Evaluation of Safflower Varieties for Resistance to the Fungal Pathogen, *Colletotrichum acutatum*

Jana VÍCHOVÁ[1], Barbora STAŇKOVÁ[1], Radovan POKORNÝ[1] and Karel VEJRAŽKA[2]

[1]Department of Crop Science, Breeding and Plant Medicine, Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic; [2]Agricultural Research, Ltd. Troubsko, Troubsko, Czech Republic

Abstract

Víchová J., Staňková B., Pokorný R., Vejražka K. (2013): **Evaluation of safflower varieties for resistance to the fungal pathogen,** *Colletotrichum acutatum.* Plant Protect. Sci., **49**: 132–136.

The influence of plant growth stage, inoculum density, and variety on the disease severity and incidence were studied. Our results indicated that growth stage of the plant had no influence on infection rate, as the pathogen attacked 100% of tested plants. When applying three different inoculum concentrations to three weeks old safflower plants, no statistical differences were recorded, disease incidence ranged 32.0–47.0%. In this test, the 3rd and 4th evaluated leaves (the youngest) were infected more intensively than were the 1st and 2nd leaves. Among 11 safflower varieties tested for resistance to this pathogen, cultivar CW 1221 manifested the highest resistance with 8.3% infection, whereas two cultivars, Sabina and AC Sunset, were found to be the most susceptible with 90.0 and 83.3% infection.

Keywords: *Carthamus tinctorius*; inoculums concentration; pathogen isolates; anthracnose; susceptibility

Colletotrichum acutatum Simmonds 1968, belonging to the phylum *Ascomycota,* is a polyphagous pathogen with ubiquitous distribution which causes significant economic losses especially to strawberry plants (anthracnose) (Timudo-Torrevilla *et al.* 2005; Smith 2008). The range of its host plants is very diverse and includes fruit plants (*Malus pumila* Mill., *Prunus cerasus* L., and *Citrus* spp.) and vegetables (*Capsicum annuum* L. and *Lycopersicon esculentum* Mill.) (Sreenivasaprasad & Talhinhas 2005; Víchová *et al.* 2012) as well as ornamental plants and conifers (Guerber *et al.* 2003). Nowadays *C. acutatum* is considered as a significant pathogen of safflower (*Carthamus tinctorius* L.) (Víchová *et al.* 2011). The pathogen infects both germinating and fully grown safflower plants, and the symptoms of the disease occur on leaves, stems, and flower parts. In early infection stages, irregular or round spots occur on leaves. These spots later merge and the leaves wither. On stems, there are also irregular, cankerous brown spots, upon which orange spore masses occur after a certain time. Seriously infected plants wither in early stages of growth. On floral parts, the infection manifests itself by rotting and no seeds formation at all or only sporadically. Post-harvest residues and seeds are the most important sources of infection (Kang *et al.* 2009). *C. acutatum* propagates by conidia and disseminates by wind and water (Than *et al.* 2008). Optimal conditions for development of the infection are at 25°C, however, infection occurs also in a wider temperature range (18–33°C), when relative humidity is high (95–100%), and when there is dampness from watering, dew or precipitation for 13 h and more. The incubation period is only a few days long (Peres *et al.* 2005).

In the Czech Republic, only one fungicide is registered against *C. acutatum* – Ortiva (a.i. azoxystrobin), for application exclusively on strawberries. For this reason, preventive and protective measures (healthy seed, choice of growing site, appropriate fertilization, sufficiency of air circulation,

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QH811029.

etc.) are regarded as very important. One possible means of *C. acutatum* effective control is breeding for resistance. For appropriate selection of materials with increased resistance, available highly virulent isolates of the pathogen (Staňková *et al.* 2011) but also rapid and reliable methods of evaluating resistance are necessary. For this reason, we focused on monitoring the influence of plants' growth stage, their leaves, and concentration of the inoculum upon the disease incidence and severity. On the basis of these methods, the resistance of eleven safflower varieties to the pathogen *C. acutatum* was evaluated.

MATERIAL AND METHODS

Isolates of the pathogen *C. acutatum*. Three safflower isolates of *C. acutatum* were used in the experiments. These were obtained from various sites in the Czech Republic during 2008 and 2009. Isolates 308 (Omice site) and 508 (Velké Němčice site) were used for evaluation of the influence of growth stage at inoculation. In other tests, isolate 1209 (from Brno site), currently serving our institution as the default standard for further testing, was used. To ensure purity, monosporic isolates were prepared from all isolates of the pathogen. The cultures were cultivated on potato dextrose agar (HiMedia, Mumbai, India) at temperature 25 ± 1°C, relative humidity of 70 ± 5%, and twelve-hour cycle of changing light and darkness.

Effect of plant growth stage at inoculation. In greenhouse experiments, the appropriate growth stage of plant for inoculation with the pathogen was tested on the Sabina variety. Inoculation was made at three growth stages, i.e. stage of the 1st and 2nd true leaf, 3rd and 4th true leaf, and at the onset of buds formation. Fifteen plants in three replication blocks were sprayed with a portable sprayer containing 20 ml suspension of spores with concentration of 10^5 conidia/ml. After inoculation, plants were wrapped in a plastic film for 48 h and placed into a cultivation chamber, where a temperature of 25 ± 1°C was maintained. After eight days, the disease incidence (frequency of plant infection) was evaluated.

Effect of inoculum density. In greenhouse experiments involving three safflower cultivars (Sabina, CW 122, and India), to determine the appropriate concentrations of inoculum, three concentrations were used: 10^3, 10^4, and 10^5 conidia/ml. Three weeks old safflower plants with 4–6 true leaves were treated with 20 ml of the inoculum using a hand sprayer. In each variant, a total of 18 plants from each variety were inoculated in three replication blocks. Cultivation conditions were identical to those of the test described in the above section. Infection of the plants was evaluated twice, on days 7 and 14 after inoculation. The disease incidence and disease severity were evaluated on the four youngest true leaves which were inoculated. For the evaluation of disease severity, a four-stage scale of infection was followed: stage 1 – healthy leaf, stage 2 – individual spots on the leaf, stage 3 – half of the leaf infected (withered), stage 4 – entire leaf infected (withered) (Staňková *et al.* 2011). Index of disease severity was calculated from degree of infection of particular plants.

Host status of safflower varieties to *C. acutatum*. Eleven safflower cultivars were selected for testing varietal resistance (Sabina, CW 1221, CW 88OL, Dincer, Quiriego 88, Sahuaripa 88, Remzibey-05, San José 89, Yenice, Pannonia, and AC Sunset). Safflower plants were pre-grown in a greenhouse. In the stage of the 4th to 6th true leaves, 18 plants of each variety in three replications were treated with 20 ml of the suspension with concentration of 8.1×10^4 conidia/ml. Cultivating conditions were the same as in the experiments describe above. On the three youngest inoculated true leaves, disease incidence and severity were evaluated after seven days. Infection gradation for disease severity was identical to that in the previous experiment. For the sake of accuracy in acquiring data, the experiment was repeated once more (the second time replication).

Statistical analysis. The results were statistically processed using multi-factor analysis of variance, with the following factors: growth stage and isolate for the evaluation of influence of plant age; concentration, variety, and leaf to determine suitable concentration; variety and leaf for evaluating resistance. Subsequently, comparison was made using Tukey's test in the UNISTAT program (Unistat, Brno, Czech Republic). The level of significance was always assumed to be $\alpha = 0.05$.

RESULTS AND DISCUSSION

Effect of plant growth stage at inoculation

The monitored growth stage of plant had no influence on infection by the pathogen. All plants

in all variants were 100% infected. According to Peres *et al.* (2005), however, growth stage can play an important role in infection by the pathogen, although this varies according to plant species. In certain cases, young tissues are infected (leaves and shoots), while in others the infection affects ripe fruits.

Effect of inoculum density

The highest disease incidence and severity were determined in the first evaluation at the concentration of 10^3 and in the second evaluation at the concentration of 10^4. However, there were no statistical differences between these two tested concentrations (Table 1). Reportedly higher concentrations are normally used by some workers. In the tests of *C. acutatum* isolates on pepper fruits, Kim *et al.* (2009) used a concentration of 5×10^5 conidia/ml. Gomes *et al.* (2009) applied 10^5–10^6 conidia/ml to olive trees, while Freeman *et al.* (2001) inoculated strawberries, tomatoes, and peppers with a suspension containing 5×10^6 conidia/ml. We decided to use the concentration of 8.1×10^4 conidia/ml as the standard for all further testing of *C. acutatum* isolates.

In the first and second evaluations examining the disease severity and incidence, the cv. CW 1221 differed statistically from cvs Sabina and India. Disease incidence in this cultivar reached at most 2.5%, while for the cvs Sabina and India it was as high as 61 and 69%, respectively. These results indicated that out of the three tested varieties, cv. CW 1221 was the least susceptible to the pathogen. From the viewpoint of disease incidence and severity of the individual leaves, the 3rd and 4th evaluated leaves (the youngest) were infected more intensively than were the 1st and 2nd evaluated leaves (Table 1). From this it can be concluded that younger plant tissues are more susceptible to the pathogen.

Host status of safflower varieties to *C. acutatum*

During the first time replication, statistically significant differences were detected among individual tested varieties both in the disease severity and incidence (Table 2). The cv. Sabina proved to be the most susceptible to the pathogen, with

Table 1. Disease severity and incidence in safflower plants inoculated at various concentrations by the pathogen *Colletotrichum acutatum*

	Evaluation 1		Evaluation 2	
	disease severity (index)	disease incidence (%)	disease severity (index)	disease incidence (%)
Cultivar				
CW 1221	1.02^{A}	1.94^{A}	1.05^{A}	2.50^{A}
Sabina	1.85^{B}	43.05^{B}	2.30^{B}	60.83^{B}
India	2.05^{B}	56.39^{B}	2.07^{B}	68.33^{B}
M.S.	11.00	28 992.60	20.38	46 758.30
Concentration				
10^3	1.68^{A}	35.28^{A}	1.88^{A}	41.67^{A}
10^4	1.64^{A}	34.44^{A}	1.95^{A}	47.22^{A}
10^5	1.62^{A}	31.67^{A}	1.93^{A}	42.78^{A}
M.S.	0.03	128.70	0.05	311.11
Leaf				
1st	1.18^{A}	16.67^{A}	1.36^{A}	27.41^{A}
2nd	1.25^{A}	21.11^{A}	1.47^{A}	35.18^{AB}
3th	1.90^{B}	44.44^{B}	2.26^{B}	53.70^{AB}
4th	2.25^{B}	52.96^{B}	2.58^{B}	59.26^{B}
M.S.	7.23	8415.74	9.62	6119.75

M.S. – mean square; significant differences at $\alpha = 0.05$ are indicated by different letters

Table 2. Resistance of varieties and infection of leaves as measured by disease severity and incidence

	Time replication 1		Time replication 2	
	disease severity (index)	disease incidence (%)	disease severity (index)	disease incidence (%)
Cultivar				
CW 88OL	1.12^{A}	10.00^{A}	1.32^{A}	20.00^{A}
CW 1221	1.12^{A}	8.33^{A}	1.18^{A}	11.67^{A}
Remzibey-05	1.17^{A}	10.00^{A}	1.32^{A}	18.33^{A}
San José 89	1.22^{A}	11.67^{A}	1.45^{A}	21.67^{A}
Dincer	1.23^{A}	11.67^{A}	1.20^{A}	15.00^{A}
Quiriego 88	1.32^{AB}	18.33^{A}	1.28^{A}	16.67^{A}
Sahuaripa 88	1.35^{AB}	13.33^{A}	1.30^{A}	16.67^{A}
Yenice	1.93^{BC}	38.33^{B}	1.55^{A}	25.00^{A}
Pannonia	2.17^{C}	55.00^{B}	2.93^{B}	66.67^{B}
Sabina	2.93^{D}	90.00^{C}	3.52^{BC}	86.67^{B}
AC Sunset	3.00^{D}	83.33^{C}	3.67^{C}	95.00^{B}
M.S.	3.10	5674.85	5.73	5797.88
Leaf				
1^{st}	1.51^{A}	31.36^{A}	1.59^{A}	26.36^{A}
2^{nd}	1.71^{A}	31.36^{A}	1.85^{A}	34.55^{A}
3^{th}	1.84^{A}	32.73^{A}	2.21^{A}	46.36^{A}
M.S.	0.61	13.64	2.16	2224.24

M.S. – mean square; significant differences at $\alpha = 0.05$ are indicated by different letters

disease incidence of 90.0%, whereas cv. CW 1221 was the least infected, with a disease incidence of 8.3%. Regarding the disease severity, cv. AC Sunset was the most intensively infected while cvs CW 88OL and CW 1221 were the least infected. In the second time replication, statistical differences in disease severity and incidence between the cvs Pannonia, Sabina, and AC Sunset versus the other cultivars were confirmed. Cultivar AC Sunset was the most infected by the pathogen, with disease incidence of 95.0% and severity of 3.7. Cultivar CW 1221 was the least susceptible, with disease incidence less than 12.0% and severity of 1.2. Thus the results obtained indicated that the cvs Sabina and AC Sunset were the most susceptible to the pathogen, while cv. CW 1221 was the least susceptible. In evaluating the disease severity and incidence of the three inoculated top leaves, no statistical difference was determined among the individual leaves in the first or the second time replication. For the 3^{rd} evaluated leaf, however, the disease incidence and severity were always higher than in the 1^{st} and 2^{nd} (older) evaluated leaves.

Only a very small number of authors have given attention to the selection of safflower materials for resistance to the significant pathogen *C. acutatum*. For example, Park *et al.* (2005) evaluated infection under field conditions and determined striking differences in infection among several cultivars. In their experiments, the cv. India was among those highly susceptible. Uniform inoculation conditions for all the tested plants within a greenhouse represent a great advantage. On the basis of our tests, therefore, we can recommend the most resistant cultivars (e.g. CW 1221, Dincer, and Quiriego 88) in the subsequent process of breeding for resistant safflower.

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Received for publication April 19, 2012
Accepted after corrections January 23, 2013

Corresponding author:

Ing. Jana Víchová, Ph.D., Mendlova univerzita v Brně, Agronomická fakulta, Ústav pěstování, šlechtění rostlin a rostlinolékařství, Zemědělská 1, 613 00 Brno, Česká republika; E-mail: jana.vichova@mendelu.cz