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Mechanical Strengths, Hydraulic Conductance and Growth of *Passiflora edulis f. edulis* Grafted on Five Different Rootstocks at Three Different Cleft Lengths and Their Susceptibility to Wind Damage in Nakuru Kenya

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Author's contribution

This work was done by the author RMG. This includes the design, implementation data collection, analysis and writing the manuscript.

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ABSTRACT

This study was carried out to determine the relationship between graft union mechanical strengths, hydraulic conductance and plant growth of *Passiflora edulis f. edulis* grafted on different rootstocks and cleft lengths and their susceptibility to wind damage in Nakuru Kenya. Vertical rupture, horizontal tensile strength, hydraulic conductance and growth of *Passiflora edulis* f *edulis* grafted on *P. edulis* f. *flavicarpa*, *P. ligularis*, *P. mollisima, P. subpeltata and P. caerulea* rootstalks with 1, 1.5 and 2 cm cleft lengths were determined 13 weeks after grafting and compared with self-grafts. Vertical rupture force was highest in *P. edulis f. flavicarpa* self grafts in all the three cleft lengths. Grafts with 1 and 1.5 cm cleft lengths had no significant difference in horizontal tensile strength. Self grafts with 1.5 cm clefts had higher hydraulic conductance than cross grafts. Scion growth was significantly high when the union length was 1.5 and 2 cm in *P. ligularis* by *P. edulis* and *P. flavicarpa* by *P. edulis* graft combinations. Vertical force generated by wire displacement showed

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that enough force was generated that could damage the vines The strength of wind needed to break the weakest was higher than the maximum recorded in Nakuru County implying that cross grafts of *P. edulis f. flavicarpa by P. edulis f. edulis* with 1.5 cm cleft lengths are not prone to wind breakages in Nakuru, Kenya. However, the growers need to graft their passion fruits with 2 cm cleft lengths since wire displacement develops enough vertical force that could damage the vines. Results indicated that the best rootstock for *Passiflora edulis* was *Passiflora flavicarpa*.

Keywords: Mechanical tensile strength; grafting; growth; passiflora; wind.

1. INTRODUCTION

For several years, passion fruit growing has been declining in many parts of the world due to *Fusarium* wilt infection [1,2]. In many places, the yellow passion fruit (*P. edulis f. flavicarpa*) seedlings are used as rootstocks because they offer short term resistance to *Fusarium* wilt. However, recently there have been many reports from Kenyan growers that *P. edulis f. flavicarpa* is no longer resistant to *Fusarium* wilt [1]. An alternative compatible rootstock with *P. edulis f edulis* and resistant to *Fusarium* wilt would be useful. This alternative rootstalk can be found if more species in the family *Passifloraceae* are screened. The family *Passifloraceae* has more than 500 species [3].

The *Fusarium* wilt pathogen gains entry into the plant directly or though wounds. The wounds on the plant can be caused by nematodes, weeding, slashing or wind [3-6]. The deleterious effects of wind have also been reported by [7,8,5]. According to [7], the extent of wind damage in apples is mainly determined by the speed of wind, the vertical rapture and horizontal tensile strengths of the plants. These strengths depend on cleft lengths [9,10]. According to [9,11], hydraulic conductance is variable amongst different graft cleft lengths. Hydraulic conductance is directly related to nutrient uptake and growth [11,12]. So far there is no information about the relationship between rootstocks resistant to *Fusarium* and their interaction with the mechanical strength: vertical shear, horizontal shear, hydraulic conductance, wind damage and growth. The objective of this experiment was to study the strength of graft unions between different graft combinations with respect to cleft lengths, wind damage and growth.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in a greenhouse in Egerton University, Njoro, Kenya.

2.2 Germination and/or Preparation of the Seed

Seedlings of *P. mollisima, P. subpeltata, P. caerulea, P. edulis* f. *flavicarp, P. edulis f. edulis* and *P. ligularis* were raised from open pollinated seeds were sown in flats in a greenhouse. Eight weeks after sowing, 12 seedlings from each seed source were transplanted into 17 cm tall and 15 cm diameter round containers and placed on benches under 50% lath shade in the greenhouse. The substrate was Metromix Maryland (Metro Inc, Maryland, USA). Fertilizer and watering were carried out as described by [13].

2.3 Grafting and Rootstocks

Eight weeks later the seedlings were grafted with purple passion fruit scions. The rootstock species were chosen to represented poor (*P. ligularis*), moderately poor (*P. mollisima*), and good (*P. mollisima*) compatible rootstocks (with respect to *P. edulis f. edulis* scions). Two compatible rootstock accessions, *P. subpeltata* and *P. caerulea* were included. The effect of three lengths of cut (1.0 cm, 1.5 cm and 2 cm) was tested. For each rootstock species and cleft length graft combination, two single plant replications were used. Also, self-graft controls i.e. *P. mollisima* on *P. mollisima*, *P. ligularis* on *P. ligularis* and *P. edulis* f. *flavicarpa* on *P. edulis* f. *flavicarpa* were included.

2.4 Evaluation of Parameters

Thirteen weeks after grafting, the following data was collected: hydraulic conductance, scion vine length, vertical and horizontal mechanical strength.

Hydraulic conductance (the speed of water movement across the graft union) was measured using the method described by [14]. Before 0800 hours, 50 x 20 cm polythene bags were weighed and placed over two randomly selected grafted plants from each rootstock-scion graft combination (24 in total). The plastic bags were secured at the graft union using rubber bands. Five un-grafted seedlings of *P. mollisima*, *P. ligularis* and *P. edulis* f. *flavicarpa* covered with plastic bags as described above were also included as controls. The grafted plants were moved out doors between 0800 and 1600 hours on a cloudless day. At 1600 hours, the plants were gently shaken to collect all the moisture on the leaves into the polythene bags. Then the bags were weighed using an analytical balance (JA 2003, Hangping, China). The transpired water was calculated by subtracting the weight of empty bags from the weight of the bags with water. The hydraulic conductance in milimiters of water per centimeter of stem length per minute (ml/cm/min) was calculated. Scion vine length was measured as previously described.

Vertical rupture strength was determined using a method similar to Rehkuger [7] but modified to measure upward force. One grafted plant from each of the fifteen graft combinations and nine self grafts were tied with a thread immediately above the graft union. The thread was tied to a spring balance mounted on an adjustable arm which was attached to a firm tripod stand. The base of the plant was also held with a clamp attached to a tripod stand. The arm holding the balance was slowly raised. The force (N) needed to break the graft union was calculated as the maximum Kg of upward force needed to break the graft union multiplied by 9.8 m/s, the conversion factor.

Horizontal rupture force was determined by a method used by Rehkurger [15,7]. All the 48 remaining plants were removed from pots and their roots washed and trimmed back to the trunk axis. Loading to failure was done and the depth and width of each sample's cross-section was measured at the point at which it failed. A graft failed when it split, shattered or snapped or when the failure occurred at or above the graft union.

The maximum bending stress (expressed as Pascals, Pa) in the test section was calculated as: $S_{max} = MC/I$; where S_{max} was the maximum stress in material at the outer fiber (Pa), M was the bending moment (N/m), C was the ½ the depth of the section in the direction of loading (m), I was the area moment of inertia of the graft cross-section (m^2), The section shape was elliptical, so the value of I/c became: $I/C = \pi bd^2/32$ where b was the graft section width (m), d the graft section depth (m), Defining P as the force (N) applied by the crosshead of the testing machine and R as the horizontal distance (m) at the loading point, then: $M = PR/2$ and substituting equation 2 and 3 into 1 gave : $S_{max} = 16PR/\pi bd^2$. This was the expression defining the maximum stress in the test specimen as a function of loading and geometry. The specimens were loaded to failure by gradually increasing the load applied at the center of the graft union. The calculated maximum stress at failure was the tensile modulus of rupture of the graft union material.

Trellising wire displacement force was determined at three points (1, 1.25 and 1.5 m along the wire) using a digital cable tension meter (Quantrol GTX, Avery Weigh-Tronix, Fairmont, MN). A trellising wire 16 gauge was mounted with fencing nails top of two posts 3 m apart and 2.5 m posts high (after installation). The displacement force (Pa) was determined by pulling the wire down using the tension meter to the furthest point. The displacement distance (m) was the vertical distance the wire moved way from the original point of loading.

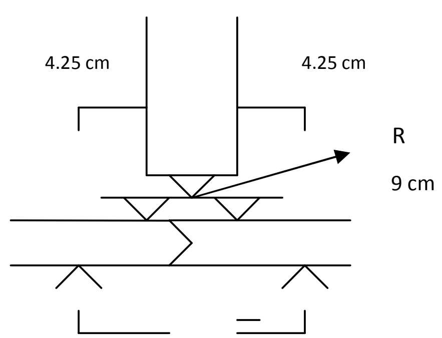

Fig. 1. The arrangement of the stem to determine graft rupture force

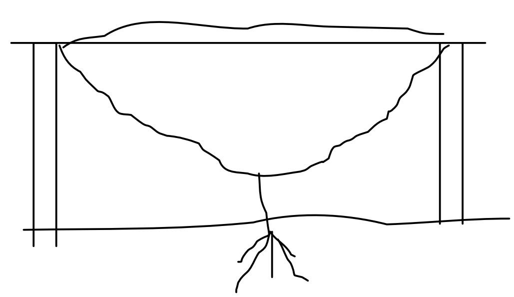

Fig. 2. The geometry and wind velocity profile for calculating stress on the graft union

Maximum wind velocity at the graft union was determined using a method similar to the one used by Rehkurger [7]. It was assumed that the plant's canopy had an inverted semi elliptical shape. The wind velocity profile was calculated relative to distance from the ground. i.e. $V = V\,max((y+a+b)/9.144)^{1/7}$; where

V = velocity of wind at any position y, Vmax = velocity (m/sec) at 9.133 m, y= position on the three as defined by coordinate, a = distance from the ground surface to the point of the graft union (m) and b = the distance from the graft union to the top of the plant (m).

The bending moment (horizontal wind force) at the graft union was determined by integrating the moment produced by the wind.

$$M = (\rho C V max^2 W)/2\int((y+a+b)/\ 9.144)^{2/7}(y+b)(y^2/H^2)^{\ 1/2}$$

C = drag coefficient (1.5)
ρ = air density (Kg/m^3)
W = plant width (m)
H = tree height minus (a+b) (m)

2.5 Experimental Design and Statistical Analysis

A randomized complete block design in two blocks was used. A plot had 11 plants and 18 plots made a block. There were 192 total grafted plants included in the experiment; the missing treatment was due to the limited number of seeds (and thus rootstocks) from the two new species (*P. subpeltata* and *P. caerulea*). Data was subjected to multivariate analysis of variance test (MANOVA) using the GLM procedure within the SPSS the version for personal computers (SPSS 15.0, SPSS Inc, University of Chicago). Means were separated using Waller-Duncan test at $p = 0.05$ level of significance. Pearson's correlation analysis for growth factors was carried out using the SPSS program. Pair wise comparisons were done at $p = 0.05$ level of significance.

3. RESULTS

3.1 Vertical Rupture Force

There was a significant graft union length by rootstock type interaction regarding vertical force needed to break the graft union ($p < 0.001$) (Table 1). Regardless of the union length, *P. flavicarpa* and *P. ligularis* self grafts had significantly greater graft union strength compared to *P. mollisima* self grafts and all the other graft combinations with *P. edulis f. edulis* scions. In all cases, the stems severed abruptly above the graft union indicating that the difference in vertical force strength was related to scion properties and not caused by weakness of the graft union.

3.2 Maximum Tensile Strength

There was no significant difference between *P. flavicarpa* self grafts and the other graft combination in the horizontal force needed to break the 1 cm and 1.5 cm graft unions except for *P. caerulea* by *P. edulis* and *P. ligularis* by *P. edulis* 1.5 cm combinations which were significantly weaker (Table 1). When the graft union length was 2 cm, *P. flavicarpa* by *P. edulis* and *P. ligularis* by *P. edulis* had significantly higher mechanical strength with regard to horizontal force than all of the three self-grafts.

3.3 Hydraulic Conductance

Hydraulic conductance was significantly reduced by grafting ($p < 0.001$). All the graft combinations and self-grafts had significantly lower hydraulic conductance than the un-grafted controls (Table 1). When the graft union was 1 cm and 1.5 cm long, all the graft combinations except *P. flavicarpa* by *P. edulis* had significantly lower hydraulic conductance than *P. flavicarpa* and *P. ligularis* self-grafts. When the union length was 2 cm, hydraulic conductance was significantly higher in only *P. ligularis* self-grafts.

3.4 Scion Growth

Grafting significantly reduced scion vine length ($p < 0.001$) except in *P. flavicarpa* by *P. edulis* 1 and 1.5 cm long union length graft combinations (Table 1). However, when the union length was 2 cm, there was no significant difference between any of the graft combinations and self-grafts.

3.5 Vertical Rupture Force

The vertical force needed to break the unions was significantly higher than when the unions were 1 cm long in all graft combinations and self grafts except *P. mollisima* 1.5 and 2 cm grafts (Table 2).

3.6 Maximum Tensile Strength

Maximum tensile strength was not significantly different in the graft combinations when the union length was 1 cm and 1.5 cm long except in *P. ligularis* by *P. edulis* and *P. caerulea* by *P. edulis* and *P. mollisima* and *P. ligularis* self-grafts (Table 2).

Table 1. The effect of the length of the cleft graft on mechanical strength, hydraulic conductance and scion growth thirteen weeks after grafting for grafted *Passiflora* plants

Scion P. edulis	Vertical rupture force [V] (Pa) Union length			Maximum tensile strength [W] (Pa) Union length			Hydraulic conductance [X] (g/cm/day) Union length			Scion length [Y] (cm) Union length		
Graft combination	**1 cm**	**1.5 cm**	**2 cm**	**1 cm**	**1.5 cm**	**2 cm**	**1 cm**	**1.5 cm**	**2 cm**	**1 cm**	**1.5 cm**	**2 cm**
Scion *P. edulis*												
P. flavicarpa	16.5c	36.4cd	37.4cd	5.7a	22.5ab	8.9a	3.0bc	3.4bc	1.9c	**23.7a**	**35.7b**	15.0a
P. ligularis	19.1c	32.8de	33.7cd	27.3a	15.6bc	7.6a	1.5c	2.8c	1.7c	11.7b	16.0cd	12.0a
P. molisima	19.6c	31.6de	33.4cd	22.6ab	23.1ab	5.8ab	1.5c	2.7c	1.7c	13.1b	15.1cd	12.2a
P. subpeltata	18.6c	30.6de	33.4cd	26.6a	23.1ab	4.8b	1.5c	2.7c	1.7c	13.1b	15.1cd	12.2a
P. caerulea	18.8c	28.7e	29.8d	26.2a	15.2b	5.2b	1.5c	2.2c	1.7c	10.6b	12 .2d	9.8ª
Self grafts												
P. molisima	19.9c	43.1b	62.2ab	26.6a	15.7bc	5.3b	1.8c	5.3a	3.9b	11.0b	26.7bc	13.7a
P. flavicarpa	9.6a	78.9a	77.1a	**28.5a**	**28.6a**	4.3b	3.7b	4.9a	2.3bc	10.7b	62.7a	8.7a
P. ligularis	30.5b	42.1c	46.1bc	15.3b	7.8c	4.7b	6.5a	4.6a	7.1a	9.30b	20.3cd	9.7a
Ungrafted												
P. molisima		**-**			**38.2**			12.2*			53.0*	
P. flavicarpa		**-**			106.1*			14.9*			**47.7**	
P. ligularis		**-**			8.7			9.9*			**44.0**	

[V] Vertical rapture force refers to the force in Pa needed to separate scion and rootstock.
[W] Maximum tensile strength measured as the horizontal force needed to break the union or scion stem.
[X] Hydraulic conductance measured as the amount of condensed transpired moisture in eight hours.
[Y] Scion length taken in cm from the start of the graft union to the top of the apical bud.
[Z] Means followed by the same letter within a column are not significantly different at p = 0.05.
** The mean is significantly higher than all other means within a column. Means in **bold** not significantly different p= 0.05*

Table 2. The effect of the length of the cleft graft on mechanical strength, hydraulic conductance and scion growth thirteen weeks after grafting for grafted *Passiflora* plants

Self grafts	Vertical rupture force [V] (Pa) Union length			Maximum tensile strength [W] (Pa) Union length			Hydraulic conductance [X] (g/cm/day) Union length			Scion length [Y] (cm) Union length		
Graft combination	**1 cm**	**1.5 cm**	**2 cm**	**1 cm**	**1.5 cm**	**2 cm**	**1 cm**	**1.5 cm**	**2 cm**	**1 cm**	**1.5 cm**	**2 cm**
Scion *P. edulis*												
P. flavicarpa	16.5b	36.4a	37.4a	5.7a	22.5a	8.9b	3.0a	3.4a	1.9b	**23.7b**	**35.7a**	15.0c
P. ligularis	19.1b	32.8a	33.7a	27.3a	15.6b	7.6c	1.5b	2.8a	1.7b	11.7b	16.0a	12.0ab
P. molisima	19.6b	31.6a	33.4a	22.6a	23.1a	5.8b	1.5c	2.7c	1.7c	13.1a	15.1a	12.2a
P. subpeltata	18.6b	30.6a	33.4a	26.6a	23.1a	4.8b	1.5c	2.7c	1.7c	13.1a	15.1a	12.2a
P. caerulea	18.8b	28.7a	29.8a	26.2a	15.2b	5.2c	1.5c	2.2c	1.7c	10.6a	12 .2a	9.8a
Self grafts												
P. molisima	19.9c	43.1b	62.2a	26.6a	15.7b	5.3c	1.8c	5.3a	3.9ab	11.0c	26.7a	13.7b
P. flavicarpa	39.6b	78.9a	77.1a	28.5a	28.6a	4.3b	3.7a	4.9a	2.3b	10.7b	62.7a	8.7b
P. ligularis	30.5b	42.1a	46.1a	15.3a	7.8b	4.7c	6.5ab	4.6b	7.1a	9.30a	20.3b	9.7b

[V] Vertical rupture force refers to the force in Pa needed to separate scion and rootstock.
[w] Maximum tensile strength measured as the horizontal force needed to the union or scion stem.
[X] Hydraulic conductance measured as the amount of condensed transpired moisture in eight hours.
[Y] Scion length taken in cm from the start of the graft union to the top of the apical bud.
[Z] Means followed by the same letter along each row are not significantly different at $p = 0.05$

3.7 Hydraulic Conductance

Hydraulic conductance was significantly higher when the graft unions were 1.5 cm in the *P. ligularis* by *P. edulis* and *P. flavicarpa* by *P. edulis* graft combinations (Table 2).

3.8 Scion Growth

Scion length on the other hand was significantly high when the union length was 1.5 and 2 cm in *P. ligularis* by *P. edulis* and *P. flavicarpa* by *P. edulis* graft combinations while in the other graft combinations, there was no significant difference.

3.9 Wire Displacement

Vertical force generated by wire displacement showed that enough force was generated that could damage the vines (Table 3).

3.10 Wind Strength

An analysis of the strength of wind on the graft union revealed that the minimum wind speed strong enough to break the weakest unions (*P. flavicarpa* by *P. edulis*, and *P. ligularis* by *P. edulis* (both graft union length 2 cm) was 150 km /h (2.46 km/h at the union) (Table 4). The maximum wind speed (9.1 m) recorded in Nakuru county i.e. 102.15 km/h (2.32 Km/h at the union) (Table 4).

4. DISCUSSION

Graft formation is most rapid when scion stem tissues are matched with those of the rootstock. Further, the tensile strength of the graft is reduced markedly when the diameter of the tissues is mismatched [16]. In the current experiment it seems that the interaction between the union length, and the rate of graft formation was responsible for the low vertical rapture forces compared to self grafts (Table 1). However, when horizontal force was applied results for the 1 and 1.5 cm graft union lengths were not significantly different except in the *P. ligularis* by *P. edulis* 1.5 cm long graft combination. This suggested that the smaller the graft union the faster and the healing of the graft union. The rate of graft healing is directly related to the strength of the union [7]. Generally there was no significant difference in the hydraulic conductance in all the graft combinations indicating that all graft combinations and union lengths affected the seedlings the same way. The same trend was seen for scion length except for *P. flavicarpa* by *P. edulis*. This finding suggested that in this graft combination the rootstock may have had a positive influence on

Table 3. Wire displacement force at three points of loading

	Loading distance from wire ends W		
	1.0 m	**1.25 m**	**1.5 m**
Wire diameter (mm)	0.50	0.50	0.50
Wire displacement (m) X	0.14	0.28	0.21
Vertical force (Pa) Y	43.30	36.30	33.30

W Loading distance refers to the point on the trellising wire where the cable tension meter was hooked.
X Wire displacement is the vertical distance (downward) the trellising wire moved away from original point before loading.
Y Vertical force refers to the force the trellising wire generated when returning to original resting position.
Z The trellising wire was 3 m long

Table 4. Maximum wind velocity and corresponding force resulting from the wind

Air Velocity at 9.144 m (Km/h)	Maximum air velocity 35 cm above the ground (Km/h)	Horizontal force (10^3) Pa
50	2.10	1.19
100	**2.32**	5.17
150	2.46	11.64 Z
200	2.56	20.70
250	2.64	32.35
300	2.71	46.58

Z the highest horizontal force 35 cm above the ground calculated from the strongest wind recorded in Njoro (Kenya) in 2014 the scion. Un-grafted plants transpired two or more times more water than the grafted plants depending on the graft combinations. Since water is the medium of transport of nutrients from the soil to the leaves [11], this limitation in water movement may have been the reasons why vine length was reduced by grafting.

Contact between the scion and the rootstock affect cambial formation between the scion and the rootstock and cambial formation can be delayed if the cambial tissue of the scion and rootstock is misaligned [9]. Also, the strength of the graft union depends on the contact surface area between the scion and rootstock which is directly related to the union length [16]. Findings in the present experiment are consistent with this observation since 1.5 cm and 2 cm union lengths needed more vertical force to break (Tables 1 & 2). However, when horizontal force was applied results were in the contrary. This suggested that in most graft combinations involving different species, cellular connections between the scion and the rootstock were first developed such that the scion was held to resist greater vertical than horizontal force. Scion growth was significantly high when the union length was 1.5 and 2 cm in *P. ligularis* by *P. edulis* and *P. flavicarpa* by *P. edulis* graft combinations. This result was consistent with those reported by Isnard [15].

The stems rupture above the graft union when vertical force was applied suggested that the graft union was stronger than the stem of the scion. An analysis of the strength of wind on the graft union revealed that the minimum wind speed needed to break the weakest unions (*P. flavicarpa* by *P. edulis*, and *P. ligularis* by *P. edulis* (both graft union length 2 cm) was 150 Km/h (2.32 Km/h at the union) (Table 4). This wind speed was higher than the maximum recorded in Nakuru County i.e. 102.15 Km/h. Wire displacement generated enough vertical force that could damage the vines. This result was consistent with those reported by Isnard [15].

5. CONCLUSIONS AND RECOMMENDATIONS

The strength of wind needed to break the weakest unions (*P. flavicarpa* by *P. edulis*, and *P. ligularis* by *P. edulis* (both graft union length 2 cm) 150 Km/h (2.32 Km/h at the union) was higher than the maximum recorded in Nakuru County i.e. 102.15 Km/h. This meant passion fruit growers in Nakuru do not need wind breaks in their orchards. However, the growers need to graft their passion fruits with 2 cm cleft lengths since wire displacement develops enough vertical force that could damage the vines. Results indicated that the best rootstock for *Passiflora edulis* was *Passiflora flavicarpa*.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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