A New Report for Downy Mildew
\([\text{(Hyaloperonospora camelinae Gãum.) Gãoker, Voglmayr, Riethm., M. Weiss & Oberw. 2003}]\) of Camelina \([\text{Camelina sativa (L.) Crantz}]\) in the High Plains of the United States

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Camelina sativa (L.) Crantz., also known as false or wild flax, is a member of the mustard family and a new oil seed crop in western Nebraska and other areas of the High Plains of the United States. It is native to Central Europe and Central Asia, and is thought to have been grown in Europe for more than 3,000 years (6). It was recently introduced into North America, where it is currently grown commercially in Montana and North Dakota (2). Camelina shows great promise for this region as a new biodiesel crop due to its drought and cold tolerance.

New Disease in a New Crop

During early June 2010, camelina plants from cultivar research trials (planted 4 April 2010 in a completely randomized block design with four replications per treatment) conducted under sprinkler irrigation at the University of Nebraska, Panhandle Research and Extension Center in Scottsbluff, NE, began exhibiting downy mildew-like symptoms consisting of upper stem distortion and signs of white, fluffy masses covering stems, seed pods and heads (Fig. 1). Incidence within plots was estimated at 20 to 25% (Fig. 2). By mid-July, stems of affected plants had turned black and roughly circular to angular oospores (34 to 35 µm in diameter) were found profusely scattered throughout infected tissues (Fig. 3). Mycelial growth was never observed on leaves, only on upper stems and heads.
These downy mildew-like characteristics had been noticed periodically since 2006 in camelina research plots, but only in one cultivar (Boa). In 2010, seven different camelina cultivars were evaluated in trials at Scottsbluff, NE (Table 1). Diseased plants were observed in all varieties throughout the trial. However, differences among varieties in disease severity and incidence were observed (Figs. 2 and 4). Ratings were made in mid-June to estimate disease severity and incidence in all plots. Ratings from each plot were recorded using a scale of 1 to 3 (1 = no or little head distortion and 0 to 25% incidence, 2 = moderate head distortion and 26 to 50% incidence, and 3 = severe head distortion and >50% incidence) (Fig. 4). Plots were harvested on 22 July 2010.

Table 1. Spring camelina varieties with downy mildew ratings and yields.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Mean downy mildew rating (1-3)&lt;sup&gt;x&lt;/sup&gt;</th>
<th>Mean seed yield (kg/ha)&lt;sup&gt;y&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blaine Creek</td>
<td>2 c</td>
<td>972 abc</td>
</tr>
<tr>
<td>Cheyenne</td>
<td>2.25 bc</td>
<td>934 bc</td>
</tr>
<tr>
<td>Sunseon</td>
<td>2.75 ab</td>
<td>922 bc</td>
</tr>
<tr>
<td>Calena</td>
<td>2.75 ab</td>
<td>923 bc</td>
</tr>
<tr>
<td>Galena</td>
<td>2.75 ab</td>
<td>998 ab</td>
</tr>
<tr>
<td>Celine</td>
<td>3 a</td>
<td>840 c</td>
</tr>
<tr>
<td>Ligena</td>
<td>3 a</td>
<td>1115 a</td>
</tr>
<tr>
<td>Trial mean</td>
<td>2.64</td>
<td>957</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>0.72</td>
<td>148</td>
</tr>
</tbody>
</table>

<sup>x</sup> Disease ratings from each plot utilized a scale of 1 to 3 (1 = no or little head distortion and 0 to 25% incidence, 2 = moderate head distortion and 26 to 50% incidence, and 3 = severe head distortion and >50% incidence).

<sup>y</sup> Seed yield estimates were determined on a dry weight basis with 10% moisture. Data were analyzed using Proc ANOVA and means were separated by LSD tests ($P < 0.05$).
Statistically significant differences in yield, as measured by seed weight, were determined among varieties; yield differences varied between 82 to 275 kg/ha (Table 1). Additionally, statistically significant differences were determined among varieties for disease severity and incidence ratings (Table 1). Interestingly, there was a lack of correlation between seed yield and disease rating; the variety ‘Ligena’ yielded significantly greater than ‘Celine,’ despite being severely infected (both had identical disease ratings of 3). This may indicate some degree of disease tolerance in ‘Ligena.’

Identifying the Pathogen

The white growth associated with symptomatic plants was examined microscopically and consisted of hyaline hyphae and conidiophores with oval to ellipsoidal conidia measuring 18.5-22 µm × 25.5-27.7 µm. Conidiophores ranged in length from 200-340 µm with 3 to 4 branches, each with 12 to 20 curved tips per branch. Similar morphological features and spore dimensions were reported recently from a downy mildew outbreak of camelina in Oregon (3).

Total DNA from affected tissue was extracted according to the manufacturer’s directions (FastDNA SPIN kit, MP Biomedical). The DNA was used as template in a PCR reaction using primers DC6 and ITS4, which are specific to the internal transcribed spacer region of ribosomal DNA (1,5). The PCR product was sequenced in two separate reactions; one using primer DC6 and the other using primer ITS4. The two overlapping sequences provided a consensus nucleotide sequence that was compared to those in the NCBI GenBank database using a BLAST search. The sequence from camelina gave 99% identity to *H. camelinae* voucher J746/01 (accession EU049217.1) with a match of 799 of 803 nucleotides.

To determine if *H. camelinae* could be detected in camelina seeds, two lots of seed (cv Cheyenne) were independently assayed by PCR. One thousand seeds were ground dry for 1 min in a Retsch mixer mill (Retsch model MM200). Two 100 µg portions of the resulting powder were put into two separate 2 ml microfuge tubes to which 600 µl Quiagen DNeasy lysis buffer and 6 µl of RNase A were added. Total DNA was extracted per directions with the DNeasy plant mini kit (Quiagen) and assayed using the DC6 and ITS4 primers. The PCR reactions were repeated with the same extracts, but diluted 1:10 and 1:50 with water. Results were negative for both seed lots at all dilutions although powdered seed samples spiked with *H. camelinae* DNA were positive. This was done as a control to confirm that no PCR inhibitors were coming from the seed extracts.
Pathogenicity Testing

To confirm pathogenicity, 3-week-old camelina plants (cv Cheyenne) were inoculated by rubbing white conidial masses of *H. camelinae* on young leaves and stems. Small cut segments of infected stems were placed in pots and sprayed vigorously with water. Water controls were included for comparison. Two incubation methods were employed for all plants: In the first method plants were incubated at room temperature in a modified mist chamber for 24 h before moving to a greenhouse maintained at 27 to 28°C. The second method consisted of covering inoculated plants with plastic bags and incubating in lighted growth chambers at 22 to 23°C for 72 h before being moved to the greenhouse. Signs and symptoms of infection similar to those from field infections developed within 7 to 8 days for both incubation methods, while no water controls developed disease symptoms.

Further Observations

A second field planting was attempted on June 27 using only cv Cheyenne; the plants emerged and became established during the first week of July. Signs and symptoms identical to those from the initial planting in April became evident by the second week of August. On August 13, disease incidence was determined to be 3.2, 6.4, 6.0, and 5.0% for the four plots. On August 18 disease incidence had increased to 41, 59, 36, and 37% for the same four plots respectively. Thus, within five days the average disease incidence had increased from 5.2 to 43.3%, demonstrating the high potential for disease spread under conducive conditions.

Conclusions

Downy mildew on camelina was reported recently from the Pacific Northwest (3) and previously from Minnesota and western Montana (2,4). This paper represents the first substantiated report of *Hyaloperonospora camelinae* in camelina in the United States from the Central High Plains and the first suggestion of the existence of naturally-occurring disease tolerance among camelina varieties. Presumptive disease tolerance was illustrated by the varieties ‘Ligena’ and ‘Galena’ yielding 275 and 158 kg/ha higher, respectively, than the variety ‘Celine’, although all had similar severity ratings of 3 or 2.75 (Table 1). More investigation is warranted to determine whether these observations are due to plant genotypes or some other factor. Downy mildew has the potential to be a major yield-limiting factor on camelina production in the High Plains.

Literature Cited