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Albugo candida staghead formation in *Brassica juncea* in relation to plant age, inoculation sites, and incubation conditions

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Maximum staghead formation was obtained in 26-day-old (growth stage [GS] 3·1) *Brassica juncea* plants by inoculating differentiating flower buds with a zoospore suspension of *Albugo candida* race 2 V; exposing apical meristem tissues by opening the flower buds with forceps proved more conducive to staghead formation. Inoculation of 35- and 45-day-old plants (GSs 4·1 and 5·0, respectively) produced fewer hypertrophies, mainly in isolated flowers. Inoculation of 7- and 13-day-old plants (GSs 1·0 and 2·1, respectively) did not produce any hypertrophied flowers, but did result in the production of hypertrophied branches at the first node on the main stem. In general, hypertrophies were initiated more readily under greenhouse conditions than in the growth chamber. Other *Brassica* hosts inoculated with *A. candida* race 2 V or 7 V at GS 31 showed similar rankings for staghead formation and leaf infection. The technique should prove useful in screening breeding lines for disease resistance, particularly staghead formation, the most damaging phase as far as yield loss is concerned.

INTRODUCTION

Albugo candida (Pers. ex Lév.) Ktze. (*A. cruciferarum* S. F. Gray) causes white rust in many members of the Brassicaceae. The disease occurs in various parts of the world and causes extensive yield losses in turnip (*Brassica rapa*) (Harper & Pittman, 1974), mustard (*B. juncea*) (Bains & Jhooty, 1979), and radish (*Raphanus sativus*) (Williams & Pound, 1963). Canadian and European cultivars of rape (*B. napus*) are considered immune from this disease (Fan *et al.*, 1983).

The fungus infects both vegetative and reproductive tissues. Infection of leaves and cotyledons results in the formation of white to cream coloured zoosporangial pustules on their lower surfaces. Flower bud infection causes extensive distortion, hypertrophy, hyperplasia and sterility, resulting in staghead formation (Verma & Petrie, 1980; Saharan & Verma, 1992). Mature stagheads consist almost entirely of oospores, which act as survival and dispersal propagules (Petrie, 1975). In addition to its epidemiological significance, the staghead phase accounts for most of the yield loss (Harper & Pittman, 1974).

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Staghead formation following artificial inoculation of flower buds has been reported previously (Verma & Petrie, 1980). However, using this technique, only 40% of inoculated plants produced stagheads (Liu & Rimmer, 1993). Lakra and Saharan (1989) and Bains (1991), were unable to produce stagheads following flower bud inoculation of susceptible *B. juncea* plants. These inconsistent results prompted us to study the effect of plant age and incubation conditions on staghead formation, and to investigate the correlation between leaf infection and staghead formation. Further, we investigated the usefulness of the flower bud inoculation technique in screening genotypes for resistance against staghead formation.

MATERIALS AND METHODS

Inoculum

Albugo candida race 2V from *B. juncea* and race 7V from *B. rapa*, obtained from Agriculture and Agri-Food Canada, Saskatoon Research Centre, were used. Zoosporangia were collected from freshly produced pustules on 24-day-old leaves and stored in sealed glass vials at −20°C. As needed, zoosporangia were suspended in deionized distilled

water, and filtered through cheesecloth to remove debris. The filtered suspension was then incubated for 6 h at 5°C to produce zoospores and adjusted to a concentration of $7{\cdot}5 \times 10^4$ zoospores per mL.

Influence of plant age and incubation conditions on staghead formation

Seeds of *B. juncea* cv. Commercial Brown were sown 2 cm deep in a modified Cornell soilless mix (Stringam, 1971) in 15-cm diameter plastic pots. Pots containing eight seeds each were placed in 7-cm deep trays in a growth chamber with an 18-h photoperiod (312 μEM^{-2} s^{-1}) and day–night temperatures of 18°C and 14°C, respectively; this range of temperature has proved optimum for excellent growth of canola/rapeseed. Seedlings were thinned to four per pot after emergence. The plants were watered daily by flooding and then draining the trays. The corresponding ages of plants and GSs (in parentheses) (Harper & Berkenkamp, 1974) at inoculation were 7 (1·0), 13 (2·1), 26 (3·1), 35 (4·1), and 45 (5·0) days after seeding. To achieve this, seeds were sown every fourth day so that at the time of inoculation there would be 20 pots, including controls, at each GS. At each GS, 40 plants in 10 pots were inoculated with a zoospore suspension of *A. candida* race 2V. The remaining 10 pots at each GS were drop-inoculated with deionized water to serve as controls. At GSs 1·0 and 2·1, the plants had no flower buds and therefore were inoculated by placing a drop (0·1 mL) of the zoospore suspension over the apical meristem region. At GS 3·1, a rosette of 20 (±2) green (differentiating) buds were inoculated by placing a drop (0·1 mL) of the zoospore suspension on them. At GS 4·1, 20 yellow (differentiated) buds on each plant were inoculated by placing 0·1 mL of the suspension onto each of them. Similarly, at GS 5·0, 20 open flowers in each plant were inoculated by placing 0·1 mL of the suspension onto each flower. Following inoculation, plants were exposed to continuous misting for 72 h with day–night temperatures of 18 and 14°C, respectively. Except for an initial 24-h dark period, an 18-h day was maintained for the duration of the experiment.

After a 72-h incubation period in the growth chamber, five of the 10 inoculated pots, and five of the controls, at each GS, were transferred to the greenhouse where the photoperiod was 16 h (light intensity variable) and day–night temperatures were 22°C (±3) and 12°C (±3), respectively. The five remaining pots at each GS were maintained in the growth chamber (312 μEM^{-2} s^{-1}) at day–night temperatures of 18 and 14°C, respectively. The 10 replicated pots of each GS and each of the two incubation conditions (growth chamber and greenhouse) were arranged in a completely randomized design. The test was repeated once.

The percentages of hypertrophied plants, flower buds and axillary branches on the main stem were recorded 30 days after inoculation. Hypertrophied axillary branches and flower buds obtained from plants inoculated at GSs 1·0, 2·1 and 3·1 were ground and the viability of oospores determined (Verma & Petrie, 1975).

Screening *Brassica* genotypes for staghead formation

Having determined the most conducive GS (see Results), further experiments determined the feasibility of using the flower-bud inoculation technique to screen genotypes for resistance against staghead formation, and also to determine the correlation between leaf infection and staghead formation. The two *B. juncea* and three *B. rapa* cultivars or genotypes in the experiments were known to have different levels of susceptibility both to *A. candida* race 2V and to race 7V. Three cultivars or genotypes of *B. napus*, known to have very high levels of resistance/immunity to both races, were included (Table 4; Anonymous, 1993).

Procedures for seeding, incubation conditions and inoculum preparation were similar to those described. For each cultivar/genotype, and for each of the two races, there were 24 replicate pots, including controls, with four plants in each. At GS 3·1, 24 plants in six pots were inoculated on the apical meristem by placing 0·1 mL of zoospore suspension onto them; the differentiating flower buds were crushed open with forceps to expose the apical meristem tissues. At GS 3·1, leaves of 24 plants in the other six pots were inoculated by spraying the zoospore suspension to runoff. The remaining 12 were either drop-inoculated (apical meristem), or sprayed (leaves) with deionized water as controls. Following inoculation, plants were exposed to continuous misting for 72 h in the growth chamber with an 18-h photoperiod (312 μEM^{-2} s^{-1}) and at day–night temperatures of 18 and 14°C, respectively. After this 72-h incubation period, pots were transferred to the greenhouse where the photoperiod was 16 h (light intensity variable) and day–night temperatures were 22°C (±3) and 12°C (±3), respectively. Pots were arranged in a completely randomized design. The test was repeated once.

Disease assessment and data analysis

The percentages of plants with hypertrophies (stagheads) and plants bearing pustules on the leaves were recorded 30 days after inoculation.

The data were analysed with Genstat 5 software (Lawes Agricultural Trust, 1987) using maximum likelihood to estimate parameters in a generalized linear model assuming bionomial errors (McCullagh & Nelder, 1989).

RESULTS

Influence of plant age and incubation conditions on staghead formation

The residual mean deviance for the plants that had hypertrophied axillary branches was close to unity, indicating a good fit of data to the model. The mean residual deviance for hypertrophies in flower buds was found to exceed unity, indicating that the assumption of binomial variation may not be realistic. This over-disperison, relative to the binomial, indicates that the variability within the growth stages with regard to hypertrophy in flower buds was not homogeneous. In fact, we found that the deviance for plants within pots was significant (analysis not shown) showing the results for observations within pots were correlated. Significance tests for hypertrophy in flower buds were performed by dividing the deviance by the heterogenicity factor (estimated by the residual mean deviance).

Inoculation at growth stage 1·0 and 2·1 had no significant effect on the percentages of plants producing hypertrophied axillary branches (Table 1). Plants inoculated at GSs 1·0 and 2·1 showed no flower bud hypertrophy but produced abundant hypertrophied axillary branches at the first node on the main stem (Fig. 1; Table 2); hypertrophied plants at these two GSs were also stunted in growth (Fig. 2). At both GSs, the frequency of hypertrophied axillary branches was significantly ($P < 001$) higher in the greenhouse (72 and 60%) than under growth chamber conditions (50 and 35%) (Table 2).

A significant ($P < 001$) effect of GS at inoculation and site of incubation was found in plants with hypertrophies in flower buds (Table 1). Inoculation on green flower buds at GS 3·1 proved most conducive for staghead formation (Table 2). Under growth chamber and greenhouse conditions, the percentage of hypertrophied flower buds decreased with the age of the plant. This trend was more pronounced in the growth chamber than in the greenhouse. The plants inoculated at GSs 3·1, 4·1 and 5·0 also differed in their morphology. At GS 3·1, infected plants were stunted (Fig. 4), and both peduncles and flowers, or entire inflorescences were hypertrophied. At GSs 4·1 and 5·0, infected plants were not stunted and hypertrophies were restricted mainly to individual flowers occurring occasionally on peduncles (Fig. 5).

Oospores obtained from hypertrophied branches (GSs 1·0 and 2·1) and hypertrophied inflorescences (GS 3·1) were viable, and 35% from each germinated to produce zoospores.

Screening *Brassica* genotypes for staghead formation

With races 2V and 7V, significant differences ($P < 001$) were found amongst cultivars and between two sites of inoculation for staghead and

Table 1 Analysis of deviance of the percentage of plants with hypertrophies in flower buds and in axillary branches at five growth stages of plants incubated under two conditions

	Hypertrophy in axillary branches		Hypertrophy in flower buds	
Factors	d.f.	Mean deviance	d.f.	Mean deviance
Site of incubation	1	23·75**	1	23·75**
Replicates per site	2	0·44	2	2·09
Growth stage	1	1·26NS	2	413·12**
Site x growth stage	1	0·1NS	2	15·12NS
Residual	34	1·34	56	6·69

**Significant at $P < 0·01$; NS, not significant.

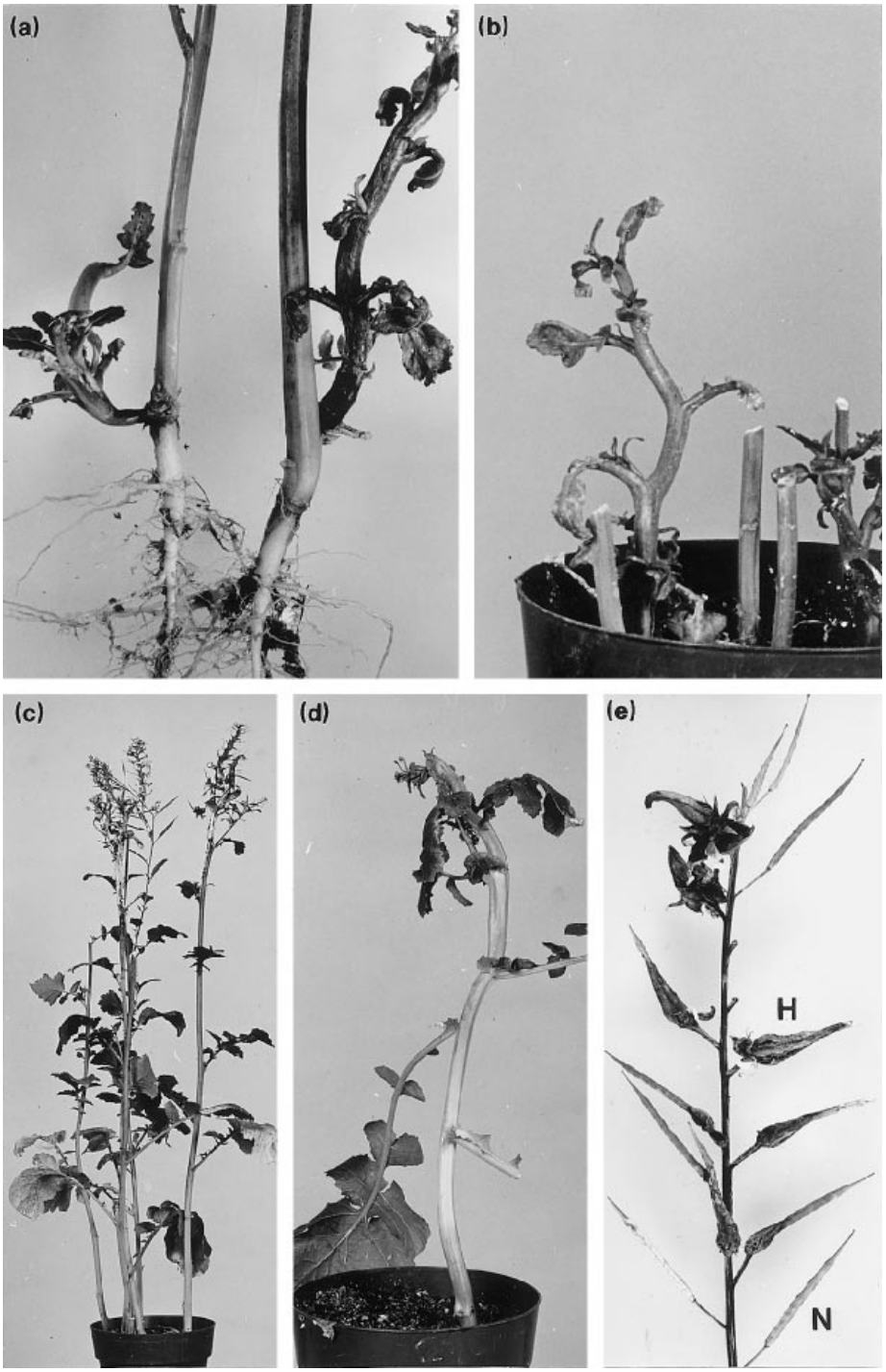

Fig. 1 Hypertrophied inflorescence and branches in *Brassica juncea* cv. Commerical Brown plants artificially inoculated with *Albugo candida* race 2V at growth stages 1·0–5·0 in the growth chamber/greenhouse. (a) Hypertrophied axillary branches from the first node on the main stem in plants inoculated at the apical meristem at GS 1·0. (b) Hypertrophied and stunted main stem in plants inoculated at the apical meristem at GS 2·1. (c) Completely hypertrophied inflorescences with apical growth terminating in stagheads on plants inoculated on differentiating flower buds at GS 3·1. (d) Hypertrophied inflorescence axis with pronounced stunting in plants inoculated on differentiating flower buds at GS 3·1. (e) Healthy (N) and hypertrophied (H) pods on the inflorescence of plants inoculated at GS 5·1.

Table 2 Mean deviance ratios (±SE) of the percentage of hypertrophied plants, flower buds, and axillary branches on the main stem of *Brassica juncea* cv. Commercian Brown, artificially inoculated with *Albugo candida* race 2V in the greenhouse and growth chamber

Growth stage at inoculation	Corresponding age in days after seeding and site of inoculation	Number of plants/parts inoculated	Hypertrophied axillary branches		Hypertrophied flower buds	
			Greenhouse	Growth chamber	Greenhouse	Growth chamber
1·0	7 (apical meristem)	40/40	72 ± 7	50 ± 8	0	0
2·1	13 (apical meristem)	40/40	60 ± 8	35 ± 8	0	0
3·1	26 (green flower buds)	40/800	0	0	70 ± 3	51 ± 3
4·1	35 (green flower buds)	40/800	0	0	41 ± 4	14 ± 3
5·0	45 (flowers)	40/800	0	0	25 ± 3	5 ± 2

leaf pustule intensity (Table 3). The cultivar–site of inoculation interactions were not significant for either race for staghead and or for leaf pustule formation. This would indicate that the degree of difference amongst cultivars does not change with the site of inoculation.

With both races, intensity of leaf pustule formation was quite low when apical meristems were inoculated; similarly, the frequency of staghead formation was very low when leaves were inoculated (Table 4).

Inoculation of apical meristems with race 7V produced stagheads in 79, 77 and 44% of plants in the *B. rapa* cvs. Torch, Tobin and Parkland, respectively (Table 4). Leaf inoculation showed a similar ranking. The *B. napus* cv. ZSN045 showed leaf pustules in 8% of the plants, following leaf inoculation.

Inoculation of apical meristems with race 2V produced stagheads in 82, 93 and 14% of plants in the *B. juncea* cvs. Commercial Brown and Cutlass, and the *B. napus* cv. ZSN046, respectively (Table 4). There was no significant difference between the two *B. juncea* cultivars for staghead and leaf pustule formation.

DISCUSSION

In the present study, artificial inoculation of flower buds on mustard plants at GS 3·1, as flower buds were being differentiated, proved most conducive to *A. candida* infection and staghead formation. The exposure of apical meristem tissues by opening the differentiating flower buds with forceps resulted in the formation of stagheads in 93% of the plants of cv. Cutlass (Table 4). Hypertrophy of peduncles on plants inoculated at GS 3·1 suggested that inoculation of differentiating meristematic tissues affects the actively dividing parenchyma cells. Overproduction of

Table 3 Analysis of deviance of the proportion of plants with stagheads, and plants with white rust pustules on the leaves assuming bionomial errors and logit link function

Source	Mean deviance stagheads			Mean deviance leaf pustules			
	d.f.	Race 2V	Race 7V	d.f.	Race 2V	d.f.	Race 7V
Cultivar	2	6·71**	26·19**	2	28·94**	2	35·04**
Site of inoculation	1	140·30**	165·95**	1	142·91**	1	161·52**
Residual	284	0·79	0·52	379	0·71	284	0·56

**Significant at $P < 0·01$.

Table 4 Mean deviance ratios of the proportion of plants in cultivars with stagheads and leaf pustules (±SE) obtained by artificial inoculation with *Albugo candida* race 7V and 2V on apical meristems and leaves at growth stage 3.1

		Apical meristem inoculation		Leaf inoculation	
Genus-species	Cultivar	Stagheads	Leaf pustules	Stagheads	Leaf pustules
		Race 7V		Race 7V	
Brassica rapa	Torch	0.79 ± 0.06	0.26 ± 0.06	0.10 ± 0.06	0.93 ± 0.04
B. rapa	Tobin	0.77 ± 0.06	0.12 ± 0.05	0.06 ± 0.03	0.84 ± 0.05
B. rapa	Parkland	0.44 ± 0.07	0.04 ± 0.03	0	0.53 ± 0.07
B. napus	ZSN045	0	0	0	0.08 ± 0.04
B. napus	ZSN046	0	0	0	0
B. napus	Excel	0	0	0	0
		Race 2V		Race 2V	
B. juncea	Comercial Brown	0.82 ± 0.05	0.25 ± 0.06	0.03 ± 0.02	0.98 ± 0.0
B. juncea	Cutlass	0.93 ± 0.03	0.11 ± 0.04	0.07 ± 0.03	0.95 ± 0.02
B. napus	ZSN046	0.14 ± 0.05	0	0	0.19 ± 0.06
B. napus	Excel	0	0	0	0
B. napus	ZSN045	0	0	0	0

auxins probably plays an important role in cellular hypertrophy in these tissues. Increased auxin content during the early staghead phase in *B. juncea* has been reported (Hirata, 1956). The significantly lower frequency of inflorescence hypertrophy in plants inoculated at GSs 4·1 and 5·0 suggested that most meristematic activity has ceased beyond the site of infection and that those metabolic activities leading to the cellular hypertrophy had been switched off before infection. The development of hypertrophies in individual flowers of *B. juncea* inoculated with *A. candida* at GSs 4·1 and 5·0 was also observed by Bains (1991).

At GS 3·1, inoculation of the apical meristem led to stunted plant growth compared to inoculation on differentiated flower buds at GSs 4·1 and 5·0. Under natural conditions, because the plant apices remain protected by the leaf primordia, the flower buds therefore seem to be the most probable pathway for *A. candida* infection for the formation of stagheads. Our observations regarding stunted growth are in agreement with those of Jones (1978) on sorghum infected with *Sclerospora sorghi*, Taylor *et al.* (1990) on peas infected with *Peronospora viciae*, and Wehtje & Zimmer (1978) on sunflower infected with *P. halstedii*. The stunted growth of tobacco plants inoculated with *P. tabacina* mycelium at the apical meristem has also been reported (Moss & Main, 1989).

At GSs 1·0 and 2·1, where plants were inoculated at the stem apex, the rapid enlargement and elongation of the cells of this region may have prevented the fungal mycelium from reaching the apical meristematic tissues. However, late in the season, when secondary branches were produced following reactivation of its cambial tissue, the symptoms were expressed in the form of hypertrophied branches of the first node on the main stem. In this instance, fungal mycelium in the stem may have reached the underlying secondary meristematic tissues before the production of secondary branches. Delayed systemic expression of *S. sorghi* in sorghum plants inoculated at the seedling stage produced similar structures and dormant fungal mycelium in the stem tissue, of apparently healthy plants, was believed to have triggered this reaction (Jones, 1978). The persistence of dormant mycelium of *A. candida* in the crown and lateral roots of perennial horseradish has also been reported (Kadow & Anderson, 1940). The hypertrophied branching from basal nodes of the main stem of *B. juncea* has not been reported previously. Under field conditions, these infected branches probably would result in increased inoculum density in the soil.

In the present study, both greenhouse and growth chamber conditions covered the range of climatic conditions likely to prevail in the field. The higher frequency of hypertrophied inflorescences and

branches in plants grown under greenhouse (day/night temperatures, 22/12°C) than under growth chamber (day/night temperatures, 18/14°C) conditions indicates that the comparatively warmer temperatures combined with low light intensity increased susceptibility to infection and subsequent formation of oospore-containing tissues (stagheads). Our results also suggest that the low temperature and high humidity in the growth chamber for the initial 72 h may be important only for the penetration and establishment of *A. candida* mycelium in the host tissues. This hypothesis is supported by Moss & Main (1989) who found that the low temperatures were important only in the early invasion of fungal mycelium in the systemic development of *P. tabacina* in tobacco plants.

The information on techniques for staghead formation will prove valuable in increasing the efficacy of screening advanced breeding lines for resistance to white rust. Also, the similar ranking of cultivars *vis-à-vis* staghead formation and leaf infection may allow the development of a rapid assay based on pustule formation.

The finding that GS 3·1 was the most conducive to staghead formation will be important in a strategy for fungicidal control of the disease, especially in countries lacking resistant varieties. Good control of staghead formation in plants sprayed with metalaxyl at the flowering stage (GS 3·1) supports this view (Stone *et al*., 1987; Saharan *et al*., 1990).

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