

Phenology and Floral Biology of Roseleaf Raspberry (*Rubus rosifolius* Sm.) under Medium and Low Elevation Conditions

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Roseleaf raspberry (*Rubus rosifolius* Sm.), a thorny weed in the highlands of Quezon province, Philippines, has shown potential in the food industry. Analysis of the phytochemical content of its fruit revealed promising medicinal properties. However, attempts to produce fruits at low elevation have failed, while the specific influence of agrometeorological conditions in lower altitudes is yet to be studied. We investigated the phenological characteristics of roseleaf raspberry plants at different elevations: in their native setting at 774 m above sea level (masl) and at low elevation (31 masl). Plants started producing flowers at the end of October and became more profuse in the later months. An observed critical feature of individual roseleaf raspberry flowers across elevations was poor overlap of anther dehiscence and stigma receptivity. Fruit set failure and flower abortion were abnormalities found to cause low fruit production as well as poor fruit quality among plants at low elevation. Excessive heat accumulation in flowers and insufficient carbohydrate supply in the shoot apices are possible causes of these abnormalities.

Key Words: altitude, flowering, fruit set, phenology, roseleaf raspberry, *Rubus rosifolius* Sm., *sampinit*

INTRODUCTION

Roseleaf raspberry (*Rubus rosifolius* Sm.) is a red-fruit raspberry that is native to Southern and Eastern Asia and is distributed in Asia, Hawaii, Australia and the Caribbean (Adams 1972; Bowen-Forbes et al. 2009; Rydberg 1913). As with all *Rubus* plants, they produce renewal shoots from the ground called canes; they have complete, hermaphrodite, white-colored, radial flowers with an apocarpous gynoecium of many carpels on a cone-like receptacle. Each of the carpels develops into a small drupelet, with the mesocarp becoming fleshy and the endocarp becoming hard and forming a tiny pit that encloses a single seed. These individual drupelets form an aggregate fruit (Graham and Woodhead 2011).

This particular raspberry species, however, has a certain degree of self-incompatibility as it has been observed to depend on pollinators for seed development (Gonçalves-Souza et al. 2008). The roseleaf raspberry has long been growing in the wild particularly in forests as well as in agricultural areas and is locally known in the Philippines as *sampinit* or *sapinit* depending on the area. Recently, locals of Dolores, in the province of Quezon, Philippines, have discovered the possibility of cultivating roseleaf raspberry as a fruit crop (DA-BAR 2012; Daffon and Daffon 2011). Interest in the plant has also grown

especially after analyses showed that its fruits contain several phytochemicals (Mojica 2010) which have numerous medicinal properties (Bulic et al. 2009; Buzzini et al. 2008; Convertino et al. 2009; Feng et al. 2008; Koleckar et al. 2008; Lü et al. 2004; Pickhardt et al. 2005; Reddy et al. 2007; Tripoli et al. 2007).

Fruit production is currently very limited as the plants only bear fruit when grown at medium elevation or higher (DA-BAR 2012). Certain combinations of agrometeorological conditions of growing areas at varying elevations may be affecting the crop and causing its failure to fruit, but their specific influence is currently uncertain due to insufficient information available on the physiology of the plant. Thus, this study was conducted to characterize the phenological development and reproductive biology of *R. rosifolius* plants grown in low and medium elevations.

MATERIALS AND METHODS

The study was conducted at the orchard of the Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, Philippines (average temperature: 26.1 °C; elevation: 31 masl, latitude: N14°09'42.0"; longitude: E121°14'58.8") for the low elevation conditions, and at Bangkong Kahoy

Valley, Kinabuhayan, municipality of Dolores, Quezon province (average temperature: 20.8 °C; elevation: 774 masl; latitude: N14°03'12.5"; longitude: E121°27'11.1"). The duration of the study was originally set from September 2013 to February 2014. However, observations were extended until April 2014 due to the late fruiting exhibited by the test plants at the Los Baños site.

Four- to five-month-old suckers of roseleaf raspberry plants grown in Bangkong Kahoy were used as test plants. Experienced farmers claimed that plants at this age were capable of flowering. The suckers were transplanted to 4.5 inch x 6 inch seedling bags using the soil from which the plants were obtained as potting medium.

The experiment at medium elevation was set up in an open area near hedges of the roseleaf raspberries in a farm in Bangkong Kahoy, while 250 plants were transferred to low elevation. These were first maintained under full shade for 1 wk to acclimatize the plants to the change in temperature before being set up in the 8 m x 12 m open area where no additional shading from trees and other tall structures could affect the plants for most of the day. The plants were irrigated thrice a week.

The phenological behavior of roseleaf raspberry plants was observed using 10 plants at each elevation. Each plant served as a replicate. The phenological growth stages were monitored on a weekly basis using the Biologische Bundesanstalt, Bundessortenamt and Chemical industry (BBCH) scale for raspberries (Schmid et al. 2001). In addition to the occurrence of phenological stages, the occurrence of flowers and fruits on 19 plants from each site was measured weekly.

For observations on floral biology, 20 branch terminals with flower buds at BBCH stage 59 were tagged from plants at both elevations. The tagged flower buds were observed twice daily, in the morning and in the afternoon, from bud opening until the stigma and stamens dried up.

The flower buds at medium elevation were used for observing pollen grain germination as well. Anthers were taken from randomly selected flowers at four different stages: half-open stage (petals cupped, anthers compact), early open stage (petals fully open, filaments elongating and separating from the center), mid-open stage (50% anthers turning brown) and late open stage (90% anthers turning brown). These were collected in Petri dish plates containing a medium consisting of 10% sucrose, 0.01% boric acid and 1.2% agar (Sharafi 2010). A Dino-Lite® Digital Microscope was used to measure and photograph the pollen germination percentage and pollen tube length in four randomized selected 'spots' 2 h after inoculation.

A 'spot' is an area on the agar surface that can be viewed within the limits of the microscope at 210x magnification. A pollen grain was considered germinated when its pollen tube length was more than the grain diameter.

The experiment was laid out in a completely randomized design. Analysis of variance and comparison of treatment means were done to determine the statistical significance of the observed variations in the parameters measured. Analysis was carried out using the Statistical Analysis System (SAS) software (SAS Institute, Inc., Cary, North Carolina, U.S.A.).

RESULTS AND DISCUSSION

Floral Biology

Development of flower buds at both elevations was observed to proceed in a similar manner. A newly emerged flower bud (BBCH stage 51) looked like a very tiny bulb at the tip of the stem with a liberal amount of trichomes sticking out of its green covering (sepals). Its peduncle then became elongated and the bud enlarged within a week. The white petals were visible through slits in the covering after about 3 d. The sepals soon broke open, exposing the petals (BBCH stage 59). Once the petals were exposed, it took less than a day before the flower fully opened. It took about 2 ½ wk for a newly emerged bud to develop into a fully open flower (BBCH stage 60).

Regardless of elevation, an open flower lasted for 3 d before it either developed into a fruit or dried up (Fig. 1). Newly opened flowers showed compact immature anthers which were bent over immature styles, similar to the observation of McGregor (1976). Anthers were first to mature though not synchronously. The filaments elongated and bent outward as they matured, exposing the stigmas at the center. Anther dehiscence proceeded from the periphery towards the center. This trend was observed as soon as the petals opened and anthers were exposed. Anthers appeared powdery during pollen shedding.

Pollen grains and pollen tubes had a near-transparent appearance. At the half-open stage, most anthers have not yet ruptured and the few pollen grains observed failed to germinate as they were not yet mature. Anthers obtained during the late open stage, on the other hand, had already lost most of their pollen. Mid-open stage anthers had the highest average number of pollen grains (37.25) and also the highest germination percentage (88.6%) (Fig. 2).

As pollen grains were being shed, the styles grew and the stigmas began to swell. By the time the stigmas were receptive, most of the pollen have already shed and the anthers already senesced, which makes it unlikely for

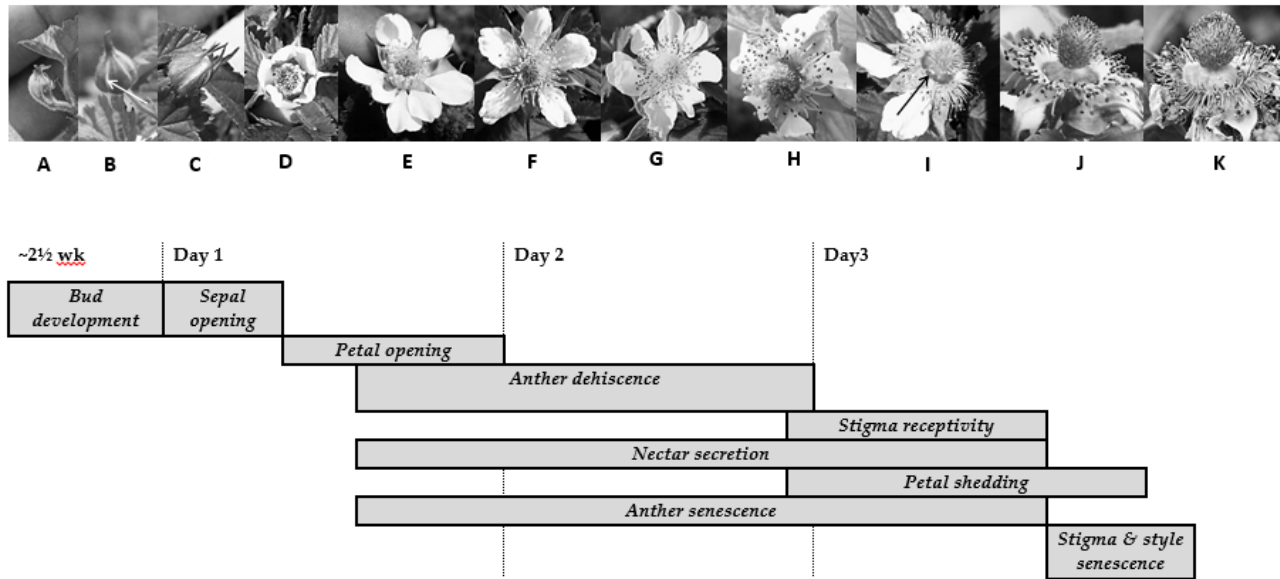


Fig. 1. Progression of flower development from bud appearance to stamen and style senescence at medium and low elevations. Buds are around 2 mm in diameter when they first become visible (A). Slits begin to appear (B, white arrow) during the week before buds open. Flowers remain open for 3 d. Sepals (C) and petals (D & E) open on day 1. Filament elongation and anther maturity at the periphery may start during the first day (F). This proceeds centripetally up to the second day (F–H). Most anthers have senesced by the end of day 2 (G & H). Stigmas may start to appear enlarged by the end of day 2 or at the start of day 3 (H). Petals begin to droop and eventually fall on day 3 (I–K). Fleshy portion at receptacle base becomes more visible (I) and may appear wet with nectar (black arrow). Stigma (J) and filaments (K) dry up by the end of day 3.

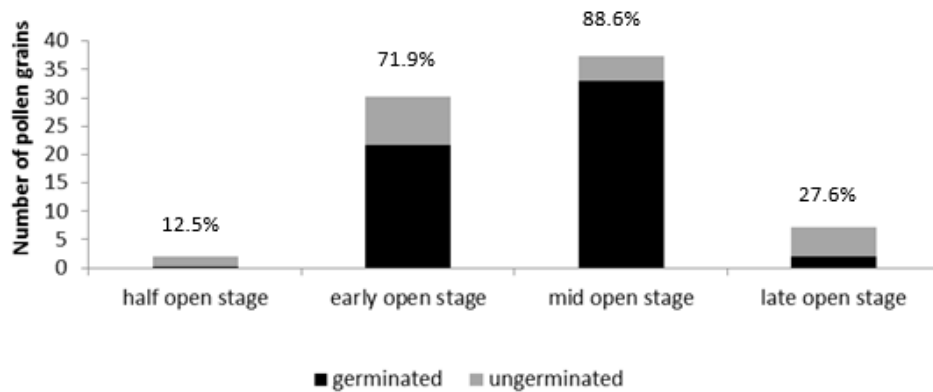


Fig. 2. Total number of germinated and ungerminated pollen grains and percent germination (written above the bars) from flowers of varying stages at medium elevation.

self-pollination to occur.

The margin around the receptacles of some flowers was observed to be particularly moist at different times. McGregor (1976) described the fleshy portion inside the ring of stamens to be the part of the flower that secretes nectar which attracts a number of insects. Nectar feeders such as bees, wasps and ants as well as pollen feeders such as flea beetles and spiders were seen visiting the flowers from the second day of flower opening.

As anthers were first to mature, they were the first to senesce as well. Browning followed right after dehiscence starting from the periphery towards the center. As this progressed, the petals began to droop and eventually fell. The stigmas and styles were the last to dry up, leaving a

green receptacle.

Phenological Development

Figure 3 gives a schematic presentation of the stages of the phenological development of roseleaf raspberry plants over the duration of the study. Branch formation often occurred at the lower nodes but for plants with main stems oriented horizontally, lateral branches formed even at the middle and upper nodes. It was noted that new leaves appeared from the branches every 1–2 wk until flower buds formed at the terminals. Number of days from branch initiation (BBCH stage 40) to flower formation (BBCH stage 51) varied across plants. Many early-emerging branches needed to grow at least six

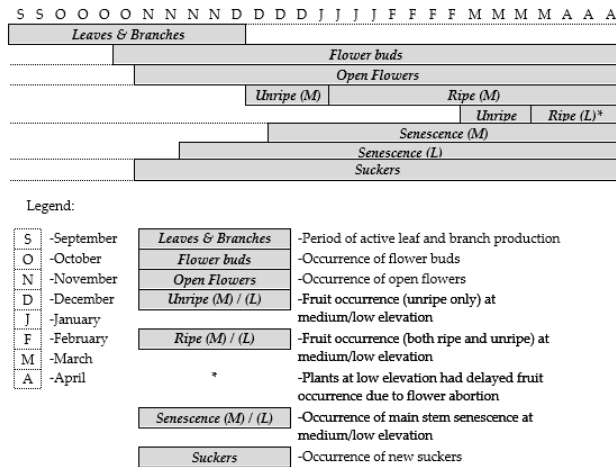
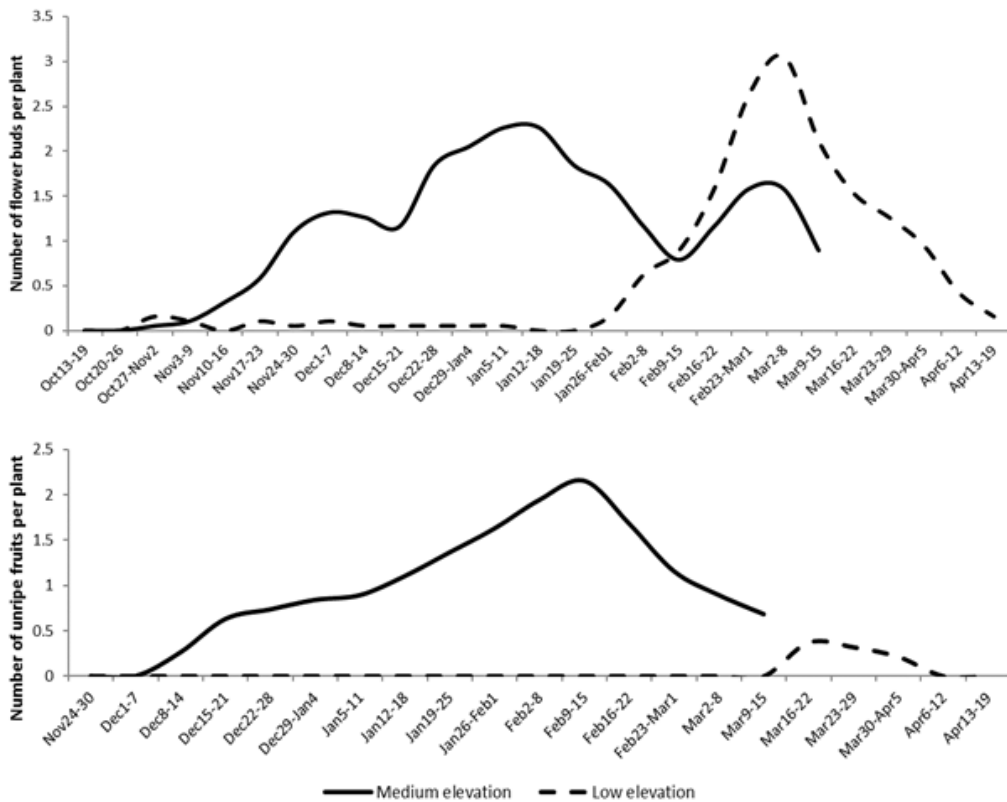


Fig. 3. Summary of the phenological development of roseleaf raspberry plants at medium and low elevations.

abortion of flower buds, especially among low elevation plants. However, a resurgence of flowers was observed among low elevation plants in February. Peak occurrence of flowers was delayed by 8 wk compared to the plants retained in their native setting (Fig. 4).

First fruits of plants at medium elevation were seen in the second week of December. A few weeks after the number of fruits at medium elevation started to decline, fruits began appearing among the plants at low elevation after 5 mo without fruit set. For this reason, the observation period at low elevation was extended until the third week of April.

Better-quality fruits – e.g., more complete setting, synchronized maturity of drupelets and fewer damaged drupelets – distinguished fruits produced at medium elevation from those produced at low elevation (Table 1).



leaves (BBCH stage 46) before a flower bud appeared at their terminals while some branches, especially those that emerged during the period of profuse flowering, produced flower buds with as few as three leaves.

Flower buds were first observed in the third week of observation on plants at both low and medium elevations. Number of flowers at medium elevation continued to increase but flowers at low elevation declined after the third week of November. There were many instances of

Ripening duration took around 5 wk before the fruits became ready for harvesting (Fig. 5). Ripe fruits readily fell off the receptacle. Incomplete fertilization of ovaries led to deformed fruits. Usually, these did not fall off easily even when ripe. If none gets fertilized, the entire flower just dried up. Many fruits that formed at low elevation were of poor quality because of incomplete setting. Also, many fruits were formed from flowers that developed under the shade of the leaves of the same plant



Fig. 5. Fruit development duration was around 5 wk. Drupelets changed color on the fourth week of development and became fully ripe on the fifth week. Ripe fruits normally fell off easily (bottom left) but deformed (incomplete set) fruits (bottom right) remained attached to the receptacle.

or of neighboring plants, often with horizontally growing basal branches.

Senescence of main stems was observed among low elevation plants starting in November. It was also exhibited by medium elevation plants starting in the second half of December (Table 2). Main stem senescence proceeded soon after the apical tip was done producing flowers and/or fruits. It also occurred when the tip was damaged. Main stem senescence progressed basipetally, leaving basal branches holding up green leaves, as also observed by Engard (1945). It was also noted that the mother plant may also produce new suckers arising from the roots for self-perpetuation. The first suckers appeared in November and then continued to appear in different plants in both elevations until the end of the experiment (Fig. 6).

DISCUSSION

The attempt to grow roseleaf raspberries at low elevation

Table 1. Total number of fruits from 19 plants that reached the ripe stage for the period October 2013 to April 2014.

Elevation	Total No. of Fruits that Reached the Ripe Stage
Medium	68 (3 of which were deformed)
Low	5 (4 of which were deformed)

Table 2. Occurrence of main stem senescence at mid observation period and on the last day of observation.

Elevation	No. of Plants Exhibiting Main Stem Senescence (N = 11)	
	Mid observation period	Last day of observation
Medium	2	10
Low	5	9



Fig. 6. A whole roseleaf raspberry plant showing a senesced main stem with replacement canes, profuse roots and formed suckers.

demonstrated that limited flowering can occur late in January coinciding with the low temperatures. Fruit formation at low elevation was very low, almost 93% less compared to production in their native habitat. The phenology and reproductive biology of the roseleaf raspberry revealed several insights regarding the low fruit production in the low lands. Morphological observations have shown that at both low and medium elevations, 4- to 5-mo-old roseleaf raspberry plants can start producing flower buds after about a month, a newly emerged bud will open in about 2 ½ wk, an open flower lasts for 3 d, and a pollinated flower takes about 5 wk for it to ripen into a harvestable fruit.

No overlap of anther dehiscence and stigma receptivity within a flower was found, hence the need for agents facilitating inter-flower pollination. Flowers are not strictly cross-pollinated, however, as flowers of the same plant may occur in a staggered manner. For fruit set to occur in such a case, the presence of pollinators and viability of pollen are required.

Experimental plants grown at medium elevation had regular bee visitors, especially because a few stingless (*Trigona* sp.) bee colonies were being cultured near the roseleaf raspberry plants. Though wasps and other pollinators were also seen visiting the flowers at low elevation, they did not appear to visit as frequently or in as large numbers as those observed at medium elevation. However, the quantity of flowers present at a given time may also be a factor affecting the frequency of pollinator visits and the number of visitors as more pollinators can be attracted to the presence of more nectar-secreting flowers.

Plants at low elevation were observed to have a high incidence of flower bud abortion. Sufficient carbohydrate supply to plant shoots is of critical importance to producing and maintaining flowers (Pescie et al. 2011; Stephenson 1981). As C₃ plants such as raspberries typically become light saturated at one-third of full sunlight, there is a possibility that the occurrence of photo-inhibition has caused a decline in carbohydrate supply (Long et al. 1994). However, as plants at both elevations were both under full sunlight, other factors may have affected the flowering of plants at low elevation.

Many fruits that formed at low elevation were of poor quality because of incomplete setting. As pollinators were seen visiting flowers regardless of elevation, other factors may have caused the variation in fruit quality. Many of the fruits from low elevation plants were observed to have developed from plants grown under shade and from flowers that were shaded by neighboring leaves (Kitma 2014). This observation brings up the possibility that prolonged exposure to full sunlight has affected the reproductive function of flowers at low elevation. However, the characteristic effect of photo-inhibition is the denaturation of proteins involved in the Photosystem II light harvesting complex, resulting in hidden hunger or no net photosynthesis (Long et al. 1994). Though this condition leads to low flower and fruit production, it still does not fully explain how flowers which did not abort led to the occurrence of incomplete fruit set.

A study by Oliveira et al. (2007) showed that annual-fruiting *R. idaeus* L., a red raspberry species belonging to the same subgenus as roseleaf raspberry (subgen. *Idaeobatus*) (Alice and Campbell 1999; Graham and Woodhead

2011), has two stages of plant development in which different carbohydrate assimilation patterns occur. The first stage begins at the start of cane development to the onset of flowering (vegetative phase). Here, the meristematic growing points are the primary sinks with starch being mobilized from the root system. In the second stage, starting from the onset of flowering onward (fruiting phase), two new processes compete for assimilates: fruiting and root carbohydrate storage. Results of an earlier study by Oliveira et al. (2004) on *R. idaeus* suggested that raspberry is a source-limited plant and that the fruit might be a weaker sink compared to the root system. It is very likely that this competition posed by roots during the period of flower and fruit development plays a major role in the abortion of roseleaf raspberry flowers in low-elevation areas.

In relation to carbohydrate partitioning, Boussadia et al. (2010) mentioned that deficiencies in nitrogen induce sink limitation within the whole plant due to decreased growth which leads, in turn, to feedback down-regulation of photosynthesis. They also mentioned that when plants sense a scarcity in mineral nutrients, they often allocate a higher proportion of their biomass to the root system than the shoots, thus highlighting the need for nitrogen during flowering.

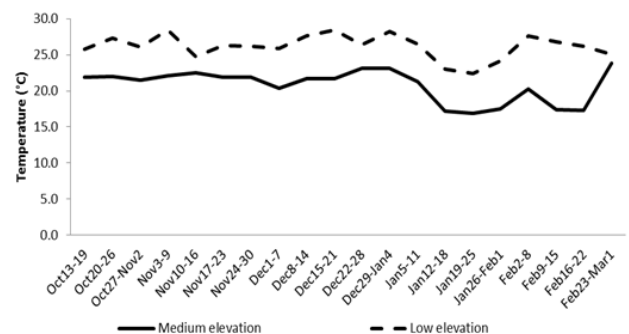


Fig. 7. Medium elevation temperatures were consistently lower than low elevation temperatures from October 2013 to February 2014. The largest observed temperature difference of 9.3 °C between the two elevations was observed in the second week of February. The month of January was observed to be the coolest during the observation period.

Aside from profuse flowering, staggered flower occurrence is also necessary in order to have a sufficient overlap of anther dehiscence and stigma receptivity among neighboring flowers (Mitchell 1994; Ohashi and Yahara 1998; Wyatt 1982). This was observed to be the case at medium elevation and also in the low elevation area during February and March. Medium elevation plants, being situated very near roseleaf raspberry

production hedges, had an even greater advantage as pollen load at the area was very high. This may have been a major factor in the higher occurrence of fruit set among plants at medium elevation.

Having a relatively low air temperature is one of the major distinguishing features of medium elevation conditions as air temperature drops by 1 °C for every 181.82 m increase in elevation (Körner 2007). Aside from direct sun radiation, the relatively high average air temperatures at low elevation may have caused excessive heat accumulation in flowers. This may have led to heat stress which resulted in abnormalities in the reproductive processes.

Temperature has been found to play a crucial role in the flowering and fruit set of *R. idaeus* plants. Carbohydrate content of plants grown at temperatures above 27 °C have been found to drop to levels too low to satisfy the crop's requirement to support the fruit development process (Carew et al. 2003; Sønsteby and Heide 2009). Carbohydrate partitioning is also affected as low temperatures reduce the export of assimilates to roots from source leaves (Farrar and Jones 2000).

Heat stress has been found to cause disorders on various crops such as poor fruiting in tomato (Peet et al. 1998) and low grain quality in corn (Wilhelm et al. 1999). In rice, this has been found to cause decreased pollen production and pollen reception in affected plants (Prasad et al. 2006). According to Prasad et al. (2008), pollen and stigma of plants do not have the potential to acclimate because of their inability to produce heat shock proteins or other types of osmolytes that can either provide protection or increase acclimation potential of these organs. They further elucidated that though both microsporogenesis (pollen development) and megasporogenesis (stigma development) are injured under heat stress, pollen is known to be relatively more sensitive to heat stress conditions. This brings the possibility that the deformity in fruit formation among roseleaf raspberry plants grown at low elevation was a result of pollen damage due to heat stress.

CONCLUSION

The study showed that regardless of elevation, poor overlap of anther dehiscence and stigma receptivity was a critical feature of individual roseleaf raspberry flowers, thus highlighting the importance of inter-flower pollination to fruit set such as profuse and staggered occurrences of open flowers and the presence of pollinators. However, aside from producing a lower number of flowers and fruits, plants at low elevation did not produce fruits until the latter part of the observation

period. The occurrence of flower abortion and failure to set fruit were found to cause delayed flower production as well as low number and poor-quality fruits among plants at low elevation. Insufficient carbohydrate supply is one of the likely causes of these abnormalities. Future studies aimed at enabling fruit production at low elevation may be focusing on the protection of flowers from heat stress and also on boosting the carbohydrate resource of the plants at low elevation conditions.

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