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**PHOMA STEM CANKER (BLACKLEG) A THREAT TO OILSEED RAPE  
(BRASSICA NAPUS L.) IN GREECE.**

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KEY WORDS: *phoma, crown canker, blackleg*

**ABSTRACT**

*Phoma stem canker is an internationally important disease of oilseed rape (Brassica napus L.), causing serious losses. This study provides evidence of phoma stem canker, caused by the species complex Leptosphaeria maculans and L. biglobosa in Greece. Infections were occurred early and resulted serious stem cankers and stem necrosis of oilseed rape before flowering. Moreover the disease caused significant yield losses on season spring varieties.*

**INTRODUCTION**

Oilseed rape (*Brassica napus* L.) is a crop which is grown mainly for its high quality oil and protein. In the face of global climate change, rapeseed could play a much more important role in the future as source of renewable energy provides versatile oil being used as a fuel and a raw material for the chemical industry. Considerable that interest seed yield is very important and diseases can cause yield losses. Phoma stem canker is one of the most economic important diseases on oilseed rape worldwide. Typical symptoms on oilseed rape are leaf symptoms, which may become visible on true leaves. The disease is caused by a complex of *Leptosphaeria* species (Mendes-Pereira et al., 2003), the most important of which is *L. maculans*, associated with damaging stem base canker. This disease has a major economic impact, with significant yield losses (West et al., 2001).

Epidemics are initiated during autumn by air-borne ascospores released from infected stubbles of previous crops. Once in contact with plants, these ascospores germinate and produce leaf lesions. The fungus then grows systemically from the leaf lesions to the stem where it produces cankers which can result in major yield loss. Infected plants are weakened and may produce smaller heads with reduced seed yield and oil.

This paper provides evidence of phoma stem canker, discussed symptoms, severity and yield loss on oilseed rape in central Greece.

**MATERIALS AND METHODS**

*Field experiments – Stem canker symptoms*

Experimental oilseed rape plots natural infected with phoma stem canker were used. Disease severity and yield loss due to phoma stem canker in *B. napus* was investigated in field, at the Technological Education Institute (TEI) of Larissa, Greece, using two resistance to stem canker oilseed varieties the Ability (a season spring variety) and Hornet (a season winter

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variety). Both varieties marked as high yielding, with high oil content. In 2008/09, the two oilseed varieties, Ability and Hornet, were sown in the second week of October and grown in a randomized block design with three replications in field conditions, at the TEI of Larissa, Greece. Each plot comprised of 2x1 m length with space of 1 m between rows.

#### Canker severity assessment

Canker severity were estimated assessing disease severity on 100 plants (Rempel and Hall, 1996), picked randomly in the field, by cutting them at the base of the stem and by sorting cankers in five classes (0 to 4) of canker (external stem canker severity). Further at harvest 50 plants were assessed for internal canker symptoms by taking a cross-section of the crown (stem base) that is discoloured by the disease and sorting canker in five classes (0%, 1–25%, 26–50%, 51–75% and 76–100%) of canker (necrosis), (Huang et al., 2009).

#### Yield loss assessment

Further we estimate yield losses on both above oilseed rape varieties Ability and Hornet. These losses are estimated based on data received from each plot experiment. Thus, yield response to variety Ability or Hornet. Yield loss to incidence of phoma stem canker is estimated as average yield in g 2m<sup>-2</sup> (Table 1.)

#### Statistical analysis

All experiment data for external, internal stem canker severity and yield were analysed to compare the differences between varieties Ability and Hornet by analysis of variance (ANOVA). Further multiple range tests (Tukey's multiple comparisons) were applied to assess differences between treatments and identify statistical differences between means, respectively; level of significance,  $P = 0.05$ .

## RESULTS

#### Stem canker symptoms

The data shows that the disease appears early in the season as small grey lesions (Fig. 1A), with black specks (fruiting bodies, pycnidia Fig 1B), found on the leaf surface (phoma stem lesions of canola plants). Later the disease produces lesions and cankers on the stem, appearing as stem cankers at the stem base (crown, Figs 2) and as upper stem lesions (Fig. 1C) second symptom appears as necrosis with yield loss. Further small circular fruiting bodies (pycnidia Fig. 1B) of the fungus are produced on the surface of the infected stem (Fig. 1A and Fig. 2). To our observations all those probably is a systemic progression of the fungus from leaves to the base of the stem where the second symptom, the canker (or necrosis), appears as reported by (West et al. 2001).

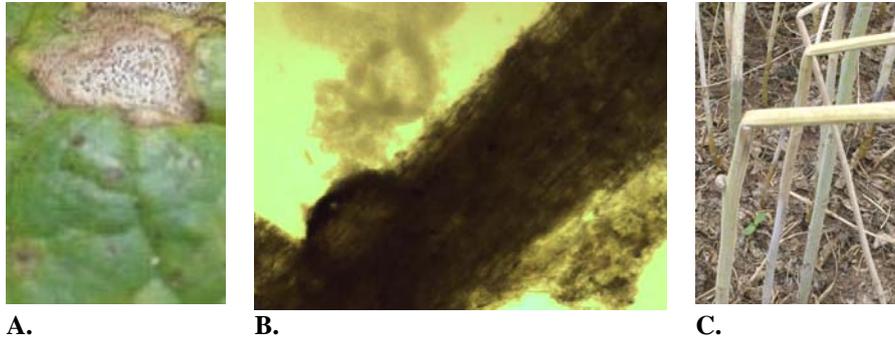
#### Canker severity assessment

Further our lab data shows that stem canker / blackleg disease is caused by the species complex *Leptosphaeria maculans* / *L. biglobosa*. *Leptosphaeria maculans*, associated with damaging stem base cankers (Fig. 2A), and *L. biglobosa*, often associated with less damaging upper stem lesions (Fig. 2B), produced a brown no pigment and a brown dark pigment mycelia (Fig 1A and B) respectively as described by Fitt et al., 2006.

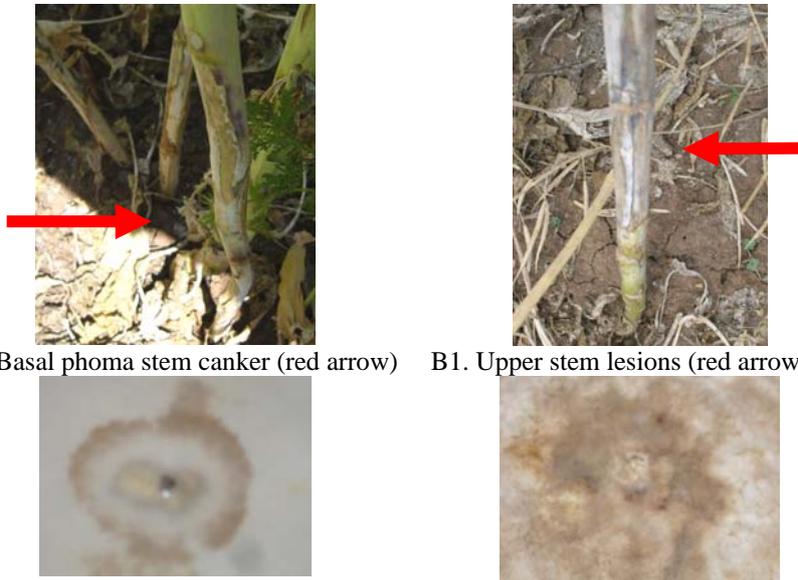
The data shows significant differences ( $P < 0.001$ ) in severity of infection between cultivars. Hornet variety showed a significant greater external and internal stem canker severity compared with the Ability variety (Fig. 3). Further the internal stem canker severities, the percentages of stem cross-sectional area with necrosis were greater on Hornet than on Ability variety (Fig. 3).

#### Yield loss assessment

The data shows that Hornet and Ability variety may infect by the same *phoma* species and have similar looking symptoms but yields are lower for Hornet variety (Table 1). The high stem canker found at the Hornet variety was high correlated with the low yield of the same variety confirmed the yield loss due the pathogen infection (Table 1). This may suggest that phoma stem canker is most serious disease on summer oilseed rape varieties.



**A.** **B.** **C.**  
 Figure 1. Symptoms of disease necrotic spots on leaves (A) and stems (C), caused by *L. maculans*. B: black specks (fruiting bodies, pycnidia) found on the infected plant tissue.



A1. Basal phoma stem canker (red arrow) B1. Upper stem lesions (red arrow)  
 A2. PDA culture of *L. maculans* B2. PDA culture of *L. biglobosa*  
 Figure 2. Symptoms of disease on stems A1: basal phoma stem canker, A2: *L. maculans* predominant species present; B1: upper stem lesions, B2: *L. biglobosa* predominant species present) of winter oilseed rape, and cultures of *L. maculans* (no pigment) or *L. biglobosa* (pigment) on potato dextrose agar (A2 and B2 respectively).

## CONCLUSIONS

Phoma stem canker (blackleg) seems to be an important disease for cropping oilseed rape (*B. napus*) in Greece. Infection occurred by two main species *L. maculans* and *L. biglobosa* as reported by Vagelas (2009). The disease severity depends on sowing variety. It would be interesting to test the effect of the relationship between canker severity and quantity of primary inoculum produced (Lo-Pelzer et al., 2009), in canker severity classes (Rempel and Hall 1996) on yield losses of numerous oilseed rape varieties (*B. napus*), (West et al, 1999).

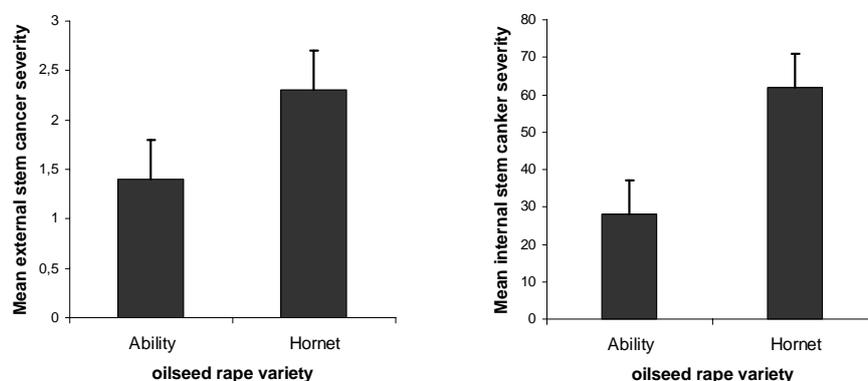


Figure 3. External and internal stem cancer severity, of oilseed rape varieties, Ability and Hornet, growing under natural infection conditions.

Table 1

Yield and yield associated with damage of Hornet and Ability variety in field experiments natural infected with phoma stem canker.

Oilseed rape variety	Yield g 2m <sup>-2</sup> (± s.e.m*)	Yield associated with damage (Pearson correlation)
Hornet	737 ± 12 a	Pearson correlation of yield and mean internal stem canker severity = <u>-0.955</u>
Ability	1070 ± 23 b	P-Value = <u>0.003</u>

\* s.e.m= standard error of mean. Values within a column followed by the same letter do not differ significantly ( $P = 0.05$ ) according to Tukey's multiple comparisons test.

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