanisms of the resistant cactus would be useful to
98 researchers, as it would allow the development of molecular markers that could be used for targeted
99 selection. However, resistance can be a complex phenomenon. Painter (1951) describes three
100 categories of resistance: tolerance, non-preference (or antixenosis), and antibiosis [32]. In plant
101 tolerance, the insect attacks the plant, but the plant has the ability to recover from the wound, and the
102 insect's biological performance is not altered and its behavior is not negatively affected [33].
103 Antixenosis describes the category of plant defense where plants have chemical or physical
104 characteristics that make it less probable that an herbivore will use this plant as a host [32, 34].
105 Insects use olfactory, gustatory, tactile, and visual cues to make host selection decisions, and anti-
106 xenotic properties have been described in numerous studies of insect-plant systems for all of these
107 cues, most of which have been described in agricultural systems [31]. Antibiosis can be defined as
108 the category of plant resistance in which plants employ mechanisms that deleteriously affect
109 herbivores once they have chosen to feed on this plant [32, 35]. Plants use a variety of antibiosis
110 mechanisms, including toxic secondary metabolites such as chemicals, proteins, mechanical
111 defenses, or combinations of these [36]. The antibiotic effects of these mechanisms can range from
112 mild to lethal and even if an individual survives, they may suffer crippling effects such as reduction
113 in body size, fecundity, and prolonged developmental periods [31]. In addition, induced responses
114 are critical components of antibiosis, where certain cues such as herbivore feeding, salivary enzymes,
115 and plant hormones cause the expression of certain defenses [31, 37].
116 Considering the enormous damage caused by this very devastating pest to cacti in Morocco and
117 worldwide and considering that this plague continues its progression in different cactus production
118 areas of the country and also the need to identify more sustainable control methods, the present study

aimed to characterize the resistance of the 10 cactus genotypes identified as resistant to *D. opuntiae* in Morocco using antexenosis and antibiosis tests in laboratory and greenhouse.

Material and Methods

Site of study and plant material

The study was conducted at the Agricultural Technical Institute of Khmiss Zemamra-Doukkala (2020-2021). The Doukkala region extends between latitudes 32°15 and 33°15 North and longitudes 7°55 and 9°15 West. It straddles the provinces of El Jadida and Safi. It is limited to the northeast by the Chaouia, southwest by the region of Abda, west by the Atlantic Ocean, and southeast by the massifs of R'hamna. The locality of Zemamra is located in a semi-arid ecological zone where annual rainfall varies between 112.6 mm and 607 mm. The annual average of 30 years is 330 mm. The temperature varies from -1 °C to 45 °C.

The ten cactus genotypes tested in this study were brought collected from an INRA (National Institute for Agricultural Research) national cactus collection. Eight of these resistant genotypes (Karama, Ghalia, Belara, Marjana, Melk Zhar, Cherratia, Angad, and Aakria) have been identified as resistant to *D. opuntiae* and are already registered in the Catalogue Officiel of Cactus in Morocco [23] (Table 1).

141 **Table 1** List of cactus genotypes resistant to *D. opuntiae* and registered in the catalog officiel of

142 cactus in Morocco [23]

Genotypes	Origin	Characteristics of cladodes	Fruit characteristics
Marjana	Dchira- Inezgane - Morocco	Intermec cladodes (without spines) for use as fodder	Fruit with light purple flesh, juicy and very sweet
Belara	Dchira - Inezgane - Morocco	Intermec cladodes (without thorns) with good forage quality	Fruit with white flesh, juicy and very sweet
Karama	Dchira - Inezgane - Morocco	Spiny cladodes with very good forage quality	Fruit with red flesh, very sweet and tasty
Ghalia	Dchira - Inezgane - Morocco	Thorny cladodes of good quality and very rich in nitrogen for livestock	Fruit with very good organoleptic quality, rich in vitamins and antioxidants, low in acidity, and very sweet
Angad	Oujda - Morocco	Very thorny cladodes with good quality and very rich in nitrogen for livestock	Fruit with dark-purple flesh, sweet, tasty, and very rich in vitamin C and antioxidants
Aakria	Bouknadel - Morocco	Thorny cladodes with good forage quality	Red fruit of small size, too acidulous, not very sweet, and appreciated in the off-season by diabetics mainly
Melk Zhar	Irradiation *O. robusta* - Morocco	Very thorny cladodes with good quality and very high nitrogen content for livestock	Fruit with very good organoleptic quality, rich in vitamins and antioxidants, low in acidity
Cherratia	Bouznika-Morocco	Very thorny cladodes with good quality and very rich in nitrogen for livestock	Fruit with very good organoleptic quality, low acidity, rich in vitamins and antioxidants, and very sweet

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144 **The cactus mealybug colony**

145 A strain colony of *D. opuntiae* was started with infested cladodes of *Opuntia ficus-indica* (L.)

146 Miller, 1768) collected from fields in the locality of Zemamra (32°37'48" N, 8°42'0" W) in the

Casablanca-Settat region, Morocco and maintained under laboratory conditions (26± 2ºC, 60± 10% RH, and 12h photophase), using a modified version of the method described by Aldama-Aguilera and Llanderal-Cazares (2003) [38]. Infested cladodes were placed in entomological cages (80× 80×80 cm) consisting of a wooden frame covered with mesh fabric to allow ventilation. Each cladode was punctured at the basal end by a wooden stake, left to heal for 24 hours under laboratory conditions (26± 2ºC, 60± 10% RH), and then suspended vertically from metal grids; other uninfested cladodes collected from the same site (Zemamra) were placed horizontally beneath for nymphs that had detached on the vertical cladodes. Healthy, uninfested cladodes were introduced weekly into the cages to maintain the colony. Portions of cotton moistened with distilled water were placed in the bottoms of inverted Petri dishes (14.5 cm diameter) and introduced into the cages to maintain humidity. In order to increase the insect numbers and monitor its age, the first instar nymphs of *D. opuntiae* (24 hours old) were transferred to another cage with the same characteristics as described above to complete their development.

Antixenosis test

Under laboratory

Under laboratory conditions at 26± 2ºC, 60± 10% RH, non-preference (antixenosis) assays were evaluated in free choice and non-choice test studies against the first nymphal stages of the scale insect. To investigate the possibility of non-preference interaction in choice tests, the ten genotypes tested cladodes (one-year-old) (n = 3) with a susceptible control were placed and arranged in a circle in entomological cages (80x80x80 cm) with the same characteristics as described above (Cactus mealybug colony section). Five cladodes of *Opuntia ficus-indica* (L.) heavily infested with *D.opuntiae* were placed in the center of each cage and equidistant from the cladodes. The number of alive insects (attracted) in each cladode was recorded 1, 3, 15, 30 days after infestation using a binocular loupe (Motic). This test had 20 replicates in a completely randomized design. For the no-

171 choice tests, we followed the same procedures as for the choice tests but this time the cladodes were
172 placed separately according to genotype in entomological cages (80× 80×80cm). Study design and
173 replication were the same for the free-choice test. The number of insects alive was measured at 1, 3,
174 15, and 30 days after infestation, similar to what was described for the free choice test.

175 **Under greenhouse**

176 To assess the preference of *D. opuntiae* among 10 cactus genotypes, we performed a multiple-choice
177 test, using a modified version of the methodology used with *Aphys glycines* Matsumura (Hemiptera:
178 Aphididae) [39, 40] and *Dichelops melacanthus* Dallas (Hemiptera: Pentatomidae) [41]. The
179 resistant genotypes' with susceptible control cladodes (one-year-old) were planted in normal polarity
180 in a plastic pot (33 cm diameter by 12 cm height), filled with a mixture of fine sand (2/3) and peat
181 (1/3), and grown until the plants reached the stage of three to five cladodes. Then the plants were
182 arranged in completely randomized rows (1 m between rows, with 5 cm spacing between plants)
183 under the greenhouse. Plants were irrigated as needed. Between the lines and approximately 50 cm
184 from each pot, an *Opuntia ficus indica* (L.) cladode that was highly infested with first and second
185 instar nymphs of *D. opuntiae* (1-15 days) was placed. These stages were chosen because the nymphs
186 do not fly and the plots used had no cover on top. The ten genotypes were evaluated using a
187 completely randomized design with twenty replicates. The number and stage of insects per plant
188 were recorded at 1, 3, 15, and 30 days after infestation with help of a handloup. The semi-field daily
189 temperature ranged 8-30 °C during this study and was recorded using thermograms, based on 6
190 measurements made with intervals of 2 h. The night temperature was determined from the 3 lowest
191 daily values.
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196 **Antibiosis Test**

197 **Under laboratory**

198 The test was performed with cladodes of similar age and under the same laboratory conditions used
199 in the previous test. However, in this study, cladodes of different genotypes were placed individually
200 inside entomological cages (80× 80×80cm) (similar to those described previously) and each cladode
201 was infested with 2 mature females of *D.opuntiae* (egg production stage). All cladodes were infested
202 with mature females of similar age and weight. After infestation, the total number of eggs laid on
203 each cladode was recorded. Eggs were placed in labeled Petri dishes (14.5 cm diameter) and
204 observed daily and the date of hatching was recorded to determine the incubation period.
205 Hatchability (%) was calculated using the equation from Abbas et al. (2012): Hatchability = All
206 neonates/All eggs [42]. After hatching, 30 first instar nymphs were left on each cladode and allowed
207 to develop. First instar nymph viability (i.e., successful development to second instar nymph) and
208 duration of each stage reached was recorded. The morphology of the different life cycle stages
209 developed on each genotype and the behavior of the insects were examined using a binocular loupe
210 (Motic) (results no-showed in this manuscript). The mealybug stages reached on each genotype
211 weight (mg) was measured using an electronic balance with a precision of 0.001 mg (OHAUS
212 CORPORATION, USA). In this experiment, each cladode represented a replicate, with 20 replicates
213 per genotype, in a completely randomized design.

214 **Under greenhouse**

215 The life cycle performance and behavior of *D. opuntiae* on 10 selected cactus genotypes were
216 evaluated in a greenhouse under the same temperature conditions as for the Antixenosis test (8-30
217 °C). The ten genotypes with a susceptible control were evaluated in a completely randomized design
218 with 20 replicates. Cactus plants offered to the mealybug were in the 3-5 cladodes stage.

219 The genotypes tested cladodes (one-year-old) were planted in plastic pots (similar in volume to those
220 used in the antixenosis test), filled with the same substrate as described above. Cactus plants were
221 infested with *Opuntiae ficus indica* (L.) cladodes heavily infested with *D. opuntiae* 1st instar nymphs
222 and placed close to the axis of each plant to allow for movement and attachment of nymphs to
223 appropriate zones on the plant. The poles were evaluated daily to assess the duration of each stage
224 reached.

225 **Morphological characterization of plants**

226 Spine and cladode measurements are important informative characters for the taxonomy of Opuntia
227 species [43]. Therefore, the methodological parameters used in this study were adapted from similar
228 methods in previous work on Opuntia morphology by Mosco (2009), Peharec et al. (2010), and
229 Musengi et al. (2021) [43, 44, 45]. The parameters measured were: total number of spines per
230 cladode, cladode surface area, number of areoles per cladode, and number of areoles per plant.

231

232 **Statistical analyses**

233 The normality of the Antixenosis and Antibiosis assays data were evaluated using the Shapiro-Wilk
234 W test. One-way analysis of variance (ANOVA) was used for the analysis of the number of insects
235 attracted to the different cactus genotypes, insect development, and nymphal viability trials to
236 compare the development of *D. opuntiae* among the host plants under laboratory and greenhouse
237 conditions with regard to the duration of instars and mortality rates. These data were examined using
238 analysis of variance, and means were compared with Tukey's LSD test ($\alpha = 0.05$).

239 **Results**

240 **Antixenosis test**

241 **Under laboratory**

242 Significant differences were observed among cactus genotypes in the four periods of attractiveness
243 assessment with *D.opuntiae* nymphs (Table 2). Generally for the two tests performed (free choice
244 and non-choice), At 1, 3, and 15 days after infestation, the genotype Aakria was the least attractive.
245 However, at 30 days after release, no differences were observed among the resistant genotypes
246 tested, and the control genotype (405-424 nymphs) was the most infested.

247 **Table 2** Mean (±SE) number of *Dactylopius opuntiae* on cactus genotypes in different periods in an
248 antixenosis experiment under laboratory conditions (26± 2ºC, 60± 10% RH, and 12h photophase)
249

Genotypes	Number of attracted insects in free-choice test[a]				Number of attracted insects in no-choice test[a]			
	1 d	3 d	15 d	30 d	1 d	3 d	15 d	30 d
Aakria	5.9 ±1.1 e	2.8 ±1.1 e	2.3 ±0.6 c	0.0 ±0.0 b	7.0 ±1.2 f	4.0 ±1.1 e	2.8 ±0.8 d	0.3 ±0.4 b
Cherratia	15.7 ±1.5 d	10.8 ±1.5 de	9.6 ±1.2 bc	0.2 ±0.4 b	28.1 ±3.8 e	21.4 ±1.6 d	12.9 ±2.9 c	0.3 ±0.5 b
Marjana	16.0 ±1.5 d	11.6 ±1.9 cde	9.9 ±1.3 bc	0.3 ±0.5 b	35.3 ±2.6 de	30.2 ±2.8 cd	14.3 ±2.4 c	0.4 ±0.5 b
Melk Zhar	26.0 ±1.9 c	15.8 ±1.5 bcd	8.7 ±1.2 bc	0.5 ±0.6 b	44.1 ±2.5 cd	38.5 ±2.4 bc	15.3 ±2.7 c	0.4 ±0.6 b
Angad	26.9 ±2.5 c	16.5 ±1.6 bcd	9.5 ±1.9 bc	0.7 ±0.8 b	47.3 ±2.4 c	40.9 ±3.3 bc	16.8 ±2.6 c	0.5 ±0.6 b
B176	30.4 ±1.7 c	21.2 ±1.8 bcd	10.9 ±1.6 b	0.8 ±0.8 b	51.8 ±2.7 bc	41.1 ±1.7 bc	18.9 ±3.1 bc	0.7 ±0.7 b
B180	30.5 ±2.0 c	22.2 ±1.9 bc	11.2 ±1.8 b	0.8 ±0.8 b	51.2 ±2.7 bc	41.7 ±1.9 bc	19.8 ±2.9 bc	0.8 ±0.8 b
Karama	32.8 ±1.6 bc	22.2 ±2.4 bc	11.2 ±1.5 b	0.9 ±0.8 b	53.2 ±1.7 bc	43.1 ±1.8 b	20.4 ±2.7 bc	0.9 ±0.8 b
Ghalia	32.9 ±1.5 bc	23.3 ±2.1bc	11.4 ±2.0 b	1.1 ±0.8 b	53.1 ±1.5 bc	44.2 ±2.3 b	20.7 ±2.5 bc	1.2 ±0.9 b
Belara	39.3 ±2.7 b	25.5 ±2.6 b	15.4 ±2.3 b	1.3 ±1.1 b	60.2 ±2.5 b	46.6 ±4.2 b	26.9 ±3.8 b	1.5 ±0.9 b
Control	176.3 ±21.8 a	319.6 ±35.2 a	361.7 ±24.4 a	405.8 ±19.9 a	197.0 ±34.8 a	340.6 ±38.3 a	374.0 ±29.6 a	424.4 ±32.9 a
Statistics	F = 937.57 df = 10, P < 0.0001	F = 1448.45 df = 10, P < 0.0001	F = 3982.03 df = 10, P < 0.0001	F = 8167.83 df = 10, P < 0.0001	F = 410.79 df = 10, P < 0.0001	F = 1242.07 df = 10, P < 0.0001	F = 2688.69 df = 10, P < 0.0001	F = 3311.98 df = 10, P < 0.0001

250

251 Means within a column followed by the same letters are not significantly different according to
252 Tukey's LSD test at $\alpha = 0.05$

253 **Under greenhouse**

254 Regarding the preference of *D. opuntiae* nymphs on different cactus genotypes, at 24 h after release,
255 Aakria (23.2 nymphs/plant) and Cherratia (45.1 nymphs/plant) genotypes were the least infected by
256 *D. opuntiae* 1st instars. No differences were observed among the different cactus resistant genotypes
257 tested at 3, 15, and 30 days after the release of the scale pest nymphs. The susceptible control
258 genotype was the most infested (Table 3).

259 **Table 3** Mean (±SE) number of *Dactylopius opuntiae* alive on cactus genotypes in different periods
260 in an antixenosis experiment under semi-field conditions (i.e., choice test experiment)

Genotypes	Time[a]			
	1 d	3 d	15 d	30 d
Aakria	23.2±4.6 f	10.6±3.5 b	6.0±1.7 b	0.0±0.0 b
Cherratia	45.1±3.9 ef	30.9±4.6 b	11.5±2.5 b	0.3±0.5 b
Marjana	62.7±5.8 de	11.6±1.9 b	11.2±1.8 b	0.7±0.8 b
Melk Zhar	77.0±6.0 cd	30.5±3.4 b	11.5±1.9 b	0.8±0.9 b
Angad	79.0±6.4 cd	32.1±4.0 b	11.9±2.0 b	0.9±1.0 b
B176	89.4±5.5 bcd	41.7±2.5 b	12.1±2.0 b	1.1±1.0 b
B180	89.8±7.6 bcd	43.9±3.7 b	13.0±2.3 b	1.2±1.0 b
Karama	94.2±5.2 bc	41.7±6.0 b	13.5±2.0 b	1.4±1.0 b
Ghalia	95.5±4.5 bc	41.6±6.5 b	13.7±1.9 b	1.4±1.0 b
Belara	112.8±11.0 b	49.6±5.4 b	14.2±1.7 b	1.7±0.9 b
Control	702.2±85.9 a	1592.8±180.0 a	1804.5±120.9 a	2029.0±99.6 a
Statistics	$F = 1022.25$	$F = 1492.85$	$F = 4382.44$	$F = 8283.89$
	df = 10, $P < 0.0001$	df = 10, $P < 0.0001$	df = 10, $P < 0.0001$	df = 10, $P < 0.0001$

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262 Means within a column followed by the same letters are not significantly different according to
263 Tukey's LSD test at $\alpha = 0.05$

264 **Antibiosis Test**

265 **Under laboratory**

266 No significant differences were observed among genotypes on the number of eggs per cladode,
267 incubation period, and hatchability percentage (Table 4). The scale pest does not reach to develop
268 definitively on the genotypes Aakria and Cherratia, and doesn't get beyond the young female stage in

all the other tested resistant genotypes. For the other genotypes, the development time of *D. opuntiae* nymphs was significantly affected by different cactus genotypes in 1st and 2nd instars.

For 1st instars, the genotypes Marjana (37 days), and Melk Zhar (36.1 days) induced the longest development times, whereas Belara induced the shortest development time for this stage. First instar nymphs fed Marjana, Melk Zhar, and A200 dying without having completed the 2nd nymphal stage. The B176 (43.5 days), B180 (42.9 days), and Karama (42 days) genotypes extended the development time of 2nd instars, compared to Belara (37.6 days) genotype. The total nymphal development time (1st instar to young female) was significantly longer when *D. opuntiae* fed on B176 (76.6 days), B180 (75.6 days), and Karama (74.3 days) genotypes and shortest when nymphs fed on Belara (65.1 days) (Table 4).

Weights of 2nd instars were significantly higher when the scale pests fed on Belara (5.5 mg) genotype compared to the other genotypes.

We recorded wide variation among genotypes in first instar nymph viability, which ranged from 0.0 % to 24.4 % (Fig 1). Higher rates of survival to second instar nymph stage were observed when first instar nymphs were fed Belara plants (24.4%). On the other hand, the genotypes Aakria (0.0%) and Cherratia (0.0%) negatively affected this parameter of the mealybug.

Table 4 The developmental parameters of *Dactylopius opuntiae* on cactus genotypes in an antibiosis experiment (i.e., no-choice test) under laboratory conditions (26± 2ºC, 60± 10% RH, and 12h photophase)

Genotypes	Number of eggs/cladode[a]	Incubation period (hours)[a]	Hatchability (%)[a]	1st instar duration (days)[a]	2nd instar duration (days)[a]	Nymphal period (1st instar-Young female) [a]	2nd instar weight (mg)[a]
Aakria	130.0±25.4 a	22.2±1.5 a	82.8±11.9 a	-	-	-	-
Cherratia	128.7±25.9 a	22.3±1.7 a	84.4±13.0 a	-	-	-	-
Marjana	129.9±25.0 a	22.3±1.6 a	83.0±11.1 a	37.0±2.2 a	-	-	-
Melk Zhar	131.9±24.7 a	21.7±1.8 a	82.9±10.6 a	36.1±2.1 ab	-	-	-
Angad	132.8±24.0 a	21.8±1.8 a	83.2±10.0 a	34.6±1.6 bc	-	-	-
B176	133.6±23.3 a	22.2±2.0 a	83.4±9.4 a	30.1±1.8 cd	43.5±1.1 a	76.6±2.1 a	4.2±0.4 b
B180	134.5±23.0 a	21.4±1.5 a	84.8±9.1 a	32.7±1.9 cd	42.9±1.5 ab	75.6±2.3 ab	4.3±0.5 b
Karama	136.9±20.4 a	21.6±1.3 a	84.7±9.8 a	32.3±1.5 d	42.0±2.2 ab	74.3±2.8 ab	4.4±0.5 b
Ghalia	137.8±19.8 a	21.2±1.4 a	84.1±9.6 a	31.9±2.5 d	41.6±2.3 b	73.5±3.9 b	4.4±0.5 b
Belara	142.8±19.4 a	20.9±1.5 a	83.4±11.2 a	27.6±3.2 e	37.6±3.1 c	65.1±3.9 c	5.5±0.6 a
Statistics	$F = 0.69$ df = 9, $P = 0.71$	$F = 1.91$ df = 9, $P = 0.05$	$F = 0.10$ df = 9, $P = 1.00$	$F = 36.29$ df = 7, $P < 0.0001$	$F = 23.64$ df = 4, $P < 0.0001$	$F = 44.08$ df = 4, $P < 0.0001$	$F = 24.41$ df = 4, $P < 0.0001$

Means within a column followed by the same letters are not significantly different according to Tukey's LSD test at $\alpha = 0.05$

Fig 1 Nymphal viability (i.e., successful development to second instar nymph) of *D. opuntiae* on cactus resistant genotypes, in an antibiosis experiment (i.e., no-choice test). Different letters indicate significant difference between treatments by the Tukey's LSD test at $\alpha = 0.05$

Under greenhouse

The results are similar in shape to those obtained in the laboratory conditions, therefore confirming them. The results have shown that under greenhouse also the scale pest does not reach to develop definitively on the genotypes Aakria and Cherratia and doesn't get beyond the young female stage in all the other tested resistant genotypes. Also, first instar nymphs fed Marjana, Melk Zhar, and A200 dying without having completed the 2nd nymphal stage (Table 5). For the other genotypes tested, the

nymphal period ranged from 54.7 to 65.5 days on average, with the longest mean development time on the genotypes B176, B180, and Karama and the shortest on Belara genotype (Table 5).

Table 5 Mean (±SE) development time of *Dactylopius opuntiae* reached stage on cactus genotypes in an antibiosis experiment under semi-field conditions (i.e., free-choice test)

Genotypes	1st instar duration (days)[a]	2nd instar duration (days)[a]	Nymphal period (1st instar- Young female) [a]
Aakria	-	-	-
Cherratia	-	-	-
Marjana	32.1±2.2 a	-	-
Melk Zhar	30.6±1.8 ab	-	-
Angad	28.9±1.9 bc	-	-
B176	27.8±2.0 cd	37.8±2.5 a	65.5±3.5 a
B180	27.3±2.0 cd	37.8±1.8 a	65.1±2.3 a
Karama	27.2±1.6 cd	37.0±2.3 a	64.2±2.6 a
Ghalia	26.7±2.6 d	36.4±2.5 a	63.1±4.0 a
Belara	22.2±3.6 e	32.5±3.0 b	54.7±4.1 b
Statistics	$F = 33.16$ df = 7, $P < 0.0001$	$F = 16.03$ df = 4, $P < 0.0001$	$F = 35.11$ df = 4, $P < 0.0001$

Means within a column followed by the same letters are not significantly different according to Tukey's LSD test at $\alpha = 0.05$

Morphological characterization of cactus genotypes

Principal component analysis of morphological traits of spines, cladodes, and whole plants showed clear groupings among cactus genotypes that correspond to their phylogenetic relationships. Karama and Ghalia shared cladode thickness and number of areoles with Belara. (Cherratia, Angad), and (Melk Zhar, Marjana), and (Aakria, B180) appeared as a distinct grouping respectively separated by the four morphometric characters tested (Fig 2).

Fig 2 A principal component analysis (PCA) using four morphometric characters (i.e. total number of spines per cladode, cladode surface area, number of areoles per cladode, and number of areoles per plant) for the ten cactus genotypes identified as resistant to *D.opuntiae* in Morocco (n = 5 plant/genotype). A236: Marjana; A202: Belara ; A205: Karama ; A206: Ghalia; B200: Angad; A180: Aakria; A176: Melk Zhar; A414: Cherratia

Discussion

Among the newly introduced enemies on cactus in Morocco, there is the mealybug "*Dactylopius opuntiae*"; a very devastating insect that is spreading in the Mediterranean countries with catastrophic damage on the cactus crop.

The management of this pest in the world, and more particularly in Morocco, is based mainly on several components, namely, i) the use of treatments by insecticides that have shown their limits in the field, ii) uprooting and burying of infested plants which is heavy, expensive and sometimes difficult to apply at the level of cactus plantations in the rough terrain or as hedges around houses, and finally, iii) scientific research. This last section focused on knowledge of this new pest, investigation of alternative methods of control, and research for genetic sources of cacti resistant to the wild scale "*Dactylopius opuntiae*". Indeed, the first two axes have allowed enrichment of scientific knowledge useful for the continuation of the work related to the control of this new pest, while the third axis on the varietal resistance of cactus to the mealybug, was the relevant and saving solution. Thus, initially, the ten genotypes identified resistant by research in Morocco, will constitute a solid foundation for the launch of the national program of recovery of cactus decimated across the country by the Ministry of Agriculture, Maritime Fishing, Rural Development, and Water and Forests [23]. Our study documents the first study on the life history of *D.opuntiae* on the ten genotypes identified as resistant in Morocco using antexenosis and antibiosis tests in the laboratory and greenhouse.

Generally, antixenotic resistance to insects occurs due to the existence of morphological and/or chemical factors [46]. The results of this study (under laboratory and greenhouse conditions) indicated that all resistant genotypes tested showed a different level of antixenosis compared to the susceptible control and Aakria and Cherratia showed a strong antexenosis effect toward *D. opuntiae*

(0-0.3 *D. opuntiae* alive 30 after infestation). The mechanisms underlying antixenosis resistance in these genotypes remain unknown. However, volatile chemical compounds may have differed between genotypes, which could determine whether a genotype will be more or less infested [47]. In addition, plants have the ability to produce multiple insecticidal compounds to defend themselves [41]. A new work published by Matos et al. (2021) that compared the chemical profile of four species of forage palms in Brazil (only one of which is susceptible to *D.opuntiae* and the others resistant), reported a total of 28 metabolites of which 18 were annotated [30]. The same authors indicated that quercetin, kaempferol, and isorhamnetin derivatives are distinguished as the main components of forage palm. Quercetin rhamnosyl dihexoside, quercitrin-3-O-2',6'-dirhamnosylglucoside, and isorhamnetin-3-sophoroside 7-rhamnoside are the biomarkers that may be associated with resistance to *D. opuntiae* [30]. However, to confirm this hypothesis, additional studies should be performed.

No influence of cactus genotypes on insect biological parameters, including number of eggs per cladode, incubation period, and hatching percentage, was observed, indicating that the scale pest does not prefer any genotype over the others for oviposition. The mealybug fails to develop successfully on genotypes Aakria and Cherratia and does not develop beyond the young female stage in all other resistant genotypes tested. Also, first instar nymphs fed Marjana, Melk Zhar and A200 died without reaching the second instar nymphal stage, in addition, all the genotypes tested prolonged nymphal development of *D. opuntiae* and adversely affected nymphal viability (<24%), indicating resistance (antibiosis and/or antixenosis) to the cactus mealybug.

Antixenosis and antibiosis typically overlap, meaning that genotypes with high levels of antixenotic determinants may also have deleterious effects on insect life history, causing similar effects to plants that express antibiosis [31, 32]. For this reason, it may be difficult to differentiate between the two categories of resistance [48, 49] and may require specific determination of the mechanisms involved in insect toxification and detoxification. To avoid this misconception, new tools have been

376 developed. For stink bugs, for example, electrical penetration graph (EPG) techniques have recently
377 begun to be used to characterize feeding behavior [50, 51] and may be used in the future to
378 characterize plant resistance categories. Second instar weights were significantly higher on the
379 Belara genotype (5.5 mg) and no differences were observed among the other genotypes. In the
380 present study, we observed that Belara leads to faster development of the nymphal stages of *D.*
381 *opuntiae* compared to the other genotypes in which the insect successfully develops to the young
382 female stage.

383 **Conclusion**

384 The results of this study showed that the genotypes Aakria and Cherratia were the least attractive to
385 *D.opuntiae*, indicating the expression of a strong antixenosis effect towards the scale pest. Under
386 multiple choice conditions, the mealybug preferred no one genotype over the others for oviposition.
387 Under laboratory and semi-field conditions, mealybug failed to develop on the genotypes Aakria and
388 Cherratia and did not grow beyond the young female stage on all other resistant genotypes tested. In
389 addition, the first instar nymphs fed on genotypes Marjana, Melk Zhar, and A200 died without
390 reaching the second instar nymphal stage. In addition, all genotypes tested prolonged nymphal
391 development of *D. opuntiae* and negatively affected their viability (<24%), indicating resistance
392 (antibiosis and/or antixenosis) to the cactus mealybug. Genotypes Aakria and Cherratia showed the
393 greatest stability of resistance as they showed a high level of antibiosis and antixenosis effect.
394 Considering the extensive damage caused by *D. opuntiae* to cactus crops worldwide, further studies
395 are needed to better interpret the resistance factors shown by certain genotypes. This information will
396 be useful for breeding programs focused on pest resistance, in addition to assisting in the
397 management of *D. opuntiae* in cactus plantations.

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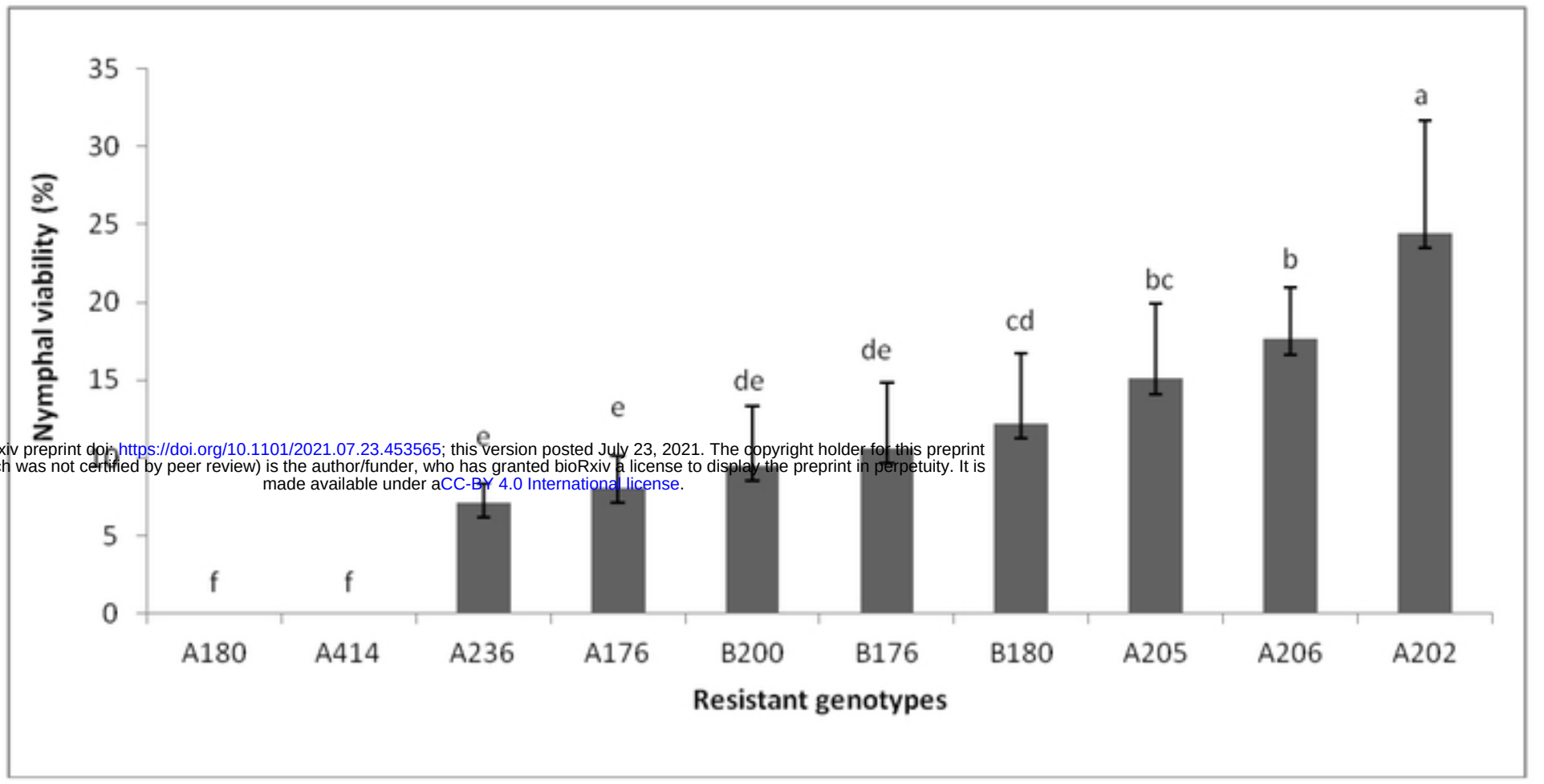

Fig. 1

Cover Letter

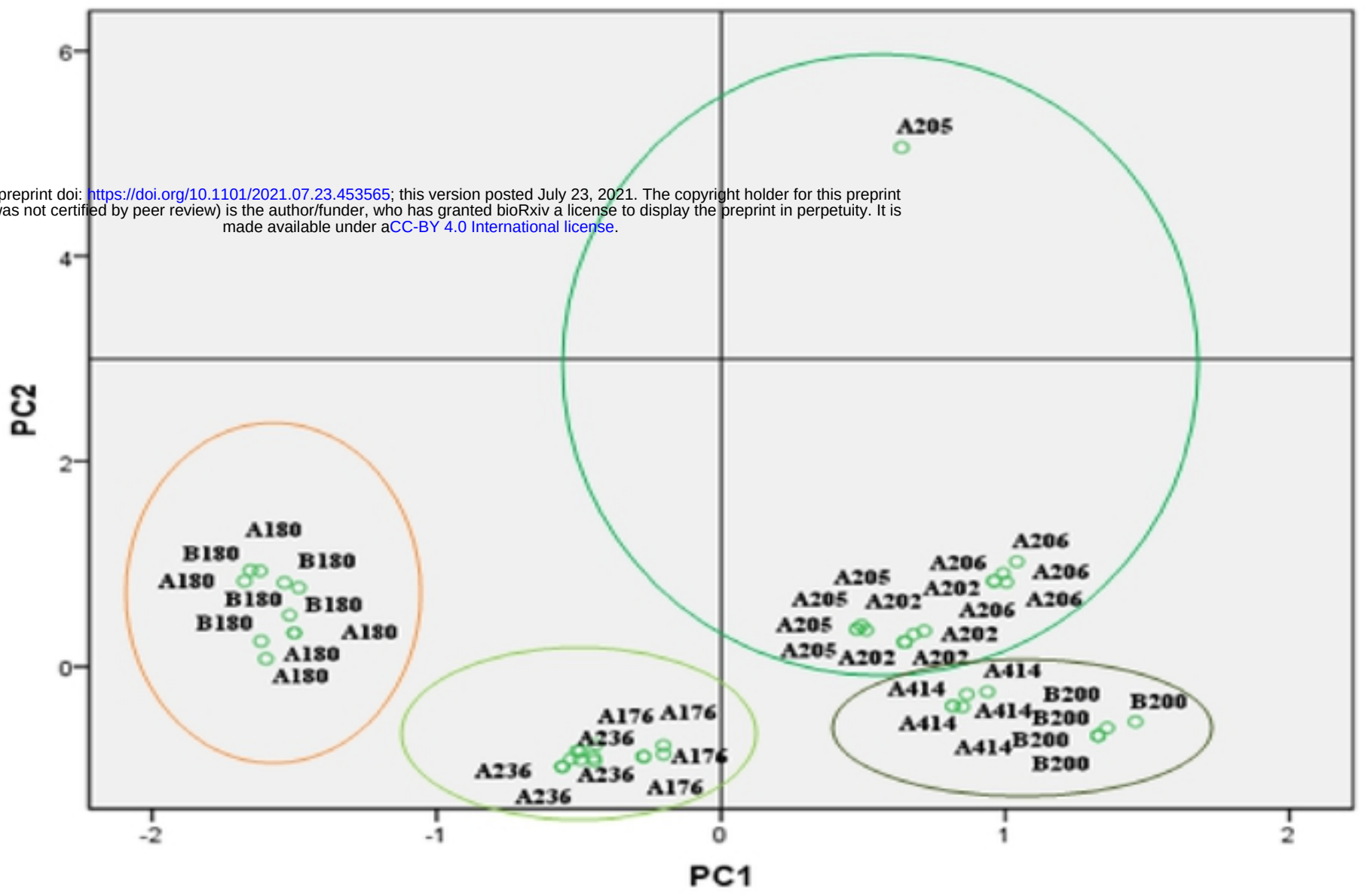

Fig. 2

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