Occurrence of Fruit-Decaying Fungi on Adult Male Mediterranean Fruit Flies (*Ceratitis capitata)* Captured in Orchards and Adjacent Vineyards

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The occurrence of adult male *Ceratitis* fruit flies and their potential to transmit fungi associated with pre- and post-harvest decay of fruit *in natura* were investigated. Sensus fruit-fly traps were installed in orchards each bordering on a vineyard on farms in the Stellenbosch region, South Africa. *Ceratitis* fruit flies were collected weekly, identified and counted to determine the fluctuations in fruit-fly population levels. Captured fruit flies were plated on a semi-selective medium and the number of flies yielding the fungi was recorded. Both the Mediterranean fruit fly (*C. capitata),* and the Natal fruit fly *(C. rosa)* were trapped. *C. rosa* seldom occurred and comprised only 1% of the total number of flies captured. Fruit-fly patterns showed that early infestation of orchards may contribute to the infestation of adjacent vineyards later in the season. At localities where flies were not trapped in the early season, infestation usually occurred in the orchards and adjacent vineyards in the late season, and well after fruits were harvested. Fruit flies from all localities yielded species of *Alternaria*, *Penicillium*, *Aspergillus*, *Rhizopus*, *Mucor* and *Botrytis cinerea* on the medium. Of the different fungi, *Alternaria* and *Penicillium* spp. were most commonly carried by the flies at the various localities. The organisms occurred in a specific pattern on flies captured at a specific locality, and in a given orchard or vineyard. The pattern of fruit-fly infestation and their fungal contamination suggest that the Mediterranean Fruit-fly has the potential to transfer fungi associated with post-harvest decay *in natura* from early-season to mid- and late-season peach and plum orchards, and to neighbouring mid- and late-season wine and table grape vineyards. This highlights the importance of fruit-fly management in order to control disease epidemics in orchards and vineyards.

Fruit-fly species in the genus *Ceratitis* (Diptera: Tephritidae) (Drew, 1989) are major pests in countries with subtropical and moderate climates (Christenson & Foote, 1960; Bateman, 1972). Two species of *Ceratitis* fruit fly occur in the Western Cape province of South Africa, i.e. the Mediterranean fruit fly (*C. capitata* Wiedemann) and the Natal fruit fly (*C. rosa* Karsch) (Myburgh, 1964; White & Elson-Harris, 1992). The Mediterranean fruit fly is probably one of the most adaptable and polyphagous of the tephritid fruit flies and has been recorded from about 300 species of fruits, nuts and vegetables (Liquido, Cunningham & Nakagawa, 1990). The Natal fruit fly, although restricted to Southern Africa and islands in the Indian Ocean, appears to have fairly similar life history characteristics to *C. capitata*. Both *C. capitata* and *C. rosa* are multivoltine, with the number of generations per year being determined by temperature (Borge & Basedow, 1997). The duration of the life cycles ranges from a few weeks to several months, depending on temperature. Long-distance flights have been recorded when fruit is unavailable. Movement to more favourable conditions is, however, restricted to a few hundred metres per week (Christenson & Foote, 1960). In the Western Cape, *C. capitata* population levels vary depending on host availability. Numbers increase during spring in orchards with early ripening fruit, such as apricots. The flies move to peach orchards in midsummer, then to vineyards in early autumn and finally, in late autumn and winter, to citrus orchards (Myburgh, 1964).

Adult fruit flies feed predominantly on ripe and wounded fruits (Hendrichs & Hendrichs, 1990). Ovipositioning takes place during the day and reaches a peak during midday in the case of *C. capitata* (Smith, 1989). Fruit-fly females lay their eggs underneath the skin of the host fruit. Direct damage to fruit, caused by females during oviposition when the fruit skin is pierced in order to lay eggs, can lead to decay by fungal pathogens (Grovè, Steyn & De Beer, 1997). The oviposition site is of critical importance, because larvae of these insects have little mobility and depend on the nutritive value of the chosen host for survival. The mortality rate of larvae in certain fruit types such as grapes is high as a result of the high juice content. Therefore, fruit-fly infestations in vineyards originate from other fruiting hosts in the vicinity, where mating and oviposition activity predominantly occurs (Hendrichs & Hendrichs, 1990). In the absence of suitable hosts, females will oviposit in hosts in which the chances of survival are low or absent (Carey, 1984).

In South Africa *Botrytis cinerea* is the major organism associated with post-harvest decay of stone fruit and grape. The pathogen is most prominent on early- and mid-season stone fruit cultivars. Other fungi, mainly *Penicillium*, *Aspergillus*, *Alternaria*, *Mucor* and *Rhizopus* spp., were also recorded on stone fruit and grapes, but their incidence was low (Fourie & Holz, 1985a; Swart & Holz; 1991, Fourie, Holz & Calitz, 2002). The ability of the Mediterranean fruit fly to vector the plant

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pathogen *Rhizopus stolonifer in vitro* was shown by Cayol *et al.* (1994). Transmission of *R. stolonifer, Alternaria* spp., *Mucor* spp. and *Geotrichum candidum* by the vinegar fly, *Drosophila melanogaster,* was also demonstrated (Butler & Bracker, 1963; Michailides & Spotts, 1990). The aim of this study was to determine the temporal occurrence of *Ceratitis* fruit flies in pome and stone fruit orchards and in vineyards. In addition, their potential for transferring fungi associated with fruit decay was studied.

MATERIALS AND METHODS

Orchards and vineyards

Fruit flies were collected in the Stellenbosch region at five different localities from stone fruit and a pear orchard bordering on vineyards. At Klein Simonsvlei A a Colombard vineyard was selected adjacent to a Santa Rosa plum orchard. Santa Rosa plums and Colombard grapes are harvested during early December and early February, respectively. At Klein Simonsvlei B a Pinotage vineyard was selected adjacent to a Blacks peach orchard. Blacks peach is an early-season cultivar and is harvested late November. Pinotage grapes are harvested early February. At Warwick a Cabernet Sauvignon vineyard was selected adjacent to a Laetitia plum orchard. Harvest dates are mid-January for Laetitia plum, and mid-February for the Cabernet Sauvignon grapes. At Simonsig a Bukettraube vineyard bordering a Packham's Triumph pear orchard was selected. Harvest dates are late January for Packham's Triumph pear, and mid-February for Bukettraube grapes. At Morgenstêr a Reubennel plum orchard (harvest late December) adjacent to a Chardonnay grape vineyard (harvest early February) was selected. Orchard and grape vineyard blocks ranged from 1 to 5 ha. No control measures against fruit flies and post-harvest decay fungi (*Penicillium*, *Aspergillus*, *Alternaria*, *Mucor* and *Rhizopus* spp.) were applied in the orchards and vineyards. In vineyards a recommended programme (De Klerk, 1985) for the control of *B. cinerea* was generally followed. Sprays against *B. cinerea* were applied at flowering, bunch closure, véraison and two weeks before harvest. Fungicides used were iprodione (Rovral Flo 25 EC, Aventis) and pyrimethanil (Scala 40 EC, Aventis).

Fruit-fly traps

Sensus fruit-fly traps (Quest Development CC, Brits, South Africa) containing the para-pheromone, Capilure (Quest Development CC, Brits, South Africa), were used to trap adult male *Ceratitis* fruit flies (Von Broembsen, 1998). The male-only lure was used as it catches more fruit flies than the female lure (A. Ware, CRI, Nelspruit South Africa, personal communication). A 6 g DDVP tablet (dichlorvos 195 g/kg) was placed in the trap to fumigate the fruit flies. Two traps per vineyard or orchard were installed at each locality. Pheromones were changed monthly. *Ceratitis* fruit flies were collected weekly from each trap and counted. The average number of flies trapped per month from each vineyard or orchard was then recorded.

Transmission of fruit-decaying fungi

Following field collection, fruit-fly species were identified (White & Elson-Harris, 1992), plated on Kerssies's *B. cinerea* selective medium (Kerssies, 1990) in Petri dishes and incubated at 22°C with a 12-h photoperiod. The active ingredients in the selective medium, which gradually degrade during incubation, initially allowed the growth of *B. cinerea* and suppressed that of fungi such as *Penicillium*, *Aspergillus*, *Alternaria*, and *Rhizopus* spp. (G. Holz, *unpublished data*). *B. cinerea* was therefore identified and recorded within 1 week of incubation, and the other fungi after 2 weeks of incubation. The mean percentage of *C. capitata* fruit flies, captured per month in an orchard or vineyard, that yielded a fruit decay-causing fungus, was then recorded.

RESULTS

Fruit-fly numbers

During the 32-week period a total of 15 439 adult male fruit flies was collected from the 20 traps placed at the five localities. Both *C. capitata* and *C. rosa* were trapped. *C. rosa* seldom occurred and comprised only 1% of the total number of fruit flies captured. Flies were consistently trapped in an orchard or vineyard when fruit approached maturity only. Their numbers then increased during the harvesting period and thereafter. The flies, however, occurred in a specific pattern in an orchard or vineyard at each locality. Patterns were similar at Klein Simonsvlei A and Klein Simonsvlei B, and at Morgenstêr and Simonsig. The mean number of *C. capitata* recorded each month is therefore given and discussed for Klein Simonsvlei B, Warrick and Simonsig only (Fig. 1). At Klein Simonsvlei B low numbers of fruit flies (3-5 per trap) were first trapped during the second last week of October in the Blacks peach orchard, and during the last week of January in the adjacent Pinotage vineyard. These records were 7 and 2 weeks prior to harvest of the peaches and grapes, respectively. Following the first catches in the peach orchard, fruit flies were intermittently caught in low numbers until 2 weeks prior to harvest, when numbers steadily increased. Fruit-fly numbers then peaked approximately six weeks after harvest. More fruit flies were caught in this orchard than in any other during the study. Fruit-fly numbers in the adjacent Pinotage vineyard, where grapes were approaching maturity, were also the highest during this study. Numbers decreased during late February and were low from March to May.

At Warrick the first flies were caught during the second week of November, when an average of one fruit fly per trap was recorded in the Laetitia plum orchard. Fruit flies were again trapped during the first week of January, whereafter numbers gradually increased. In the adjacent Cabernet Sauvignon vineyard an average of five flies was caught during the second week of January and the first week of March. In both the orchard and the vineyard, the highest mean numbers were recorded during April.

At Simonsig fruit flies were first caught during the first week of February 2001 in the orchard and vineyard. Numbers then steadily increased during March and peaked in May. Fruit-fly numbers were, however, markedly lower in the pear orchard compared to the peach and plum orchards at Klein Simonsvlei and Warwick.

Transmission of fruit-decaying fungi

Fruit flies from all localities yielded species of *Alternaria*, *Penicillium*, *Aspergillus*, *Rhizopus*, *Mucor* and *B. cinerea* on the selective medium. The organisms occurred in a specific pattern on fruit flies caught at a specific locality, and in a given orchard or vineyard. Patterns were similar at Klein Simonsvlei A and Klein Simonsvlei B, and at Morgenstêr and Simonsig. Therefore, the mean percentage of fruit flies yielding the different fungi each month is given and discussed for Klein Simonsvlei B, Warrick and Simonsig only (Fig. 2).

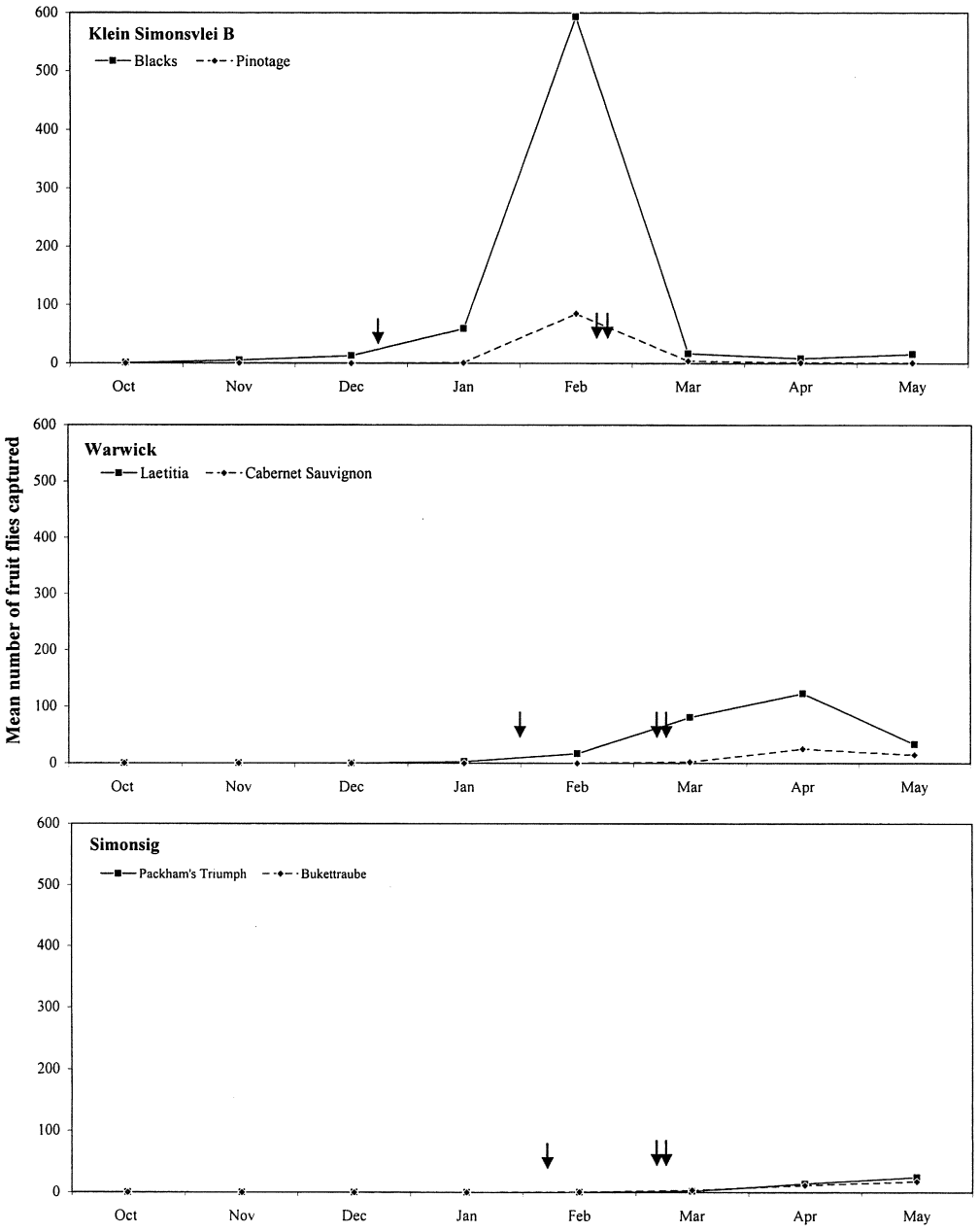

FIGURE 1

Mean number of *Ceratitis capitata* fruit flies captured per month in orchards and adjacent vineyards in different localities. Harvest dates of fruits are indicated by arrows (orchards ↓; vineyards ⇊). Fruit cultivars were peach (Black), plum (Laetitia), pear (Packham's Triumph) and grape (Pinotage, Cabernet Sauvignon, Bukettraube).

Alternaria and *Penicillium* spp. were the fungi isolated most frequently from fruit flies. At Klein Simonsvlei B the proportion of flies yielding *Alternaria* was high during the 32-week study period. In the Blacks peach orchard *Alternaria* was isolated from 53% to 85% of the fruit flies caught from November to May. In the Pinotage vineyard these values ranged from 27% to 65% during the same period. Therefore, fruit flies commonly carried this fungus when fruit approached maturity. In the Laetitia plum orchard at Warwick, the proportion of flies carrying *Alternaria* increased during the first two weeks of December, then remained at approximately 67%. In the Cabernet Sauvignon vineyard the proportion of fruit flies with *Alternaria* increased from the beginning of February and peaked at 84% in April. For both the orchard and vineyard these sudden increases in the proportion of flies contaminated with *Alternaria* occurred prior to harvest. At Simonsig a sudden surge in the proportion of flies contaminated with *Alternaria* occurred in both the Packham's Triumph orchard and Bukettraube vineyard during the first two weeks of January.

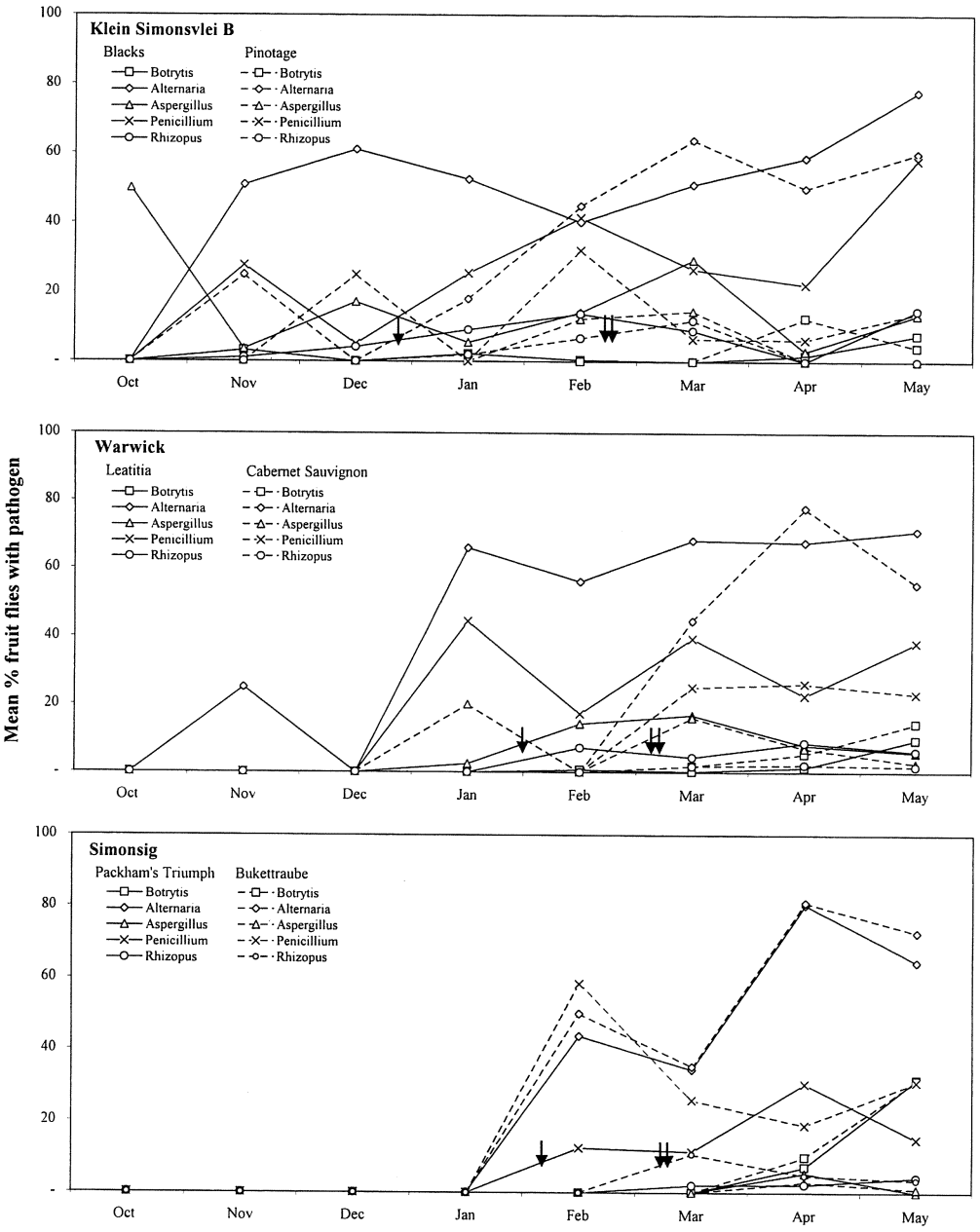

FIGURE 2

Mean percentage of *Ceratitis capitata* fruit flies captured per month in orchards and adjacent vineyards that yielded fruit-decaying fungi after incubation on Kerssies's medium. Harvest dates of fruits are indicated by arrows (orchards ↓; vineyards ⇊). Fruit cultivars were peach (Black), plum (Laetitia), pear (Packham's Triumph) and grape (Pinotage, Cabernet Sauvignon, Bukettraube).

The flies also frequently carried *Alternaria* prior to harvest.

Fruit flies caught at Klein Simonsvlei B carried *Penicillium* as early as October. However, the proportion of contaminated fruit flies fluctuated during the 32-week period. At Warwick and Simonsig, the proportion of fruit flies contaminated with *Penicillium* was high at fruit maturity and during the harvest. *Aspergillus*, *Rhizopus* and *Mucor* species usually developed on the agar plates from fruit flies caught from January onwards. These fungi were less frequently associated with the flies.

In all orchards and vineyards *B. cinerea* developed mainly from fruit flies caught after harvest. In addition, the proportion of contaminated fruit flies increased during March only and reached a peak during April to May. During this period the percentage of fruit flies contaminated with *B. cinerea* usually varied from 1% to 20%. The exception was in the vineyards and orchards at Klein Simonsvlei, which were already infested with fruit flies early in the season. In the Blacks peach and the Santa Rosa plum orchards, *B. cinerea* was sporadically isolated from fruit flies

caught during mid-October and mid-November. In both orchards the fruit flies also carried the pathogen when the fruit approached maturity. Fruit-fly numbers were usually very low during this period.

DISCUSSION

In stone fruit and pear orchards and grape vineyards in the Western Cape province fungi associated with fruit decay (*B. cinerea* and *Penicillium*, *Aspergillus*, *Alternaria*, *Mucor* and *Rhizopus* spp.) (Fourie & Holz, 1985a; Swart & Holz, 1991; Fourie *et al.*, 2002) were carried by adult male Mediterranean fruit flies (*C. capitata).* Furthermore, early infestation of stone fruit orchards by the fruit fly have contributed to the infestation of adjacent vineyards later in the season. For example, in both the peach and plum orchards at Klein Simonsvlei fruit flies were consistently trapped early in the season. Both orchards were heavily infested by the flies soon after harvest early in December. Adjacent vineyards were infested soon thereafter, at a stage when grapes were approaching maturity. At Warwick, Simonsig and Morgenstêr, where flies were not trapped early in the season, infestation usually occurred in the orchards and adjacent vineyards late in the season, well after fruits were harvested. Therefore, the pattern of fruit-fly infestation and their fungal contamination suggested that the Mediterranean fruit fly has the potential to transfer fungi associated with post-harvest decay *in natura* from early-season to mid- and late-season peach and plum orchards, and to neighbouring mid- and late-season vineyards. This implies that it is important to control fruit-fly populations in order to manage disease epidemics. The common practice to install fruit-fly traps in stone fruit orchards should also be implemented in vineyards.

Throughout this study no pre-harvest decay or bunch rot was noted during visual fruit inspections in the orchards or vineyards. In spite of this, varying proportions of fruit flies yielded fruit decay-causing pathogens when trapped. Therefore, the fruit flies could either have been contaminated with the fungi from rotting fruit that went unnoticed, or more likely, from rotting fruit remaining in neighbouring orchards or vineyards after harvest. Species of *Alternaria* and *Penicillium* occur in vineyards as early as blossom and increase gradually (Hewitt, 1974). Hewitt (1974) also found higher *Rhizopus* and *Penicillium* incidences in vineyards adjoining plum orchards. These fungi appeared to increase as the plums ripened and began to drop. This may explain the high level of fruit flies contaminated with both species of *Penicillium* and *Alternaria.*

The finding that fruit-fly numbers, and the proportion flies that vectored a fruit decay-causing fungus, tended to increase during the season in orchards and vineyards suggests that fruit flies can be an important source of primary fungal inoculum. In a study done by Cayol *et al.* (1994) the external and internal mode of transmission of *R. stolonifer* by *C. capitata* was proved *in vitro.* The external mode of transmission involved the mechanical transfer of conidia on the fruit fly's body. Scanning electron microscopy showed that the spores of *R. stolonifer* were carried on the proboscis, head, tarsus and legs of the fruit fly. The internal mode of transmission, involving partial (regurgitation) or total (faeces) transit through the digestive tract, was defined as semi-persistent (spores remain viable after passage through the digestive tract) (Harris & Maramorosch, 1980). Louis *et al.* (1996) demonstrated the ability of the vinegar fly, *Drosophila melanogaster* Meig, to vector *B. cinerea.* Conidia of the fungus can be carried on the fly surface and through intestinal transit, as reported for *Rhizopus* transmission by *D. melanogaster* (Louis *et al.*, 1989) and for *B. cinerea* transmission by grape berry moth larvae (Fermaud & Le Menn, 1989; Fermaud & Giboulot, 1992). Long-term *D. melanogaster/B. cinerea* relationships were obtained in the fly crop during the life of the vinegar fly. Conidia germinated in the insect crop, developed into mycelium, and differentiated into microsclerotia (fungal survival structures) that the flies can carry for their entire life (Louis *et al.*, 1996). Since *D. melanogaster* overwinters as an adult, Louis *et al.* (1996) concluded that it could play a role in winter conservation of *B. cinerea* inoculum. *C. capitata* also overwinters in the adult stage during moderate winters (Fletcher, 1989). The possibility for inoculum transfer of fungal pathogens associated with fruit decay by infested fruit flies from the one season to the next is therefore likely.

The epidemiological consequences of Mediterranean fruit-fly infestations may lead to increased pre- and post-harvest decay in stone fruit and grape. Fruit-fly adults need carbohydrates, lipids and proteins to perform the biological activities necessary for survival and reproduction (Bateman, 1972). Adult fruit flies feed predominantly on ripe and wounded fruits (Hendrichs & Hendrichs, 1990), since they acquire carbohydrates from feeding on, among other things, fruit juices. In the case of *B. cinerea* and *R. stolonifer*, wounding has been regarded as a major entry site for the pathogen on stone fruit (Fourie & Holz, 1985b). On grapes injury has also been regarded as a major entry site for *B. cinerea* (Nair, Emmett & Parker, 1988) and *A. alternata* (Swart, Lennox & Holz, 1995). However, on grapes a combination of fresh wounds, freshly deposited *B. cinerea* conidia and free water on the berry surface is necessary for successful wound infection (Coertze & Holz, 2002). Synchronisation of these events may not occur commonly in the vineyard. However, insects may play a very prominent role in this context. Fermaud and Le Menn (1989) showed that larvae of *Lobesia botrana* carried viable conidia of *B. cinerea* externally or internally. According to Fermaud and Le Menn (1992), the introduction of conidia into wounds by *L. botrana* is important in the initiation of rot during the stages before véraison. Insects can therefore be considered as playing a primary role in disease outbreaks in stone fruit orchards and grape vineyards. They can act as suppliers of inoculum to wounds, and as initiators of the disease cycle under conditions generally unfavourable for disease development. Inocula packages consisting of clusters of conidia or/and mycelia, which might also be placed into wounds, would be more aggressive than single conidia that land near the wound (Coertze & Holz, 2002). However, more information is needed on vectoring and deposition of fungal inocula on fruit by the Mediterranean fruit fly, *C. capitata,* to confirm its role in pre- and post-harvest decay.

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