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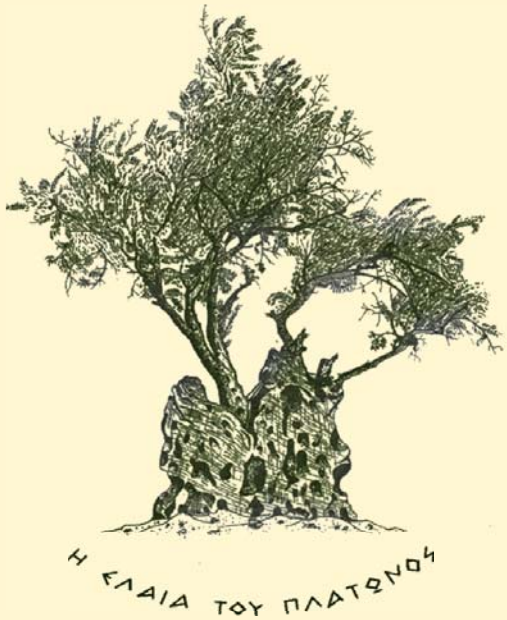

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Survival and germinability of *Rhynchosporium secalis* conidia exposed to solar radiation

E. Al-Shehadah, A. Al-Daoude and M. Jawhar*

Summary *Rhynchosporium secalis*, the causal agent of barley scald disease, is a fungus commonly found in the environment. Disease spread within a field and between fields occurs through the aerial dispersal of the fungal spores. However, not much is known about the survival potential of fungal conidia exposed to solar radiation. In the present study, detached conidia of *R. secalis* were exposed simultaneously in the field to direct sunlight or placed in an adjacent ventilated enclosure in the dark for periods ranging from 0.5 to 8h. In addition, conidia were either exposed or not exposed to UV-C light (254 nm) for periods ranging between 0.5 and 60 min in the laboratory. After exposure, conidia were placed on water agar Petri dishes and allowed to germinate for 24h. Germinability of conidia was reduced by up to 94% after 8h of exposure to solar irradiance (670-860 Wm^{-2}) in the field in comparison to the non-exposed control. Germinability of conidia in the laboratory was reduced up to ~100% by doses of UV-C light of 3.2±0.7 Wm^{-2}. The results of this study will contribute to a better understanding of the relationship between climatic conditions and barley scald epidemics.

Additional keywords: barley scald, climate, spore survival, UV-C light, virulence

Introduction

Aerial dispersal of inoculum is considered an important factor in the epidemiology of many fungal plant diseases (Aylor, 2003; Stanosz *et al.,* 2016). *Rhynchosporium secalis* (Oudem) J. J. Davis, the causal agent of scald disease, is an important pathogen of barley (*Hordeum vulgare* L.) worldwide (Zhan *et al.,* 2008). *R. secalis* is considered economically important because it can cause marked reduction in crop yield and quality (Yahyaoui, 2003). The appearance of scald disease in a field with no previous history of barley cropping can be attributed either to the use of infected seed or to the transportation of airborne putative ascospores from sources outside the field. Once infection is established in a crop, conidia and/or putative ascospores can then be easily dispersed throughout the crop by rain splashing or wind (Fountaine *et al.,* 2010). Therefore, the recent resurgence of the scald in new barley cultivation areas due to the arrival of more aggressive isolates of the pathogen underscores the need for renewed and improved management of the disease. Clearly, forecasts could be improved if they included knowledge of the presence or absence of the pathogen in an area.

Not much is known whether *R. secalis* conidia could be transported to a field from an outside source, and, if transported, whether they would initiate disease. Several researchers have determined the potential of *R. secalis* for *in vitro* spore production (Stedman, 1980; Avrova and Knogge, 2012). However, to our knowledge, there have been no published studies related to the escape of *R. secalis* conidia in the air and their capacity to survive exposure to direct sunlightand incite disease.

The purpose of the present work was to investigate the survival potential of *R. secalis* conidia exposed to solar radiation in the field, as measured by their ability to germinate after exposure to direct sunlight for various lengths of time. The effect of UV-C light on the germinability of *R. secalis* conidia under controlled conditions was also evaluated.

Department of Molecular Biology and Biotechnology, AECS, P.O. Box 6091 Damascus, Syrian Arab Republic.
* Corresponding author: ascientific3@aec.org.sy

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Materials and Methods

Experimental material

Rhynchosporium secalis pathotype Rs 22, which is one of the most virulent Syrian pathotypes to all barley genotypes available so far (Arabi *et al.,* 2010), was used in this study. At the end of the growing season, symptomatic barley leaves, naturally infected by pathotype Rs22, were collected from the field. In the laboratory, leaf sections measuring 1.5 cm x 1 cm bearing well-developed scald lesions were cut, placed at 18 °C in the dark and wetted twice a day using a high-pressure sprayer in order to stimulate the production of conidia. Sporulation on leaf lesions was checked under a light microscope. After 7 days, produced conidia were transferred from diseased leaf sections to coverslips (18 x 18 x 0.14 mm) by lightly pressing the coverslip on the surface of a sporulating lesion. The coverslips were then placed (conidia facing up) on a plastic window screen stretched on a lightweight wooden frame (20 cm^2). The coverslips were fixed on the screen with a small piece of transparent, double-sided adhesive tape (Aylor and Sanogo, 1997).

Exposure tests in the field

The experiments were performed under field conditions during the summer of two consecutive years (2013-2014). Samples (coverslips carrying the conidia) were exposed either to direct sunlight or to darkness for time periods ranging from 0.5 to 8 h. For each exposure period, two sets of samples were used: one set was exposed to direct sunlight and another one to darkness (control). For exposing the samples to direct sunlight, the plastic window screen carrying the coverslips with the conidia was placed in the field 1.1 m above the ground under full sunlight conditions. For exposing the second set of samples to darkness in the field, the window screen with the coverslips was placed in a darkened enclosure with ventilation. Six coverslips per treatment were used as replicates. At the beginning of each exposure time, conidia were collected from each replicate coverslip, placed immediately on 1.5% water agar Petri dishes, incubated at 20-22°C in the dark for 24h, and served as non-exposed controls (G_0). The temperature of the coverslips carrying conidia was determined with a Multi-thermometer apparatus with copper thermocouple wires fixed to the bottom of two additional coverslips without conidia (one coverslip for each treatment) placed in the same conditions as those that carried the conidia.

Exposure tests in the laboratory

Conidia produced on barley leaves, using the method described above, were transferred to coverslips and were handled as described for the field tests. The coverslips with conidia were exposed in the laboratory at room temperature (22 to 25°C) to short-wavelength (254 nm) UV light (UV-C light) emitted from ultraviolet tubes (TUV-30 W/G T8-UV-C; Philips, The Netherlands) for 0.5, 5, 15, 30, and 60 min. The distance between the conidia and the UV-C light tubes was ~15 cm. The UV irradiance at the level of the conidia was 3.2±0.7 Wm^{-2} as measured with a UVX-CR radiometer with a UVX-25 sensor (Ultraviolet Products, Cambridge, UK). Three replicate coverslips were used for each exposure period and treatment (with and without exposure to UV-light). The non-exposed to UV-C light coverslips (control) were kept under room temperature. The experiment was repeated three times.

Assessment of conidial viability

At the end of each exposure time in both the field and the laboratory tests, exposed and non-exposed coverslips with conidia were collected and placed (conidia facing down) on 1.5% water agar medium contained in 9-cm plastic Petri dishes (6 Petri dishes per treatment) and incubated at 20-22°C in the dark for 24h to allow germination. Germinated conidia were counted in random fields at x 100 magnification with a light microscope. A total of 100 to 200 conidia was examined on each coverslip. A conidium was considered germinated if the length of the germ tube was greater than or equal

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to the length of the conidium (12.5-14.3 µm) (Manners, 1966). For calculating the percentage of conidial germination, the following formula was used:

Germination (%) = (Number of germinated conidia x 100)/total number of conidia examined.

Meteorological data

Solar irradiance, air temperature, relative humidity (RH) and wind speed were measured in the field during the whole experimental period. Solar irradiance (Wm^{-2}) was measured with a pyranometer (LI 200 SB, Li-COR. Int., Lincoln, NE 68504) located at a height 1.1m above the ground. Air temperature and RH were measured with a platinum resistance-thermometer sensor (Pt100) of DIN EN 60751, Class B (ES Electronic Sensor GmBH, Germany) and wind speed with a cup anemometer (model 014A, COMBILOG, Theodor Friendrichs & Co. Hamburg, Germany). All instruments were located at the same height above the ground as the coverslips carrying the conidia (i.e. 1.1 m). The meteorological data were recorded at 1h intervals using a data logger (COMBILOG 1020, Theodor Friendrichs & Co. Hamburg, Germany).

Virulence tests

The virulence of conidia exposed for 8h to solar radiation and those that were not exposed was tested in the field using three barley cultivars; WI 2291 from Australia, and Arabi Abiad and Tadmor from Syria. Those cultivars were selected for their different levels of resistance to scald disease (Arabi *et al.*, 2010). The experiment was located at a site with favorable environmental conditions for the development of the disease. Seeds were sown under rainfed conditions (500 mm annual rainfall) in a completely randomized block design, with three replicate plots for each treatment (50 plants per replicate and cultivar). Each replicate plot was 1 × 1 m, with 1m wide buffer zone around it. Each plot consisted of five rows, 25 cm apart, with 10 seeds sown per row. Inoculum of pathotype Rs22 was prepared from conidia produced on 2- to 3-week-old cultures grown on Lima Bean Agar (LBA) medium. Conidia were suspended in water and their concentration was adjusted with a hematocytometer to 0.5×10^6 conidia/mL. A surfactant (polyoxyethylene-20-sorbitan monolaurate) was added (100 µL/L) to the conidial suspension to facilitate dispersion of the inoculum over the leaf surfaces.

Inoculations were timed to correspond with periods of free moisture when possible. Three inoculations were performed using a fine mist hand-held sprayer. The first inoculation was applied when plants began to tiller (GS 31) and the last when plants were at least 30 cm height (Zadoks *et al.,* 1974). Non-inoculated plants (control) were sprayed with distilled water. Disease severity was assessed as a percentage of the second leaf lamina showing symptoms, 17 days post-inoculation, according to the method used by Ceolini (1980).

Statistical analysis

Data were analyzed using the STAT-ITCF programme (MICROSTA, realized by ECOSOFT, 2nd Version, Institut Technique des Céréales et des Fourrages Paris). Analysis of variance (Newman-Keuls test) was conducted to test for differences among exposure periods in test sets. The germination for conidia exposed to sunlight was calculated as G_S/G_0, and for conidia that were not exposed to sunlight (controls) as G_{NS}/G_0 where, G_S: the average of absolute germination percentage for exposed conidia, G_{NS}: the average of absolute germination percentage for non-exposed conidia, and $G_{0:}$ the average of absolute germination percentage for non-exposed conidia at the beginning of the exposure period (time 0). The germinability of conidia exposed to sunlight was compared to that of conidia not exposed to sunlight using the formula: G_{NS}-G_S/G_{NS}.

Results and Discussion

The environmental conditions that prevailed in the field during the exposure of *R. secalis* conidia to direct sunlight are presented

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in Table 1. Germinability of *R. secalis* conidia was significantly (P<0.001) reduced by up to 94% after 8h of exposure to direct sunlight in comparison to the non-exposed controls (Table 2). Results showed a highly significant decrease in the germination of conidia with increasing exposure time to direct sunlight (Table 2). In addition, significant differences (P<0.05) in the virulence between exposed and non-exposed to sunlight conidia were observed (Table 3).

Survival of conidia is of paramount importance in the build-up of *R. secalis* inoculum, as conidia transported from infected plant residues *via* wind or rain can germinate and infect healthy barley plants. Very little is known about the effect of sunlight in the survival of these conidia. In the present work, conidia collected on coverslips and either exposed or not exposed to solar radiation were used to represent conidia naturally deposited on the upper surface of a barley leaf. Although there may have been differences in the ability of conidia of different ages to germinate the germination of non-exposed conidia were used as a baseline (control) to compensate these differences. Under the conditions of our study, germinability of *R. secalis* conidia was reduced significantly by exposure to solar radiation. These results agree with those of earlier studies that have demonstrated a significant effect of light on the germinability of plant pathogens in other pathosystems (Rotem and Aust, 1991; Ben-Yephet and Shtienberg, 1994; Braga *et al.,* 2015; Cordo *et al.,* 2017).

Our findings clearly indicate that solar radiation significantly affects *R. secalis* conidial viability, which mainly depends on the duration of exposure. Considering the impacts of these findings on the epidemiology and the spread of *R. secalis* conidia with a potential of causing infection at a given location, the later could be reasonably predicted through detailed analyses of the available solar radiation data along with the travel path of the conidia (Isard *et al.,* 2005). On the other hand, conidial pigmentation has been reported to play an essential role in solar radiation protective mechanisms (Swan, 1974; Ignoffo and Garcia, 1992; Butler and Day, 1998; Fuller *et al.,* 2015), which might be a possible explanation of *R. secalis* tolerance to solar exposure up to 8 h.

UV-C exposure (3.2±0.7 Wm^{-2}) significantly (P<0.001) reduced the germinability of *R. secalis* conidia (Fig. 1). The reduction in germinability increased with increasing time of exposure to UV irradiation. It is well known that the UV-C wavelength is highly efficient

Table 1. Environmental conditions that prevailed in the field during the exposure of *Rhynchosporium secalis* conidia to direct sunlight.

Variable	Range (Unit)
Air temperature	26-38 (°C)
Relative humidity	30-47 (%)
Average wind speed at sample height	0.9-4.1 (m/s)
Irradiance of incident solar radiation	670-860 (Wm^{-2})

Table 2. Germination (%) of *Rhynchosporium secalis* conidia exposed (Gs) and not exposed (G_{NS}) to direct sunlight in the field for periods ranging from 0.5 to 8 h.

Treatment	Time (h)								
	0.5	1	2	3	4	5	6	7	8
G_S	A83.3b[x]	B74.4b	C65.7b	C60.3b	D50.1b	E37.9b	E32.2b	E25.6b	F11.1b
G_{NS}	A94.3a	A94.6a	A97.5a	A98.5a	A98.8a	A98.9a	A98.9a	A98.1a	A98.3a
$(G_{NS}-G_S/G_{NS})$	0.12	0.21	0.32	0.39	0.49	0.62	0.67	0.74	0.89

[x] Means preceded by different capital letters (column) and followed by different lowercase letters (row) differ significantly at P<0.001 according to Newman-Keuls test.

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Table 3. Mean scald disease severity (% of the second leaf lamina showing symptoms) 17 days after the inoculation of three barley (*Hordeum vulgare*) cultivars with *Rhynchosporium secalis* conidia, previously exposed (Gs) or not exposed (G_{NS}) to direct sunlight for 8 h under field conditions.

Cultivar	Disease severity (%)[x]	
	G_{NS}	G_S
WI2291	A89.3a[y]	A77.57b
Arabi Abiad	B76.7a	B57.43b
Tadmor	C17.1a	C11.10b

[x] Mean of three replicates.
[y] Means preceded by different capital letters (column) and followed by different lowercase letters (raw) are significantly different at (P<0.05) by Newman-Keuls test.

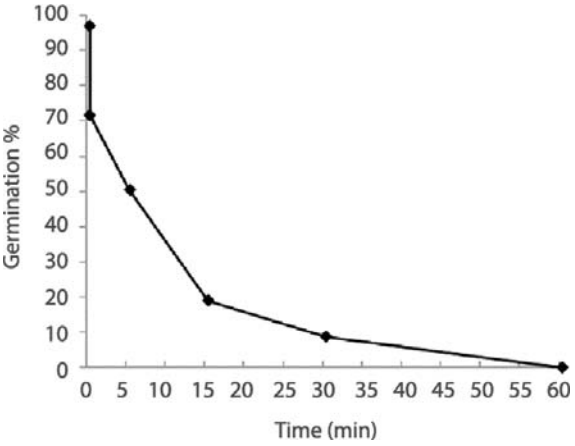

Figure 1. Germination (%) of *Rhynchosporium secalis* conidia exposed to UV-C light (254 nm) for different time periods at room temperature (22-25°C). The UV irradiance at the level of conidia was 3.2±0.7 Wm^{-2}.

in killing most micro-organisms. This method has been used extensively in sterilization procedures with low energy germicidal lamps, which emit radiation principally at 254 nm. These results are consistent with reports on other fungi, such as *Aspergillus carbonarius*, *Aspergillus niger*, *Cladosporium herbarum*, *Penicillium janthinellum, Alternaria alternata* and *Venturia inaequalis* (Aylor and Sanogo, 1997; Valero *et al.,* 2007).

Overall, the present study, which is part of an on-going project on the epidemiology of scald disease on barley in Syria, provides new information on the effect of solar radiation on the survival of *R. secalis* conidia during their aerial dispersal. Solar radiation affects the viability of *R. secalis* conidia, and thus, it is an important factor in their survival while being airborne. This knowledge is important because dispersal limitation of the *R. secalis* population may drive very interesting ecological dynamics. Models of airborne dispersal of plant pathogens show that the rate of long-distance dispersal depends strongly on the longevity of propagules in the atmosphere (Aylor, 2003; Wilkinson *et al.,* 2012). The influence of environmental factors, such as cloud cover, wind, etc. on solar radiation and their effect on conidia viability need to be also considered.

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Επιβίωση και βλαστικότητα κονιδίων του μύκητα *Rhynchosporium secalis* μετά από έκθεση στην ηλιακή ακτινοβολία

E. Al-Shehadah, A. Al-Daoude and M. Jawhar

Περίληψη Ο φυτοπαθογόνος μύκητας *Rhynchosporium secalis*, που προκαλεί στο κριθάρι την ασθένεια ρυγχοσπορίωση, απαντάται συχνά στο περιβάλλον. Η εξάπλωση της ασθένειας εντός του αγρού ή μεταξύ των αγρών επιτυγχάνεται μέσω των αερομεταφερομένων σπορίων του μύκητα. Εντούτοις, δεν υπάρχουν επαρκείς πληροφορίες σχετικά με την επιβίωση των κονιδίων του μύκητα που εκτίθενται στην ηλιακή ακτινοβολία. Στην παρούσα μελέτη, κονίδια του *R. secalis* εκτέθηκαν ταυτοχρόνως και σε γειτνιάζουσα θέση στον αγρό είτε απευθείας στο ηλιακό φως είτε στο σκοτάδι (εντός ενός επαρκώς αεριζόμενου κλειστού θαλάμου), για περιόδους που κυμαίνονταν από 0,5 έως 8 ώρες. Επιπροσθέτως, κονίδια του μύκητα εκτέθηκαν ή δεν εκτέθηκαν (μάρτυρας) σε UV-C ακτινοβολία (254 nm) για περιόδους που κυμαίνονταν από 0,5 έως 60 λεπτά, στο εργαστήριο. Μετά την έκθεση, τα κονίδια τοποθετήθηκαν σε τρυβλία Petri που περιείχαν άγαρ και αφέθηκαν να βλαστήσουν για 24 ώρες. Η βλαστικότητα

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των κονιδίων που εκτέθηκαν για 8 ώρες στην ηλιακή ακτινοβολία (670-860 Wm^{-2}) στον αγρό μειώθηκε έως και 94% σε σχέση με εκείνη του μη εκτεθειμένου μάρτυρα. Σε συνθήκες εργαστηρίου, δόση UV-C ακτινοβολίας 3.2 ± 0.7 Wm^{-2} μείωσε τη βλαστικότητα των κονιδίων του μύκητα έως και ~100%. Τα αποτελέσματα της παρούσας μελέτης θα συμβάλουν στην καλύτερη κατανόηση της σχέσης μεταξύ των κλιματικών συνθηκών και της εμφάνισης επιδημιών της ασθένειας.

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SHORT COMMUNICATION

First record of parasitoids associated with insects inhabiting capsules of *Papaver rhoeas* in Greece

F. Karamaouna[1*], M. Samara[1], V. Kati[1] and M.-D. Mitroiu[2]

Summary A faunistic complex of chalcidoid parasitoids (Hymenoptera: Chalcidoidea) associated with cynipids and cecidomyids (Hymenoptera: Cynipidae; Diptera: Cecydomyiidae) inhabiting capsules of the annual weed *Papaver rhoeas* L. (corn poppy) was recorded in Amynteo, Northern Greece (2012) and Orchomenos, Voeotia, Central Greece (2013). The parasitoids are *Idiomacromerus papaveris* (Forster, 1856), *Idiomacromerus* sp., *Pseudotorymus papaveris* (Thomson, 1876) (Torymidae), *Aprostocetus epicharmus* Walker, 1839 (Eulophidae), and *Cyrtoptyx* sp. (Pteromalidae). *Aprostocetus epicharmus* was recorded only in Amynteo while *Idiomacromerus* spp. and *Cyrtoptyx* sp. only in Voeotia. This is the first record of these parasitoid species in corn poppy capsules in Greece. All parasitoids except the eulophid, which probably parasitizes Cecydomyiidae, are most likely parasitoids of *Aylax papaveris* (Perris, 1840) (Cynipidae).

Additional keywords: *Aprostocetus epicharmus*, *Aylax papaveris*, *Cyrtoptyx* sp., gall, *Idiomacromerus papaveris*, *Pseudotorymus papaveris*

A complex of chalcidoids (Hymenoptera: Chalcidoidea) was recovered from mature, dry capsules of the winter annual weed *Papaver rhoeas* L. (corn poppy, common poppy) (Papaveraceae), which were collected from Amynteo, Florina, Northern Greece in 2012, and Orchomenos, Voeotia, Southern Greece in 2013. The parasitoids are *Idiomacromerus papaveris* (Förster, 1856), *Idiomacromerus* sp., *Pseudotorymus papaveris* (Thomson, 1875) (Torymidae), *Aprostocetus epicharmus* Walker, 1839 (Eulophidae), and *Cyrtoptyx* sp. (Pteromalidae). *Aprostocetus epicharmus* was recorded only in Amynteo while *Idiomacromerus* spp. and *Cyrtoptyx* sp. only in Voeotia. Identifications of the parasitoids have been made based on Bouček and Rasplus (1991) and Medvedev (1978). To our knowledge, this is the first record in Greece of these parasitoids occuring in corn poppy capsules. *Chalcimerus borceai* Steffan and Andriescu, 1962 (Hym.: Torymidae) is the only parasitoid species associated with corn poppy capsules that has been previously reported in Greece (Askew *et al.,* 2006).

Adult females and males of *P. papaveris* were recorded upon emergence from the *P. rhoeas* capsules in both regions. In the capsules from Amynteo, together with the parasitoid specimens, we obtained an adult female of *Aylax papaveris* (Perris) (Hym.: Cynipidae) and found infestation by Cecidomyiidae larvae (Diptera), most likely *Dasineura papaveris* Winnertz.

Pseudotorymus papaveris is probably a parasitoid of *A. papaveris* and the cecidomyiid while *A. epicharmus* is a parasitoid of the cecidomyiid (Figure 1). *Pseudotorymus papaveris* has a Palaearctic geographical distribution (Nikol'skaya and Zerova, 1978; Grissell, 1995; Noyes, 2017). Popescu (2002) reported the emergence of *P. papaveris* from *A. papaveris* and *D. papaveris*, infesting *P. rhoeas* and *P. dubium* L. capsules, and from their predator *Lestodiplosis callida* Winnertz (Diptera: Cecidomyiidae). *Aprostocetus epicharmus* has been reported (as *Tetrastichus epicharmus* (Walker)) parasitising the bras-

[1] Benaki Phytopathological Institute, 8 St. Delta str., GR 145 61 Kifissia, Attica, Greece.
[2] Alexandru Ioan Cuza University of Iasi, Faculty of Biology, 700505 Iasi, Romania.
* Corresponding author: f.karamaouna@bpi.gr

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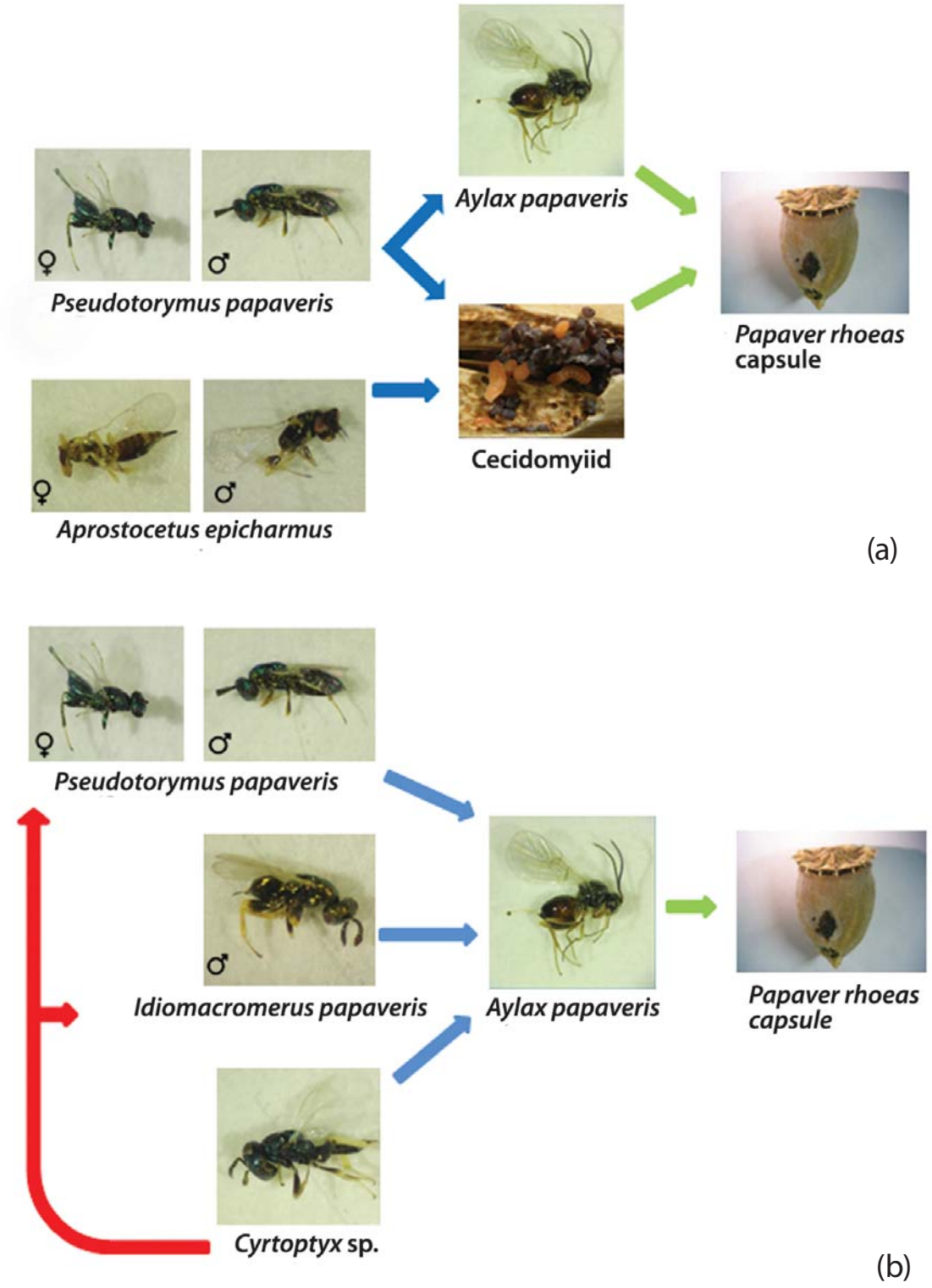

Figure 1. Suggested interrelations a) between the parasitoids *Pseudotorymus papaveris* and *Aprostocetus epicharmus*, and the herbivores *Aylax papaveris* and a cecidomyiid (samples from Northern Greece) and b) between the parasitoids *Pseudotorymus papaveris, Idiomacromerus papaveris* and *Cyrtoptyx* sp., and the host *A. papaveris* (samples from central Greece), in *Papaver rhoeas* capsules. Colored arrows indicate the various associations (green for herbivory, blue for parasitism, red for hyper-parasitism) (Images by M. Samara, V. Kati).

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sica pod midge, *Dasineura brassicae* (Winnertz) (Diptera: Cecidomyiidae), in Poland (Czajkowska, 1978) and the raspberry cane midge, *Resseliella theobaldi* (Barnes) (Diptera: Cecidomyiidae), in Hungary (Vètek *et al*., 2006).

Aylax papaveris is one of the three Cynipidae species inducing capsule galls in *P. rhoeas*, *P. dubium*, *P. argemone* L. and *P. somniferum* L., the other two species being *Barbotinia oraniensis* (Barbotin) and *Aylax minor* Hartig, 1840 (Pujade-Villar, 2015; Gómez *et al*., 2017). *Aylax papaveris* forms light yellow fused galls, irregular oval or globular in shape, highly variable in size, including a few dozen larval chambers arranged perpendicularly to the vertical capsule axis. The individual gall chambers originate from the transformation of the septum and seeds, usually causing deformation and enlargement of the capsules (mainly in *P. rhoeas* and *P. dubium*). Internal septa and seeds disappear (Pujade-Villar, 2015). *Dasineura papaveris* is a monophagous bivoltine gall midge of *Papaver* spp. capsules, which have a normal appearance after infestation and only when opened the clearly swollen septa are visible (Skuhravá and Skuhravý 1997; Popescu 2002).

In the capsules from Voeotia, we encountered galls by the cynipid gall wasp *A. papaveris* (Figure 2) and the parasitoids *Idiomacromerus* spp. (three male specimens, probably *I. papaveris*, although it is difficult

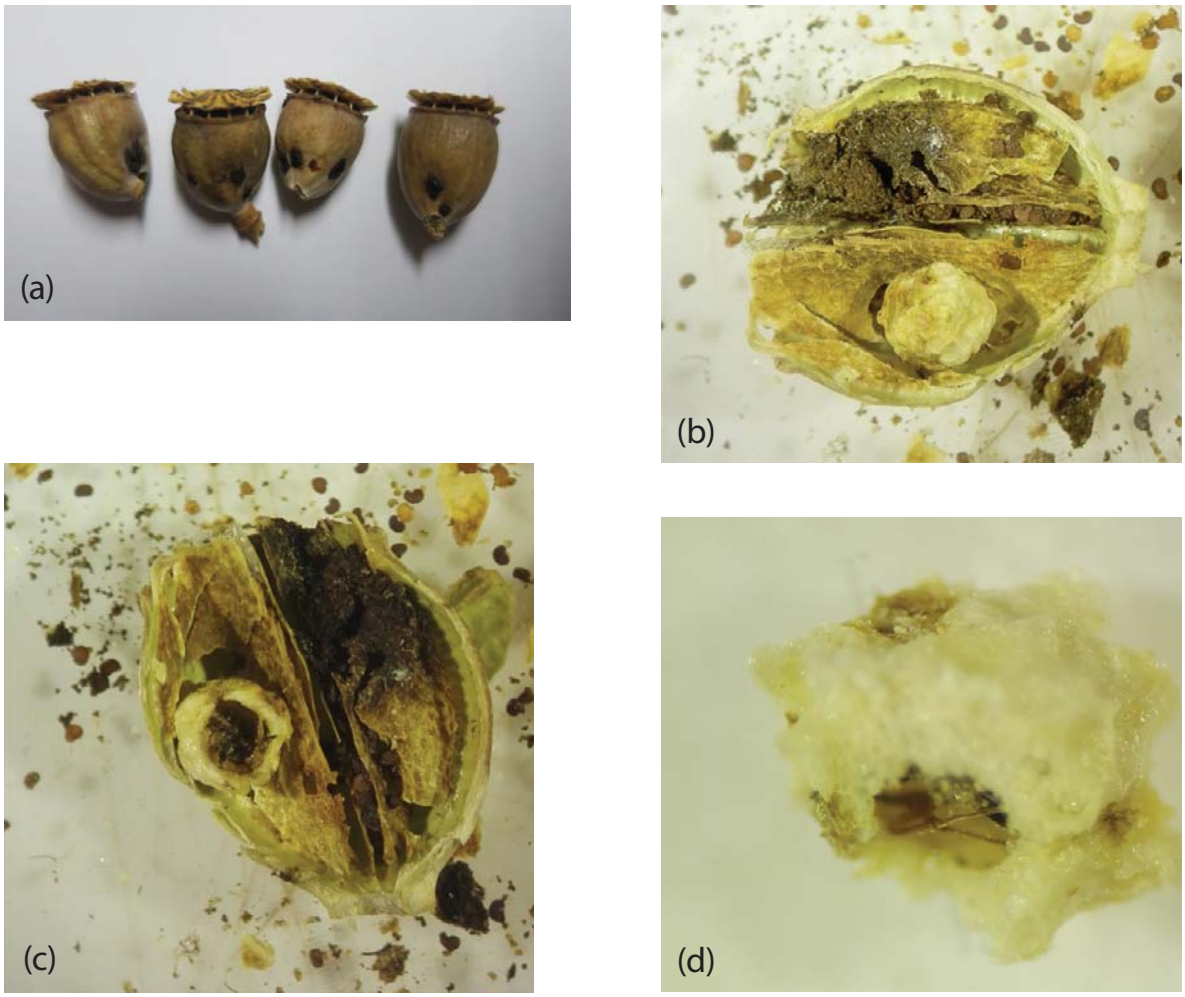

Figure 2. *Papaver rhoeas* capsules a) infested by cynipid gall wasp, b) capsule with the gall (10x), c) gall with the cynipid gall wasp (10x) and d) *Aylax papaveris* (Hym.: Cynipidae) before emergence (40x) (Images by M. Samara).

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to be certain without any females, *Idiomacromerus* sp. (1 male) (different from *I. papaveris* in the coloration of tibiae), and *Cyrtoptyx* sp. (1 male).

A possible scenario is that *P. papaveris*, *I. papaveris* and *Cyrtoptyx* sp. are parasitoids of *A. papaveris*, although no *Cyrtoptyx* sp. has ever been reared from *Aylax* (Figure 1). *Cyrtoptyx* sp. might as well be a hyperparasitoid of the torymids. *Idiomacromerus papaveris* has been reported to parasitise *A. papaveris* (in Croatia, France, Hungary, Romania, Spain) and *A. minor* (in Andora, France, Romania, Spain) in *Papaver* galls (Askew *et al.*, 2006). *Idiomacromerus papaveris* is widely distributed from Iran to U.K. (Noyes, 2017). Some *Cyrtoptyx* species have been reported to parasitise cynipid gall wasps e.g. *Cyrtoptyx robustus* (Masi, 1907) on *Cynips disticha* Hartig, 1840 on *Quercus* (Nieves Aldrey, 1982; Bellido and Pujado-Villar, 1999) in Spain; others are parasitoids of fruit flies, e.g. *Cyrtoptyx latipes* (Rond.) on the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) (Baratella, 2008).

The sampled parasitoid species and their hosts are associated with the natural vegetation in agricultural systems of different intensity in terms of cultivation and pesticide use, affecting the abundance and species diversity of spontaneous plants: *P. rhoeas* plants were located in uncultivated land around the grape-producing area of Amynteo and in the intensively cultivated arable crop area of Voeotia. Our findings highlight the importance of natural or semi-natural areas for the maintenance of these species. Further studies are necessary to clarify the exact trophic relations among the Hymenoptera pararasitoids and the hosts found in the *P. rhoeas* capsules, as well as their potential role in ecosystem services through the biological control of the corn poppy.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή παρασιτοειδών σε έντομα Cynipidae και Cecidomyidae σε κάψες παπαρούνας, *Papaver rhoeas*, στην Ελλάδα

Φ. Καραμαούνα, Μ. Σαμαρά, Β. Κατή και M.-D. Mitroiu

Περίληψη Στην εργασία παρουσιάζεται ένα σύμπλεγμα παρασιτοειδών σε έντομα Cynipidae και Cecydomyiidae που προσβάλλουν τις κάψες του ετήσιου ζιζανίου *Papaver rhoeas* (κν. παπαρούνα), όπως καταγράφηκε στις περιοχές του Αμύνταιου Φλώρινας (2012) και του Ορχομενού Βοιωτίας (2013). Τα παρασιτοειδή ανήκουν στα είδη *Idiomacromerus papaveris* (Forster, 1856), *Idiomacromerus* sp., *Pseudotorymus papaveris* (Thomson, 1876) (Torymidae), *Aprostocetus epicharmus* Walker, 1839 (Eulophidae), και *Cyrtoptyx* sp. (Pteromalidae). Το *A. epicharmus* καταγράφηκε μόνο στο Αμύνταιο ενώ τα *Idiomacromerus* spp. και *Cyrtoptyx* sp. στη Βοιωτία. Πρόκειται για την πρώτη καταγραφή των παρασιτοειδών αυτών σε κάψες παπαρούνας στην Ελλάδα. Όλα τα παρασιτοειδή, εκτός από το Eulophidae, το οποίο πιθανόν παρασιτεί δίπτερα της οικογένειας Cecydomyiidae, φαίνεται να είναι παρασιτοειδή του υμενόπτερου *Aylax papaveris* (Perris, 1840) (Cynipidae).

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SHORT COMMUNICATION

First record of *Coptotriche angusticollella* (Duponchel, 1843) (Lepidoptera: Tischeriidae) on the oil-bearing rose in Turkey

O. Demirözer[1], A. Uzun[1]*, S. Erbaş[2] and F. Can[3]

Summary *Coptotriche* spp. are known to cause damage on plants of Rosaceae and Fagaceae. *Coptotriche angusticollella* (Duponchel, 1843) (Lepidoptera: Tischeriidae) was recorded for the first time infesting the oil-bearing rose, *Rosa damascena*, at the last half of May 2017 in Isparta, Turkey. It should not be ignored that *C. angusticollella* can be a potential risk posed to the oil-bearing rose crop.

Additional keywords: pest, *Rosa damascena,* rose oil, trumpet leafminers

Cultivation of the oil-bearing rose, *Rosa damascena* Mill. (Rosaceae), has an important economic position in agricultural production of Turkey. Furthermore, approximately 90% of the world's oil-bearing rose cultivation is located in Turkey and 50% of the world's rose oil is provided from this country (Baydar and Kazaz, 2013).

*R*osa *damascena* is a host of several insect pests, including three Lepidoptera, *Cnaemidophorus rhododactyla* (Denis and Schiffermüller) (Pterophoridae), *Archips rosana* (L.) (Noctuidae) and *Notocelia rosaecolana* (Doubleday) (Tortricidae) (Demirözer *et al.,* 2011; Demirözer, 2012). In this study, *Coptotriche angusticolella* (Duponchel, 1843) (Lepidoptera: Tischeriidae) was collected for the first time on leaves of oil-bearing rose in Isparta, Turkey (Figure 1a) adding a trumpet leafminer to the lepidopteran pests of the oil-bearing rose.

Trumpet leafminers (Lepidoptera: Tischeriidae) are known as the smallest moths, with a wingspan of only 5–11 mm. Their larvae make usually trumpet-shaped mines or blotch mines, on a variety of host plants (Kobayashi *et al.,* 2016). Pupation occurs in the leaf mines and adults are diurnally active.

Main hosts of Tischeriidae belong to the plant families of Rosaceae, Fagaceae and Asteraceae (Puplesis and Diškus 2003; Stonis *et al.,* 2014). Until this time, 115 *Tischeria* species have been reported from different parts of the world. *Coptotriche* spp. have been associated with fruit and ornamental trees: *Coptotriche* spp. on *Carpinus* (Betulaceae) and *Quercus* (Fagaceae) trees in Japan (Sato, 2011); *C. citrinipennella* and *C. zelleriella* on *Quercus* sp.; *C. castaneaeella* on *Quercus imbricaria* (Anonymous, 2018). *Coptotriche angusticollella* has been reported in Japan (Hokkaido, Honshu), Europe (Slovenia), Tunisia, Caucasus, Turkmenistan, South Korea, the Russian Far East. The plant hosts recorded were *Rosa multiflora*, *R. wichuraiana*, *R. canina*, *Rosa* spp. and other Rosaceae (Kollar and Hrubik, 2009; Lesar and Govedik, 2010; Kobayashi *et al.,* 2016). *Coptotriche angusticolella* had been previously reported in Turkey but hosts were not fully defined then and Rosaceae were reported as potential hosts for this species (Kobayashi *et al.,* 2016).

In the present study, infestation of *R. damascena* by *C. angusticollella* was recorded at Ardıçlı Village (37 47'51.0 N, 30 11'22.1 E, 974 m) in the district of Keçiborlu, which holds a considerable amount of the oil-

[1] Department of Plant Protection, Faculty of Agriculture, Suleyman Demirel University, Isparta, Turkey.
[2] Department of Field Crops, Faculty of Agriculture, Suleyman Demirel University, Isparta, Turkey.
[3] Department of Plant Protection, Faculty of Agriculture, Mustafa Kemal University, Hatay, Turkey.
* Corresponding author: asiyeuzun@sdu.edu.tr

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bearing rose cultivation areas (11.700 acres) in Turkey (Tüik, 2018). *Coptotriche angusticollella* was observed during the last half of May 2017 in a three year old conventional oil-bearing rose plantation (8 acres), where one third of the plants were infested. Larvae caused an opaque white gallery becoming a blotch mine on the upper or, occasionally, lower epidermis surface of the leaves (Figures 1a, 1b). Infested leaf samples were placed in paper bags and transferred to the laboratory in cold chain. The leaves were then placed into plastic boxes (long side 22-cm x 15-cm wide x 15-cm high) and kept in a climate-controlled room at 25±1°C, 60 % RH, and 16: 8 h photophase: scotophase. Larvae (Figure 2a) were easily visible within the translucent mines. The pupal stage was completed in the mines (Figure 2b). The emerged moths (Figure 2c) were identified by Dr Erik J. Van Nieukerken and kept in EMIT (Entomological Museum of Isparta, Turkey).

After flowering of the oil-bearing rose (15 May-end of June), observations were repeated during post harvest period (July-October) in the Ardıçlı village at other rose fields as well as Kermes Oak (*Quercus coccifera* L.) but no damage symptoms were found. Moreover, no natural enemy was obtained from the infested samples.

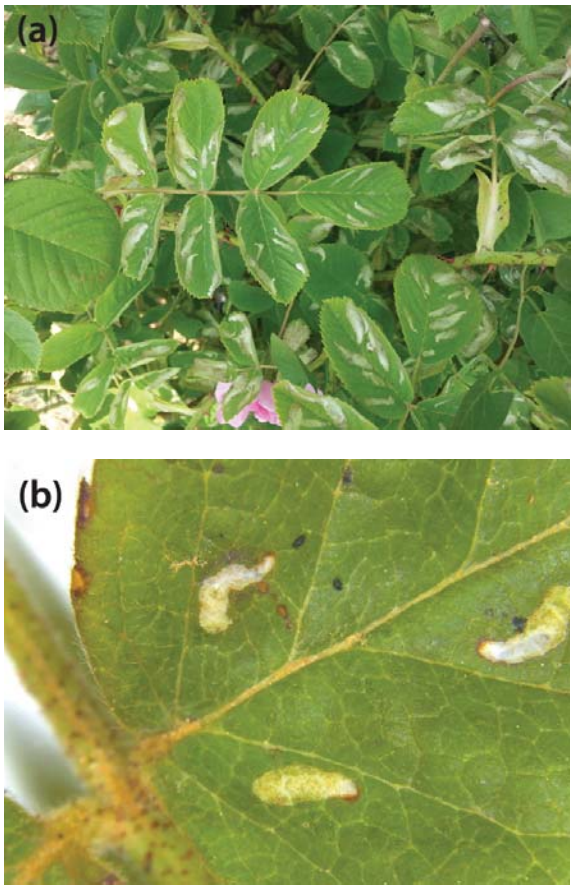

Figure 1. Infestation by *Coptotriche angusticollella* larvae on leaves of *Rosa damascena*: infested leaves (a); opaque white galleries (b).

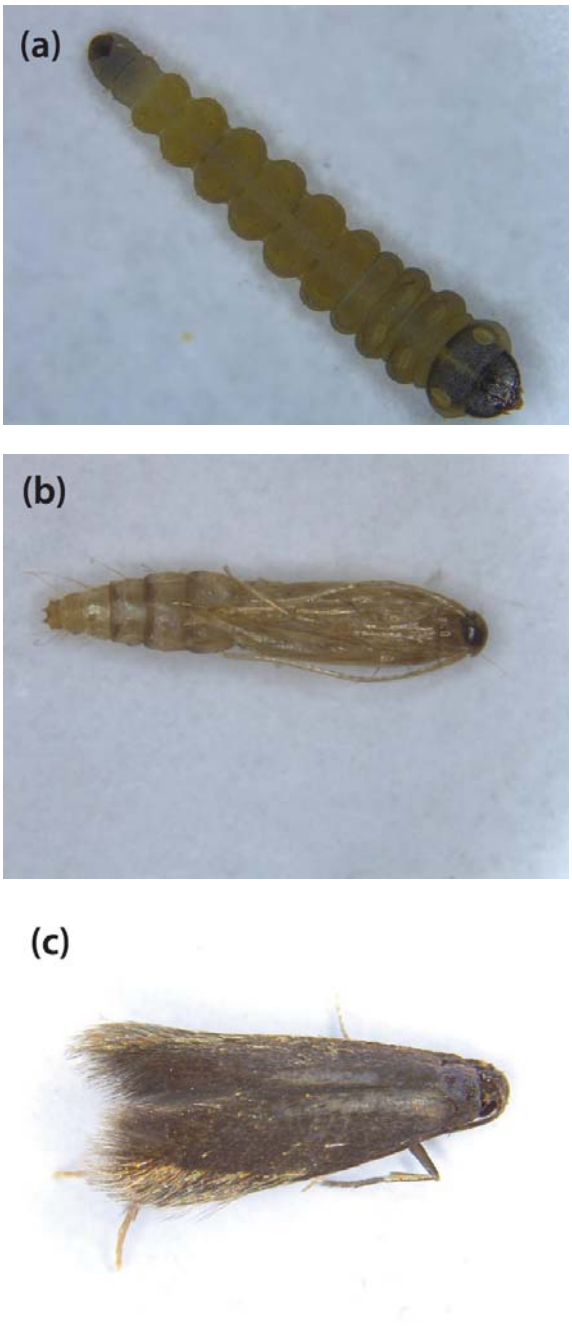

Figure 2. Larva (a), pupa (b) and adult (c) of *Coptotriche angusticollella.*

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We would like to draw the attention of researchers, growers and advisers to an intensive monitoring for the presence of symptoms from this minute leafminer in oil-bearing rose cultivations in Turkey during the next season in order to prevent an outbreak of the pest.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του *Coptotriche angusticollella* (Duponchel, 1843) (Lepidoptera: Tischeriidae) στο ρόδο *Rosa damascena* στην Τουρκία

O. Demirözer, A. Uzun, S. Erbaş and F. Can

Είδη του γένους *Coptotriche* spp. είναι γνωστό ότι προσβάλλουν φυτά των Οικογενειών Rosaceae και Fagaceae. Η εργασία αυτή αποτελεί την πρώτη καταγραφή του μικρολεπιδόπτερου *Coptotriche angusticollella* (Duponchel, 1843) (Lepidoptera: Tischeriidae) στο φυτό *Rosa damascena*, κοινώς ρόδο της Δαμασκού ή εκατοντάφυλλη τριανταφυλλιά, το Μάιο του 2017, στην περιοχή της πόλης Isparta της Τουρκίας. Το έντομο μπορεί να αποτελέσει εν δυνάμει κίνδυνο για την παραγωγή του ροδέλαιου, το οποίο παράγεται από την καλλιέργεια του φυτού.

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Lethal and sublethal effects of ten insecticides, used in date palm production in Saudi Arabia, on the parasitoid *Trichogramma cacoeciae*

M. Jamal Hajjar* and M. Al-Masoud

Summary Lethal and sublethal effects of ten insecticides commonly used in date palm production in Saudi Arabia were assessed in the laboratory against adults of *Trichogramma cacoeciae*, an important egg parasitoid of the dried fruit moth *Ephestia calidella*. Bioassays were conducted according to the standard protocol of the International Organization for Biological Control IOBC/WPRS/Working Group 'Pesticides and Beneficial Organisms'. Our results showed that cypermethrin, deltamethrin, malathion, phenthoate, methomyl, and carbosulfan were moderately harmful (IOBC Class 3) to the parasitoid. The botanical insecticides azadirachtin and matrine were moderately harmful (IOBC Class 3) and slightly harmful (IOBC Class 2), respectively. The insect growth regulator pyriproxyfen was slightly harmful, whereas bistrifluron was harmless (IOBC Class 1). Regarding sublethal effects, the parasitism ratios compared to control were reduced by pyriproxyfen and azadirachtin to 49.0% and 58.0%, respectively; hence they are classified as slightly harmful insecticides (IOBC Class 2). Bistrifluron and matrine were harmless (IOBC Class 1) as parasitism ratios were reduced by 9.2% and 27.6%, respectively. Longevity of adults exposed to bistrifluron and matrine (3.6 and 3.3 days, respectively) and to pyriproxyfen and azadirachtin (1.7 and 1.3 days, respectively) was significantly lower than that in control (4.67 days). In semi-field tests, residues of most insecticides on leaves of tomato, a common host plant of lepidopteran pests parasitized by *T. cacoeciae*, were considered moderately harmful to harmful based on parasitoid mortality at 24 h post-treatment whereas they were slightly harmful at 7 and 14 days post-treatment.

Additional keywords: longevity, mortality, parasitism, sublethal effects, *Trichogramma cacoeciae*, viability

Introduction

Species of *Trichogramma* (Hymenoptera: Trichogrammatidae) are important parasitoids in natural and agricultural ecosystems and act as effective biocontrol agents of lepidopteran pests in important crops such as tomato, soybean and cruciferous plants (Godfray, 1994; Beserra and Parra 1994; Moezipour *et al*., 2008; Polaszek, 2010).

The dried fruit moth *Ephestia calidella* (Guenée) (Pyralidae) is a pest of economic importance on date palm in Saudi Arabia, attacking dates during harvest, storage and packinghouse processing (El-Shafie *et al.,* 2017). Development of the pest and its damaging impacts on date palms are restricted by the use of the parasitoid *Trichogramma cacoeciae* Marchal (Rubeai *et al.,* 2003; El-Shafie *et al.,* 2017), which is commercially available and the most commonly used natural enemy in biological control programs (Hassan, 1993; Hassan *et al*., 2000).

Nevertheless, application of insecticides for the control of date palm pests cannot be excluded and sustainable use is foreseen in the frame of an Integrated Pest Management plan. Therefore, there is an urgent need to assess and quantify the risks of pesticides against natural enemies of date palm pests in order to minimize any adverse effects. Pesticides may cause lethal effects to non-target organisms in addition to sub-lethal behavioral and development effects, such as changes in rates of parasitism, longevity, sex ratio, and adult emergence (Desneux *et al.,* 2007; Firake and Khan, 2010; Firake *et al.,*

College of Agricultural and Food Sciences, King Faisal University, Saudi Arabia.
* Corresponding author: jamalnoura@yahoo.com

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2012; Blibech *et al.,* 2015).

Toxicity of different classes of insecticides to *Trichogramma* spp. vary. Synthetic pyrethyroid insecticides appear to be the most harmful class (Youssef *et al.,* 2004; Abdulhay and Rathi, 2014; Sohrabi and Amini, 2015; Thubru *et al.,* 2016); Furthermore, the effect of deltamethrin on pupae of *Trichogramma oleae* (Voegele and Poitale)*, T. cacoeciae*, and *T. bourarachae* Pintureau and Babault persisted for 30 days following exposure (Blibech *et al.,* 2015).

The neonicotinoid insecticide acetamiprid was harmful as regards adult emergence and rates of parasitism of *Trichogramma evanescens* Westwood (Jiu-Sheng *et al.,* 2010). Thiacloprid was harmful to *T. cacaoeciae* adults but harmless to larvae and pupae (Schuld and Schmuck, 2000).

Organophosphate insecticides have been shown to be harmful to eggs of *T. evanescens* and *Trichogramma platneri* Nagarkatti (Jiu-Sheng *et al.,* 2010; Brunner *et al.,* 2001) and moderately harmful to *T. cacoeciae* (Youssef *et al.,* 2004).

Insect growth regulators and insect growth inhibitors (fenoxycarb, diflubenzuron and lufenuron) have been reported not to be harmful to adults, pupae or eggs of *T. cacoeciae* (Hassan *et al.,* 1998; Brunner *et al.,* 2001; Abaar *et al.,* 2010). Consoli *et al.* (2001) found that lufenoron and triflumuron did not affect parasitism efficiency in *T. galloi* Zucchi, although they were harmful when applied to larvae and caused 100% death rate in adults when applied to eggs.

Abdelgader and Hassan (2012) reported that azadirachtin was harmful to *T. cacoeciae* adults, which were exposed to residues on glass plates and slightly to moderately harmful regarding adult emergence when applied to parasitized host eggs at different time intervals.

Herein, we studied lethal, sublethal and persistence effects of ten insecticides, which are commonly used in date palm production in Saudi Arabia, on *T. cacoeciae*. Adults of the parasitoid were exposed to dry residues of the highest recommended rates of the test insecticides thus at very high risk (Hassan *et al.,* 2000), likely resulting in the greatest degree of harm. The outcome of lethal/sublethal effects together with the results on the insecticide persistence will contribute to the selection of insecticides that pose lower risk to the parasitoid and to determine the conditions of safe use in IPM programs in date palm plantations (Grutzmacher *et al.,* 2004).

Material and Methods

Trichogramma cacoeciae cultures

Trichogramma cacoeciae was selected among *Trichogramma* spp. for the bioassays as it is easy to rear and handle in the laboratory (Hassan 1998). *Trichogramma cacoeciae* was obtained from a colony maintained at the Excellence Research Center for palms and dates at the King Faisal University. The colony originated from parasitized Lepidopteran eggs on leaves of cruciferous, tomato and other Solanaceae plants cultivated at Al-Hassa fields, Eastern province of Saudi Arabia. Adults of *T. cacoeciae* were reared on eggs of *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) (Suh *et al.,* 2000), in an insect growth chamber maintained at 23 ±2 ºC and 75 ±5% relative humidity (RH) with a 16:8 L:D cycle.

Insecticides

Lethal and sublethal effects on *T. cacoeciae* were assessed for 10 commonly used and commercially available insecticides in Saudi Arabia against pests of date palms. The insecticides are presented in Table 1 and contain active substances which belong to different chemical groups (pyrethroids, organophosphates, carbamates, Insect Growth Regulators and plant extracts).

Lethal effects of insecticides

Lethal effects of insecticides were assessed on *T. cacoeaciae* adults after exposure to dry residues on glass plates using the highest recommended application rates (Hassan *et al*., 2000). Glass tubes (2.5 x 20 cm) were treated by adding 5 ml of an aqueous insecticide solution which covered the inner surface; the tubes were then emptied and left to

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Table 1. List of insecticides used in the study.

Insecticides	Class	Recommended application rate (ml/L)	Manufacturing company
Cypermethrin (Hi power10% EC)	Pyrethroid	0.50	Sulphur mills limited- India
Deltamethrin (Flotron 2.5% EC)	Pyrethroid	0.35	Sulphur mills limited- India
Malathion (Sulmathion 57% EC)	Organic phosphorus	0.75	Sulphur mills limited- India
Phenthoate (Peston 50% EC)	Organic phosphorus	0.80	Astrachem - Saudi Arabia
Methomyl (Metho900 SP)	Carbamate	0.20	BASF Corporation – Germany
Carbosulfan (Marshal 25% WP)	Carbamate	0.50	Astrachem - Saudi Arabia
Bistrifluron (Hanaro 10% EC)	(IGR)	1.00	Astrachem - Saudi Arabia
Pyriproxyfen (Muligan 10% EC)	(IGR)	0.75	Parabolan- Spain
Matrine (Kingbo 0.6% EC)	Plant Extract	2.00	Beijing Kingbo Biotech- China
Azadirachtin (Amen 1.0% EC)	Plant Extract	2.50	Ecopheosides – India

dry for 3 h. Control tubes were treated with distilled water. Five replicates of glass tubes per insecticide treatment were used. Twenty adults of *T. cacoeciae*, which were 24 hours old, were transferred to each treated tube after drying, using a fine feather. The tubes were then sealed using cotton previously dipped in 50% honey solution and were kept in an incubator at 26 ±2°C, RH 75 ±5% RH and 16:8 L:D cycle. After 24 h of exposure to the dry insecticide residues, the effect on adult mortality was recorded. The classification system of the International Organization for Biological Control (IOBC) was used for the classification of the insecticide toxicity as: harmless (<30% mortality, Class 1); slightly harmful (30-79% mortality, Class 2); moderately harmful (80-99% mortality, Class 3); or harmful (>99% mortality) (Hassan *et al.,* 2000).

Sublethal effect on parasitism

Sublethal effect on parasitism were assessed on adult females of *T. cacoeciae*, which had survived the 24 h exposure to insecticide residues (bistrifluron 10% EC; pyriproxyfen 10% EC; matrine 0.6% EC; azadirachtin 1.0% EC, and control). Females were placed in glass tubes (2.5 x 20 cm), 6 individuals per tube, along with 100 ± 10 eggs of *E. cautella*, which were glued on cardboard tape (1cm^2). The females were provided with food through a cotton wool, which had been dipped in 50% honey solution, and served also as a closure of the tubes. Five tubes (replicates) were used per insecticide treatment and the control and were kept at 26 ±2°C, RH 75 ±5% RH at a 16:8 L:D cycle. The eggs of *E. cautella* were removed from the glass tubes after 24 h exposure to the parasitoids and placed in labeled Petri dishes corresponding to the replicates, in the incubator. After 9 days of incubation, the number of parasitized eggs was counted and parasitism ratios of the insecticide treatments were compared with the control. The IOBC classification (Hassan *et al.,* 2000) was used for the classification of insecticide toxicity and the data were corrected using the Abbott's formula (Abbott 1925).

Sublethal effects on parasitoid longevity

Sublethal effect of insecticides on parasitoid longevity was also assessed on adult females which survived 24 h exposure to the insecticide treatments. Female individuals were placed individually in clean glass tubes sealed with cotton wool, which had been dipped in 50% honey solution, and were incubated as described before. Five tubes (replicates) per insecticide treatment and the control were used. Longevity of the parasitoid as number of days until death was recorded.

Persistence effects on parasitoid mortality

The persistence effect of insecticides, ap-

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plied to tomato leaves, on *T. cacoeciae* adults was assessed. The experiment was conducted on tomato leaves because it is a common host for lepidopteran insects parasitized by *T. cacoeciae*. For this purpose, 75 tomato seedlings of the local cultivar Alhassawi were transplanted in five replicate plots, each measuring 2 x 3 m. Plants were grown in a mixture of peat moss (75% by vol.), native fine sand, perlite and vermiculite.

Each insecticide was applied to plants in four replicate plots, while the control (water) was applied to the fifth plot. Following treatment with insecticide or water, the plants were left to dry for three hours. Five tomato leaves, measuring 5-6 cm long, were randomly collected from the upper third of the plant in each plot of each insecticide treatment. Each set of five leaves, representing one replicate per treatment, was placed in a separate bag and transferred to the laboratory. Leaves were cut into 1 cm^2 pieces and placed in a test tube (5 x 1.5 cm) along with 20 x one day old adults of *T. cacoeciae* (Suh *et al*., 2000). The tubes were then sealed with cotton wool and kept in an incubator for 24 h, before mortality was recorded at 1, 2, 3, 7, 14 days post-treatment. Mortality ratio data were corrected using the Abbott's formula (Abbott, 1925).

Statistical analysis

The experiment was designed as a randomized complete block design using five replicates per treatment in addition to the control. Corrected percent mortality was calculated (Abbott 1925) and data were analyzed using Analysis of Variance (ANOVA). Separation of the means was conducted using the Fisher's protected Least Significant Difference test (PLSD), ($P < 0.05$) (Steel *et al.,* 1997). The analyses were performed using SPSS 12.0 Windows (SPSS Inc., 2003).

Results and Discussion

Lethal Effects

Survival and corrected mortality of adult females of *T. cacoeciae* after 24 h exposure to dry insecticide residues differed among the insecticides (Table 2). Cypermethrin, deltamethrin, malathion, phenthoate, methomyl and carbosulfan, were moderately harmful

Table 2. Survival and corrected mortality of adult females of the egg parasitoid *Trichogramma cacoeciae*, when exposed to dry residues of insecticides on glass surfaces treated at the recommended application rates.

Treatment		Adult survival	Corrected mortality (E%)	IOBC Classification
Insecticide	Class			
Cypermethrin (Hi power)	Pyrethroid	2.00 ± 0.77 gh	98.00	3
Deltamethrin (Flotron 2.5%)		4.33 ± 0.26 efg	95.67	3
Malathion (Sulmathion 57%)	Organophosphate	1.00 ± 0.45 h	99.00	3
Phenthoate (Peston 50%)		3.33 ± 0.26 fgh	96.67	3
Methomyl (Metho900)	Carbamate	1.00 ± 0.45 h	99.00	3
Carbosulfan (Marshal 25%)		5.00 ± 0.45 ef	95.00	3
Bistrifluron (Hanaro 10%)	IGR	92.67 ± 0.7 b	7.33	1
Pyriproxyfen (Muligan 10%)		40.33 ± 0.68 d	59.76	2
Matrine (Kingbo 0.6%)	Plant Extract	50.00 ± 0.89 c	50.00	2
Azadirachtin (Amen 1.0%)		6.67 ± 1.37 e	93.33	3
Control		100.0 ± 0.00 a		-
LSD ($P > 0.05$) = 28.639				
F value = 0.041				

All values are means of 5 replicates ± SE. Means in the same column followed by the same letter are not significantly different (Fisher's test, $P > 0.05$). IOBC insecticide classification, where Class 1: harmless ($E < 30\%$); Class 2: slightly harmful ($30\% < E < 79\%$); Class 3: moderately harmful ($80\% < E < 99\%$); and Class 4: harmful ($E > 99\%$).

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(IOBC class 2) to the parasitoid *T. cacoeciae*, according to the IOBC classification system (Hassan *et al.,* 2000). Similar moderate to high toxicities of organophosphorus and synthetic pyrethroid compounds to adults of *T. cacoeciae* and other *Trichogramma* spp. have also been reported in other studies (Brunner *et al.,* 2001, Youssef *et al.,* 2004; Jiu-Sheng *et al.,* 2010, Zhu *et al.,* 2009; Abaar *et al.,* 2011; Sohrabi and Amini, 2015; Thubru *et al.,* 2016).

The plant extract azadirachtin (Amen 1.0%) was moderately toxic, supporting data from Thubru *et al.* (2016) on another egg parasitoid, *Trichogramma brassicae* (Bezdenko).

The insect growth regulator (IGR) pyriproxyfen (Muligan 10%) and the plant extract matrine (Kingbo 0.6%) are slightly harmful (IOBC class 3) to the parasitoid *T. cacoeciae* while the IGR bistrifluron is harmless (corrected mortality did not exceed 7.3%; IOBC class 1).

Sublethal effects

Parasitism ratio of *E. cautella* eggs by female parasitoids of *T. cacoeciae*, which had survived 24h exposure to dry insecticide residues, was significant lower compared to the control, while it differed among the tested insecticides (Table 3). Similarly, longevity of adult females of *T. cacoeciae* that survived initial 24h exposure to the insecticide residues was significantly lower than that of the control but there were variations in the effect among the insecticides (Table 3).

Based on parasitism ratios of *T. cacoeciae* on *E. cautella,* the IGRs pyriproxyfen and bistrifluron are classified as slightly harmful (IOBC class 2) and harmless (IOBC class 1) to *T. cacoeciae*, respectively. The plant extracts azadirachtin and matrine are classified as slightly harmful (IOBC class 2) and harmless (IOBC class 1) to the parasitoid, respectively. In terms of effect on longevity, the most harmful insecticides to the parasitoid were pyriproxyfen and azadirachtin (Table 3).

Regarding IGRs, Consoli *et al.* (1998) and Hassan *et al.* (1998) found that lufenuron was slightly harmful to *T. cacoeciae*. However, Abaar *et al.* (2010) reported that fenoxycarb, diflubenzuron, and lufenuron were not harmful to pupae or toxic to *T. cacoeciae* eggs and the low toxicity levels of IGR in *T. cacoeciae* larvae have been reflected in the high rates of adult emergence compared to the control.

Azadirachtin, although a botanical insecticide, was found to be moderately harmful to *T. cacoeciae* regarding mortality and slightly harmful regarding parasitism ratio. Our results are in conflict with those reported by Thubru *et al.* (2016), who found that surface contact toxicity of azadirachtin was

Table 3. Parasitism ratio of *Trichogramma cacoeciae* on eggs of *Ephestia cautella* and longevity of adult females of the parasitoid, which survived 24 h exposure to dry residues of insecticides on glass plates treated at the recommended application rates.

Treatment		% hatching after nine days ± SE	Reduction in parasitism rate (E%)	IOBC Classification	Longevity (days)
Insecticide	Class				
Bistrifluron (Hanaro 10%)	IGR	83.80 ± 4.40 b	9.21	1	3.60 ± 0.20 b
Pyriproxyfen (Muligan 10%)		47.13 ± 4.21 d	48.98	2	1.67 ± 0.12 c
Matrine (Kingbo 0.6%)	Plant Extract	66.73 ± 2.61 c	27.64	1	3.27 ± 0.12 b
Azadirachtin (Amen 1.0%)		40.93 ± 3.52 e	57.95	2	1.33 ± 0.12 c
Control		92.33 ± 4.04 a			4.67 ± 0.58 a
LSD (P > 0.05) = 25.345					LSD (P > 0.05) = 1.622
F value = 0. 046					LSD (P > 0.05) = 1.622

All values are means of 5 replicates ± SE. Means in the same column followed by the same letter are not significantly different (Fisher's test, P > 0.05). IOBC insecticide classification, where Class 1: harmless (E < 30%); Class 2: slightly harmful (30% < E < 79%); Class 3: moderately harmful (80% < E < 99%); and Class 4: harmful (E > 99%).

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slightly harmful to adults of *T. brassicae* mortality, while it was harmful to parasitism ratio and longevity of the parasitoid. These differences may be due to differences in sensitivity to insecticides among *Trichogramma* species as well as the host species upon which the parasitoid species are reared (Brunner *et al.,* 2001).

To summarize, the two IGRs (bistrifluron, pyriproxyfen) and the plant extract matrine were the least harmful insecticides to adults of *T. cacoeciae* in terms of lethal effect (mortality after 24h exposure) as well as sublethal effects (parasitism ratio on *E. cautella* and parasitoid longevity). Bistrifluron and pyriproxyfen have been classified in the IOBC database as harmless to *T. cacoeciae* (IOBC, 2005). Our results support this classification for bistrifluron but not for pyriproxyfen, which was classified as slightly harmful. This difference in classification may be due to variability in sensitivity to pesticides among *Trichogramma* spp., caused by host rearing conditions e.g. size of host egg that affects the growth and development of parasitoids (Suh *et al.,* 2000; Goulart *et al.,* 2008; Hegazi and Khafagi, 2001).

Insecticide Persistence

Mortality of the parasitoid after exposure to aged residues of the tested insecticides on tomato leaves differed among the insecticides and different age of residues (Table 4). Cypermethrin and phenthoate resulted in 100% mortality of adult females exposed to one day old residues and were classified as harmful (IOBC class 4) to *T. cacoeciae*. One day old residues of deltame-

Table 4. Mean % survival of adult females of the egg parasitoid *Trichogramma cacoeciae*, when exposed to aged insecticide residues (persistence of insecticide effect).

Insecticide	Class	Mean adult survival (%)					IOBC Classification
		Days after treatment					
		1	2	3	7	14	
Cypermethrin (Hi power)	Pyrethroid	0.00 d	5.00 d	14.00 d	31.00 e	39.00 de	4
Deltamethrin (Flotron 2.5%)		4.00 cd	6.00 d	16.00 d	29.00 ef	42.00 d	3
Malathion (Sulmathion 57%)	Organophosphate	2.00 cd	9.00 d	18.00 d	26.00 ef	34.00 ef	3
Phenthoate (Peston 50%)		0.00 d	7.00 d	15.00 d	24.00 fg	24.00 g	4
Methomyl (Metho900)	Carbamate	7.00 cd	8.00 d	13.00 d	25.00 efg	31.00 fg	3
Carbosulfan (Marshal 25%)		7.00 cd	12.00 a	15.00 d	25.00 efg	36.00 def	3
Bistrifluron (Hanaro 10%)	IGR	1.00 b	83.00 b	84.00 b	86.00 b	94.00 a	1
Pyriproxyfen (Muligan 10%)		77.00 b	77.00 b	49.00 b	80.00 c	85.00 b	1
Matrine (Kingbo 0.6%)	Plant Extract	41.00 d	45.00 c	47.00 c	49.00 d	52.00 c	2
Azadirachtin (Amen 1.0%)		9.00 c	10.00 d	13.00 d	19.00 g	26.00 g	3
Control		20a					
LSD (P > 0.05)		25.503	20.533	24.093	27.348	17.855	
F value		0.007	0.249	0.041	0.044	0.041	

All values are means of 5 replicates. Means in the same column followed by the same letter are not significantly different (Fisher's test, P > 0.05). IOBC insecticide classification where Class 1: harmless (E < 30%); Class 2: slightly harmful (30% < E < 79%); Class 3: moderately harmful (80% < E < 99%); and Class 4: harmful (E > 99%).

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thrin, malathion, methomyl, azadirachtin, and carbosulfan, causing 96, 98, 93, 91 and 93% adult mortality, respectively, as compared with the control, are classified as moderately harmful (IOBC class 3) to *T. cacoeciae*. Our results corroborate those reported by Youssef *et al.* (2004) for *T. exgium* on olive leaves, and Blibech *et al.* (2015) who found that deltamethrin residues on olive leaves affected parasitism in *T. oleae, T. cacoeciae*, and *T. bourarachae*, 31 days post-treatment. Nevertheless, Suh *et al.* (2000) showed that deltamethrin has short-term persistence on cotton leaves, and this difference in persistence may be attributed to variation among *Trichogramma* spp. sensitivity to pesticides (Goulart *et al.,* 2008) and pesticide interactions with plant leaf types or climate conditions (Bueno *et al.,* 2008).

One day old residues of the IGR insecticides, bistrifluron and pyriproxyfen, caused 19% and 23% mortality of *T. cacoeciae*, respectively, and were classified as harmless. One and 14 days post-treatment residues of the botanical insecticide matrine caused 59% and 48% mortality, respectively, and were classified as slightly harmful. According to Brunner *et al.* (2001) the benzoylhydrazin IGRs, tebufenozid and methoxyfenozide, on treated Oregon spur apple tree leaves at leaf-disk bioassays produced no sublethal effect on *Colpoclypeus florus* (Hym.: Eulophidae) and *Trichogramma platneri*, which are potential biological control agents of leafrollers in apple orchards.

Overall, toxicity of insecticide residues on tomato leaves to *T. cacoeciae*, from one to 14 days after treatment varied among the insecticides (Table 4). However, most of them were considered moderately harmful to harmful 24 h post-treatment. Residue toxicity reduced with time and became moderately harmful, 2 and 3 days after treatment and slightly harmful at 7 and 14 days. Zhu *et al.* (2009) also noted that persistence of insecticide residues on leaf surfaces differed among pesticides and that the effects on the natural enemy *T. evanescens* in their study decreased with time. Also, the understudy IGR aged residues were harmless at all time points after -application and these results are consistent with those reported by Hassan *et al.* (1998).

In conclusion, most of the tested insecticides were moderately harmful to harmful to *T. cacoeciae*, except for the IGR insecticides, which can be considered further for use in a sustainable IPM programme against major Lepidopteran pests of date palm.

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Επίδραση δέκα εντομοκτόνων που εφαρμόζονται στην καλλιέργεια της χουρμαδιάς στη Σαουδική Αραβία, στο παρασιτοειδές *Trichogramma cacoeciae*

M. Jamal Hajjar and M. Al-Masoud

Η επίδραση δέκα εντομοκτόνων, που εφαρμόζονται συνήθως στην καλλιέργεια της χουρμαδιάς στη Σαουδική Αραβία, αξιολογήθηκε στο εργαστήριο σε ενήλικα άτομα του *Trichogramma cacoeciae*, ενός σημαντικού ωοπαρασιτοειδούς του λεπιδόπτερου *Ephestia calidella*. Οι βιοδοκιμές διεξήχθησαν σύμφωνα με το πρωτόκολλο του Διεθνούς Οργανισμού IOBC/WPRS/Ομάδα Εργασίας 'Pesticides and Beneficial Organisms'. Τα αποτελέσματα έδειξαν ότι τα σκευάσματα με δραστικές ουσίες cypermethrin, deltamethrin, malathion, phenthoate, methomyl και carbosulfan ήταν μέτρια επιβλαβή (κλάση IOBC 3) στο παρασιτοειδές. Από τα βοτανικά εντομοκτόνα, το σκεύασμα με δραστική azadirachtin ήταν μέτρια επιβλαβές (κλάση IOBC 3) ενώ αυτό με τη δραστική matrine ήταν ελαφρώς επιβλαβές (κλάση IOBC 2). Ο ρυθμιστής ανάπτυξης των εντόμων pyriproxyfen ήταν ελαφρώς επιβλαβής ενώ το σκεύασμα με δραστική bistrifluron ήταν αβλαβές (κλάση IOBC 1). Όσον αφορά στις έμμεσες αρνητικές επιδράσεις των εντομοκτόνων, το ποσοστό παρασιτισμού σε σύγκριση με το μάρτυρα μειώθηκε από τα σκευάσματα με δραστικές pyriproxyfen και azadirachtin σε 49,0% και 58,0%, αντίστοιχα, επομένως τα εν λόγω εντομοκτόνα ταξινομούνται ως ελαφρώς επιβλαβή (κλάση IOBC 2) στο *T. cacoeciae*. Τα σκευάσματα με δραστικές bistrifluron και matrine ήταν αβλαβή (κλάση IOBC 1) καθώς τα ποσοστά παρασιτισμού μειώθηκαν κατά 9,2% και 27,6% αντίστοιχα. Η μακροβιότητα των ενήλικων ατόμων του παρασιτοειδούς που εκτέθηκαν σε bistrifluron και matrine (3,6 και 3,3 ημέρες, αντίστοιχα) και σε pyriproxyfen και azadirachtin (1,7 και 1,3 ημέρες, αντίστοιχα) ήταν σημαντικά χαμηλότερη από αυτή του μάρτυρα (4,67 ημέρες). Σε δοκιμές σε συνθήκες ημι-υπαίθρου, τα υπολείμματα των περισσότερων εντομοκτόνων σε φύλλα τομάτας, κοινό φυτό-ξενιστή λεπιδοπτέρων που παρασιτούνται από το *T. cacoeciae*, ήταν μέτρια επιβλαβή έως επιβλαβή με βάση τη θνησιμότητα του παρασιτοειδούς, 24 ώρες μετά την εφαρμογή, ενώ ήταν ελαφρώς επιβλαβή, 7 και 14 ημέρες μετά την εφαρμογή.

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Changes in salicylic acid content and pathogenesis - related (*PR2*) gene expression during barley - *Pyrenophora teres* interaction

A. Al-Daoude*, M. Jawhar, E. Al-Shehadah, A. Shoaib, M. Orfi and M.I.E Arabi

Summary Net blotch (NB), caused by the necrotrophic fungal pathogen *Pyrenophora teres f. teres*, substantially reduces barley grain yield and quality worldwide. The role of salicylic acid (SA) signaling in NB resistance has been poorly documented. In this study, SA levels as well as the expression of the SA-responsive gene *PR2* were monitored in infected leaves of two barley genotypes, Banteng (resistant) and WI2291 (susceptible), at different time points of infection. SA signaling was activated in bothgenotypes 24 hours post infection (hpi) as compared with non-inoculated plants. However, with or without pathogen pretreatment, SA significantly increased (P=0.001) in Banteng comparing with WI2291. RT-PCR analysis revealed that *PR2* expression increases in the resistant and susceptible genotypes over the inoculation time points, with maximum expression (6.4 and 1.99-fold, respectively) observed 6 dpi. *PR2* expression was paralleled by an increase in leaf SA content as shown by the test coincidence ($F_{3, 32} = 4.74$, $P = 0.001$). Based on barley genotype resistance levels, our data strengthen the idea that SA signaling and *PR2* play a role in barley NB reduction.

Additional keywords: barley, *Pyrenophora teres*, *PR2* gene expression, RT-PCR, salicylic acid

Introduction

Net blotch, caused by the fungal pathogen *Pyrenophora Drechs. teres* Smedeg. (anamorph: *Drechslera teres* [Sacc.] Shoem. f. *Teres* Smedeg.), is a common foliar disease of barley (*Hordeum vulgare* L.), a disease responsible for heavy crop losses (Liu *et al.,* 2011; Wang *et al.,* 2015). Various mechanisms for NB resistance and susceptibility appear to operate in barley. *Pyrenophora teres* activates different defense responses which are regulated through different plant signaling pathways, including plant hormones such as SA and pathogenesis-related (PR) proteins (Wang *et al.,* 2011; Bogacki *et al.,* 2008).

A number of studies have demonstrated that SA signaling pathways play important roles in resistance against fungal pathogens in plants (Trusov *et al.,*2009; Zwart *et al.,* 2017). Therefore, discovery of SA targets and the understanding of their molecular modes of action in physiological processes could help in the dissection of the SA signaling network, confirming its important role in plant responses to fungal diseases (Vásquez *et al.,* 2015). After a pathogen attack SA levels often increase and lead to the induction of PR expression and the development of systemic acquired resistance and hypersensitive response. Furthermore, SA appears to regulate the delicate balance between pro- and after- cell death functions during hypersensitive response (Dorey *et al.,* 1997; Alvarez, 2000).

Barley plants produce enzymes that digest fungal cell walls to stop fungal penetration. However, since all true fungi contain chitin as a primary structural component of their cell walls, the chitinase family of PR proteins is of particular importance (Wessels, 1994). Chitin in fungal cell walls can be hydrolyzed by chitinases into smaller oligomers or monomers (Bishop *et al.,* 2002), so PR proteins such as *PR2* are known to play a major role during plant–pathogenic fungus interactions (Collinge *et al.,* 1993; Dangl and Jones, 2001).

Quantitative PCR (qPCR) is now a well-

Department of Molecular Biology and Biotechnology, AECS, P.O.Box 6091 Damascus, Syria
*Corresponding author: scientific@aec.org.sy

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established method forquantifying the relative expression level of a particular transcript and determines its expression after being exposed to a specific alteration, such as pathogen infection (Kralik and Ricchi 2017). In the present work, we studied the defense responses of two barley genotypes Banteng and WI 2291, which are integrated in international breeding programs aimed at developing NB resistant barley genotypes. Banteng was described as a highly resistant to *P. teres* (Arabi *et al.,* 2003), i.e. exhibited a lower level (compared with WI2291) of NB symptom development. We thus hypothesized that SA-triggered defenses could drive contrasted levels of resistance in Banteng and WI2291, inoculated by the same pathogen isolate. Thus, the aim of the current study was to evaluate the changes in SA content and induction of *PR2* gene expression in two barley cultivars with different resistance to *P. teres*.

Materials and Methods

Plant materials and pathogen inoculation

The German genotype Banteng has proved to be the most resistant genotype to all NB isolates available so far under field and greenhouse conditions for over fifteen years (Arabi *et al*., 2003). For this reason, it was chosen and used in this study. A universal susceptible control genotype (cv. WI2291) from Australia was also included in the experiments. The *P. teres* single conidium isolate (NB4) tested was the most Syrian virulent pathotype to all barley genotypes available up to now (Arabi *et al*., 2003). The fungus was incubated in Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI, USA) for 8 days at 20-22°C in the dark. Conidia were collected in 10 mL sterile distilled water and the suspension was adjusted to 2 x 10^4 conidia/mL using hemacytometer. A surfactant (polyoxyethylene-20-sorbitan monolaurate) was added (100 µL/L) to the conidial suspension to facilitate dispersion of the inoculum over leaf surfaces. *Pyrenophora teres* inoculum preparation, inoculation, and post-inoculation were similar to those described by Abu Qamar *et al.* (2008). Barley plants were grown in the greenhouse and inoculated at the two- to three-leaf stage with the second leaf fully expanded.

SA quantification

Pooled samples containing the fourth leaf of 20 independent plants/genotype where used for SA quantification. Pooled samples were prepared from leaves taken 24, 48 and 72 hpi, respectively. For each time case studied, six pooled sample replicates were used for quantification. SA was extracted from approximately 200 mg of freshly ground leaves in 1.5 ml tubes following the method described by Trapp *et al.* (2014), with minor modifications. Briefly, 100 mg of plant material were dried overnight in a freeze drier at −42°C. The extraction was achieved by adding 1.0 mL of ethyl acetate, dichloromethane, isopropanol, MeOH:H_2O into each tube containing dry plant material. Samples were shaken for 30 min and centrifuged at 16,000 g and 4°C for 5 min. The supernatant was transferred into a new 1.5 micro-centrifuge tube and dried in a speed vac. After drying, 100 µL of MeOH was added to each sample, homogenized under vortex and centrifuged at 16,000 g and 4°C for 10 min. The supernatant was analyzed by a high-performance liquid chromatography coupled mass spectrophotometer (HPLC-MS/MS) system (Agilent Technologies, Böblingen, Germany). Changes in SA content were compared to the control for each time point. Six independent repetitions were performed for each time point. Data were analyzed using the standard deviation and t-test methods.

RNA isolation and cDNA synthesis

Primary leaves from three individual biological replicates were collected at 24, 48 and 72 hpi, and homogenized with a tube pestle in liquid nitrogen. mRNA was extracted with the Nucleotrap mRNA mini kit (Macherey-Nagel, MN, Germany) following the

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manufacturer's instructions. RNA was used for cDNA synthesis with the QuantiTect Reverse Transcription Kit (Qiagen) following the manufacturer's instructions and the resulting cDNA was stored at –20°C. At the same time points, samples from mock inoculated plants were collected as controls.

Semi quantitative RT-PCR

PCR primers for *PR2* were designed based on the cDNA sequences of barley available at NCBI (http://www.ncbi.nlm.nih.gov) database (Id:M23548.1) using Primer 3 software (5′ CAGCGAATGCTCCAATGAAGA 3′ and 5′ TACCCTGCCGTGAACATCAAG 3′). PCR reactions were performed in a 50-µL final volume including 1µL of ten times diluted cDNA template, 5 µL of 10X amplification buffer (Thermo Scientific, USA), 1 µL of 200 µM deoxynucleotide triphosphates (Thermo Scientific, USA), 1 µL of 10 pico-molar of each primer, 0.2 µL (1 U) of Taq DNA polymerase (MBI Fermentas, York, UK) and 40.8 µL of PCR grade water. PCR reactions were performed on a thermocycler (Biometra) with the following program: an initial denaturing step at 94°C for 4 min, followed by 30 cycles of 94°C for 30 s, 57°C for 30 s, 72°C for 1 min with a final extension at 72°C for 10 min. PCR products were separated using 1% agarose gels, stained with ethidium bromide and observed on a UV transilluminator. PCR was performed three times for each primer using the same cDNA sample in order to confirm the reproducibility of the results.

qRT-PCR assay

Quantitative real-time PCR (qPCR) was performed using the method described by Derveaux *et al.* (2010). Data was checked by qRT-PCR dissociation curve analysis using stepone software (v2.3). The fluorescence readings of six replicated samples were averaged and the blank value (without DNA control) was subtracted. *PR2* relative expression levels were determined using the average cycle threshold (CT). Average CT values were calculated from the triplicate experiment conducted for each gene, with the ΔCT value determined by subtracting the average CT value of genes from the CT value of *EF1α* gene. Finally, the equation $2^{-\Delta\Delta CT}$ was used to estimate *PR2* relative expression level (Livak and Schmittgen, 2001). Standard deviation was calculated from the replicated experimental data. The statistical analysis was conducted through the Tukey's test at the 0.05 level. The assumption of coincidence was tested using the ANOVA procedure implemented in the software package Statistica 6.1.

Results and Discussion

In this study, we used two barley genotypes with different resistance to *P. teres* infection. As shown in Figure 1, *P. teres* produced net-like striated lesions surrounded by chlorosis or necrosis, and these symptoms were more severe on the susceptible genotype 'WI2291' after 10 days of infection. These results are in agreement with our previous observations under natural field conditions (Arabi *et al.,* 2003).

Further studies of barley-*P. teres* interactions by measuring changes in the leaf SA content and *PR2* gene expression at four early time points after pathogen challenge, showed that SA levels of infected barley leaves increased 24hpi in comparison with non-inoculated plants (Fig. 2). With or without pathogen pretreatment, the tolerant genotype Banteng contained three-fold or greater total SA than the susceptible genotype WI2291 (24hpi). It was found that Banteng contained significantly (P=0.001) higher levels of total SA thanWI2291 a teach time point investigated (Fig. 2), which might reflect the expected role of SA in signaling events during *P. teres* infection. This result could support the findings published by Häffner *et al.* (2014), stating that the endogenous SA level in a plant is the main cause of susceptibility *versus* resistance in barley, since pathogen infection may induce plant responses regulated by SA. In addition, SA accumulation has been widely used as a reliable marker of elevated defense responses

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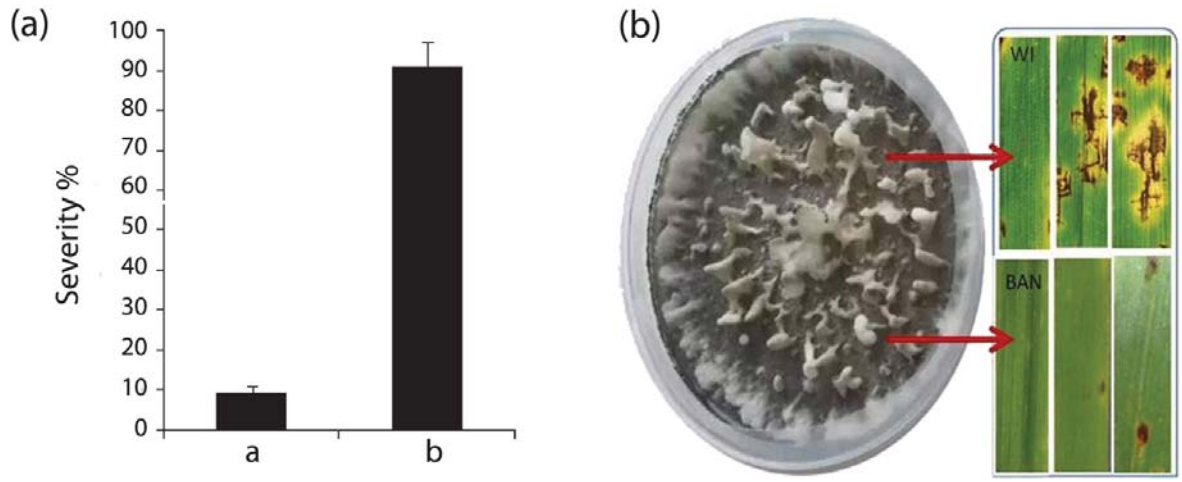

Figure 1. a) Frequency of disease reactions incited on barley (a) resistant cv. Banteng and (b) susceptible cv. WI2291, 10 days after *Pyrenophora teres* infection. b) Disease symptoms on the resistant (BAN) and susceptible (WI) barley genotypes, which were measured using the scale described by Abu Qamar *et al.* (2008).

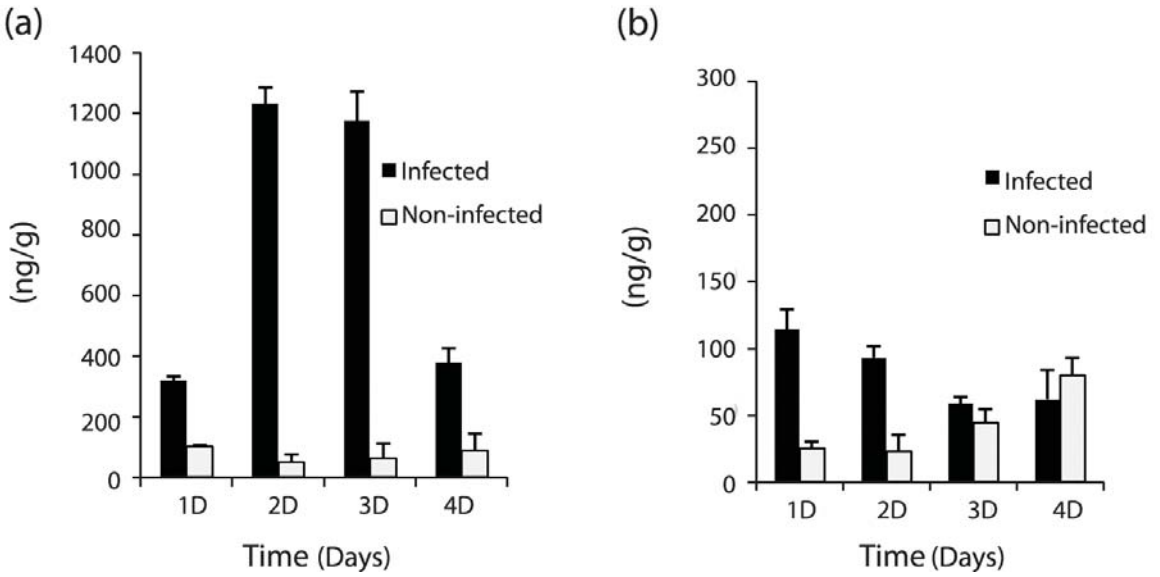

Figure 2. Quantification of total salycilic acid in barley leaves 1, 2, 3 and 4 days post inoculation with *Pyrenophora teres* in (a) the resistant cv. Banteng and (b) the suscepitible cv. WI2291. Error bars represent the stantard error of the means ($n = 3$).

and is closely associated with redox homeostasis, hypersensitive response, or systemic acquired resistance (Alvarez, 2000; Dong, 2004).

Semi quantitative RT-PCR analysis demonstrated that attack of barley by *P. teres* induced *PR2* accumulation in infected plants as compared with the un-infected controls and it was inversely regulated 24h post inoculation i.e, it was repressed in the susceptible cultivar WI2291 while being induced in the tolerant genotype Banteng (Fig. 3). Moreover, RT-PCR expression analysis revealed that the *PR2* expression increased in the resistant and susceptible genotypes over the inoculation time points, with the highest expression (6.4 and 1.99–fold for Banteng and WI2291, respectively) observed at 6 dpi. *PR2*

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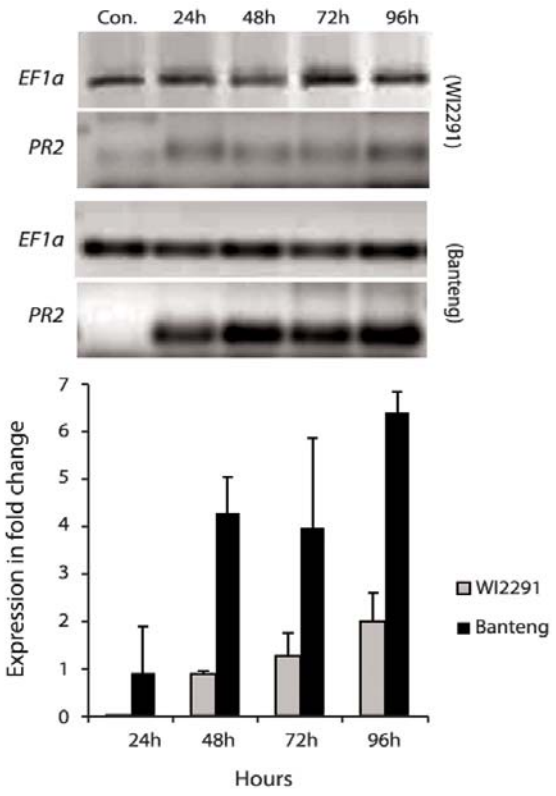

Figure 3. Relative expression profiles of *PR2* gene in the resistant cv. Banteng and in the susceptible cv. WI2291, 24, 48, 72 and 96 hours after infection by *Pyrenophora teres*.

encodes for a 1,3-ß-glucanase (Simmons, 1994), belonging to the glycoside hydrolases family (Opassiri *et al*., 2010). 1, 3-ß-glucanase hydrolyses the ß-*O*-glycosidic bond of ß-glucan in plant cell walls, resulting in cell wall loosening and expansion (Akiyama *et al*., 2009). This phenomenon may be the cause of barley cell wall leakage during *NB* infestations.

Our data showed that *PR2* gene exhibited a differential expression (P=0.01) in the tolerant and susceptible barley genotypes and was closely related to the increase of the SA level. The SA marker *PR2* was upregulated 3-fold in infected leaves of the tolerant Banteng than in the susceptible WI2291 (Fig. 2). *PR2* expression was paralleled by an increase in leaf salicylic acid (SA) content as shown by the coincidence test ($F_{3, 32} = 4.74$, $P = 0.001$). This is supported by previous works indicating that SA is involved in the regulation of induced immunity in barley through the induction of PR proteins with chitinase, β-1, 3-glucanase and peroxidase enzyme activity, both locally and systemically (Bindschedler *et al.,* 1998).

Our data show that the contribution of the SA pathway to the resistance response appears to depend on the plant genotype. The NB tolerant genotype Banteng used for this study was proved to be the most resistant genotype to all *P. teres* isolates available so far. The higher activities of the selected defense genes such as *PR2* and higher level of SA in infected Banteng leaf tissues compared with the susceptible genotype WI 2291 may explain its high level of resistance.

This study provides information about the role of SA in resistance of barley against the necrotrophic foliar pathogen *P. teres*. In addition, it highlights that SA may increase in response to *P. teres* infection in different barley genotypes. It is also noteworthy that *PR2* has a higher constitutive expression and faster induction in the tolerant genotype as compared with the susceptible one. Our results suggested that not only SA is important for the induction of defense-like responses but, in the absence of pathogen attack, SA may sustain basal expression levels of genes associated with resistance responses and may keep the defense system primed.

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Μεταβολές στην περιεκτικότητα του σαλικυλικού οξέος και στην έκφραση του σχετιζόμενου με την παθογένεια γονιδίου *PR2*, στο κριθάρι κατά την αλληλεπίδραση με το μύκητα *Pyrenophora teres*

A. Al-Daoude, M. Jawhar, E. Al-Shehadah, A. Shoaib, M. Orfi and M.I.E Arabi

Περίληψη Η ασθένεια του κριθαριού που είναι γνωστή διεθνώς ως «net blotch» και προκαλείται από το μύκητα *Pyrenophora teres f. teres*, μειώνει σημαντικά την απόδοση και την ποιότητα των σπόρων κριθαριού παγκοσμίως. Ο ρόλος της σηματοδότησης του σαλικυλικού οξέος (SA) ως προς την αντοχή στην ασθένεια δεν έχει τεκμηριωθεί επαρκώς. Σε αυτή τη μελέτη, καταγράφηκαν τα επίπεδα του SA καθώς και η έκφραση του σχετιζόμενου με την σηματοδότηση του SA, *PR2* γονιδίου σε μολυσμένα φύλλα δύο γονότυπων κριθαριού, του Banteng (ανθεκτικό) και WI2291 (ευαίσθητο), σε διαφορετικά χρονικά σημεία της μόλυνσης. Η σηματοδότηση του SA ενεργοποιήθηκε και στους δύο γονότυπους, 24 ώρες μετά τη μόλυνση (hpi) σε σχέση με το μάρτυρα. Ωστόσο, με ή χωρίς την εφαρμογή παθογόνου, το SA αυξήθηκε σημαντικά (P = 0,001) στον ανθεκτικό γονότυπο Banteng συγκριτικά με τον ευαίσθητο WI2291. Η ανάλυση με RT-PCR αποκάλυψε ότι η έκφραση του *PR2* αυξάνει στους ανθεκτικούς και ευαίσθητους γονότυπους μετά την εφαρμογή του παθογόνου, με τη μέγιστη έκφραση (6,4 και 1,99 φορές, αντίστοιχα) να παρατηρείται στις έξι ημέρες μετά την εφαρμογή του παθογόνου. Η έκφραση του *PR2* συνοδεύτηκε με παράλληλη αύξηση της περιεκτικότητας του SA στα φύλλα του κριθαριού (coincidence test , $F_{3, 32} = 4.74$, P = 0,001). Τα αποτελέσματα ενισχύουν την άποψη ότι η σηματοδότηση του SA και το γονίδιο *PR2* σχετίζονται με τη μείωση της ασθένειας «net blotch» στο κριθάρι.

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