Viruses in spring-sown dual-purpose canola in the high rainfall zone of southern Victoria

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Abstract
Canola (*Brassica napus*) is an important oilseed crop and viruses are one of the yield limiting factors. A field survey was undertaken in the Glenelg Hopkins region of southern Victoria, canola samples were collected and tested for viruses using tissue blot immunoassay (TBIA) and aphids monitored. The viruses tested in this study were: *Turnip yellows virus* (TuYV), *Cauliflower mosaic virus* (CaMV) and *Turnip mosaic virus* (TuMV). Survey results indicated that TuYV, CaMV and TuMV were wide spread in spring-sown canola. The incidence of TuYV was high in nine of the 11 crops infected. CaMV incidence was high in four of the 10 crops infected, while TuMV was detected in six crops with low incidence. Apterous (wingless) turnip aphid (*Lipaphis pseudobrassicae*) colonies were found in eight of the 12 crops sampled in March-April. The number of alate (winged) aphids was low in autumn-sown crops. Results indicate that spring-sown dual-purpose (grain/graze) canola in the High Rainfall Zone (HRZ) of southern Victoria can harbour aphids and viruses. In years with favourable conditions, this may provide a 'green bridge' for emerging autumn-sown canola crops.

Keywords
Graze and grain, green bridge, aphids, *Brassica napus.*

Introduction
Canola (*Brassica napus*) is the third most important winter grain crop following wheat and barley in Australian dryland farming systems. In southern Victoria, canola has gained acceptance in mixed farming enterprises as a dual-purpose grain/graze crop. Particularly, the introduction of high yielding canola from Europe with winter dormancy has provided the opportunity for spring sowing. This extends the period for grazing over the summer and into the autumn filling the feed gap at these times of harsh summer conditions, with minimum grain yield penalties (Paridaen and Kirkegaard 2015). However, the adaptation of fodder canola and turnip crops into southern Australia is a matter of concern because they may host pests and diseases (Kirkegaard 2007) especially viruses.

Three significant viruses are reported in canola grown in Australia. They are TuYV (Aftab et al. 2015), CaMV and TuMV (Coutts and Jones 2000). The most important and wide spread of these viruses is considered to be TuYV (Dreyer et al. 2001). TuYV is a persistent virus that belongs to the *Polerovirus*es within the family of *Luteoviruses*. In field experiments, early infection of TuYV caused yield losses of up to 46% (Jones et al. 2007). While field observations made during the 2014 TuYV epidemic have seen early infections cause up to 75% yield loss (Coutts et al. 2015). TuYV is a phloem-limited virus that disrupts the transport of assimilates which is an important factor in determining seed yield (Mendham et al 1981). Earlier infection with TuYV can result in high yield loss, but yield loss due to TuYV infection decreases at later stages of crop development (Coutts et al. 2006). However, canola remains susceptible until mid-podding. Infection after mid-podding can affect oil quality, but yield loss is minimal (Jones et al. 2007).

CaMV a non-persistent virus, genus *Caulimovirus* family *Caulimoviridae*, which has been shown in field trials to reduce seed yield per plant by 92% (Walsh and Tomlinson 1985). In glasshouse experiments, TuMV, a non-persistent virus genus *Potyvirus* family *Potyviridae*, reduced seed yield by 64% (Walsh and Tomlinson 1985). Transmission of canola viruses is exclusively through their aphid vectors. The green peach aphid (*Myzus persicae*) is the principal vector of TuYV (96% transmission efficiency), while the cabbage aphid (*Brevicoryne brassicae*) can also transmit at a lower rate (14% transmission efficiency) (Schliephake et al. 2000) as can turnip aphid (*Lipaphis pseudobrassicae*) (van Leur et al. 2013). Autumn is the critical time for virus infection in canola, as aphids move from the 'green bridge' into the earliest-sown

crops. Infections can also occur with spring aphid flights, but these probably have little effect on yield (Marcroft et al. 2008).
The potential for carryover of canola viruses on self-sown canola and alternative hosts from one season to the next on the 'green bridge' has been identified in south-western Australia (Coutts and Jones 2000). Despite this, little research has been undertaken to determine the potential for carryover of canola viruses on spring-sown dual-purpose canola in southern Victoria. The aim of this study was to determine virus incidence in the 'green bridge', to assess the potential for the carryover of viruses, and subsequent level of transmission to autumn-sown crops by aphid vectors.

Methods

Canola field survey

A survey of 12 canola crops was conducted during March and April 2016 to detect virus that may be present in crops acting as a 'green bridge' (Figure 1). These crops were sown in spring 2015 for use as dual-purpose crops. One hundred random leaf samples were collected from each canola crop by walking in an "M" pattern and removing individual leave petioles at random every 5 metres to avoid bias. To assess the possible spread of viruses by aphids from the 'green-bridge' into new season (2016) autumn canola crops, 10 random leaf samples from each of six nearby crops (<1.5 km) were collected in mid-August 2016 and tested for all three viruses.

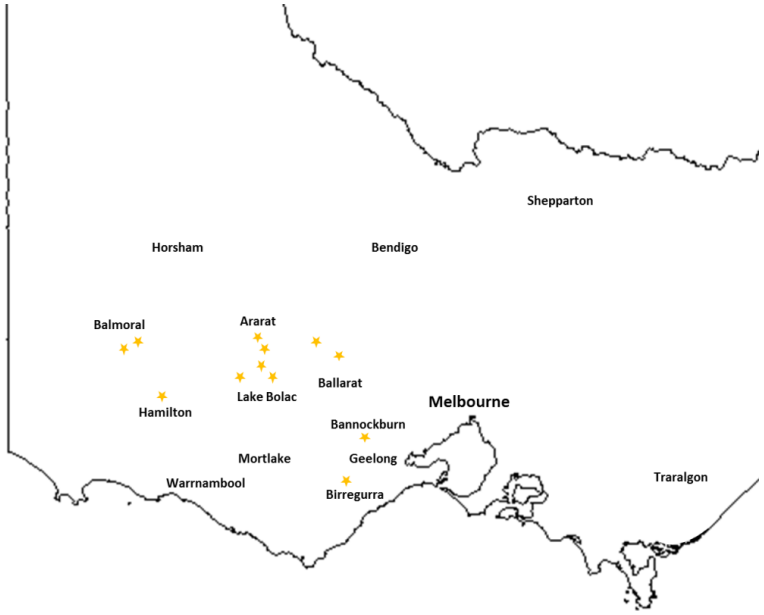

Figure 1. Location of 12 paddocks of dual-purpose winter canola sown in spring 2015 and surveyed for canola viruses in the autumn of 2016, across south-eastern Victoria.

Sample processing and virus detection

Sap from canola leaf petioles or stems were blotted onto nitrocellulose membranes in bundles of 10. The bundles were blotted on three copies of membrane and the blots were tested for TuYV, TuMV and CaMV using tissue blot immunoassay (TBIA) as described by Freeman et al. (2013). The primary antibodies for CaMV and TuMV were obtained from DSMZ Germany. The strains of *Beet western yellows virus* that infect oilseed rape are now referred to as TuYV (Stevens et al. 2008) but antisera to detect TuYV is not available. Instead, Luteovirus group antisera was used to detect TuYV in TBIA. This antisera was obtained from DSMZ Germany.

Aphid monitoring

During both the autumn and spring virus surveys, the same plants sampled for TBIA virus identification were also assessed for the presence of aphids within each paddock. In live colonies, only turnip aphid was identified. Additionally, aphid flights were monitored from June to November 2016 fortnightly during the growing season using four sticky traps on the edge of a canola crop near Hamilton.

Results

Virus incidence in spring-sown canola crops acting as a 'green bridge'

Viruses were widespread in spring-sown dual-purpose canola crops assessed in autumn 2016 (Table 1). TuYV was detected in 92% of crops sampled, with an average in-crop incidence of 47%. TuMV was found in 50% of crops surveyed and CaMV was detected in 83%, however, the in-crop incidence was low with 2% and 8% of plants infected respectively. Combinations of viruses were also detected. TuYV in combination with TuMV was found in 50% of crops and with CaMV in 75% of crops. TuMV and CaMV were found together in 42% of crops. All three viruses were found together in 42% of crops.

Virus incidence in autumn-sown crops
In mid-August 2016, TuYV was detected in three autumn-sown canola crops with an average in-crop incidence of 7%. TuMV and CaMV were not detected.

Aphid monitoring
Aphids were counted and identified to species level during the autumn survey. Turnip aphid was the only aphid detected in canola crops during 2016. It was found in eight of the twelve crops surveyed. Turnip aphid colonies were also found in eight of the spring-sown canola crops. On yellow sticky traps green peach aphid, turnip aphid, oat aphid (*Rhopalosiphum padi*), blue green aphid (*Acyrthosiphon kondoi*), ornate aphid (*Myzus ornatus*) and corn aphid (*Rhopalosiphum maidis)* were trapped in low numbers (data not shown).

Table 1. Detection of *Turnip Yellows Virus* (TuYV), *Turnip mosaic virus* (TuMV) and *Cauliflower mosaic virus* (CaMV) in 12 spring-sown dual purpose (graze/grain) canola crops in southern Victoria in autumn 2016. One hundred plants were tested for virus per paddock.

Virus	Number of crops infected	Virus incidence	In-crop virus infection	
		%	Average (%)	Range (%)
TuYV	11	92	47	0-92
TuMV	6	50	2	0-7
CaMV	10	83	8	0-29
TuYV & TuMV	6	50	-	-
TuYV & CaMV	9	75	-	-
TuMV & CaMV	5	42	-	-
TuYV & TuMV & CaMV	5	42	-	-

Discussion
TuYV, TuMV and CaMV occur throughout grain production areas in Australia (Hertel et al. 2004), but TuYV is considered the most important virus in south eastern Australia due to its high incidence (Aftab et al 2015), wide host range (Coutts and Jones 2000) and persistent transmission. Autumn is the critical period for virus infection because aphids invade crops from the weed hosts and self-sown canola, and canola is more susceptible to yield loss at seedling stage (Coutts et al. 2006). Therefore, dual-purpose canola crops growing over the summer period are considered to be high risk management option for virus due to 'green bridge' for infection of autumn crops. Results from the survey confirm spring-sown canola provides a virus reservoir for not only TuYV, but also for TuMV and CaMV (Table 1). Some crops also had multiple virus types, the implications of which need further investigation as TuMV and CaMV are considered more reducing canola yield by up to 90% (Coutts and Jones 2000) while TuYV reduced yields by 46% (Jones et al. 2007). In this study no yield loss studies were conducted.

There was no clear evidence for the movement of viruses from spring-sown dual-purpose canola to the autumn-sown canola. Possible factors that could contribute to the lack of transmission are (i) the presence of turnip aphid in spring-sown dual purpose canola which is considered a less efficient vector (van Leur et al 2013) and (ii) a low number of alate (winged) green peach aphids in newly sown autumn canola crops. The low number of green peach aphids detected during the crop survey and subsequent low virus transmission was probably related to dry summer (decile 4-7) and autumn (decile 4-7) conditions (Bureau of Meteorology 2016). This agrees with Coutts et al. (2006) who found regional differences in TuYV incidence was related to the amount of rainfall in the 2 months preceding the growing season, with low rainfall leading to low aphid flights and hence low virus transmission.

Conclusion
There was a large reservoir of viruses, particularly the more common TuYV, detected within the 12 crops of spring-sown (2015) dual-purpose canola surveyed. Although spread of viruses into autumn-sown canola was

not detected, probably due to the dry summer and autumn conditions, there is a high risk of transmission under more conducive conditions to aphid survival in summer (decile 8-9) in combination with an autumn favourable for aphid multiplication. This could significantly reduce yield and oil quality in autumn-sown crops if the 'green bridge' is not managed and autumn-sown canola is not protected from transmission of viruses by aphids. Further work will focus on the factors that lead to yield loss from CaMV and TuMV.

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