

## Regulatory information

### Ampelomyces quisqualis - AQ 10

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*Ampelomyces quisqualis* isolate M-10 is a naturally occurring hyperparasite of powdery mildews. This parasitism reduces growth and may eventually kill the mildew colony. The mycoparasite is not restricted to powdery mildews. *In vitro* work indicates that it can be parasitic on *Botrytis cinerea* Pers. Ex. Fr., *Alternaria solani* (Ell. & Mart.) Sor., *Colletotrichum coccodes* (Wallr.) Hughes, and *Cladosporium cucumerinum* Ell. & Arth (Jarvis & Slingsby, 1977). There is no information indicating that *A. quisqualis* shows infectivity or pathogenicity to any organisms beyond this relatively narrow taxonomic range of fungal pathogens. Indeed, its life history strategy as a hyperparasite, would tend to preclude a wide host range.

It infects and forms pycnidia (fruiting bodies) within powdery mildew hyphae, conidiophores (specialised spore-producing hyphae) and cleistothecia (closed fruiting bodies of powdery mildews). Having penetrated into the mildew hyphae, the fungus produces pycnidia, in which form the pathogen can survive adverse periods e.g. winter, in and around the host plants of the mildew fungi. The pycnidia produce spores, which require favourable conditions for successful germination e.g. in terms of temperature, moisture and in particular the presence of the appropriate host. Overwintering pycnidia can also be produced saprophytically in vascular plants e.g. in mildewed clover leaves and in cucumber leaves (Yarwood, 1939). The infectivity of the spores produced by the pycnidia rapidly diminishes under field conditions (e.g. 24 to 48 hours), although this can be extended under appropriate conditions.

Sundheim and Krekling (1982) demonstrated that *A. quisqualis* produced specialised appressorium-like penetration structures on powdery mildew. Penetration of the host cell was probably due to mechanical and enzymatic processes. Furthermore, enzymatic digestion played a major role in the invasion of the host cell and the destruction of cytoplasm. Invading hyphae penetrated the host cells through the septal pores of the host. Beuther *et al* (1981) studied the effects of extracts from the hyperparasite on growth, sporulation and conidial germination of the host. They found no evidence of toxin production.

In terms of long-term exposure, the spores are unlikely to remain viable for long periods of time. Thus, for successful germination the spores need both favourable conditions (e.g. high humidity or moisture, temperature around 25°C) and the presence of the host. Without the host, viability is rapidly lost e.g. within a few days. While the spores can survive for longer under appropriate conditions (low humidity or lack of moisture and low temperatures), these are unlikely to be the prevailing conditions at the time of application or at least for any prolonged period. However, the *A. quisqualis* pycnidia, which will be produced from the infected mildew, are more resilient and may persist in the environment for relatively long periods (at least into the next season). These in turn, may give rise to viable spores when conditions become favourable again.

The toxicological profile of *A. quisqualis* is environmentally sound. The product shows no adverse effect on humans with no pathogenic or infective effects (acute or chronic) in the different routes of application (oral, dermal, inhalation), tested through experimental studies. No irritating or sensitizer effect on the tested animals and no genotoxicity or mutagenicity activity observed in vitro tests.

Regarding the effect on non target organism, *A. quisqualis* shows very low toxicity to birds. No signs of infectivity or pathogenicity were observed in either of the two species tested.

The generated information indicates that *A. quisqualis* is of low toxicity to non-target organisms, aquatic organisms (fish and aquatic invertebrates) and honey bees. The results of the studies also showed no signs of any infectivity or pathogenicity to the organisms tested.

These findings are consistent with the known mode of action of this fungal parasite, invading the mildew hyphae and penetrating the host cell walls, probably by enzymatic action, resulting in slow death of the host. There is no evidence of any toxin production, which could in turn have effects on other, non-target organisms. In addition, these findings are consistent with the life history strategy of a hyperparasite, having a relatively narrow host range, which in this case is restricted to powdery mildews as well as some other fungal species. On this basis, it would be unlikely to find infectivity or pathogenicity extending to other, non-host groups particularly those that are taxonomically unrelated e.g. birds, mammals, fish, aquatic invertebrates, algae, non-target arthropods and earthworms.

One final consideration that needs to be taken into account, is that *A. quisqualis* is a naturally occurring organism that has been reported from most parts of the world. It is therefore likely that it is present at endemic levels wherever its hosts (the powdery mildews etc) are found with reasonable abundance/persistence. The only difference that the application of AQ-10 is likely to make to this situation, is to the pattern of exposure i.e. short-term increases in the levels of exposure. Taking this into account, suggests that it is even less likely that there will be any significant risk to non-target organisms.

AQ-10, is a water dispersible granule formulation containing the fungal hyperparasite *A. quisqualis* isolate M-10 as the active ingredient (58% viable spores and 42% inert co-formulants). The minimum viable spore content guaranteed is  $5.0 \times 10^9$  / gram of product.

AQ-10 should be applied 2 to 12 times a season at intervals of 7 to 14 days, depending on disease pressure. Earliest application is during shoot emergence, which depending on the region varies from March to April. It is applied at a rate of 35 - 60 g/ha in an adequate volume of water sufficient to have an good coverage of the vegetation. The optimum germination temperature is 25°C and above 30°C germination decreases and eventually stops at 37°C.

The advantage of using AQ10 is multiple. Firstly, the original mode of action, different from any other fungicide, reduced the risks of selection of resistant strains, mostly appearing with sterol inhibitors and strobilurins products. Another important factor is related to the fact that the product shows a higher activity than sulphur at lower temperatures, never determining phytotoxicity on the vegetation.

On extensive field trials, AQ10 demonstrate also an activity against cleistothecia, over-wintering stage of the powdery mildew. Due to the absence of residue, National Authorities did not set any maximum level of residue (MLR) and consequently did not apply a post harvest interval (waiting period). Another relevant aspect is the no interference on the making and quality of musts and wines.

For effective powder mildew control AQ10 can be applied alone, but it can also be included in IPM strategies, which provide for applications of both conventional agrochemicals and the bio control agents. AQ10 can be applied with any conventional spray equipment on grapes, strawberry, cucurbits, tomatoes, pepper and roses at low powdery mildew infestation levels.