COMPARISON OF SURVIVAL RATES OF UNTREATED, PSHB INFESTED *PLATANUS X ACERIIFOLIUM* TREES VS *PLATANUS X ACERIIFOLIUM* TREES TREATED WITH PSHB FUNGICIDE L10670 – A 3 YEAR TRAIL – M. VIVIERS

INTRODUCTION:

This is a report on the efficacy of PSHB Fungicide, Emergency Registration L10670 - 13/6/2019, comparing the health and survival of treated and untreated ***Platanus x acerifolium*** trees.

Platanus x acerifolium were chosen as the main experimental subject because:

- They are what is known as "**Breeder** Trees" – thus favoured by the PSHB beetles
- They are abundant and 54 trees of the same age, locality and assumedly infection date could be selected
- The beetle can complete its life cycle in a **Breeder** tree, thus VASTLY increasing their numbers in a tree and axiomatically a vastly increased fungal infection on which the beetles feed
- According to FABI: "The majority of reproductive hosts eventually succumb to the disease symptoms caused by the fungus" (1) I challenge this statement
- I also challenge the current mindset from California which states that "Trees that are severely infested (with more than 150 beetle attacks and ISHB-related branch dieback) are not likely to recover from the infestation (2)
- The list of trees cited in this study were NOT DNA sequenced to prove beyond all doubt that ***Fusarium euwallaceae*** was present. In the face of the approximately 1.8 million PSHB infested trees in Johannesburg, they were assumed to be more of same. We fully realise that a few cases might have been some other beetle/fungus than PSHB and its related symbionts, but in the absence of any warning from FABI, we deem this risk negligible.

AIM:

1. To demonstrate that PSHB Fungicide ER L10670 can eradicate ***Fusarium euwalleace, Graphium euwallaceae*** and ***Paracremonium pembeum***, (All associated with ***Euwallecea fornicatus*** infestation(3)) ***and*** maintain the health of ***Platanus x aceriifolium*** trees regardless of the number of entry holes

2. To verify whether treatment with PSHB Fungicidal treatment only, it will be possible to also eliminate or control beetle activity. In other word, whether both beetle and fungal infestation could be eradicated or controlled by the removal of the fungus.
3. To demonstrate that Crown health (%) and not "Stage of infection(2) should be used as treatment initiator.
4. So far, no effective preventative chemical treatments have been reported (4). However, this assertion is invalid, as the PSHB Fungicidal has been registered under Act 36 for this purpose, and the results of this study aims to confirm its relevance and status.

HYPOTHESES:

- PSHB Fungicide ER L10670 can control/eradicate the degenerative results of the unchecked development of ***Fusarium euwallaceae*** in PSHB infested trees.
- PSHB Fungicide ER L10670 is effective in both breeder trees and in non-breeder trees.
- Crown Health and not number of entry holes should be used as an indicator of treatment initiation.

SYNOPSIS:

Euwallaceae cf fornicatus (There is still taxonomic confusion about this name) was first reported as such in 2017 in Pietermaritzburg by Dr Trudie Paap of FABI (1). Reading this paper at the time and realizing the severe impact it could have on the South African ecologies, Pan African Farms decided to do research and try and find a solution to the problem using their unique liposomal technologies. A product was formulated (PSHB Fungicide) and submitted to: The Registrar of Agricultural Products under Act 36 of 1947. Emergency Registration L10670 was given on 13/6/2019.

The most serious limitation was an experimental design that would reflect the efficacy of the fungicide. ***In Vitro*** experimentation gave 100% success, but we needed something that would reflect the efficacy of the fungicide ***In Vivo***. The step was from Petri Dish to an enormous 30 metre high, living organism.

Both the beetle and its symbiont fungi and their initial combined impacts on the tree are hidden from sight. Any assessment using criteria like "Tree health, % crown death" although very visible, would be subjective and not replicable. Using the above would give vastly variable results if just "Age of infestation" was added as a variable. Core drilling and cultivation of ***Fusarium*** on a Petri dish would at most give a yes/no answer at an eye-watering price. What was needed was a more definite assessment.

Another bugbear was the issue of replication. Although more than 122 species of trees have been sprayed, few species were abundant enough to generate replicated and comparative data. I felt that the performance of one species could not be compared to a different unrelated species. Issues like different susceptibilities to PSHB attack, age, size and being a breeder tree or not would make interpretation of data difficult.

To overcome this hurdle I selected a street with 54 ***Platanus x aceriifolium*** trees. This would stabilize

- Site conditions
- Planting age
- Species uniformity
- Landscape orientation

It would also override observations like

- Tree height
- Bole diameter at breast height

Finally it was decided that the only irrefutable data, that could be be sustainable and used in the final analysis, would be

- Decrease in expression of ***active*** wounds on the surface of the tree
- The comparison of % survival of treated trees vs untreated trees.

These results cannot be DUPLICATED as such, but also it cannot be falsified. The trees are in the landscape and as such can be resurveyed. It would satisfy some of the rigorous proofs demanded by science. Death is the ultimate parameter – there are varying degrees of health, but only one expression of death.

The survival rate of the sprayed trees in Ashford Road was compared to randomly selected plane trees in Jan Smuts Avenue. Location was between Smits and Hume streets – both sides of the road. This locality was convenient because of a parking facility. An unexpected complication here was that the plane trees were all of the "Black Bark" variety which makes observing and counting the entry wounds virtually impossible. At most a small grey fungal stain can be observed – Photo 1.

All the trees for this locality were recorded for their biological status. However to get an indication of the infestation pressure that caused this 60%+ mortality, I drove up and down Jan Smuts Avenue and recorded the first 15 smooth bark Plane trees.

PHOTO 1 - the grey discoloration on a black barked plane

METHODOLOGY:

1. The fungicide concentration is 5ml per liter ratio
2. Currently There are two spraying methods used;
    - Backpack Spraying – wetting the stem up to 3-4 metres. Initially 3 times at one week intervals and there-after at 6 week intervals.
    - A total crown spray in which the aim is to wet in entirety the whole crown This is done once a year only by:
        - Climbing the tree and spraying from within the crown
        - Spraying from within a Cherry Picker
        - Using a high pressure pump with a reach of up to 25 metres
        - Using a drone
        - Using a spraying plane
        -

OBSERVATIONS:

Many observations indicate that there is a definite reaction between the exudate of the various trees and the fungicide. This in itself is not currently used as a measure of success. The fact that there is a reaction, can be with more observation, be used as an indicator of success after a spray. I will discuss them in depth under the discussion portion:

1 Exudates stop
2 Exudates lose their typical colouring
3 Exudates lose their texture i.e. Pliable gum exudates go hard and resinous
4 Bite wounds can either recede and be grown over or
5 Bite wounds can form a bulging lesion and be expelled
6 Over time after spraying, bite wounds reduce in numbers as the scar tissue heals itself and the tree bark reassume its pre-infestation nature.

RESULTS:

The results of this study is tabled in:

TABLE 1– Ashford Rd,

TABLE 2 Jan Smuts Rd

TABLE 3 Jan Smuts – Random selected Trees

TABLE 4 Trees Sprayed

- 100% of the treated plane trees in Ashford road survived with an intact crown
- 44% of the untreated planes in Jan Smuts Avenue survived – all with various degrees of crown damage
- On average Ashford Road trees had >500 bites per tree and Jan Smuts Avenue trees had 278 bites per tree. Ashford sitting at a much higher infestation rate
- 122 species of trees were treated in this period. They were a mixture of indigenous/exotic and breeder trees/non breeder trees.
- All the treated trees in Table 4, survived and is alive and uncompromised at the time of writing this report.

TABLE 1

ASHFORD STREET PLANES

No	Circumference (cm) at 1.8 metres (2021)	Active bites 2018 from groundlevel corrected to 4 metres	Active bites 2021 from groundlevel to 4 metres	Crown Health % @Interface with Sky
1	217,6	>500	0	100%
2	166,4	>500	3	100%
3	166	>500	0	100%
4	242	>500	0	100%
5	215,8	>500	0	100%
6	189,2	>500	0	100%
7	220	>500	0	100%
8	221,4	>500	1	100%
9	266,4	>500	0	100%
10	256,4	>500	0	100%
11	290,4	>500	0	100%
12	211,8	>500	0	100%
13	275,2	>500	0	100%
14	154	>500	1	100%
15	212,8	>500	0	100%
16	177,2	>500	0	100%
17	195,4	>500	0	100%
18	287	>500	0	100%
19	250,8	>500	0	100%
20	179,4	>500	0	100%
21	175	>500	1	100%
22	190,4	>500	0	100%
23	220,8	>500	0	100%
24	227	>500	0	100%
25	207,8	>500	0	100%
26	268	>500	0	100%
27	177	>500	2	100%
28	186,2	>500	0 - Turn	100%
29	206,4	>500	2	100%
30	198,4	>500	0	100%
31	145	>500	0	100%
32	231,4	>500	0	100%
33	133,2	>500	0	100%
34	138	>500	5	100%
35	240,2	>500	0	100%
36	117,6	>500	0	100%
37	146,4	>500	0	100%
38	242,8	>500	0	100%
39	104,2	>500	0	100%
40	172,8	>500	2	100%
41	177,4	>500	0	100%
42	164	>500	5	100%
43	200	>500	0	100%
44	226	>500	0	100%
45	157,2	>500	2	100%
46	166,2	>500	0	100%
47	220,8	>500	0	100%
48	204,4	>500	4	100%
49	179,2	>500	5	100%
50	284	>500	1	100%
51	220,8	>500	1	100%
52	174	>500	1	100%
53	260	>500	0	100%
54	235	>500	0	100%
Total Bites		>27000	36	
Chorisia speciosa	310		0	100%

TABLE 2

JAN SMUTS AVENUE PLANES
Between Smit and Hume Streets

No	Circumference (cm) at 1.8 metres	Active bites from groundlevel to 3 metres	Crown Health %
1	183,46	Dead	Dead
2	167,3	Dead	Dead
3	236,2	102	40%
4	236,4	164	75%
5	186,2	160	60%
6	241,9	Dead	Dead
7 - Turn	226,2	Dead	Dead
8	376,9	Dead	Dead
9	185,3	Dead	Dead
10	144,5	Dead	Dead
11	238,8	Dead	Dead
12	285,8	Dead	Dead
13	109,9	Dead	Dead
14	254,4	194	50%
15	166,5	Dead	Dead
16	150,8	Dead	Dead
17	178,4	289	60%
18	179,4	172	70%
		66%	

TABLE 3

RANDOMLY SELECTED TREES WITH WHITE BARK

No	Circumference (cm) at 1.8 metres (2021)	Active bites 2021 for a 1 metre Section	Active bites 2021 corrected for a 4 metre section
1	117	61	244
2	108	56	224
3	190	82	328
4	217	62	248
5	108	62	248
6	156	80	320
7	262	82	328
8	164	62	248
9	230	84	336
10	185	63	252
11	129	70	280
12	276	65	260
13	107	64	256
14	284	63	252
15	239	87	348
Average			278

TABLE 4

SPRAYED TREES - STILL ALIVE

Acacia melanoxylon
Acca (Feijowia) sellowiana
Acer buergerianum
Acer macrophyllum
Acer negundo
Acer palmatum
Adansonia digitata
Aesculus X
Afrocarpus (Podocarpus) falcatus
Ailanthus altissima
Albizia adianthifolia
Albizia julibrissin
Alnus sp.
Archontophoenix cunninghamianum
Bauhinia galpinii
Bauhinia natalensis
Bauhinia purpurea
Betula pendula
Boscia albitrunca
Brachychiton aceriifolia
Brachychiton discolor
Buddleja saligna
Caesalpinia ferrea
Calodendrum capense
Calpurnia aurea
Camellia japonica
Carya illinoinensis
Castanospermum australe
Ceiba pentandra
Celtis africana
Cinnamomum camphora
Citrus aurantiifolia
Citrus limon
Citrus x sinensis
Combretum erythrophyllum
Combretum krausii
Cordia caffra
Corymbium (Eucalyptus) ficifolia
Cussonia spicata
Diospyros dichrophylla
Diospyros glabra
Diospyros lycioides
Diospyros whyteana
Dombeya rotundifolia
Dovyalis caffra
Ekebergia capensis
Eriobotrya japonica
Erythrina caffra
Erythrina lysistemon
Ficus carica
Ficus elastica
Ficus natalensis
Ficus sur
Fraxinus americana
Fraxinus exelsior
Galpinia transvaalica
Gleditsia triacanthos
Grewia occidentalis
Gymnosporia buxifolia
Halleria lucida
Harpephyllum caffrum
Ilex cornuta
Ilex mitis
Jacaranda mimosifolia
Kiggelaria africana
Lagerstroemia indica
Ligustrum lucidum
Liquidambar styraciflua
Macadamia sp.
Magnolia grandiflora
Malus domestica
Melia azedarach
Milettia grandis
Mimusops caffra
Mimusops zeyherii
Morus alba
Nuxia floribunda
Olea europea subsp. pungens
Olinia ventosa
Pearsea americana
Peltophorum africanum
Pittosporum viridiflorum
Platanus x acerifolia
Populus fremontii
Populus simonii
Prunus cerasifera "rubrum"
Prunus dulcis
Prunus nigra
Prunus persica
Psidium guajava
Ptaeroxylon obliquum
Quercus palustris
Quercus palustris
Quercus robur
Quercus suber
Rapanea melanophloeos
Rauvolfia caffra
Ricinus communis
Robinia pseudoacacia
Salix babylonica
Salix mucronata
Schinus molle
Schotia brachypetala
Schotia latifolia
Searsia (Rhus) chirindensis
Searsia (Rhus) lancea
Senegalia (Acacia) burkei
Senegalia (Acacia) galpinii
Sideroxylon innerme
Sterculia murex
Sysigium cordatum
Taxodium distichum
Taxodium distichum
Terminalea phanaerophlebia
Tipuani tipu
Trichelia dregeana
Ulmus parvifolia
Vachellia (Acacia) karroo
Vachellia (Acacia) sieberiana var. woodii
Viburnum sinensis
Virgilia divaricata
Wisteria floribunda
Yucca sp.

DISCUSSION

The 54 Plane trees in Ashford Road, Photos 2 & 3 – the avenue, that received an annual spray with PSHB fungicide $_{ER\ L10670}$, from 2018, survived 100% without any observation of terminal necrosis. I use terminal necrosis as the ultimate treatment initiation or withholding there-of.

If you compare the average number of bites in Ashford Street (>500/tree) and their health status, to the average number of bites per tree on Jan Smuts Avenue (278/tree) and their corresponding health status, it is clear that as long as the terminal canopy is still intact, regardless of the number of bites, a tree can be salvaged. According to Google Earth, Ashford Road and Jan Smuts avenue are less than 500 metres apart as the bird flies.

PHOTO 2 Ashford Avenue

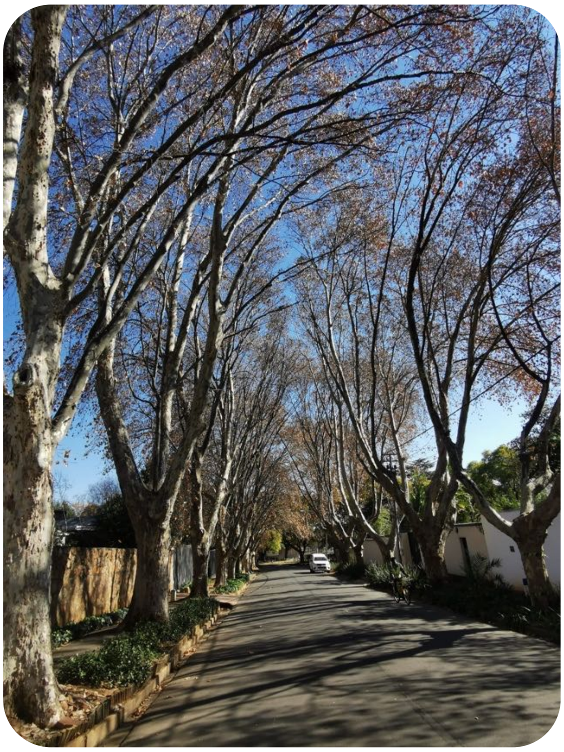

PHOTO 3 Ashford Avenue

It is also clear that the Californian observation that "Trees that are severely infested (with more than 150 beetle attacks and ISHB-related branch dieback) are not likely to recover from the infestation $_{(2)}$ is incorrect in the face of the availability of PSHB fungicide $_{ER\ L10670}$, Photo 4. This is a third of 686 bites in the first 4 metres from ground level.

PHOTO 4 - a third of 686 bites

PHOTO 5 - Bright wet stain on plane

Almost in all cases, after the spraying of PSHB fungicide ER L10670, the expression of a ***Fusarium*** infected tree stops and changes. An active seeping bite on a plane is a bright, wet seep. Photo 5.Within two months of it being treated it becomes grey and slightly sunken into the bark of the tree.

PHOTO 6 - 3 sunken, Grey bites about 3 months after spraying

PHOTO 7 - Top right is what is left once the sunken bite is expelled

It appears that there are now 2 reactions to the ***Fusarium*** invasion: The slightly sunken lesion in Photo 6 is expelled in Photo 7 and regenerate back to smooth bark in the next year, or a lesion is formed, Photo 8.

PHOTO 8 lesion being expelled - notice plastic marker being engulfed

PHOTO 9 - Lesion on the bark of a plane tree

There is active growth – notice the plastic marker being engulfed, The lesions are also expelled revealing the smooth bark underneath, but this is a 2-3 year process. However ugly the lesion looks, if it is lifted, the phloem/cambium region below the lesion shows no ***Fusarium*** staining. It is as if the tree encapsulates the lesion. and then expels it. (Photo 9) is a lesion on the bark of a plane tree. Photo 10 is exactly the same lesion, with the loose "erupting" bark lifted off by hand. Photo 11 is the same lesion as portrayed in photos 8 and 9. Half of the mound has been removed with a wood chisel to expose the cambium and phloem, showing the absence of ***Fusarium*** staining. Over time the lesion will be expelled completely and the bark will resume its former smoothness.

PHOTO 10 - the same lesion with the "erupting" bark removed by hand

PHOTO 11 - the tissue underneath the same old lesion as in photos 8, 9, 10, lifted to reveal cambium and phloem and display the lack of Fusarium staining

Photo 12 shows a completely expelled lesion – this is the side which interfaces with the bark. The bark is now smooth with a slight indentation. Photo 13 shows the original bite hole.

PHOTO 12 - a completely expelled lesion.

PHOTO 13 - the flip side of the lesion with the original bite hole

It is clear that we can control the beetle by controlling its food source ***Fusarium euwallaceae***. In Gauteng I do not spray liposomal Cypermethrin because of the extent of our infestation and additional cost. Johannesburg with an estimated 1.8 million infested trees (depending on which source you want to believe) exudes roughly 472 million beetle a year which calculates to 1.2 million beetles a day. In the face of authorities doing nothing, the extra cost is not justified. This approach is not advised for other centres in the country.

Furthermore, once a tree has been given a crown spay with PSHB fungicide $_{ER\ L10670}$, there is an exodus of beetles and larvae. Photos 14, 15, 16 and 17. Logical reasoning leads me to believe that:

PHOTO 14- exodus of PSHB beetles 24 hours after spray

PHOTO 15 - PSHB beetles collected 24 hours after spray

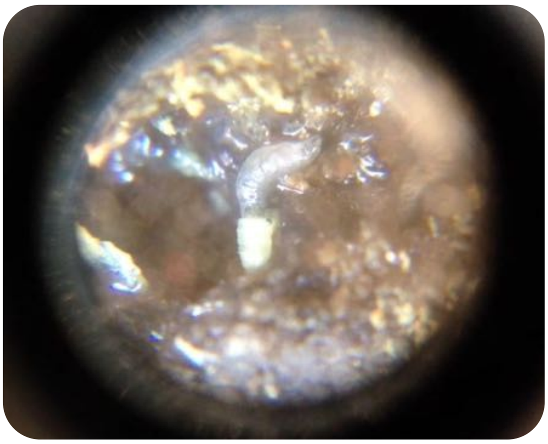

PHOTO 16 - exodus of PSHB beetle larvae after spray

PHOTO 17 - exodus of PSHB beetle larvae after spray

- The only reason why larvae should leave the tree is because of lack of food. This is the most sensitive stage in the cycle of the beetle and should have been completed in the tree. Keep in mind that PSHB fungicide ER L10670 is a FUNGICIDE not an insecticide.
- The males are doomed. They are flightless and can't get back in the tree.
- The unfertilized females are doomed, because they need to be fertlised in the tree before they take flight.
- This leaves us with the fertile females. I have not seen them take flight within the hour and they amble around aimlessly. However, technically speaking they can fly to another tree and colonise it.

A thought here is, given the penetrability of the liposomes, could it be that the fungal spores in the mandibular mycangia of the female beetles are sterilised?. Fungal spores themselves are all microscopic, some as small as two micrometres in size[6] . The liposomal base of the Fungicidal formulation is much smaller (average 50 nanometers) and can therefore easily penetrate into these microspores and destroy same.

The issue of whether one should spray in winter (especially with deciduous trees) raises its head. In an article edited by Ute Sass-Klaasen [9] it is suggested that tree metabolism can slow down by 50 to 60% in winter. It does not stop however. A tree without biological processes is a dead tree.

Usheda and Paine[7] argued that the beetle's distribution and spread could be predicted by temperature. They found that the minimum **AVERAGE** temperature was 13°C and the maximum **AVERAGE** was 33°C, respectively. PSHB development rate was calculated to be optimal near 28 °C. The number of degree days required for PSHB to complete development from egg to adult was estimated to be approximately 398 degree days. Looking at Johannesburg weather data[8], and using above temperatures, it is clear that the beetle will be slowed down during June – September, but will still breed. The degree days are reachable, even in winter. By default I assume that the ***Fusarium*** fungus must then also adhere to these temperature ranges. No such work has been done, but the fungus MUST survive if the beetle survives due to their intricate symbioses.

The IMPORTANT implication here is that the fungus continues growing, albeit slower, in the winter months. I sprayed Ashford in 2018's winter. 2018's spring saw a large portion of the planes in Jan Smuts dead. I can only surmise that the fungus finally throttled the vascular systems of the trees during our mild 2018 winter.

Incidental data:

"E. fornicatus attacks a very wide range of host trees, usually attacking cut, stressed or dying trees, small stems and branches, but primary attacks on healthy plants also occur. Healthy plants can sometime resist attack by exuding gum or latex in which the beetle can become entrapped".[5]

Certainly in Johannesburg the ***E. fornicatus*** has infected a host of healthy, mature trees which has given rise to table. All the trees in this table has been taxonomically ID'ed and ID'ed for the presence of ***Fusarium euwallaceae.*** They are noted but not taken up in the data base because of the lack of repeatability like with ***Platanus x aceriifolium***. They occur either singly or in twos or threes. It is stated categorically that every one of those trees are still alive and healthy. ***Persea americana*** is encountered often and treated with the same results.

The Chorisia/Ceiba inserted in table 1, was sprayed with PSHB fungicide $_{\text{ER L10670}}$, after it was injected twice, by a local arborist. This happened in 2018. One treatment of liposomal Cypermethrin evicted about 150 beetles overnight. The tree has been part of the annual Ashford spray programme. A recent spray (2021) with liposomal Cypermethrin flushed no beetles. Three years ago the tree had less than 100 flower. This year it was covered in flowers. Photo 18

All the trees in table 3 have been Id'ed and sprayed for ***Fusarium euwallaceae.*** They are noted but not taken up in the data base because of the lack of repeatability like with ***Platanus x aceriifolium***. They occur either singly or in twos or threes. It is stated categorically that every one of those trees are still alive and healthy. ***Persea americana*** is encountered often and treated with the same results.

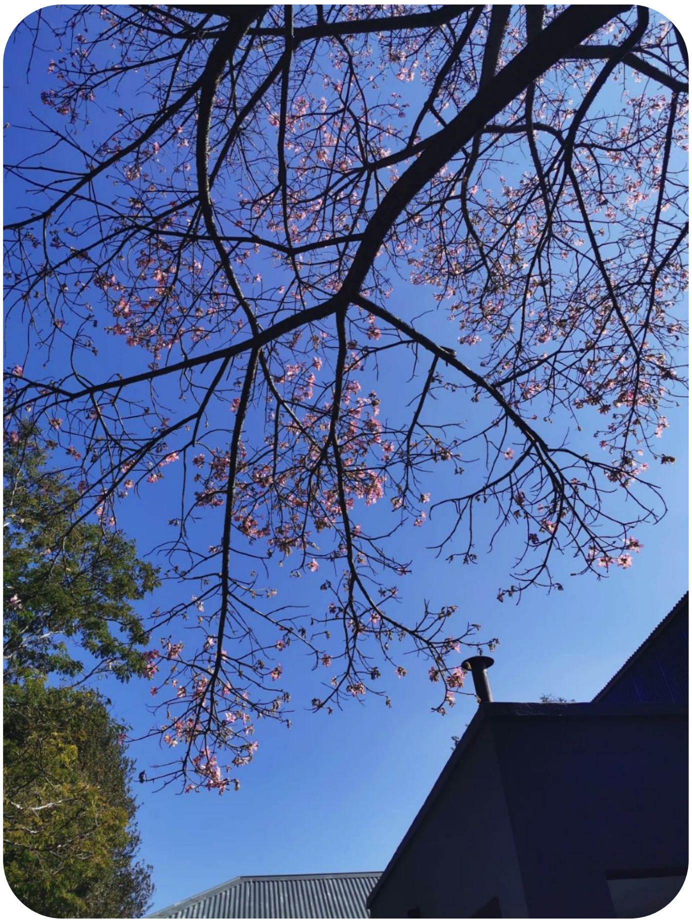

PHOTO 18 - Flowering Chorisia/Ceiba

Many of my clients have reported renewed vigour of their treated trees of which the Chorisia/Ceiba above is an example. Comments were typically:

- Restored or more flowering
- Restored or more fruiting
- Restored or denser tree canopy

I realise these are subjective observations, but leeway should be made for a home owner that lives with a tree.
Certainly this can be tested in an agricultural setting comparing fruit crop volume from infested trees in one year to the next after they have been sprayed normalizing for

- Cultivar
- Age
- Infestation
- Terroir

This would be almost impossible in an urban setting.

CONCLUSION:

PSHB fungicide $_{ER\ L10670}$ is an effective fungicide for the controlling of ***Fusarium euwallaceae*** and Polyphagous Shothole Borer Beetle in shrubs and trees. I have found no tree in the treated list, that was inure to the fungicide.

It is believed that the fungicide embues a 12 month "immunity" to the tree, and an annual respray is recommended.

If we can devise a test to differentiate between "New Infestation" vs "Old Exiting" scar marks a better understanding can be garnered.

Various scenarios can be drummed up for "collapsing" the beetle infestation in South Africa. However, I have never believed that chemical control can be efficient in open ecologies. At this moment the PSHB fungicide $_{ER\ L10670}$ can be used with great effect and eco sensitivity in cities and in agriculture.

M. Viviers
28 June 2021.

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