PHENOLOXIDASE AND MELANIZATION TEST FOR MANGO SEED WEEVIL*

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Abstract

This project was initiated to determine whether the phenoloxidase test successfully developed for fruit flies would be applicable to mango seed weevil, *Sternochetus mangiferae* (Fabricius). Mango seed weevil represents a quarantine impediment to the entry of mangoes to mainland USA and some other countries. It is not a destructive pest and rarely causes fruit damage even in late maturing varieties in which adults can emerge from ripe fruit. The main problem with the weevils come from nursery propagators who are concerned about possible effects on germination. It is questionable whether this is adequate justification for the level of quarantine importance with which this pest is currently regarded. It should not be confused with the mango pulp weevil *Sternochetus frigidus* Fabricius which does damage all infested fruit.

1. INTRODUCTION

This project was initiated to determine whether the phenoloxidase test successfully developed for fruit flies [1], [2], [3] would be applicable to mango seed weevil, *Sternochetus mangiferae* (Fabricius). Mango seed weevil represents a quarantine impediment to the entry of mangoes to mainland USA and some other countries. It is not a destructive pest and rarely causes fruit damage even in late maturing varieties in which adults can emerge from ripe fruit. The main problem with the weevils come from nursery propagators who are concerned about possible effects on germination. It is questionable whether this is adequate justification for the level of quarantine importance with which this pest is currently regarded. It should not be confused with the mango pulp weevil *Sternochetus frigidus* Fabricius which does damage all infested fruit.

Irradiation is the preferred disinfestation treatment, but mango seed weevil is slow to die so a method is required to identify irradiated individuals which may be present and alive at a post treatment inspection. The phenoloxidase test relies on reaction of the insect enzyme phenoloxidase with the substrate 2-METHYL-DOPA (2-methyl-3(3,4-dihydroxyphenyl)-DL-alanine). It can be assessed qualitatively as a spot test in which macerated insect tissue is brought into contact with the substrate on a background which will allow color change recognition or tissue homogenate can be added to substrate solution and color development measured quantitatively with a spectrophotometer. In tephritid fruit fly larvae irradiated as eggs or 1st instars, irradiation reduces or eliminates melanization that normally occurs after death, and typically greatly reduces phenoloxidase activity in third instars [1], [2], [3].

2. MATERIALS AND METHODS

There is no laboratory method for rearing mango seed weevil, and infestation takes place when mangoes are part grown. Thus, infested fruit had to be collected in the field from localities where there was known to be a high incidence of infestation of the weevil. Infested fruit can be recognized with reliably by the presence of overgrown oviposition scars. For these trials fruit was collected from trees of "Common" and "Kensington" varieties at Bowen, a major coastal production area for mangoes in Queensland (20^0 S).

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Irradiation was done on fruit of commercial maturity with equal numbers of each cultivar bulked to single samples for all three tests. Doses were 0, 38, 75, 150, and 300 Gy applied on 5 December 1996 with a Gammacell 220 at a dose rate of 260 Gy/hr. Twenty fruit were treated at each dose with an equivalent number of untreated fruit for each dosed sample. Fruit was then held in a temperature controlled insectary at 27°C until seeds were dissected 2 or 4 weeks after treatment. Larvae, pupae, and adults dissected from seeds, put into an ultra deep freeze at -40°C and held until reagents and a suitable spectrophotometer could be accessed.

The tests detailed by Nation et al. on *Anastrepha suspensa* (Loew) [1] and Mansour and Franz on *Ceratitis capitata* (Wiedemann) [2], [3] were used in my tests on larvae, pupae, and teneral adults of *S. mangiferae* to determine melanization, reaction to a colorimetric spot test, and spectrophotometric assays to the extent of the specimens of each stage available for each dose of irradiation.

Spectrophotometric assays were conducted with a "DMS 100" spectrophotometer operating at a wavelength of 475nm. Larvae, pupae, and adult specimens for assay were macerated individually in 5 ml of 0.1 M phosphate buffer pH 6.5 [1] and centrifuged at 2000g for 10 minutes. The supernatant was filtered through a cotton wool plug. The substrate used was 2-methyl-3(3,4-dihydroxyphenyl)-DL-alanine (DOPA) which reacts with phenoloxidase by giving a characteristic deep red color that can be measured spectrophotometrically, or visually as a spot test. Nation et al. used a 1% solution of 2-METHYL-DOPA but we were unable to dissolve more than 0.05% even after prolonged agitation. Nevertheless, this gave a satisfactory color reaction.

3. RESULTS

Spectrophotometric assays (Table I) revealed no reduction of phenoloxidase activity attributable to irradiation. Larvae, pupae, and adults had approximately equal titers but there were some anomalously high results and some in which the activity was zero. This was corroborated by phenoloxidase spot tests where 2 adults, one untreated, gave no response. In neither test was there any apparent relationship to the irradiation dose. Inspection of the incidence of melanization (Table II) showed mostly low level but recognizable development of melanin in larvae and pupae. In adults sclerotization would have masked any exhibition of melanin.

4. DISCUSSION

The mango seed weevil is a difficult pest for disinfestation with irradiation. While assurance of quarantine security based on the criterion of inability to reproduce following irradiation at an appropriate dose is clearly achievable with irradiation at doses that can be tolerated by fresh mangoes at suitable levels of maturity, death of the various stages of the weevil in mango seeds is slow. This is almost certainly because of the time spent in each life stage during the extended (1 year) development span. Even with fruit fly larvae, death does not always occur until after the change to a subsequent stage such as the pupa [4]. The phenoloxidase test appears to be highly reliable for fruit flies [1], [2]. However fruit flies can develop from egg to pupa in as few as 5 days and from egg to first instar in 1 to 2 days, with similar time intervals between instars, so nullifying the phenoloxidase system as a result of irradiation in one stage will result in its absence in succeeding stages. However, it has been shown that irradiation early in the third instar resulted in only approximately halving of phenoloxidase levels at an assay later in the same stage [1] because the enzyme was synthesized prior to the irradiation.

The rapid, short life stages of tephritid fruit flies contrast with the long duration of the life stages of the mango seed weevil. Most of the irradiated individuals would not be expected to have changed stage in the 6- or 14-day intervals after irradiation that were sampled, although on the basis of diminution of phenoloxidase levels in fruit flies irradiated early in the third instar some spectrophotometric differences would not have been unexpected. The exact time of synthesis of phenoloxidase is the critical factor, however, in whether irradiation can inhibit its synthesis, since in fruit flies the activity of previously synthesized phenoloxidase is not altered by irradiation. Although

color development was strong in spot tests, no differences were apparent in these or in spectrophotometric results. Longer sampling intervals were not possible because fruit ripened and began to deteriorate at the holding temperature. Use of lower temperatures such as the 13°C holding temperature used commercially would slow development of the weevil stages.

Even at the highest dose of 300 Gy there was no apparent diminution of phenoloxidase activity attributable to irradiation in larvae pupae or adults. Responses were neither dose nor stage dependent. This dose was higher than that used for fruit fly. The results for spectrophotometric assay, spot testing and melanization were in general agreement.

In the absence of a test for irradiated mango seed weevil, greater emphasis will need to be placed on harvesting mangoes from seed weevil-free orchards for the few markets that refuse fruit with seeds containing live larvae. This can ensure that infested mangoes will not be present in export consignments. Although this alone may not satisfy the quarantine security requirements of some markets, in combination with a generic irradiation dose of 300 Gy it should satisfy the highest security levels required by any market. Most orchards are free of mango seed weevil when they first come into production, and provided that there are no infested trees in the near vicinity, they can be maintained pest free for some years with minimal quarantine precautions.

TABLE I. Spectrophotometrically assayed concentrations of phenoloxidase with L-2-METHYL DOPA 0.05% w/v in water as a substrate for macerated larvae, pupae, and unemerged teneral adults of mango seed weevil 6 and 14 days after gamma irradiation in mangoes at 0-300 Gy followed by freezing at -40°C. Where no value is recorded, the stage was absent from the sample.

	6 days			14 days		
Dose (Gy)	Larvae	Pupae	Adults	Larvae	Pupae	Adults
0	0.075	0.074	0.023	0.193	0.027	0.012
38		0.025	0.043	0.053	0.044	0.000
75	0.061	0.029	0.006			
150		0.027	0.013	0.063	0.025	0.032
300	0.080	0.000		0.103	0.000	0.021

TABLE II. Melanization of larvae and pupae of mango seed weevil thawed following freezing at -40°C 6 or 14 days after gamma irradiation at 38 - 300 Gy. (P = Present; N = Absent; ? = Uncertain; No entry = stage not present in fruit sample).

	6 days		14 days	
Dose (Gy)	Larva	Pupa	Larva	Pupa
0	P	N	P	?
38	?	N	N	N
75	?			
150	P	?	N	P
300	P	P	P	

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