TECHNICAL NOTES FOR ROMEO®

SUMMARY

ROMEO is an approved professional fungicide – a host plant defence inducer- a biofungicide.

- Based on Cerevisane, a purified extract of a *Saccharomyces cerevisae* strain LAS117. ROMEO mimics a disease attack on the plant and acts to strengthen the plant against disease.
- Can be used alone, in a programme with or in tank mix with chemical fungicides.
- Use for resistance management. Use to increase spray programme performance.
- Use in sustainable organic production, subject to certification body approval.

Approved for use on crops grown under permanent protection with full enclosure –
Botrytis: aubergine, strawberry and tomato. Powdery mildew: cucumber and other named cucurbits. Downy mildew: lettuce.

Approved for use on outdoor crops –
Botrytis, powdery mildew and downy mildew: table and wine grapes.

EAMU*s allow use on a range of other protected and outdoor crops including ornamental plant production, named herbs and named fruit crops, see table 1, page 8 for more detail.

Label recommended rates are summarised in table 1 and range from 0.25 to 0.75 kg per ha.

ROMEO must be applied preventatively as it has no direct effect on plant disease.

ROMEO offers protection 24 hours after application. Applications should be made every 7-10 days with a maximum of 8 treatments for most crops and 10 treatments for table and wine grapes per annum. In case of high disease pressure or disease development ROMEO should be used with other fungicides either by tank mix or alternation.

Mixable with many chemical plant protection products. Details of known compatibilities are summarised in table 2 page 12.

Use plant protection products safely. Always read the label and product information before use.
Further product information, including warning phrases and symbols, is included in the main product manual.

VERSION CONTROL

Version	Publication Date	Change from previous version
12/18	December-2018	New product.
03/21	March 2021	Addition of grapes, change in MAPP number and marketing company.
07/21	July 2021	Additional extensions of authorisation for minor use EAMUs

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These notes are designed to support the main product manual insert on ROMEO and advise on how to obtain the best possible disease control from the product.

INTRODUCTION

WHAT IS ROMEO?

- ROMEO is an approved fungicide MAPP number 19170.
- ROMEO contains 941 g/kg Cerevisane, a purified extract of the cell wall of a specific strain LAS117 of yeast *Saccharomyces cerevisiae*, it is non - living.
- Cerevisane is a plant defence inducer which acts to strengthen the plant against disease. It has no direct activity on fungal pathogens.
- ROMEO is effective in induction of defence against a broad spectrum of fungal diseases.
- ROMEO's defence induction takes about 24 hours to develop and lasts for 7-10 days after treatment.

WHY USE ROMEO?

- Another way to control downy mildew, powdery mildew and *Botrytis*.
- Use to increase spray programme performance.
- Use for resistance management.
- Can be applied alone or in a programme.
- No phytotoxicity has been seen.
- No residues.
- Usable in organic systems.

Figures 1 to 4 below demonstrate activity.

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Figure 1: ROMEO on strawberry against Botrytis.

Percent incidence of *Botrytis* on strawberry flowers and fruits. Greenhouse trial Italy. Natural infection of *Botrytis.* 4 foliar applications applied at 10 day intervals.

Treatments:

First bars: Flowers.
Second bars: Fruits on plant.
Third bars: Fruits in store.
Blue bars: - untreated control, Orange bars: - fenhexamid, Grey bars ROMEO 0.5 kg/ha, Yellow bars ROMEO 0.75 kg/ha.

Good efficacy on flowers and fruits (64-80% efficacy). Dose effect 0.75 kg > 0.5 kg /ha. Statistically similar to fenhexamid reference product. *Agrauxine SA).*

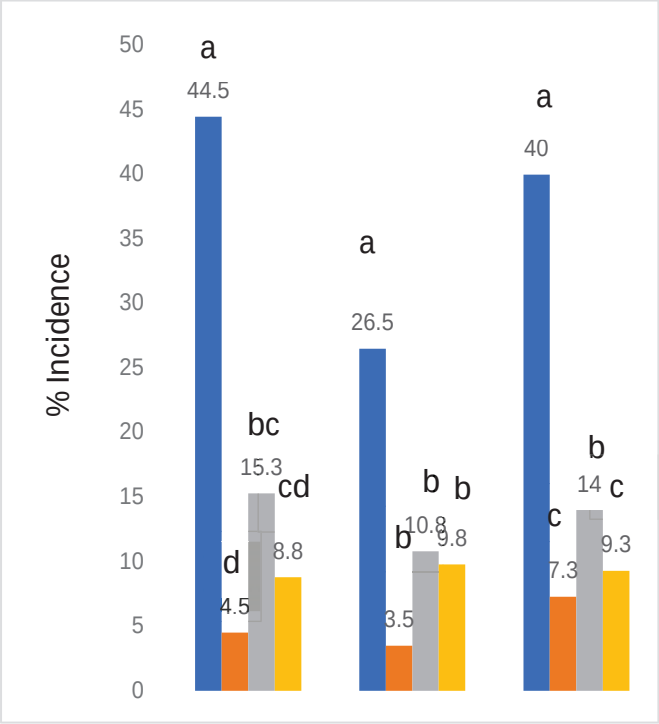


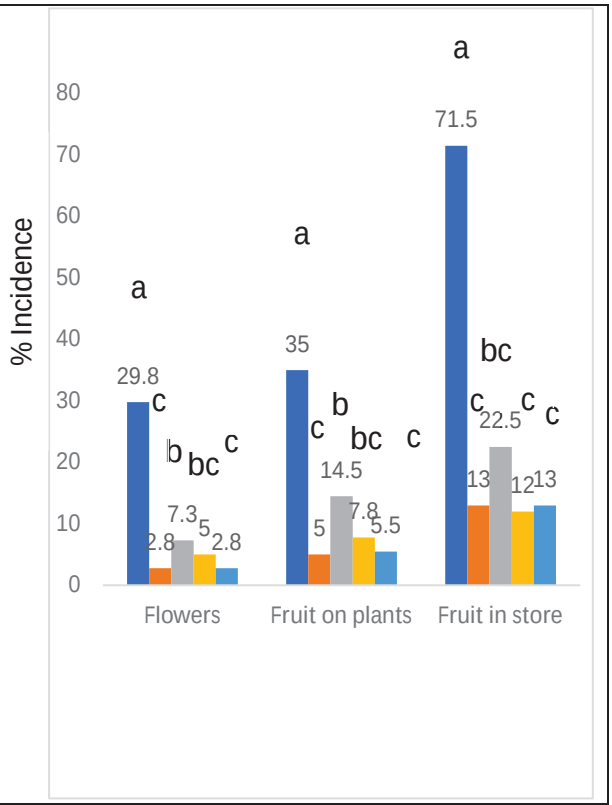
Figure 2: ROMEO on tomato against Botrytis.

Greenhouse trial Italy. 5 foliar applications 10/14 day intervals. Natural infection. Comparison with iprodione as a chemical reference.

Treatments:

First bars: *Botrytis* - on flowers.
Second bars: *Botrytis* - fruit on plant.
Third bar: *Botrytis* - fruit in store.
Blue bars: untreated control.
Orange bars: iprodione.
Grey bars: ROMEO at 0.25 kg/ha.
Yellow bars: ROMEO at 0.5 kg/ha.
Pale blue bar: ROMEO at 0.75 kg/ha.

Good intrinsic efficacy on flower and fruits around 82-91% efficacy. Dose effect 0.75 kg/ha better than 0.5kg/ha better than 0.25 kg/ha. Similar to iprodione reference. (*Agrauxine SA*).



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Figure 3: ROMEO on cucumber against powdery mildew. Comparing efficacy with sulphur. Alternated or tank mix with sulphur fungicide.

Greenhouse trials Italy. Comparison with tank mix and alternation.
First bars: tank mix treatment.
Blue bar: untreated control.
Orange bar: sulphur at 0.5 kg/100 L.
Third bar: ROMEO 0.5 kg/ha plus sulphur at 0.5 kg/100l.
Second bars: Alternated treatment.
Blue bar: untreated control.
Orange bar: sulphur at 2 kg/ha (treatment B & D only).
Gray bar: ROMEO 0.5 kg/ha (treatment A, C & E) alternated with sulphur fungicide 2 kg/ha (treatment B & D).
Good efficacy in tank-mix and alternation with sulphur.
(*Agrauxine SA*).

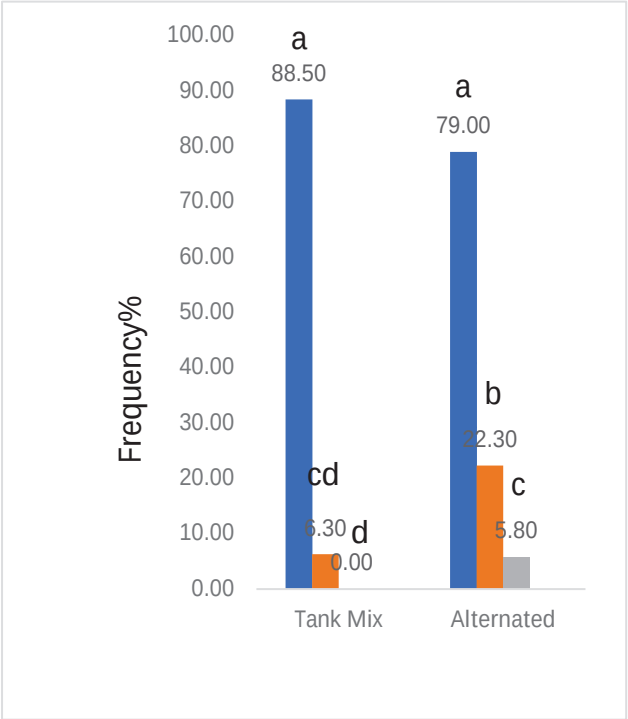
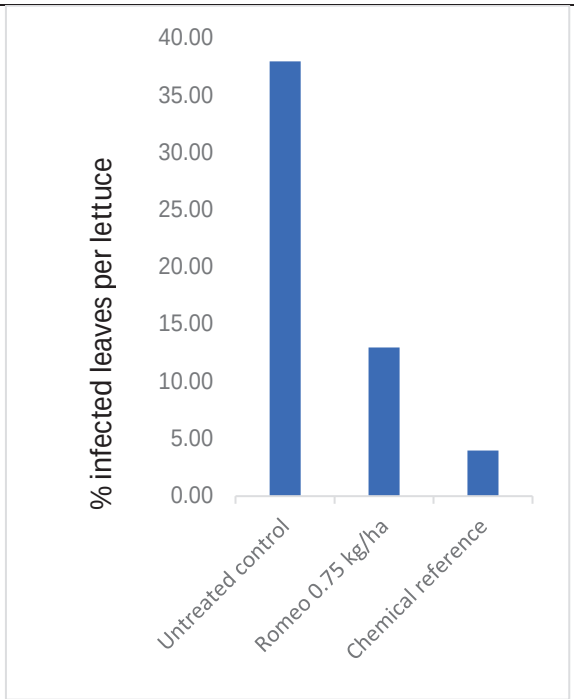


Figure 4: ROMEO on lettuce against downy mildew.

Greenhouse trials. 4 -7 foliar applications every 7-14 days at 400 – 1000 l/ha. Based on 9 trials in France, Italy, Spain and Denmark, 2010-2015.
High disease pressure. 37% leaves infected per lettuce in the control.
Treatments:
First bar: Untreated control.
Second bar: ROMEO at 0.75kg/ha.
Third bar: Chemical reference programme.
ROMEO gave 69% efficacy, slightly lower than the chemical reference programme (85%).
(Agrauxine SA).



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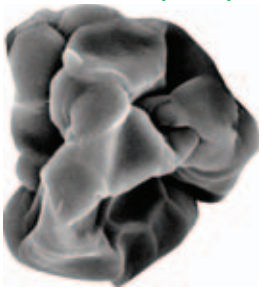
WHAT DISEASES DOES ROMEO CONTROL?

ROMEO has been shown to have activity against powdery mildew, downy mildew and *Botrytis*. Activity has been reported in a limited trial against *Rhizoctonia* on lettuce.

HISTORY AND DEVELOPMENT

ROMEO contains the first microbial fraction activating the physical and biochemical defence mechanisms. The active Cerevisane® was patented in 2006 by Agruaxine Lessaffre Plant Care and the active ingredient approved in the EU on 23/04/2015. It is classified as a low risk substance. On 18/01/2016 Cerevisane was added to annex IV of EC 396 /2005, so no maximum residue limits (MRL)for Cerevisane are required. The product has been developed with over 300 trials and approved in parts of EU for various crops under the development code ALD1901.

Figure 5: Cerevisane microscopic structure. (SEM)



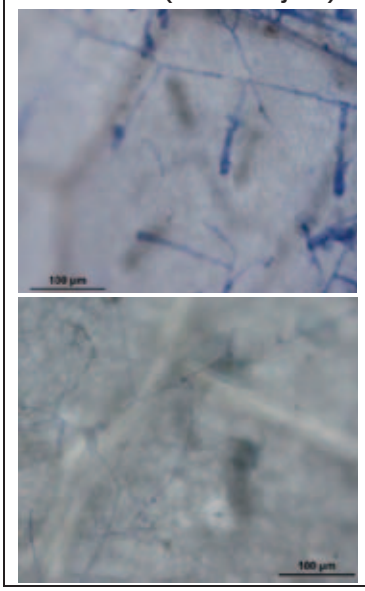
At the time of writing ROMEO is approved in France, Italy, Belgium, Holland, Germany, parts of the USA and the UK. Approval is pending in a number of other European countries.

HOW DOES ROMEO WORK?

ROMEO acts as a host plant defence inducer and has no direct effect on plant pathogens.

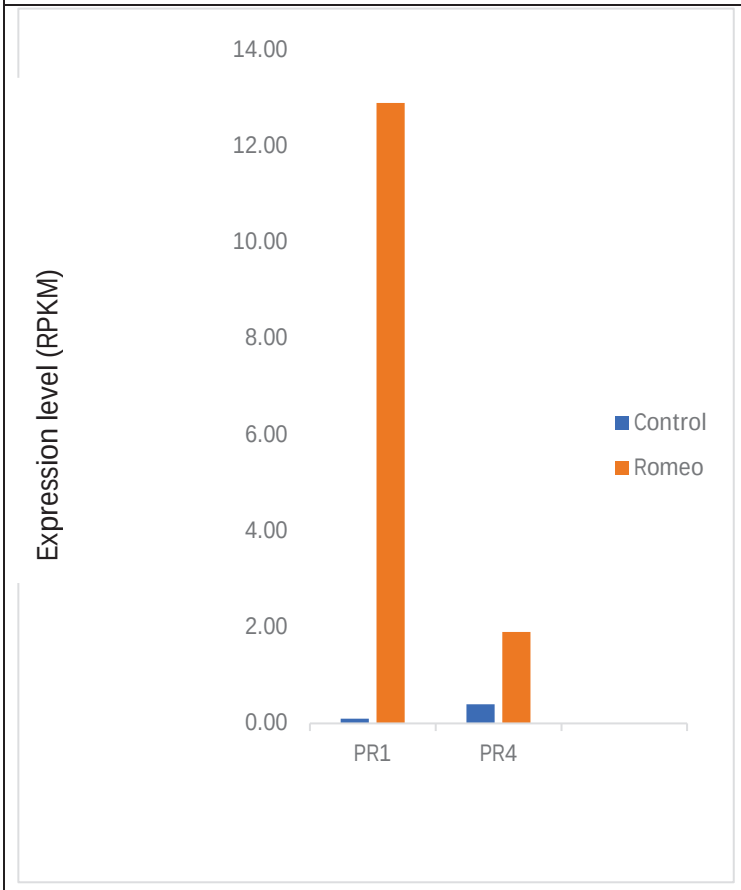
- The active mimics a microbial attack on the plant.
- It activates the cell signalling cascade, inducing many defence genes including those dependent on the salicylic acid protection mechanism, (powdery mildews, downy mildews) and the defence mechanism dependant on jasmonic acid and ethylene protection mechanism against necrotrophic pathogens (kill host cell and live on the contents) eg *Botrytis.*
- Metabolic changes in the plant result in the stimulation of phytoalexins (antimicrobial compounds) strengthening cell walls and leaf cuticles.
- Efficacy lasts for 7-10 days and is typically effective 1 day after application.

Figure 6: Effect of ROMEO on internal colonisation of powdery mildew. Top -untreated, bottom - treated. (INRA Dijon)



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Figure 7: Induction of defence genes. Induction of PR1 and PR4 marker genes.



Gene expression level RPKM of PR1 and PR4 marker genes 1 day after application of ROMEO.

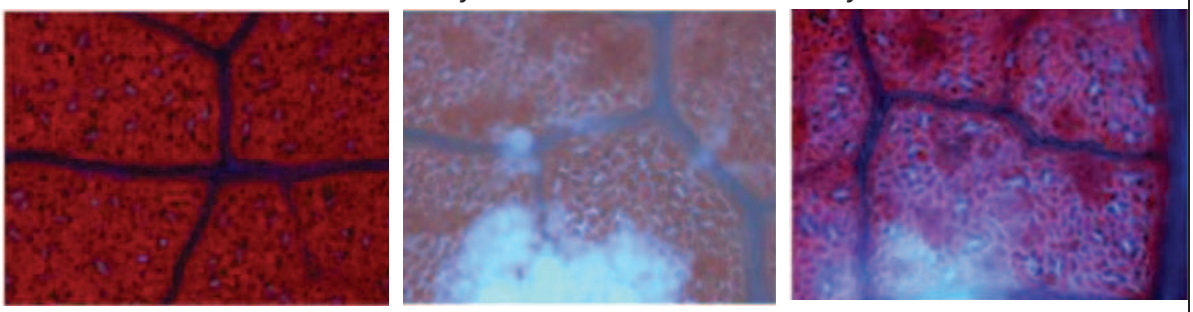
PR1 is a marker gene of the salicylic acid (SA) defence pathway against biotrophic pathogens eg Powdery mildew.

PR4 is a maker gene of the jasmonic acid and ethylene (JA/ET) pathway against necrotrophic pathogens) eg Botrytis.

Source: University Bari (It)

FIgure 8: Stimulation of phytoalexins (fluorescent antimicrobial compounds).

Untreated control | 3 days after treatment | 7 days after treatment



Source: INRA (Dijon)

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ON WHAT CROPS AND IN WHAT SITUATIONS CAN ROMEO BE USED?

In the UK, at the time of writing ROMEO is approved for use on permanently protected crops with full enclosure of: Strawberry, lettuce, tomato, aubergine, cucumber, melon watermelon, winter squash and pumpkin, courgette and summer squash, gherkin and on outdoor table grapes and wine grapes. Use on a number of other crops is allowed in the UK by way of Extension of Authorisations for Minor Uses (EAMU*s). For the crop list see the following table. The EAMUs allow a maximum rate of 0.75 kg/ha and are for the control of botrytis, downy mildew and powdery mildew.

CROP LIST

Table 1: Crop list.

Crop	Label or EAMU*	Situation	Rate kg/ha	Disease on label
Aubergine	On label	permanent protection with full enclosure	0.5	botrytis
Baby leaf crops	1443 of 2021	permanent protection with full enclosure and outdoor		
Basil	1443 of 2021	permanent protection with full enclosure and outdoor		
Bay	1443 of 2021	permanent protection with full enclosure and outdoor		
Bilberry	1449 of 2021	protected and outdoor		
Blackberry	1449 of 2021	protected and outdoor		
Blackcurrant	1449 of 2021	protected and outdoor		
Blueberry	1449 of 2021	protected and outdoor		
Chives	1443 of 2021	permanent protection with full enclosure and outdoor		
Coriander leaves	1443 of 2021	permanent protection with full enclosure and outdoor		
Courgette	On label	permanent protection with full enclosure	0.5	powdery mildew
Cranberry	1449 of 2021	protected and outdoor		
Cress	1443 of 2021	permanent protection with full enclosure and outdoor		
Cucumber	On label	permanent protection with full enclosure	0.5	powdery mildew
Endive	1443 of 2021	permanent protection with full enclosure and outdoor		
Gherkin	On label	permanent protection with full enclosure	0.5	powdery mildew
Gooseberry	1449 of 2021	protected and outdoor		
Herbs (others)	1443 of 2021	permanent protection with full enclosure and outdoor		
Lamb's lettuce	1443 of 2021	permanent protection with full enclosure and outdoor		

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Lettuce	1443 of 2021	permanent protection with full enclosure and outdoor		
Lettuce	On label	permanent protection with full enclosure	0.75	downy mildew
Loganberry	1449 of 2021	protected and outdoor		
Melon	On label	permanent protection with full enclosure	0.5	powdery mildew
Mint	1443 of 2021	permanent protection with full enclosure and outdoor		
Oregano	1443 of 2021	permanent protection with full enclosure and outdoor		
Ornamental plant production	1455 of 2021	permanent protection with full enclosure, protected and outdoor.		
Parsley	1443 of 2021	permanent protection with full enclosure and outdoor		
Pumpkin	On label	permanent protection with full enclosure	0.5	powdery mildew
Raspberry	1449 of 2021	protected and outdoor		
Redcurrant	1449 of 2021	protected and outdoor		
Rocket	1443 of 2021	permanent protection with full enclosure and outdoor		
Rosemary	1443 of 2021	permanent protection with full enclosure and outdoor		
Rubus hybrids	1449 of 2021	protected and outdoor		
Sage	1443 of 2021	permanent protection with full enclosure and outdoor		
Spinach	1443 of 2021	permanent protection with full enclosure and outdoor		
Strawberry	1449 of 2021	protected and outdoor		
Strawberry	On label	permanent protection with full enclosure	0.75	botrytis
Summer Squash	On label	permanent protection with full enclosure	0.5	powdery mildew
Table Grapes	On label	outdoor	0.25	botrytis, powdery mildew, downy mildew
Tarragon	1443 of 2021	permanent protection with full enclosure and outdoor		
Thyme	1443 of 2021	permanent protection with full enclosure and outdoor		

Continued on page 10

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Tomato	On label	permanent protection with full enclosure	0.5	botrytis
Watermelon	On label	permanent protection with full enclosure	0.5	powdery mildew
Wine Grapes	On label	outdoor	0.25	botrytis, powdery mildew, downy mildew
Winter Squash	On label	permanent protection with full enclosure	0.5	powdery mildew

**These extensions of authorised use (EAMUs) provide for the use of ROMEO in respect of crops and situations, other than those included on the product label. No efficacy or phytotoxcity data have been assessed and as such the 'extension of use' is at all times done at the user's choosing, and the commercial risk is entirely theirs.*
Before use of any pesticide under an extension of authorisation, users must have a copy of the document, which is available for the UK from the HSE/CRD EAMU database. Documents are frequently revised and therefore it is important to check that the documentation is current and correct before any use.

APPLICATION MIXING AND SPRAYING

WHEN SHOULD ROMEO BE APPLIED?

ROMEO acts preventatively and prepares the plant to defend itself against fungal attack. ROMEO must be applied before the risk of disease appearance.

Application should be made every 7-10 days ensuring a minimum interval of 7 days is maintained and the maximum number of applications of 8 treatments per year is not exceeded (10 for table grapes and wine grapes).

The first application may be made at BBCH 12, two true leaf stage with leaves unfolded to BBCH 89, beginning of fruit abscission stage).

In case of disease development or under high disease pressure ROMEO should be used with other fungicides either by tank mix or alternation.

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HOW BEST TO APPLY ROMEO?

What is the recommended water volume for dilution?

Use sufficient water to ensure uniform coverage of the crop, without causing run-off. The label advises 100 to 1000 litres per hectare.

ADJUVANTS:

Adjuvants are not necessary for ROMEO and do not increase efficacy.

APPLICATION WITH ULV EQUIPMENT

At the time of writing ULV application has not been tried on UK crops.

IS THERE A LIMIT ON THE ACIDITY OR ALKALINITY OF THE SPRAY SOLUTION?

No problems have been reported.

IS THERE ANY BENEFIT FROM PRE-MIXING?

There is no requirement to pre-mix in water. Agitation in the spray tank is required.

WHAT TREATMENT INTERVAL IS RECOMMENDED?

Apply every 7 to 10 days with at least two successive treatments.
A maximum of 8 treatments may be applied to most crops but for wine grapes and table grapes a maximum of 10 treatments may be made.

HARVEST INTERVAL

A pre-harvest interval of 1 day is specified under the restrictions section of the label.

ARE THERE ANY ISSUES OF PLANT PHYTOTOXICITY?

No phytotoxicity has been observed in any of the trials.

IS IT POSSIBLE TO OVER INDUCE THE PLANTS DEFENCE?

There is a maximum point of plant stimulation beyond which the plant fails to respond any more.

COMPATIBILITIES

TANK MIXING OR POSITION IN CROP MANAGEMENT PROGRAMME

The label states that ROMEO is compatible with most plant protection products (fungicides, insecticides) and fertilisers. In case of new mixtures, please initially test physical compatibility in a small container. It is also not possible to guarantee the biological and physical compatibility of all spray mixtures.

Table 2, below, lists some experience generated outside the UK. Some of the products listed may not be approved in the UK on the labelled crops. The formulations listed appear to be the same as the UK product listed but this may not be the case.

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Users are advised to test a small sample first to check physical compatibility and then test treat a small trial area. Liability may cannot be accepted for any issues with regard to tank mixing.

Table 2: Tank mixing		
Active	**UK Example**	**Key**
Fungicides		
Ametoctradin + dimethomorph	Percos	Y
Amisulbrom	Shrinkon	Y
Ampelomyces quisqualis	AQ 10	N
Azoxystrobin	Amistar	Y
Bacillus amyloliquefaciens subsp. plantarum strain D747	Amylo-X WG	Y
Bacillus subtilis strain QST713	Serenade (not UK formulation)	
Boscalid	Filan	Y
Boscalid + pyraclostrobin	Signum	Y
Bupirimate	Nimrod	Y
Chlorothalonil	Bravo	Y
Cyflufenamid	Cyflamid (no data Takumi)	Y
Difenoconazole	Plover	Y
Fenhexamid	Teldor	Y
Fenpyrazamine	Prolectus	Y
Fludioxonil + cyprodinil	Switch	Y
Fluopyram+trifloxystrobin	Luna sensation	Y
Flupicolid+propamocarb	Infinito	Y
Fosetyl aluminium + propamocarb	Previcur Energy	Y
Kresoxim methyl	Stroby	Y
Mancozeb	Dithane NT WDG	Y
Meptyldinocap	Kindred	Y
Metalaxyl plus mancozeb	Fubol Gold	Y
Metrafenone	Vivando	Y
Penconazole	Topas	Y
Potassium bicarbonate	Karma / commodity substance	N
Proquinazid	Talius / Justice	Y
Pyrimethanil	Scala	Y
Sulphur	Kumulus DF	Y
Sulphur	Microthiol Special	Y
Trifloxystrobin	Flint (no data on Swift)	Y
Insecticides:		
Bacillus thuringiensis ssp kurstaki	Dipel DF	Y
Esfenvalerate	Sumi-Alpha	Y
Adjuvants:		
Silwet L77		Y
Nu-Film P		Y

Y: ROMEO has been successfully tank mixed with ROMEO added first.
N: Problems in tank mixing have been reported, be careful.

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COMPATIBILITY WITH OTHER COMPONENTS OF A CROP PRODUCTION PROGRAMME OR WITH OTHER BENEFICIAL FUNGI OR ORGANISMS?

In efficacy trials to date no deleterious effect has been observed on insects or mites.

IS ROMEO USABLE IN ORGANIC SYSTEMS?

ROMEO is used in organic growing systems according to EU 889/2008. It is suggested that certification body approval is obtained before use.

WHAT IS THE SHELF LIFE AND WHAT ARE THE RECOMMENDED STORAGE CONDITIONS?

ROMEO has a shelf life of 2-years from manufacture when stored in a cool dry location. Do not freeze.

HOW IS ROMEO PACKAGED?

ROMEO is packed in 1 kg plastic containers in outers of 8.

This product is to be used only in accordance with the recommendations and instructions given on the labels provided with the pack. Use in any other circumstances is entirely at the user's own risk.

APPROVAL HOLDER AND MARKETING COMPANY

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49070 Beaucouze France
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All other registered trademarks acknowledged.

Marketed in the UK by Fargro Ltd. Pack Size: 1 kg (outer 8).
Safety data sheet available on request.
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