Project Title:	Apple: Investigation on survival and viability of cankers of *Nectria galligena* following removal from the tree and pulverisation on the orchard floor
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Apple: Investigation on survival and viability of cankers of *Nectria galligena* following removal from the tree and pulverisation on the orchard floor

Headline

- Pruned-out cankers pulverised or unpulverised on the orchard floor can produce fruiting bodies (perithecia) for at least 16 months after being removed from the trees and are therefore a potential canker risk to apple trees. However, pulverised cankered prunings left in the grass alleyway decay more rapidly than those in the tree row.

Background and expected deliverables

Canker, caused by the fungus *Nectria galligena*, is one of the most important diseases of apple and pear. The fungus attacks trees in the orchard, causing cankers and die- back of young shoots, resulting in loss of fruiting wood and increasing pruning costs. Apple canker can be particularly damaging in young orchards where, in some years, up to 10% of trees can be lost annually in the first few years of orchard establishment as a result of trunk cankers. *Nectria* also causes a fruit rot that can result in significant losses, as high as 10% or more in stored fruit. *Nectria* rot. This rot, which is often at the fruit stalk end, is difficult to spot on the grading line but becomes obvious during marketing leading to rejection of fruit consignments.

The fungus produces two spore types, conidia in the spring and summer and ascospores in the autumn and winter. These enter shoots and branches on the tree through wounds, either natural such as bud-scale scars, leaf scars, fruit scars or artificial such as pruning wounds. Thus inoculum and points of entry on the tree are available all year round and the only limiting factor is rain, which is essential for spore production, spread, germination and infection. Autumn leaf fall is usually the main infection period and wet autumns are usually followed by a high incidence of shoot dieback the following spring and summer due to autumn canker infection.

Currently, canker is controlled by a combination of cultural methods to remove canker lesions and the use of protectant fungicides. Effective fungicides are limited. Generally, copper fungicides are used at autumn leaf fall and before budburst to protect leaf scars and bud-scale scars and carbendazim is applied during the spring and summer.

Up until the 1970s, it was normal orchard practice to remove prunings from the orchard and burn them. Any cankers pruned out would therefore have been eliminated from the orchard. Removal and burning of prunings from orchards is now rare, most being pulverised in the tree alleyways. What is not clear is the effect of this practice on canker survival and viability and the likely risk to trees from spores generated by canker debris on the ground. Previous studies by UK and Dutch scientists in the late 1970s and early 1980s, which have focused on canker infection in the trees, have indicated a minimal risk. Despite this there are still concerns among growers.

The overall aim of this project was to assess the risk of pruned-out cankers left in the tree alleyway as sources of canker spores for tree infection by investigating:

- The survival period of the pulverised cankers
- The spore production period

Summary of project and main conclusions

The trials were set up in two Gala orchards located at Rocks Farm, East Malling, and on a commercial farm at Teynham where canker had been a significant problem since planting. The orchards were visited in February 2005 and cankered one-year-old shoots collected from the Gala trees and distributed among sprout nets, which were then placed back out in the orchard, in the tree row. The bags were held in place with metal pins and the positions noted so that they could be relocated for future sampling. Cankered two, three, four and older wood was collected from trees and similarly distributed among sprout nets after pulverising with a tractor-trailed standard orchard pulveriser. The bags were then placed back out in the orchard, either in the tree row (East Malling) or in the grass alleyway (Teynham) and held in place with metal pins and the positions noted so that they could be relocated for future sampling.

The orchards were visited, initially at monthly intervals, and the state of the cankers assessed in terms of state of decay. Labelled bags containing pieces of canker from the pulverised wood or the one-year-old cankers were collected from the orchards and examined carefully for signs of sporing either white pustules (conidia) or red fruiting bodies (perithecia). Where present they were checked for spores. All assessments of cankers were conducted at East.Malling

Pulverised or unpulverised pruned-out cankers continued to produce perithecia for at least 16 months after being removed from the trees. The cankers produced conidia for a much shorter period of time. Perithecia were produced more abundantly on pruned-out cankered young shoots. Decay of prunings appeared to take place more slowly in the tree row. Pulverised prunings left in the grass alleyway decayed more rapidly and were also overgrown by the grass but despite this perithecia could still be found almost twelve months after the pulverising.

Previous studies in Belgium and the UK indicated a minimal risk from cankered prunings dumped in the grass alleyway. However, this study shows that pulverised cankers can continue to pose a threat to apple trees for more than a year after pulverising. In areas where conditions favour canker it would ideally be desirable to return to the practice of collecting prunings and burning to minimise the risk. This however, may not always be practical. The best alternative would be to dump all prunings, including young shoots, in the grass alleyway and pulverise. Decay is more rapid and repeated mowing of prunings would increase the break down.

Conclusions

- Pulverised or unpulverised pruned-out cankers can produce perithecia for at least 16 months after being removed from the trees.
- The cankers continued to produce conidia for only a few months.
- Perithecia were produced more abundantly on pruned-out cankered young shoots than on older wood.
- Decay of prunings appears to take place more slowly in the tree row than in the grass alley.

- Pulverised prunings left in the grass alleyway appeared to decay more rapidly and were also overgrown by the grass. Despite this, perithecia could still be found almost twelve months after the pulverising.

Financial benefits of the project

Apple canker is one of the most difficult disease problems facing the apple industry, mainly because of the difficulties in achieving successful control of the problem. The risk posed by pulverised apple prunings and excised cankers has always been of concern to growers. Previous studies have focused on assessing *Nectria* infection on apple trees from cankers introduced into the tree alleyway. This study has concentrated on the activity of the actual canker debris left in the alley and has clearly shown that this is a source of inoculum production from this source. By following the Action Points for Growers growers should be able to reduce the infection risk and thus the losses caused by canker.

Action points for growers

- In areas where conditions favour canker, consider a return to the practice of collecting prunings and burning to minimise the risk.
- Alternatively dump all prunings, including young shoots, in the grass alleyway and pulverise to ensure rapid decay and where repeated mowing of grass and prunings will increase the speed of break down.
- Avoid dumping young cankered shoots in the tree row as they can generate more inoculum.

Science Section

Introduction

Canker, caused by the fungus *Nectria galligena*, is one of the most important diseases of apple and pear. The fungus attacks trees in the orchard, causing cankers and die- back of young shoots, resulting in loss of fruiting wood and increasing pruning costs. Apple canker can be particularly damaging in young orchards where, in some years, up to 10% of trees can be lost annually in the first few years of orchard establishment as a result of trunk cankers. *Nectria* also causes a fruit rot that can result in significant losses, as high as 10% or more in stored fruit. *Nectria* rot. This rot, which is often at the fruit stalk end, is difficult to spot on the grading line but becomes obvious during marketing leading to rejection of fruit consignments.

The fungus produces two spore types, conidia in the spring and summer and ascospores in the autumn and winter. These enter shoots and branches on the tree through wounds, either natural such as bud-scale scars, leaf scars, fruit scars or artificial such as pruning wounds. Thus inoculum and points of entry on the tree are available all year round and the only limiting factor is rain, which is essential for spore production, spread, germination and infection. Autumn leaf fall is usually the main infection period and wet autumns are usually followed by a high incidence of shoot dieback due to canker the following spring and summer.

Currently, canker is controlled by a combination of cultural methods to remove canker lesions and the use of protectant fungicides. Effective fungicides are limited. Generally copper fungicides are used at autumn leaf fall and before budburst to protect leaf scars and bud-scale scars and carbendazim is applied during the spring and summer.

Up until the 1970s, it was normal orchard practice to remove prunings from the orchard and burn them. Any cankers pruned out would therefore have been eliminated from the orchard. Removal and burning of prunings from orchards is now rare, most being pulverised in the tree alleyways. What is not clear is the effect of this practice on canker survival and viability and the likely risk to trees from spores generated by canker debris on the ground. Previous studies by van der Scheer

(1978, 1981) and Swinburne and Souter (1984) have indicated a minimal risk. Apple canker is one of the most difficult disease problems facing the apple industry, mainly because of the difficulties in achieving successful control of the problem. The risk posed by pulverised apple prunings and excised cankers has always been of concern to growers. The previous studies have focused on assessing Nectria infection on apple trees from cankers introduced into the tree alleyway. This study will concentrate on the activity of the actual canker debris left in the alley and should therefore give a clear idea on likely inoculum production from this source. This will provide growers with a good assessment of the risk posed by pulverising cankers and whether alternative methods of canker disposal need to be identified.

Overall objective:

To investigate the risk posed by pulverised apple canker prunings left in the tree alleyway as sources of inoculum for infection of adjacent apple trees.

Specific objectives

1. To investigate the time of survival of pulverised and unpulverised canker prunings on the orchard floor.

2. To investigate production of spores (conidia and ascospores) by pulverised canker prunings on the orchard floor.

Materials and Methods

Site

Two orchard sites were selected for the study. One orchard site was located at Rocks Farm, East Malling, (TL161) and consisted of a solid block of cv. Gala on M9 rootstock, planted in 1999. The second orchard was located on a commercial farm – Elverton Farm - at Teynham (Marsh Gala) and consisted of Gala with Cox pollinators both on M9 rootstock. The second site was managed by FAST Ltd. In both orchards canker had been a significant problem since planting.

Experimental details

East Malling

The orchard was visited in February 2005. Approximately 110 cankered one-year-old shoots were collected from the Gala trees in each of four pairs of rows and placed in black sacks, giving a total of about 440 cankered shoots. Similarly, approximately 110 cankered two, three, four and older wood was collected from each of the same rows and placed in black sacks, giving a total of around 440 lumps.

At least 10 one-year-old cankered shoots were placed in each of 44 sprout nets, giving four replicates of 11 nets. These were then placed back out in the orchard, in the tree row of each of 4 rows, 11 bags per row. The bags were held in place with metal pins and the positions noted so that they could be relocated for future sampling.

The older cankers were spread out on a concrete pad and pulverised by a tractor-trailed standard orchard pulveriser twice, in two different directions. All pulverised prunings were then collected up and divided into 4 equal replicate lots. Each lot was then divided up into 11 sprout nets, giving 44 in total. These were then placed back out in the orchard, in the tree row in each of 4 rows, 11 bags per row. The bags were held in place with metal pins and the positions noted so that they could be relocated for future sampling.

Elverton Farm

A similar procedure was followed for canker collection as that at East Malling. Similarly one-year old cankers were laid out in sprout nets in the tree row.

A similar procedure was followed for mature cankers collected. These cankers were then spread down the grass alley way in the orchard and pulverised by a tractor-trailed standard orchard pulveriser twice, in two different directions. All pulverised prunings were then collected up and divided into four equal replicate lots. Each lot was then divided up into 10 sprout nets, giving 40 in total. These were then placed back out in the orchard, in the grass alley way in each of four rows, 10 bags per row. The bags were held in place with metal pins and the positions noted so that they could be relocated for future sampling.

Assessments

Initially at monthly intervals the orchards were visited and the state of the cankers assessed in terms of state of decay. One of the labelled bags containing pieces of canker from the pulverised wood was collected from each replicate in the orchard.
In the laboratory each canker piece was examined carefully for signs of conidial masses or perithecia. Where present they were checked for spores.

If no fruiting bodies were present then the canker pieces were incubated in damp chambers after wetting to encourage sporing.

Estimates were made of numbers of fruiting bodies present. If appropriate, cankers were washed to remove spores and the spores counted using a haemocytometer slide to give a measure of canker activity with sampling time.

Cankers on one-year-old pruned wood were similarly collected and similarly assessed.

All assessments of cankers were conducted at East Malling.

Results and Discussion

East Malling

Rain fell in most 7-day periods from 1 January 2005 (Table 1). Driest conditions were in July and August 2005 and June 2006 when there were three 7-day periods without rain. Conditions were therefore favourable for canker sporulation throughout the spring and early part of the summer with conditions becoming less favourable in July and August, which were exceptionally hot in 2005.

Netted samples of one-year-old cankered shoots and pulverised cankers on older wood were collected from the orchard and checked for sporulation at roughly monthly intervals from March 2005 until July 2005. Thereafter, samples were collected and examined at longer intervals until June 2006 (Table 3). Initially non-sporing cankers in the netted samples were damp incubated to check canker viability. However, the damp incubated cankers were soon overrun by secondary saprophytic fungi, making it impossible to check cankers for sporulation. It was therefore decided not to continue damp incubation after the first sample. Similarly washing and counting spores was too time consuming and did not add any additional information to the study.

The netted one-year shoots were unpulverised and mostly retained their bark throughout the assessment period. The dead wood was rapidly colonised by various saprophytic fungi, including Botryosphaeria and Diaporthe which were present on many of the twigs examined from the first assessment in March 2005 onwards. By the final assessment in June 2006, the shoots were becoming very dry, rotted and easily broken, although the original cankered area was still obvious and perithecia easily visible. On average more than 60% of cankers were sporing at each sampling. Perithecia containing mature or immature ascospores were present in abundance on these cankered shoots at each sampling. This is surprising as perithecia are normally associated with mature cankers rather than those on young shoots. Conidia were present in samples checked in April and May 2005 but were not observed in subsequent samplings (Table 3).

The netted pulverised cankers had mostly been debarked during the pulverising process and as with the one year shoots by June 2006 were becoming very dry and colonised by various saprophytic fungi. The original cankered areas were less easily distinguished with time. Initially most perithecia or conidia observed were associated with the barked areas of the prunings, but in later samplings perithecia were equally found on debarked areas also. Numbers of cankers observed with conidia or perithecia present were on average half of that recorded on the one year old unpulverised prunings (Table 4). Conidia were only recorded on samples collected in April 2005. Perithecia were recorded on the pulverised prunings at every sampling and 43% of cankers were still producing perithecia 16 months after pulverising (Table 4).

Elverton Farm

Rain fell in most 7-day periods from January 1 2005 (Table 2) but there were more dry weeks than recorded at East Malling. Driest conditions were in July and August 2005 when there were three 7-day periods without rain. Conditions were therefore favourable for canker sporulation throughout the spring and early part of the summer, with conditions becoming less favourable in July and August which were exceptionally hot in 2005.

Netted samples of one-year-old cankered shoots and pulverised cankers on older wood were collected from the orchard and checked for sporulation at roughly monthly intervals from March 2005 until July 2005. Thereafter samples were collected and examined at longer intervals until December 2005 (Table 3).

The results for netted one-year shoots were similar to those for East Malling. The unpulverised twigs mostly retained their bark throughout the assessment period. The dead wood was rapidly colonised by various saprophytic fungi, including Botryosphaeria and Diaporthe which were present on many of the twigs examined from the first assessment in March 2005 onwards. Assessments were only continued up to October 2005 as later samples could not be located in the orchard. By the final assessment the shoots were becoming very dry, rotted and easily broken, although the original cankered area was still obvious and perithecia easily visible. On average more than 60% of cankers were sporing at each sampling. Perithecia containing mature or immature ascospores were present in abundance on these cankered shoots at each sampling except the first one in March 2005 (Table 3). This is surprising as perithecia are normally associated with mature cankers rather than those on young shoots. In contrast to the East Malling site conidia were present in samples up to the final assessment in October 2005 (Table 3). Perithecia however, were usually more abundant.

The netted pulverised cankers had mostly been debarked during the pulverising process. In contrast to the East Malling site, the netted samples had been placed in the grass alleyway. Consequently at each sampling the pulverised prunings were much wetter, were more colonised by saprophytic fungi and generally decaying more rapidly. It was also noted that the grass was rapidly growing over the netted samples, increasing the general decay of the wood. This more rapid decay was reflected in the incidence of perithecia found on the prunings which was usually less than half of that found on pulverised prunings at the East Malling site. At the last sampling in December 2005, perithecia were found on less than 10% of cankers (Table 3). Conidia were only observed on cankers during the first two samplings in March and April 2005.

Table 1. Weekly rainfall totals from 1 January 2005 for East Malling

Start date	No. rain days	Total rain in 7 days mm	Start date	No. rain days	Total rain in 7 days mm
1 Jan	4	4.6	12 Nov	4	2.0
8 Jan	3	7.8	19 Nov	4	2.2
15 Jan	3	2.4	26 Nov	3	14.0
22 Jan	6	8.2	3 Dec	4	7.2
29 Jan	4	2.2	10 Dec	2	0.6
5 Feb	6	7.0	17 Dec	2	1.4
12 Feb	3	6.4	24 Dec	5	13.0
19 Feb	5	10.2	31 Dec	4	4.4
26 Feb	6	29.6	7 Jan	5	6.6
5 Mar	4	2.4	14 Jan	4	7.8
12 Mar	1	0.2	21 Jan	4	2.2
19 Mar	4	5.4	28 Jan	1	0.8
26 Mar	2	13.2	4 Feb	1	1.4
2 Apr	1	3.8	11 Feb	6	21.6
9 Apr	4	14.8	18 Feb	6	34.2
16 Apr	3	10.0	25 Feb	2	2.0
23 Apr	5	20.6	4 Mar	5	13.8
30 Apr	4	3.4	11 Mar	1	0.2
7 May	4	7.0	18 Mar	2	3.4
14 May	3	12.2	25 Mar	7	22.8
21 May	4	1.8	1 Apr	3	10.2
28 May	5	9.6	8 Apr	4	39.8
4 June	2	1.6	15 Apr	3	4.4
11 June	2	1.4	22 Apr	3	3.8
18 June	1	0.8	29 Apr	2	12.6
25 June	3	3.0	6 May	5	15.4
2 July	5	4.2	13 May	5	23.2
9 July	0	0	20 May	7	34.8
16 July	0	0	27 May	3	3.6
23 July	4	27.6	3 Jun	1	0.2
30 July	4	11.8	10 Jun	4	2.2
6 Aug	0	0	17 Jun	2	0.4
13 Aug	4	23.0	24 Jun	3	5.6
20 Aug	4	22.0	1 Jul	3	3.4
27 Aug	1	0.2			
3 Sep	5	3.8			
10 Sep	6	13.6			
17 Sep	3	1.0			
24 Sep	6	14.0			
1 Oct	2	1.4			
8 Oct	3	10.6			
15 Oct	6	21.0			
22 Oct	6	26.8			
29 Oct	5	25.6			
5 Nov	5	9.4			

Table 2. Weekly rainfall totals from 1 January 2005 for Teynham

Start date	No. rain days	Total rain in 7 days mm	Start date	No. rain days	Total rain in 7 days mm
1 Jan	2	5.0	12 Nov	1	3.0
8 Jan	2	9.0	19 Nov	1	1.0
15 Jan	2	2.0	26 Nov	3	20.0
22 Jan	4	9.0	3 Dec	3	23.0
29 Jan	2	2.0	10 Dec	0	0
5 Feb	2	4.0	17 Dec	1	3.0
12 Feb	4	7.0	24 Dec	4	21.0
19 Feb	6	25.0			
26 Feb	7	37.0			
5 Mar	2	4.0			
12 Mar	0	0			
19 Mar	1	2.0			
26 Mar	2	15.0			
2 Apr	2	5.0			
9 Apr	3	14.0			
16 Apr	2	7.0			
23 Apr	5	12.0			
30 Apr	2	3.0			
7 May	4	15.0			
14 May	3	15.0			
21 May	1	4.0			
28 May	2	18.0			
4 June	1	1.0			
11 June	0	0			
18 June	1	8.0			
25 June	1	1.0			
2 July	4	18.0			
9 July	1	5.0			
16 July	0	0			
23 July	5	46.0			
30 July	2	11.0			
6 Aug	0	0			
13 Aug	3	18.0			
20 Aug	4	28.0			
27 Aug	0	0			
3 Sep	1	2.0			
10 Sep	4	29.0			
17 Sep	0	0			
24 Sep	5	17.0			
1 Oct	1	1.0			
8 Oct	3	8.0			
15 Oct	5	21.0			
22 Oct	3	23.0			
29 Oct	4	38.0			
5 Nov	3	12.0			

Table 3. Numbers of cankers present on netted one-year old unpulverised pruned twigs and percentage of these cankers with conidia or perithecia present at various sample dates from orchard sites at East Malling or Elverton Farm in 2005 or 2006

Sample date	Site	Rain mm in 14 days pre sample	Total number branch bits examined	Total number of cankers	% cankers sporing	% cankers sporing conidia	% cankers with perithecia
23 March 2005	East Malling	5.6	9	9	27.8	0	100
21 March	Elverton Farm	1.0	11	11	9.1	100	0
20 April	East Malling	28.6	8	5	62.5	55.0	65.0
	Elverton Farm	24.0	10	10	70.0	100	14.3
20 May	East Malling	19.2	9.5	9	50.0	61.1	72.2
	Elverton Farm	28.0	15	15	46.7	71.4	100
24 June	East Malling	2.2	10	9.5	57.9	0	100
	Elverton Farm	8.0	18	18	33.3	0	100
20 July	East Malling	0.2	9.5	8.3	66.7	0	100
28 July	Elverton Farm	46.0	19	18	66.7	91.7	41.7
3 October	East Malling	16.2	9	8.5	73.5	0	100
12 October	Elverton Farm	13.0	23	21	57.1	4.3	100
21 December	East Malling	6.6	11	9	88.9	0	100
23 March 2006	East Malling	3.0	11	11	54.5	0	100
29 June 2006	East Malling	6.2	11.8	7.3	61.6	0	100

Table 4. Numbers of cankers present on netted mature pulverised pruned cankers and percentage of these cankers with conidia or perithecia present at various sample dates from orchard sites at East Malling or Elverton Farm in 2005 or 2006

Sample date	Site	Rain mm in 14 days pre-sample	Mean number branch bits examined	Mean number of cankers	% cankers sporing	% cankers sporing conidia	% cankers with perithecia
23 March 2005	East Malling	5.6	47.5	47.5	5.3	0	100
21 March	Elverton Farm	1.0	108	108	0.3	0.3	0
20 April	East Malling	28.6	66.3	66.3	6.0	31.3	75.0
	Elverton Farm	24.0	75.8	75.8	2.3	42.9	57.1
20 May	East Malling	19.2	61.0	42.0	28.6	0	100
	Elverton Farm	28.0	53.8	34.0	5.1	0	100
24 June	East Malling	2.2	63.8	59.8	26.3	0	100
	Elverton Farm	8.0	44.3	39.8	10.7	0	100
20 July	East Malling	0.2	51.0	30.8	39.0	0	100
28 July	Elverton Farm	46.0	55.3	39.8	14.4	0	100
3 October	East Malling	16.2	63.8	26.5	84.0	0	100
12 October	Elverton Farm	13.0	56.5	49.0	11.2	0	100
21 December	East Malling	6.6	47.0	34.0	32.4	0	100
15 December	Elverton Farm	39.0	60.0	56.3	8.3	0	100
23 March 2006	East Malling	3.0	43.0	43.0	26.7	0	100
29 June 2006	East Malling	6.2	37	22.5	43.3	0	100

General discussion

It is clear from this study that pruned-out cankers pulverised or unpulverised can produce perithecia for at least 16 months after being removed from the trees. The cankers continued to produce conidia for a much shorter period of time, but it is the perithecia that pose the risk to canker infection on trees as ascospores can be shot out from perithecia during wet weather similar to the release of scab ascospores from fruiting bodies (pseudothecia) surviving on leaf litter. These ascospores of N galligena then can be carried by air currents up to infect trees. Perithecia were produced more abundantly on pruned-out cankered young shoots. These are often just dumped in the tree row rather than the alleyway and left unpulverised. Decay of prunings appears to take place more slowly in the tree row. Pulverised prunings left in the grass alleyway appeared to decay more rapidly and were also overgrown by the grass. Despite this perithecia could still be found almost twelve months after the pulverising.

Previous studies by van der Scheer (1978, 1981) and Swinburne & Souter (1984) have indicated a minimal risk from cankered prunings dumped in the grass alleyway. However, this study shows that pulverised cankers can continue to pose a threat to apple trees for more than a year after pulverising. Ideally in areas where conditions favour canker it would be desirable to return to the practice of collecting prunings and burning to minimise the risk. This however, may not be practical. The best alternative would be to dump all prunings, including young shoots, in the grass alleyway and pulverise. Decay is more rapid and repeated mowing of prunings would increase the break down.

Conclusions

- Pruned-out cankers pulverised or unpulverised can produce perithecia for at least 16 months after being removed from the trees.
- The cankers continued to produce conidia for only a few months.
- Perithecia were produced more abundantly on pruned-out cankered young shoots.
- Decay of prunings appears to take place more slowly in the tree row.
- Pulverised prunings left in the grass alleyway appeared to decay more rapidly and were also overgrown by the grass. Despite this, perithecia could still be found almost twelve months after the pulverising.

Future work

- The results can be incorporated as best practice in to the canker best practice project.

Technology transfer

Growers have been informed of the project, but no data has been presented except to the HDC Top Fruit committee.

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