PERFORMANCE OF THE CABBAGE APHID *BREVICORYNE BRASSICAE* (HEMIPTERA: APHIDIDAE) ON CANOLA VARIETIES

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Summary

The cabbage aphid *Brevicoryne brassicae* L. (Hemiptera: Aphididae) is one of the most abundant canola pest insects, causing economic damage to flowering and podding crops. Cabbage aphid performance (abundance, fecundity, development, longevity and generation time) in canola, juncea canola, and canola-mustard was studied under glasshouse conditions. The three canola varieties tested in this study are highly susceptible to cabbage aphid damage. There were no significant differences between canola-mustards and conventional canola in attracting cabbage aphids. Twenty one days after the initial aphid infestation, numbers of winged adults and wingless adults were similar among the canola varieties ($p>0.05$). Within a *Brassica* variety, cabbage aphids responded differently to plant parts. In the life table study, there was a significant difference in fecundity ($p=0.04$), finite rate of increase λ ($p=0.048$) and doubling time DT ($p=0.032$) of cabbage aphids reared on mature leaves among the canola varieties. The highest fecundity (55.93 ±3.35 nymphs/female) and intrinsic rate of increase r_m (0.364±0.013) were observed on canola-mustard. However, no significant differences were found in the nymphal development period, longevity, survival and mean generation time of cabbage aphids on the canola varieties tested. Assessing the ability of mustard and canola varieties to resist aphid infestation in the drier and warmer regions of Australia is critical with new canola varieties being released, and the increasing climatic variability in the cropping regions of NSW due to human-induced climate change.

Key words life table parameters, canola-mustard, climate change, plant structure

INTRODUCTION

Among the various insect pests invading *Brassica* crops, the cabbage aphid *Brevicoryne brassicae* L. (Hemiptera: Aphididae) is considered one of the most destructive, being widely distributed in temperate and warm regions around the world (CABI 2013). Aphids transmit 50% of all insect-borne plant viruses (Nault 1997). Virus transmission is increasing with warming conditions and changing precipitation regimes (Finlay and Luck 2011). With the Australian continent warming by 0.75°C since 1910 (CSIRO-ABM 2012), and predictions for a dryer continent by 2030 (CSIRO 2007), it is important to understand the effects on population dynamics (Andrew 2013) including at the microclimate level (Andrew *et al.* 2013a). Also understanding changes in nutrition for different plant varieties is critical in studying associated pest species (Nguyen *et al.* 2014; Chanthy *et al.* 2012).

The glucosinolate content of canola (*Brassica napus*) affects the taste and quality of the canola oil product. Australian plant breeders actively select canola and mustard (*Brassica juncea*) plants with the aim of reducing the glucosinolate content as much as possible. Mustards, despite higher glucosinolate levels than canola, germinate faster and are more tolerant to moisture stress (drought), traits which are desirable for growing in dryer regions

(Holland 2002). Plant breeders are now producing near-canola mustard plants with glucosinolate levels comparable to that found in canola (Burton *et al.* 2003), thereby enabling canola quality mustards (canola-mustard) to be grown in dryer regions, such as northern New South Wales.

Glucosinolates play an important role in the host plant-insect (pest) relationship. Glucosinolates, in combination with flavonoids and isothiocyanates (mustard oils) are responsible for attracting and stimulating the feeding and oviposition of pest species, e.g. diamondback moth (*Plutella xylostella*), cabbage butterfly (*Pieris* spp.), *Helicoverpa* spp., cabbage aphids (*Brevicoryne brassicae*), and turnip aphids (*Lipaphis erysimi*). Conversely, glucosinolate is toxic to many insect species and thus responsible for deterring and repelling many potential pests (Hopkins *et al.* 2009). Few studies report on aphid population dynamics on canola–mustard, mustard and conventional canola *B. napus* in the dryer regions of northern NSW, where cabbage aphids are an important pest of *Brassica* crops.

The main objectives of this study were to assess attractiveness of three varieties of canola: (1) canola (Pioneer® hybrid 45Y77) (*Brassica napus*), (2) Juncea canola 'Oasis' (*B. juncea*) and (3) Canola-mustard 'Kaye' (*B. juncea*) to aphids, and performance of aphids on each variety. The results of this study may provide useful guidelines for decision making in canola crop management in northern New South Wales. The physiological differences between canola and canola-mustards may result in differences the pest aphid densities and nutrition.

MATERIALS AND METHODS

The study was conducted in the Zoology glasshouse complex, University of New England, Armidale, Australia, from January to June, 2012.

Cultivation of canola varieties

The seeds of three canola varieties (Table 1), (1) canola (Pioneer® hybrid 45Y77) (*B. napus*), (2) juncea canola 'Oasis' (*B. juncea*) and (3) canola-mustard 'Kaye' (*B. juncea*), were obtained from New South Wales Department of Primary Industries, Tamworth. Twenty seeds of each canola variety were sown in a germination tray (25 x 35cm) filled with potting compost on 10th February 2012. Three-week-old seedlings were transplanted individually to conically shaped plastic pots (15cm diameter) filled with the same potting compost. One plant was grown in each plastic pot. Plants were kept in a glasshouse compartment at 18-25°C and relative humidity (RH) of 60-75%. All plants were watered daily without adding fertilizers or chemical controls.

Aphid colony

To establish a laboratory culture, free-living cabbage aphids *B. brassicae* were collected from broccoli plants in Armidale, New South Wales and were then maintained on new broccoli plants. Aphid stock cultures were maintained in cages (1m x 0.7m x 0.7m) in a glasshouse (18-25°C, 60-75% RH) to produce a suitable population of aphids for experimental design. After two or three generations, two-day-old females were used for the experiments under glasshouse conditions.

Aphid performance on canola varieties

Experiment 1 –Assessing population growth of cabbage aphids on canola varieties

Ten plants from each canola variety were kept in the glasshouse under the conditions of 18-25°C, 60-75% RH and natural light. We measured cabbage aphid performance on five-week old plants of each of the three canola varieties. Six plants at the same growth stage of each variety were selected for the experiment. Ten two-day-old wingless mature females from the stock cabbage aphid colony were placed on each plant using a paintbrush. Three plants of each canola variety were then placed in a cage (1m x 0.7m x 0.7m) with two cages per canola variety.

Aphid numbers (nymphs + wingless adults + winged adults) on plants of each canola variety were counted on days 3, 15 and 21 from the start of aphid infestation. At higher aphid densities when direct counting became difficult, the aphid population was estimated by carefully counting (without disturbing) the number of aphids on a measured part of the plant and then the estimated population was extrapolated for the whole plant. Aphid numbers were counted separately on the leaves, the flowers and on the rest of the plant. Total numbers of cabbage aphids on the whole plant were also calculated based on aphids counted on the separate plant parts. After three weeks the number of wingless and winged aphids on each canola variety was counted and recorded separately. Relative susceptibilities of the different canola varieties to aphid infestation were assessed by comparing average abundance of aphids on the plants.

To assess the performance of cabbage aphids on the different plant structures of each canola variety, aphid numbers on the top leaves (two leaves per plant), central stem, and inflorescence of each plant were counted and recorded on the last observation day of the experiment (21 days). All flowers were removed from the plants and placed on white paper to count the number of cabbage aphids directly.

Experiment 2 - The reproductive performance of cabbage aphids reared on different canola varieties

The reproductive performance of cabbage aphids was studied on three canola varieties: canola, juncea canola and canola-mustard in a glasshouse at 18-25^{0}C, 60-75% RH and natural light using clip cages (3cm diameter and 1.5cm depth fitted with mesh lids) established on leaves of each plant at the 4-6 true leaf stage. To establish a cohort of first instar nymphs (<24h old), viviparous wingless aphid adults were transferred individually to the underside of a predetermined mature leaf (below the top) of the plant for each canola variety. Wingless aphids were placed individually on each canola leaf and then confined in a clip cage. After 16-18h (overnight), one first instar nymph was left in each clip cage, while the wingless adult aphid and other newborns were removed. Fifteen replicate clip cages were established for each canola variety (2-3 clip cages per plant) (Figure 1). Daily observations were conducted to measure: survival rates of the nymph until adult emergence; and development period of immature stage of cabbage aphids.

To measure fecundity on canola varieties, a viviparous aphid (< 2 days old) was reared from the immature stage and transferred to a new canola leaf of the same variety, and then confined with a clip cage as described previously. The cages were observed daily to record numbers of offspring laid on the leaf inside the clip cage. Nymphs were removed from the cages after counting. If the aphid mother died within the first 24h, it was replaced with a newly emerged adult. Daily observations were recorded until wingless adults died (up to 28 days).

Data analysis

The data were analysed using Datadesk 6.3.1 and R statistical software (version 2.14.1). In experiment 1, cabbage aphid abundance (log x+1 transformed) among canola varieties and plant parts were analysed using a 2-way analysis of variance (ANOVA). In experiment 2, effects of different canola varieties on survival rate of the pre-reproductive stage of the cabbage aphid were analysed using generalized linear model (GLM). Fecundity and life table parameters of cabbage aphids reared on three canola varieties were also analysed using a one-way ANOVA. The differences between means for ANOVA was compared with least significant difference tests ($\alpha = 0.05$).

The following equations were used to measure net reproductive rate (R_0) and mean generation time (T) (Birch 1948; Laughlin 1965): Reproductive rate: $R_0 = \sum_x l_x m_x$; Generation time: $T = \sum_x l_x m_x x / R_0$; intrinsic rate of increase $r_m = \ln(R_0)/T$; finite rate of increase $\lambda = e^{rm}$; population doubling time $DT = ln(2)/ R_0$, where x is the age of the immature and mature stages in days, l_x is survival of the immature and mature stages until x, and m_x is the number of born progeny at age x.

RESULTS

The population growth of the cabbage aphid *Brevicoryne brassicae* on canola varieties

Total aphid number/canola plant: The population growth of cabbage aphids reared on different varieties of canola in all three observations is shown in Figure 2. Mean number of cabbage aphids did not differ significantly among canola varieties, but did increase significantly over time (Figure 2, Table 2). There was no interaction between canola variety and time (Table 2).

Wingless and winged cabbage aphids on canola varieties: There was no significant difference in the number of alate and wingless adult aphids feeding on the whole plant of three canola varieties. However, there were significantly more wingless individuals (Figure 3, Table 3) and an interaction with a significant difference between alate individuals on canola compared to canola-mustard (p=0.0426).

Cabbage aphid reproductive performance on top leaves, stems and flowers of canola plants: Aphid abundance was significantly different between canola varieties, plant structures, and their interaction (Figure 4, Table 4). Aphids were significantly in higher abundance on the top leaves of canola compared to the stems of the same host plant, and non-existent on the flowers. Abundance was also high for canola when compared to canola-mustard and juncea canola. Aphids on the stems of canola were significantly less abundant than on the stems of other varieties. On canola-mustard, aphids were significantly more abundant on the flowers compared to stems and top leaves of the same species.

Effects of canola varieties on cabbage aphid *Brevicoryne brassicae* survival and reproductive performance

Survival rate of immature stage: Survival rate (%) of the cabbage aphid (from birth to adult emergence) in clip cages on tested canola varieties is shown in Table 5. Canola variety had no significant impact on survival rate (%) of nymphal stage from birth to adult emergence (p=0.098). Immature stages of cabbage aphids passed through four nymphal instars to reach the adult stage. All of the 1st instar nymphs survived.

Development, longevity and fecundity: There were no significant differences in immature development periods and longevity of cabbage aphid winged adults reared on different canola varieties ($F_{(2,42)}$=2.63, p=0.085). The aphid nymphs passed through four instars to reach maturity, with total time ranging from 7.78 days on canola-mustard to 8.58 days on canola. Fecundity of the cabbage aphid was affected by canola varieties with 55.93±3.35 nymphs/wingless female on canola-mustard, 46.83±5.53 nymphs on canola and 42.60±3.39 nymphs on juncea canola ($F_{(2,42)}$=3.52, p=0.04) (Table 6).

Life-history parameters: The intrinsic rate of increase (r_m) ($F_{(2,42)}$=3.39, p=0.044), population growth rate per day (λ) ($F_{(2,42)}$=3.29, p=0.048) and doubling time (days) ($F_{(2,42)}$=3.78, p=0.032) were significantly higher on canola-mustard compared to canola and juncea canola (Table 7). However, mean generation time (T) of the cabbage aphid in clip cages among canola varieties was not significantly different under glasshouse conditions ($F_{(2,42)}$=1.94, p=0.158).

DISCUSSION

This study demonstrates that three tested canola varieties, namely canola (*B. napus*), canola-mustard Kaye and juncea canola are very susceptible to cabbage aphid infestation under glasshouse conditions. In experiment 1, there were no significant effects of canola variety on the population growth of cabbage aphids at each assessment day. The cabbage aphid performance, however, indicates different responses to plant parts within a canola species. Cabbage aphids performed well (based on abundance) on the topmost young leaves of canola, whereas on canola-mustard and juncea canola more aphids were found on stems, leaves and flowers. Holland *et al.* (2003), reported that mustard has a higher content of glucosinolates than canola and juncea canola. Moreover, glucosinolates occur differently across parts of the plant and their total concentration decreases as the leaf tissue matures (Lambdon *et al.* 2003), and variation of glucosinolates also occurs on small spatial scales within leaves (Shelton 2005). On yellow mustard (*Sinapis alba*), cabbage aphids prefer young parts of the growing stems, possibly to compensate for the presence of glucosinolates elsewhere on the plant (Hopkins *et al.* 1998). Previous studies investigating the distribution of glucosinolates show considerable variation among plant organs and even plant development stages. The highest glucosinolate levels are found in youngest leaves (Lambdon and Hassall 2005), and in reproductive tissues of flowers and seeds (Brown *et al.* 2003; Smallegange *et al.* 2007).

In a previous study, Cole (1997a), found that the population growth rate of cabbage aphids reared on a wide range of wild and cultivated *Brassica* varieties has a strong relationship with a combination of four glucosinolates (sinigrin, gluconapin, progoitrin and napoleiferin). Additionally, the physical structures of host plants could affect the oviposition preference of aphids (Fathi *et al.* 2011). The information on morphological features of canola varieties indicates that canola-mustard and juncea canola are early-flowering and more attractive to cabbage aphid feeding. The harder stem of canola however, could limit feeding behaviour of cabbage aphids (Table 4). In this study, our results showed that numbers of wingless adult cabbage aphids at the last observation (21 days) were not different among canola varieties, but marginally higher number of alates occurred on canola. Other studies have pointed out that nutritional quality of aphid diets has a correlation with the production of winged morphs (Mittler and Kleinjan 1970; Vereschagina and Shaposhnikov 1998). A review by Müller *et al.* (2001), showed

that poor nutritional quality of host plants is not always related to increased production of winged morphs in aphids. The production of winged aphid adults may be affected by different factors such as environmental cues, density, unfavourable abiotic conditions, interactions among aphid species, or even maternal effects. Here, our results suggest that crowding within cabbage aphid populations on the canola varieties is likely to induce the production of winged aphids.

In experiment 2, the canola varieties tested had no significant effects on the survival rate and duration of immature development of the cabbage aphids in clip cages. The nymphs developed through four nymphal instars ranging from 7.78 days to 8.58 days with a high survival rate (80-100%). Similar trends were observed in the longevity of wingless adults and mean generation time (from birth to first oviposition), both of which did not vary among canola varieties. The presence of trichomes on mustard leaf surfaces had non-significant effects on the reproductive performance of the cabbage aphids in clip cages. However, experiment 2 showed that fecundity and the population growth parameters such as r_m, λ and doubling time (DT) of the cabbage aphid are affected by canola varieties when cabbage aphids were kept in clip cages on mature leaves. Fecundity of the cabbage aphid was lowest when females were reared individually on leaves of juncea canola and highest when reared on canola-mustard. Compared with the study of Mirmohammadi *et al.* (2009), undertaken on oilseed rape varieties, fecundity and r_m in this experiment are higher and show significant difference between the tested varieties. However, Ulusoy and Ölmez-Bayhan (2006), showed that mustard was resistant to cabbage aphids based on the low values of r_m and fecundity which were measured on excised leaves under laboratory conditions. Indeed, different environmental conditions or rearing techniques could lead to different life-history parameters of aphid individuals. Cole (1997b), suggested that various concentrations of glucosinolates in some *Brassica* varieties could cause changes in the values of r_m. Moreover, in experiment 2, the use of clip cages without knowledge of aphid performance on various parts of the plants might be giving an over or underestimate of r_m.

Mustard and canola appear to be suitably adapted to parts of Australia with dry and warm conditions. These crop species may have an important role due to their superior drought resistance characteristics, and consequently higher yields in the harsh climates (Spenceley *et al.* 2003). Environmental stresses such as drought and changing temperature can have profound effects on the biochemical composition of host plants and subsequently affect aphid communities. These stressors may be amplified with the predicted warming and drying climate over the coming decades (CSIRO-ABM 2012). Such changes may alter the ecology, physiology and behaviour of aphids (Andrew *et al.* 2013b), with some unpredictable effects. This may then lead to further plant stress via reducing plant nitrogen uptake from the soil (Katayama *et al.* 2014) and proliferation of aphid-borne viruses (Finlay and Luck 2011). In this study under glasshouse conditions, no significant differences were found between tested canola varieties and aphid abundance. Further research to assess the ability of mustard and canola varieties to resist aphid infestation in the drier and warmer regions of Australia would be beneficial.

In conclusion, three canola varieties: canola, juncea canola and canola-mustard were very susceptible to cabbage aphids. Total aphid numbers did not vary significantly among tested canola species over the assessment period under glasshouse conditions. Cabbage aphids responded differently to different parts of the plant on different varieties. A higher population of the cabbage aphids was observed on the topmost leaves of canola. However, on

canola-mustard aphids were significantly more abundant on the flowers compared to stems and top leaves of the same species. Unlike free-living aphids on plants, analysis of variance showed that the canola varieties affected some life-history parameters of cabbage aphid individuals confined in clip cages on mature leaves. No significant differences were found in the nymphal development period, survival longevity and mean generation time of cabbage aphids among canola varieties. Further research is required to investigate the performance of other aphid species and insect pests attacking new canola varieties in northern NSW. Studying aphid responses to environmental stress-induced changes in conventional and new canola varieties is also needed and may have scientific significance in canola breeding programs.

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Figure. 1. Canola plant specimen with clip cages

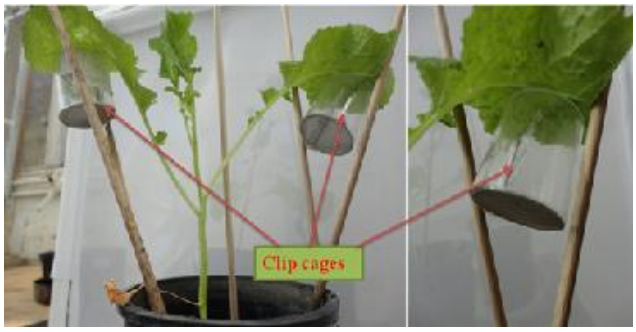

Figure 2. Boxplot showing population growth of the cabbage aphid under glasshouse conditions among three canola varieties (canola, canola mustard, and juncea canola). Initial population size (Day 0 = 10). The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, dotted line marks the mean, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentiles.

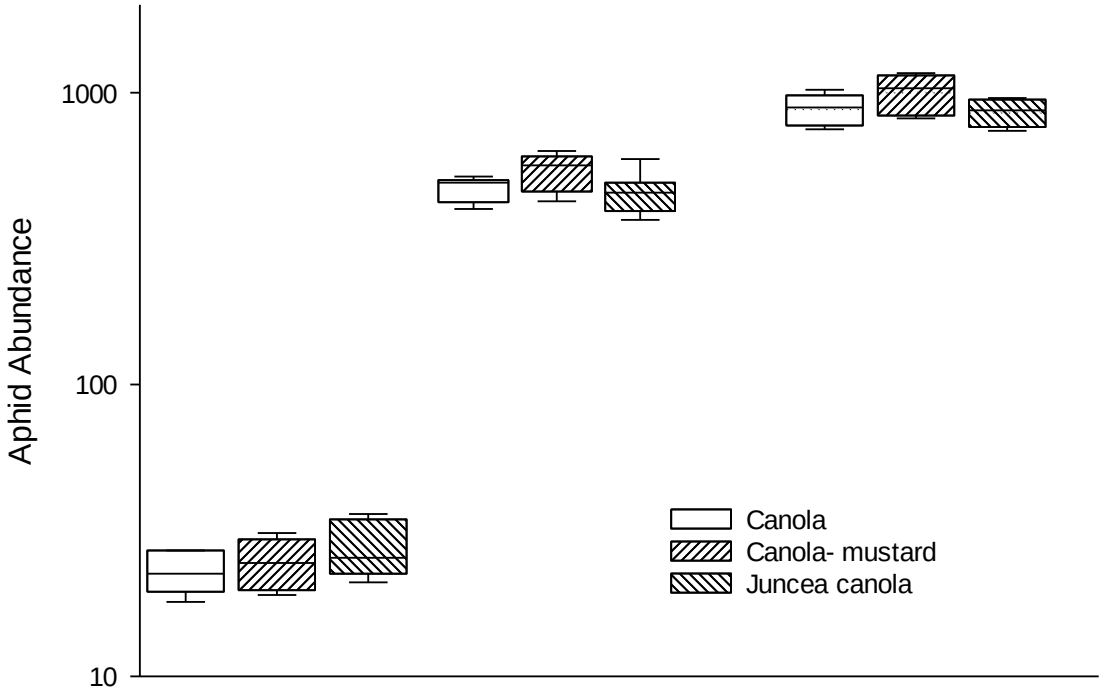

Figure. 3. Mean number of alate and wingless adult cabbage aphids on three canola varieties (canola, canola mustard , and juncea canola). The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, dotted line marks the mean, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentiles.

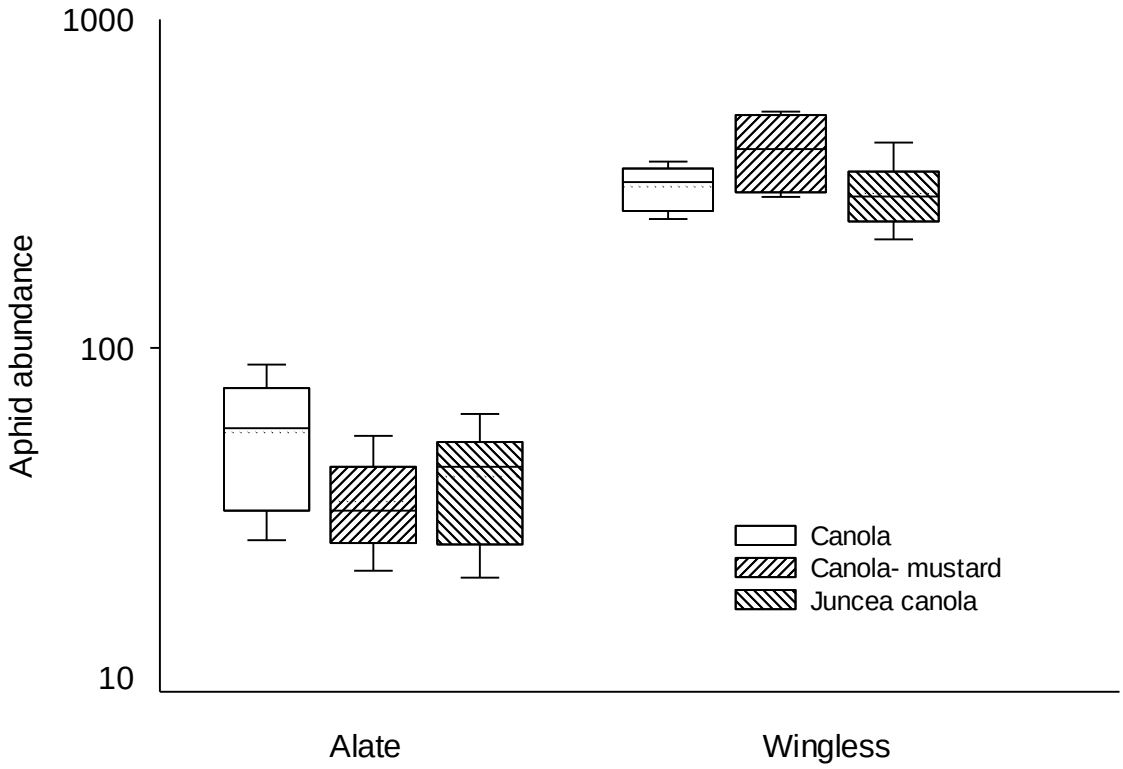

Figure 4. Numbers of cabbage aphids on different plant structures (top leaves, strems, flowers) of canola varieties (canola, canola mustard, and juncea canola). The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, dotted line marks the mean, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentiles.

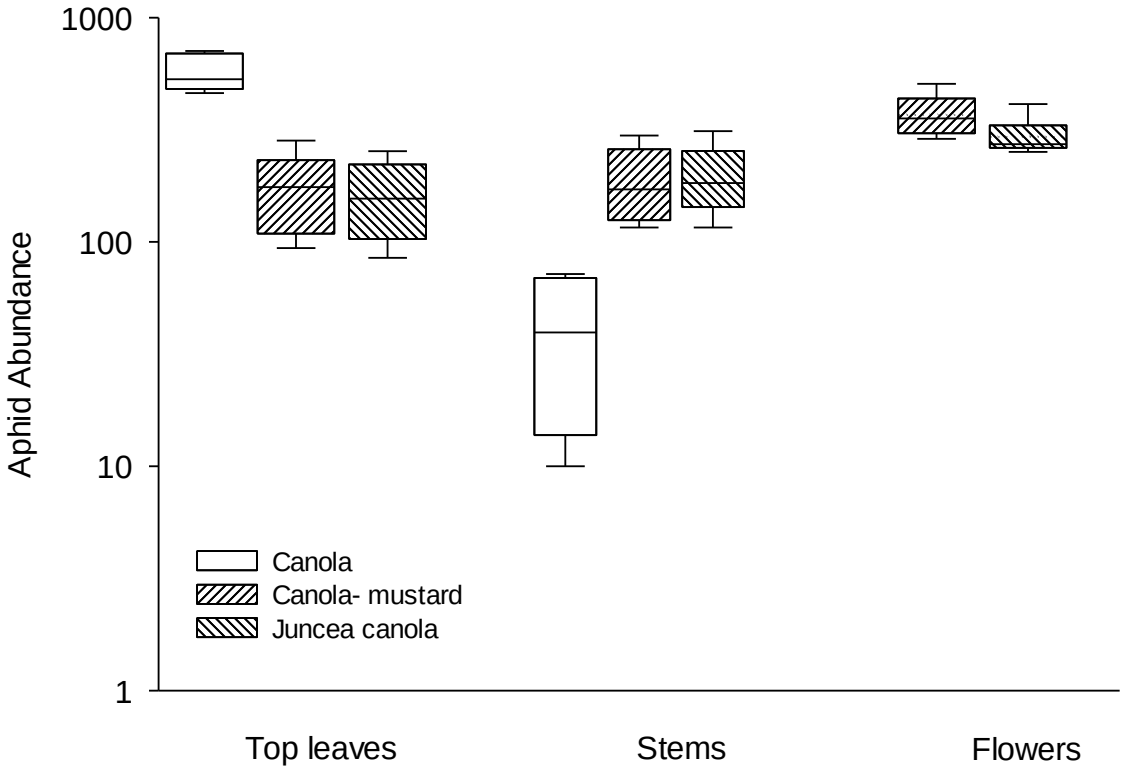

Table 1. Canola varieties tested in aphid feeding experiments and their morphological characteristics.

Canola variety	Morphological characteristics
Canola	Waxy green foliage, short and harder stem
Juncea canola	Dark green foliage, long and soft stem, early-flowering
Canola-mustard	Dark green foliage, young leaf surface has high trichome density, long and soft stem, early-flowering

Table 2. ANOVA output assessing population growth of the cabbage aphid on three canola varieties (Canola) and at three times (Day). Significant values in bold.

Factor	df	SS	MS	F	*p*
Canola	2	0.12	0.06	2.45	0.098
Day	2	130.84	65.42	2770.30	**<0.0001**
Canola*Day	4	0.16	0.04	1.65	0.1783
Error	45	1.06	0.02		
Total	53	132.18			

Table 3. ANOVA for significant effect of canola varieties (Canola) on a number of alate and wingless adult cabbage aphids (Winged). Significant values in bold.

Factor	df	SS	MS	F	*p*
Canola	2	0.18	0.09	0.88	0.4266
Winged	1	38.94	38.94	382.61	**<0.0001**
Canola*Winged	2	0.72	0.36	3.51	**0.0426**
Error	30	3.05	0.10		
Total	35	42.88			

Table 4. ANOVA for significant effect of canola varieties (Canola) on abundance of cabbage aphids on different structures of host plants (Structure). Significant values in bold.

Factor	df	SS	MS	F	*p*
Canola	2	52.06	26.03	173.84	**<0.0001**
Structure	2	23.83	11.91	79.56	**<0.0001**
Canola* Structure	4	100.52	25.13	167.84	**<0.0001**
Error	45	6.74	0.15		
Total	53	183.14			

Table 5. Survival of immature stage of the cabbage aphid in clip cages on three canola varieties under glasshouse conditions (n = 15).

Canola varieties	% survival in nymphal instars				
	1st	2nd	3rd	4th	PRD*
Canola	100	93.33	80	80	80
Canola-mustard	100	93.33	93.33	93.33	93.33
Juncea canola	100	100	100	100	100

(*) PRD: Adults during pre-reproductive delay

Table 6. Reproductive period, adult longevity (days ± SE) and fecundity (nymphs per wingless adult) of the cabbage aphid in clip cages on three canola varieties. No significant difference in means (p >0.05) among stage among varieites within stages indlicated with the same letter.

Host plant / Stage	Canola variety		
	Canola	Canola-mustard	Juncea Canola
Immature period (days)	8.58 ± 0.26[a]	7.78 ± 0.19[a]	8.06 ± 0.26[a]
Longevity of wingless aphid	12.83 ± 0.44[a]	12.07± 0.61[a]	13.60 ± 0.63[a]
Numbers of nymphs/female	46.83 ± 5.53[b]	55.93 ± 3.35[a]	42.60 ± 3.39[b]

Table 7. Population growth parameters of the cabbage aphid in clip cages on three canola varieties (mean ± SE). No significant difference in means (p >0.05) among stage among varieites within stages indlicated with the same letter.

Host plant / Parameters	Canola variety		
	Canola	Canola-mustard	Juncea Canola
Intrinsic rate of increase (r_m)	0.316 ± 0.015b	0.364 ± 0.013[a]	0.325 ± 0.013[b]
Mean generation time T (days)	12.16 ± 0.38[a]	11.14 ± 0.39[a]	11.53 ± 0.31[a]
Finite rate of increase λ (day^{-1})	1.374 ± 0.021[b]	1.441 ± 0.018[a]	1.386 ± 0.019[b]
Doubling time DT (days)	2.241 ± 0.1[a]	1.934 ± 0.068[b]	2.176 ± 0.080[a]