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The effect of fruit fly larval density on some quality parameters of mango

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Key words: *Bactrocera invadens,* fruit flies**,** larval infestation, fruit quality, Total Soluble Solids, Percentage Titratable Acidity.

1 SUMMARY

Three varieties of mango, *Jaffna*, *Palmer* and *Kent* were infested with different first instar larval densities (10, 20 and 30) of the invasive fruit fly species, *Bactrocera invadens* Drew, Tsuruta & White. This was to determine the effect of each of the densities on three fruit quality parameters. The parameters included Total Soluble Solids (TSS), Percentage Titratable Acidity (% T.A.) and pH, of which TSS and pH showed decreasing trends while % T.A. showed an increasing trend with time. In the controlled treatments where fruits were either held intact or punctured but with no introduction of larvae, there was an increase in pH and TSS and a decrease in %T.A.. The shelf-life, which was taken as the period from the first day of treatment till signs of damage were observed, saw the three varieties infested with 10 larvae lasting up to 6 days, while fruits infested with 20 and 30 larvae lasted for 3 days. The shelf life of the controlled treatments for all the varieties lasted till the 15th day.
The presence of fruit fly larvae in fruits causes a number of changes in internal quality parameters such as the TSS, pH, % T.A. and the internal damage area. While pH and TSS decreased with storage time, % T.A. increased with storage time compared with the controlled fruits. Total damage area of fruits was also increased with increase number of larvae introduced. pH, TSS and % T.A. of *Jaffna* and *Kent* on the 3rd day of storage were almost the same as their control treatments. Despite the differences, the presence of fruit fly larvae in fruits (irrespective of number), will have negative impact on the internal quality parameters after the 3rd day of storage and reduce the market (export and locally) value of the fruits.

2 INTRODUCTION

The mango industry provides both employment and income to farmers and exporters alike (Ekesi & Billah, 2006), and explains the recorded continuous growth in both the domestic and foreign markets during the past years (Stefan et al., 2003). Mango is the only fruit imported in significant quantities by both the developed and developing countries and, after pineapple, considered as the second most important tropical fruit traded internationally. In 2003, a new invasive fruit fly, *Bactrocera invadens* Drew, Tsuruta and White, originating from Asia was detected in Kenya and was reported to be spreading across tropical Africa (Lux et al., 2003b; Mwatawala et al., 2004; Drew et al, 2005; Vayssieres et al., 2005; Billah et al., 2006). Prior to its invasion of Sub-Saharan Africa, the major pest of mango was *Ceratitis cosyra* (Walker) whose average damage range was 20-30% (Lux et al., 2003a, b). Damage assessment

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of *B. invadens* on mango in Benin showed average losses from 10-57 % between the months of April and June (Vayssieres et al., 2005), while losses in Ghana were estimated at 60-85% depending on cultivar and season (Billah et al., 2006). It has been estimated that of the 1.9 million tonnes (MT) of mangoes produced annually in Africa in the 2000s, about 40% is lost to fruit flies (Lux et al., 2003a, b). Damage occurs through oviposition on the fruits/pods, resulting in the formation of black or brown necrosis (lesions) around the puncture marks, followed by decomposition of the fruit especially from the internal feeding of the larvae (CABI, 1999). The larvae tunnel inside the fruit, contaminate the pulp with frass, which predisposes the fruit to fungi and bacteria attack (Pena & Mohyuddin, 1997). These losses deprive communities of an important source of nutrition (particularly vitamin A) and lead to the loss of highly valuable market shares when quarantine-sensitive importing countries refuse produce because of the potential threat of these flies. This study was therefore aimed at ascertaining some of the quality parameter changes that take place when different larval loads are introduced into mango fruits.

MATERIALS AND METHODS

Fruit collection and incubation: Mango fruits were collected from different localities across three regions in Ghana (Greater-Accra, Eastern and Volta) from December 2008 to May, 2009. Fruits were placed on plastic racks with perforated openings at the bottom and incubated in wooden cages over a thin layer (2-5 cm) of moistened heat-sterilized sand (at 100°C for 1 hour) (Plate 1A). This served as a pupariation medium in the laboratories of the West African Sub-Regional Centre of the African Regional Postgraduate Programme in Insect Science (ARPPIS). The wooden cages had netting material of fine mesh size to prevent exiting larvae from escaping. The sand was checked for puparia every 3 days by sieving and after 2-3 weeks of incubation, rotten fruits were dissected to retrieve any hidden larvae or puparia. Collected puparia were held in petri dishes lined with moistened filter paper till fly and/or parasitoid emergence, with laboratory conditions of 27-32 °C and 64-80% relative humidity.

A. B. C.

Plate 1: Laboratory setup and treatment of fruits. **A** = Fruit incubation in plastic trays **B** = Fly holding cages **C** = Experimental mango fruits individually wrapped in grocery bags for observation.

Adult fly rearing and collection of eggs: Emerged flies were transferred to rearing cages. The top and bottom of the cages were made of plastic containers, with fine gauze fabric (muslin cloth) for ventilation at the sides. Also on the side was a zip to allow access into the cages (Plate 1B). Flies were fed on a mixture of sugar and yeast (3:1 w/w) by putting a small quantity of diet on a petri dish placed inside the cage. Cotton wool soaked with water on a petri dish was used as a medium of water for the flies. Oviposition domes made from mango fruits cut into two halves and the pulp scooped out were placed in filter paper-lined petri dishes and introduced into the rearing cages. To facilitate oviposition, domes were punctured with No. 2 entomological pins. Each morning, eggs were collected by means of a soft camel hair brush and a wash bottle into petri dishes lined with a dark

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moistened muslin cloth (to show visibility and contrast of the eggs) and held till hatching.

Treatment of experimental fruits: Different numbers of first instar larvae (10, 20, and 30) were introduced separately into the different varieties of mango (*Kent, Palmer and Jaffna*) (Plate 2). Holes in the fruits were made with the tip of Swiss-made hard forceps. Control set ups were in forms; 1) Punctured fruits with no larvae, and 2) Unpunctured/whole fruits with no larvae. All fruits were individually wrapped in large brown grocery bags to prevent entry of any insects or microorganisms (Plate 1C) and held under ambient conditions of 30-32°C and 68-80% R.H. for observation. Each set up consisted of thirty (30) fruits and the experiments were repeated three times. At 3-day intervals (3d, 6d, 9d, 12d, 15d etc), five fruits from each category (variety and different larval density levels) were randomly picked, cut and quality parameter readings such as pH, Total Soluble Solids **(**TSS) and percentage Titratable Acidity (T.A.) determined (Table 1.). They were also observed for signs of damage to see how long they lasted (shelf life) under each of the infestation levels. Fruit pH of was determined by using a pH meter (Omega Engineering Inc., US).The pH meter was standardized with a pH 4.0 buffer solution. 50-75gm (v/v) of well-mixed proportion of the mango juice was placed in a 100-ml beaker and read on the pH meter. Total soluble solids (or sugar content) were measured using a refractometer (SHIBUYA Manufacturing Company, Japan). A representative sample of the well-mixed clear juice was placed on a refractometer prism and read directly at 20°C. The percentage soluble solid was calculated using the formula;

% Soluble solids = % solids by refractometer x (100%) (Ruck, 1969).

Total acidity was determined by filtering sample juices through Whatman® filter paper and a 10 ml aliquot diluted with 100 ml of distilled water for titration against 0.1N NaOH to a pH of 8, using a pH meter. Four (4) drops of phenolphthalein was added to the solution as an indicator, which changed from orange to pink at the endpoint. Percentage total acidity was calculated using the formula by Ruck (1969).

$$\% \text{ Total acid} = \frac{1}{10} \times \frac{\text{equivalent weight of acid x normality of NAOH x titer}}{\text{weight of sample}}$$

Shelf life of fruits: The shelf life of fruits was obtained by observing the condition of fruits infested with different number of first instar larvae for a period of 15 days by dissection. In cases where fruits were not totally damaged after the 15th day, they were further monitored for 7 days before discarding. In doing this, the levels of internal damage caused by the feeding larvae and total discolored areas were also observed and the areas determined by measurement with venier calipers for discussion purposes (data not shown).

RESULTS

Table 1 shows the effect of fruit fly larval density on pH, Total soluble solids and Titratable acidity in the three varieties of mango studied.

Plate 2: A treated mango fruit (Palmer) showing punctured points where first instar larvae were introduced into the fruits in the Laboratory.

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Table 1: Effect of fruit fly larval density on pH, total soluble solids and titratable acidity of three mango varieties.

Mango Variety	Treatment	Storage Time (Days)														
		3	6	9	12	15	3	6	9	12	15	3	6	9	12	15
		pH					TSS					TA				
Jaffna	10 Larvae	4.4	4.2	4.0	3.9	3.6	8.30	9.33	9.67	7.67	7.00	0.18	0.20	0.23	0.31	0.44
	20 Larvae	4.3	4.1	3.8	3.6	3.5	8.00	9.67	7.33	4.00	4.33	0.18	0.24	0.34	0.41	0.48
	30 Larvae	4.2	4.0	3.6	3.5	3.3	11.33	9.67	6.00	5.00	4.33	0.35	0.42	0.46	0.52	0.52
	Intact + No Larvae	4.7	4.8	5.0	5.1	5.2	11.00	13.00	13.67	14.00	15.33	0.22	0.19	0.14	0.12	0.10
	Punctured + No larvae	4.7	4.8	4.9	5.0	5.1	11.67	13.67	14.33	14.68	15.41	0.18	0.15	0.13	0.10	0.09
Palmer	10 Larvae	4.3	4.2	4.1	3.7	3.5	10.67	12.33	13.33	8.00	5.67	0.36	0.37	0.38	0.41	0.43
	20 Larvae	4.1	3.9	3.8	3.6	3.4	11.00	13.67	14.00	9.00	6.67	0.26	0.28	0.35	0.36	0.45
	30 Larvae	4.0	3.7	3.5	3.5	3.4	11.00	13.00	12.67	9.00	8.30	0.32	0.34	0.36	0.37	0.47
	Intact + No Larvae	3.7	3.9	4.2	4.7	5.0	11.67	15.33	15.67	17.00	17.80	0.19	0.15	0.14	0.11	0.10
	Punctured + No larvae	3.3	4.1	4.5	4.6	4.7	13.33	15.00	16.33	17.32	18.10	0.18	0.14	0.12	0.11	0.09
Kent	10 Larvae	4.6	4.6	4.5	4.3	4.0	10.00	12.67	13.67	12.00	10.67	0.34	0.35	0.35	0.37	0.40
	20 Larvae	4.5	4.5	4.4	4.1	3.6	10.33	12.32	12.67	12.00	11.67	0.33	0.34	0.36	0.39	0.44
	30 Larvae	4.8	4.4	4.1	3.6	3.5	12.33	13.46	13.92	13.00	11.58	0.36	0.38	0.39	0.43	0.48
	Intact + No Larvae	3.6	4.2	4.6	4.8	4.9	13.67	14.76	15.33	16.33	17.67	0.37	0.32	0.18	0.13	0.11
	Punctured + No larvae	3.9	4.4	4.5	4.6	4.9	15.00	16.67	17.33	17.67	18.33	0.32	0.29	0.20	0.14	0.10

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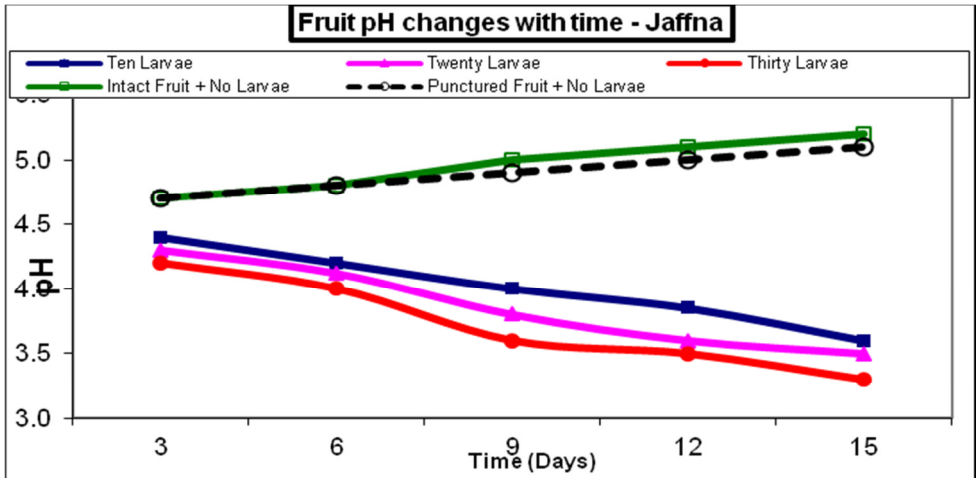
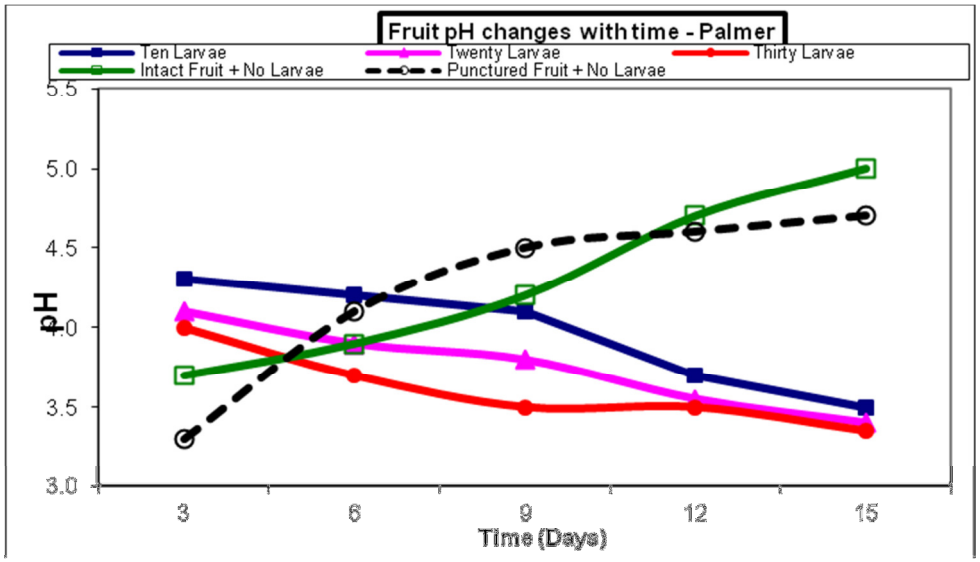
Fruit pH: *Jaffna* - pH of the two control treatments (punctured fruits with no larvae and unpunctured/whole fruits with no larvae) increased with storage time, ranging from 4.7 on the 3rd day of storage through to 5.1 on the 15th day. On the contrary, fruits infested with larvae had decreasing pH with time, from 4.4-3.3, 4.3-3.5 and 4.5-3.8 from the 3rd to the 15th day for the 10-, 20- and 30-larval infestation levels (Fig. 1A).



A.



B.

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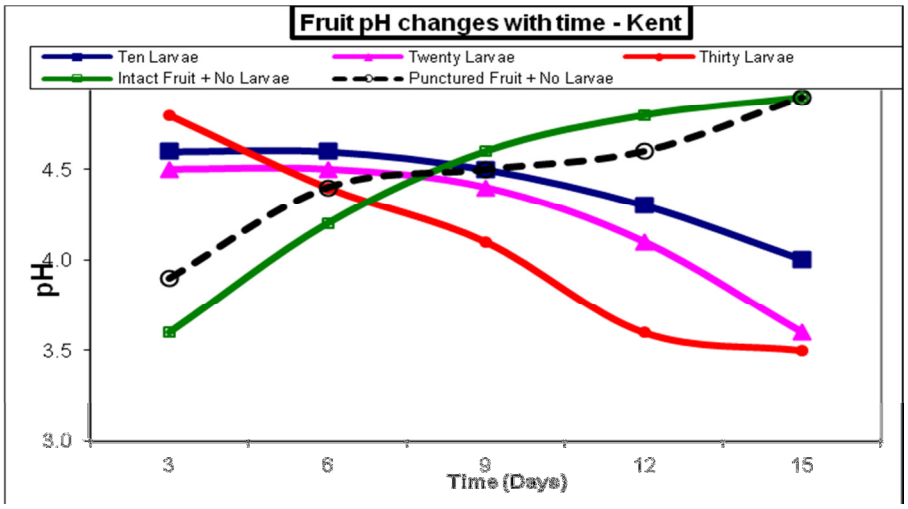


C.

Figure 1: Fruit pH changes with time. A = *Jaffna*, B = *Palmer*, C = *Kent*.

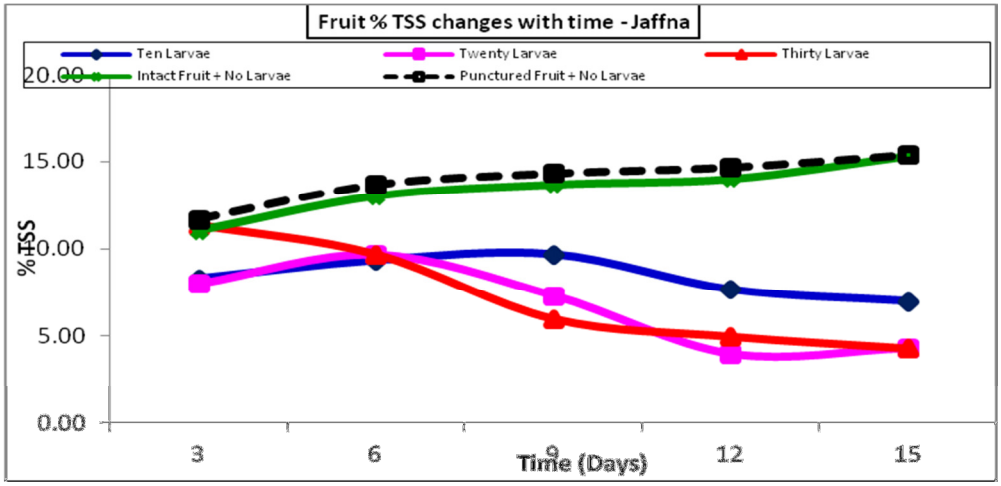
Palmer - The pH range for control treatments increased from 3.3 - 5.0, with the 10-, 20- and 30-larval infestation levels recording 4.0-3.5, 4.1-3.4 and 4.3-3.4, respectively from the 3rd through to the 15th day of storage. Infested fruits on the other hand, decreased in pH with storage time (Fig. 1B).

Kent - The pH range for the control treatments increased from 3.6-4.9, while those for 10-, 20- and 30-larval infestations decreased from 4.6 to 4.2, 4.5-3.6 and 4.8-3.5 with storage time. (Fig. 1C).

Percentage (%) TSS: *Jaffna* - There was a general increase in TSS from 11.0-15.3 with storage time for the control treatments, and a decrease with increasing number of larvae in the fruits. Sugar content ranged from 8.3-7.0, 8.0-4.0 and 11.0-3.7 for the 10-, 20- and 30-larval infestation levels, respectively (Fig. 2A).

Palmer – While TSS for control treatments increased with storage time from 11.0-17.3, those for infested fruits increased to certain peak levels and started decreasing with time. For the 10-, 20- and 30-larval levels, the trends were 10.7, 13.3, 5.7; 11.0, 14.0, 6.7 and 11.0, 13.0, 8.3, respectively for the 3rd, 9th and 15th days (Fig.2B).

Kent - TSS for control treatments was higher than those of infested fruits, and ranged from 13.7 to 18.3. There was also a similar trend in infested fruits, where sugar levels increased with infestation and later reduced. The 10-, 20- and 30-larval infestation levels recorded sets of TSS levels of 10.0, 13.7, 10.7; 10.3, 12.7, 11.7 and 12.3, 12.9, 10.8, respectively for the 3rd, 9th and 15th day observations (Fig.2C).



A.

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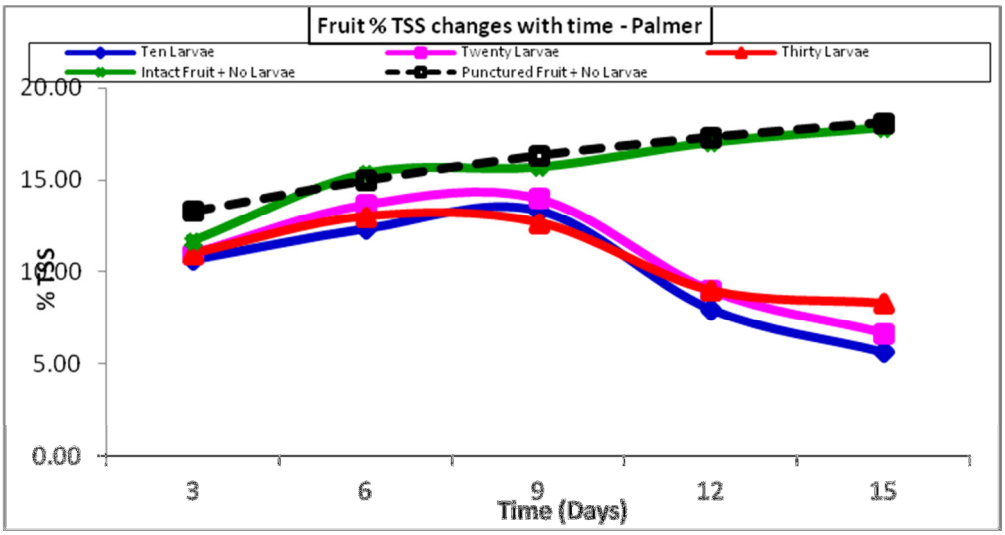

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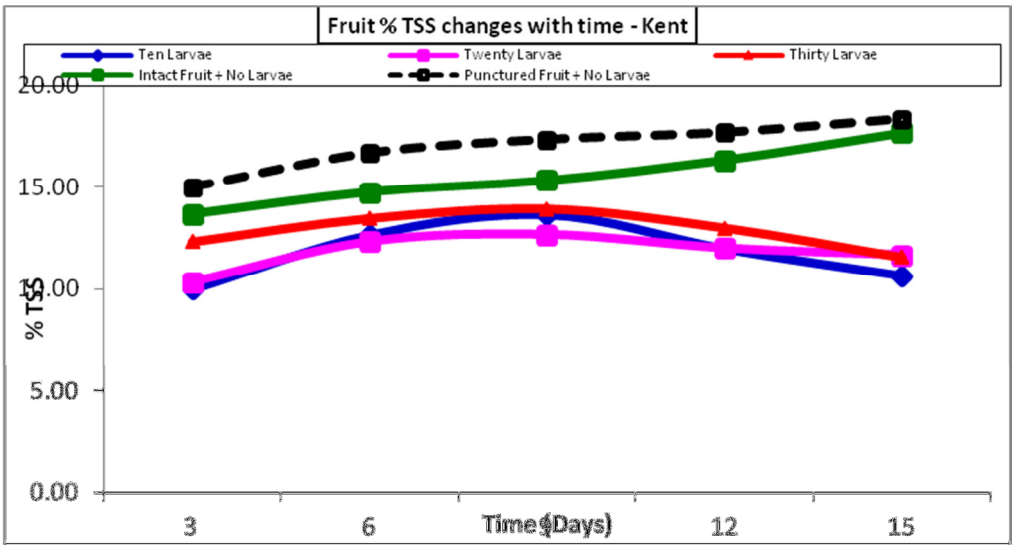

C.

Figure 2: Fruit % TSS changes with time. A = Jaffna, B = Palmer, C = Kent.

Percentage (%) TA: Percentage Acidity decreased with storage time for the two control treatments and increased with fruits infested with larval infestation.

Jaffna - There was a higher increase in percentage acidity in fruits infested with 30 larvae, followed by those with 20 and 10 larvae. Decrease in acidity was gradual for the two control treatments (Fig. 3A).

Palmer - The percentage total acidity for the control treatments decreased with storage time whilst for fruits infested with larvae it increased with storage time. The increase in percentage acidity was higher for fruits with 30 larvae, followed by fruits with 20 and 10 larvae (Fig. 3B).

Kent - There was a decrease in % T.A. for the control treatments and an increase in % T.A. for fruits infested with larvae. The % T.A. for the two control treatments decreased from the 3rd to the 15th day. The increase in % T.A. for fruits infested with larvae increased sharply from the 3rd to the 9th day, and increased slightly from the 9th to the 15th day of storage (Fig. 3). Increase in % T.A. for fruits infested with 30 larvae was higher, followed by fruits infested with 20 and 10 larvae (Fig 3C).

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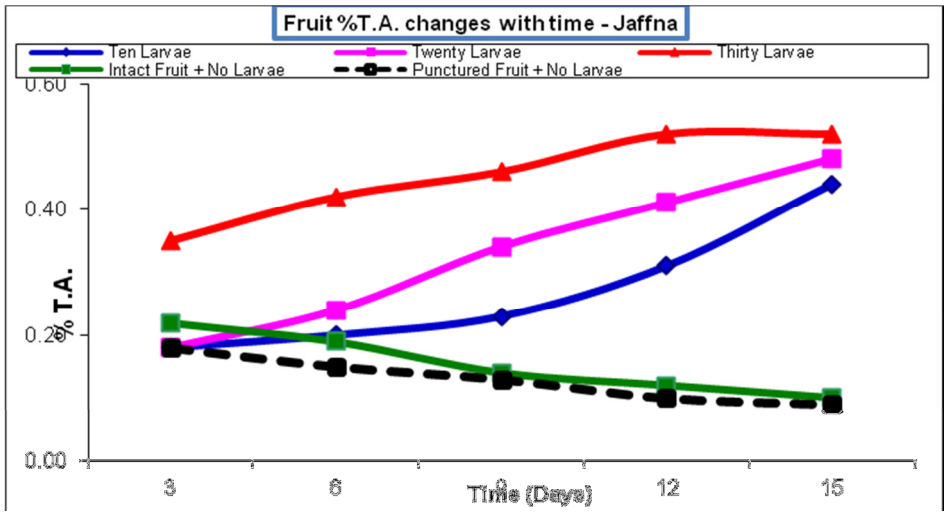

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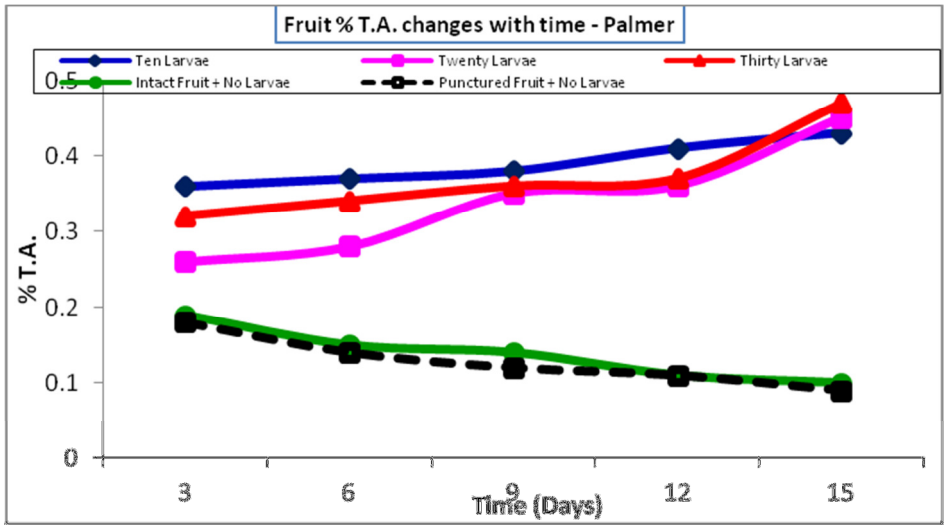

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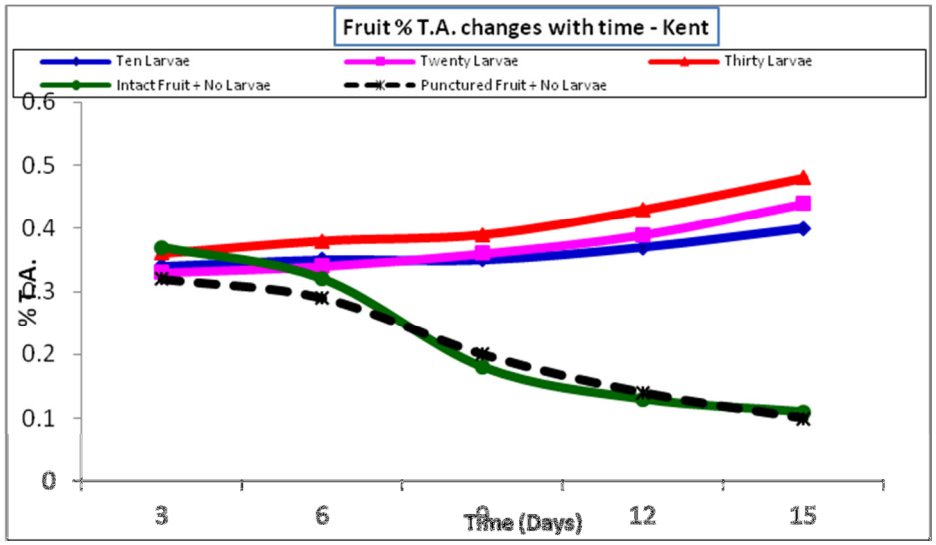

C.

Figure 3. Fruit % T.A. changes with time. A = Jaffna, B = Palmer, C = Kent.

Shelf life of fruits at different infestation levels: The shelf life of the three mango varieties for the control treatments was longer than those infested with larvae. Though shelf life of the two control treatments was longer than infested fruits, those without punctures lasted longer than those with punctures. For the treatments with larvae, the shelf life of fruits with 10 larvae was longer than those with 20 and 30 larvae.

DISCUSSION

In general, pH has been observed to increase with increasing storage time in several studies (Ruck, 1969; Alaka et al., 2003; Dea et al., 2010). This is because as fruits mature, there is a general decrease in total acidity and increase in pH as a result of the conversion of carbohydrates into sugars. The pH for fruits infested with larvae decreased with increased storage time, while total acidity increased with storage time. The decrease in pH was lower with higher infestation i.e. for 30, 20 and 10 larvae per fruit for all the three mango varieties. The increase in acidity was higher for fruits with 30 larvae, followed by those with 20 and 10. The differences in pH on fruits with different densities of larvae could be attributed to the number and stage of larvae surviving in the fruit when the fruits were cut open. From observations made during the experiment, there were large numbers of surviving larvae in fruits infested with 30, 20 and 10 larvae. It could be concluded that the larger the number of larvae present in a fruit, the greater the damage, the lower the pH and the higher the total acidity. The reduction in pH from the 3rd to the 15th day of storage could be as a result of the feeding activity of the larvae which converted most of the carbohydrates in the fruit to acid (Dea et al., 2010). From the 9th to the 15th day, all fruits infested with first instar larvae had internal colour of the fruits changed from yellow to brown - an indication that fruits were severely damaged. This increased the acid content of the fruits (% T.A.) with storage time for the infested fruits. The death of most of the larvae could be due to the higher acidic content of the fruits towards the end of the storage period. Results of the pH and %T.A. on the three varieties also indicates that puncturing of the fruits did not have much effect on damage since the punctured control fruits with no larval infestation did not have any negative effect on the pH and %T.A. of the fruits, compared with the infested fruits. Most of the punctures made on the controlled fruits with no larval infestation dried up from the third day of storage with no exudates from the fruits. This implies that if punctured fruits can be detected early in the fields, they can be harvested and processed into fruit juices without much negative influence on the fruit quality. During the period of storage, no larvae or insects were recorded from the control fruits, showing that the brown grocery bags protected the fruits from any external attacks, and could support the use of such bags in wrapping of high value crops in backyard orchards to minimize fruit fly damage.

The shelf life for the two controlled treatments was longer than those of infested fruits. The number of larvae present in the infested fruits might have led to a further rapid ripening of the fruits, whereas the larval tunnels might have provided entry points for bacteria and fungi that cause the fruits to rot.

CONCLUSION

The presence of fruit fly larvae in fruits causes a number of changes in some internal quality parameters such as the TSS, pH, % T.A. and the internal damage area. pH and TSS of decreased with storage time as compared with the control treatments, which increased with time. The % T.A. increased with storage time compared with the controlled fruits, while the damage areas of fruits with 30 larvae were bigger, followed by those with 20 and 10 larvae. In terms of the varieties, pH, TSS and % T.A. of Jaffna and Kent on the 3rd day of storage were almost the same as their control treatments, with some fruits recording very low or no internal damage. In conclusion, the presence of fruit fly larvae in a fruit, irrespective of the number from the 3rd day of storage, will have a negative impact on the internal quality parameters which would further reduce the market (export and locally) value of the fruit.

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