

Luis F. Roca, Juan Moral, José R. Viruega,
Arantxa Ávila, Rodrigues Oliveira and Antonio Trapero.

Department of Agronomy,
ETSIAM, University of Córdoba, Córdoba, Spain.
E-mail: lfroca@terra.es

Summary

Copper compounds are effective in controlling major fungal foliar and fruit diseases of olive, mainly olive leaf spot or peacock spot caused by *Spilocaea oleagina*, anthracnose incited by *Colletotrichum spp.*, cercosporiose due to *Pseudocercospora cladosporioides*, and olive knot, caused by the bacterium *Pseudomonas savastanoi pv. savastanoi*. However, their effectiveness depends a great deal on the specific disease, time of application, and persistence on olive leaves and fruits. The commercial formulations of copper fungicides markedly influence their persistence, but the type of copper salt has no influence on persistence. The negative effects of copper fungicides are being evaluated in olive orchards, although no phytotoxic effects have been observed on leaves and flowers. Some copper fungicides may induce systemic acquired resistance (SAR) in olive trees and experiments are being carried out to study this phenomenon and its use in an integrated disease management system.

Introduction

Copper fungicides are used extensively to control fungal diseases affecting olive trees. The most important foliar and fruit diseases controlled by copper fungicides are olive leaf spot or peacock spot caused by *Spilocaea oleagina*, anthracnose incited by *Colletotrichum spp.*, and cercosporiose due to *Pseudocercospora cladosporioides*. Those three diseases may cause heavy defoliation which results in reduced tree vigor, decreased yields and lower quality of oil. Peacock spot is the best known and most important disease of this complex in southern Spain, although in some olive cultivars, such as 'Hojiblanca' or 'Picudo', anthracnose is of the greatest importance. Copper products also are recommended to control olive knot, caused by the bacterium, *Pseudomonas savastanoi pv. savastanoi*. This disease may cause severe infections on trees in some olive orchards (Trapero and Blanco, 2004).

Field diagnosis of these diseases is easy due to their characteristic symptoms which develop under environmental conditions that are favourable for disease development. Under unfavourable conditions, infections remain latent and symptoms or signs of the disease are not expressed for long periods of time, which make early detection of these diseases very important. Latent infections of olive leaf spot can be detected by the NaOH method, which consists of dipping the leaves in a 5% NaOH solution for 20-30 minutes. *Spilocaea oleagina* infections appear as circular, brown to black spots on the leaves. There are no easy and reliable methods to detect the other diseases mentioned above, although molecular techniques that are under investigation could change this situation in the near future (González et al., 2002; Martín et

al., 2002; Bertolini et al., 2003; Ávila, 2005).

An integrated control program for these diseases has several common aspects, which relate to the importance of high humidity that is required for infection, multiplication and dissemination of the pathogens (Trapero and Roca, 2004). These include avoiding humid sites and dense plantings of trees, and pruning trees to improve ventilation. High nitrogen fertilization and potassium deficiency seem to increase the susceptibility of trees to olive leaf spot (Bohórquez, 1997). Early harvest and the removal of diseased leaves and rotted fruits are measures recommended to control anthracnose. Branches showing olive knot tumors must be removed in order to decrease the amount of inoculum. The use of resistant cultivars is a desirable practice in the sites where these diseases are endemic and/or conditions are favourable for infection and disease development.

Fungal diseases of olive are controlled by applying protective fungicides, mainly copper-containing products, because of their low price, compatibility with other products, and efficacy. Copper products are authorized in organic agriculture, according to European Union regulation (R(CEE) 2092/1991). Although these products have been used in olive orchards since their discovery by Millardet in 1885, information about their efficacy in controlling the major fruit and foliar diseases or their secondary effects on olive trees is limited. For this reason, the Plant Pathology Group of the University of Córdoba initiated a research program in 1997 to study these problems. Results of these studies regarding time of fungicide application, efficacy against different foliar and fruit pathogens, control of infection, persistence and secondary effects on host tissues, and induced resistance in the plant are presented below.

Time of application

Due to the protective character of these chemical treatments, the timing of application is crucial. The number of treatments and the time of applications vary depending on the persistence of the fungicides, the disease, environmental conditions, susceptibility of the cultivar, and level of infection (Trapero and Blanco, 2004). A disease forecasting system is being developed for these foliar pathogens (Aldebis and Trapero, 2002). However, early fall and late winter spray treatments are recommended, which coincide with the main periods of infection of *S. oleagina* (Alvarado and Benito, 1975). Fall treatments also are useful in protecting the fruits against infection by *Colletotrichum spp.*, although several spray treatments may be required, particularly when falls are wet and cultivars are late maturing. The treatments used to control olive leaf spot are also important in controlling *P. cladosporioides*, but these treatments may fail when environmental conditions are favourable for disease development (Tjamos et al., 1993). The fall and winter treatments are also recommended for control of olive knot (Teviotdale and Krueger, 2004).

At least one application of a copper fungicide is recommended during the spring, particularly if the weather is cool and wet, in order to protect the young leaves which are more susceptible to infection by *S. oleagina*. Spring infections remain latent until fall, when they become a main source of inoculum for the fall-winter infections (Viruega and Trapero, 1999; Trapero and Roca, 2004). The spring treatment would also be useful against early infections of young fruits by *Colletotrichum spp.*, and young leaves to infection by *P. cladosporioides* and *P.s. savastanoi*.

Efficacy against the pathogens

Copper compounds inhibit the germination of fungal spores (Campillo, 1998). This effect depends on the concentration of the copper ion Cu^{2+} . Differences observed among products at the same concentration of copper in the inhibition of conidia are due to differences in the solubility of the copper compounds (Sánchez, 1999). Sensitivity of the olive pathogens to copper varies greatly, depending on the product and the pathogen. Thus, great differences have been observed in the IC50 (copper dose that inhibits germination of 50% of the conidia) of *S. oleagina*, ranging from 1 to 100 ppm, although usually below 30 ppm. IC50 values are notably lower for *P. cladosporioides* (0.1-1 ppm) and *Colletotrichum spp.* (9-22 ppm). Given the high toxicity of the copper ion, it is rare to encounter fungal strains that are resistant to copper products. Nevertheless, copper resistant strains of *P.s. savastanoi* have been found (Teviotdale and Krueger, 1998).

An additional effect of copper fungicides may be the eradication or reduction of inoculum that develops in lesions. This effect has been evaluated in foliar lesions caused by *S. oleagina* under controlled conditions. Foliar treatments with copper fungicides reduced germination of conidia formed in the lesions by 95% (Viruega et al., 2002). Under field conditions, a significant reduction in the populations of *P.s. savastanoi* in diseased olive tissues treated with copper fungicides has been observed (Quesada et al., 2004).

Efficacy against the infection of olive trees

Evaluation of the efficacy of copper products against the four olive diseases has been tested under field conditions where constraints related to the time of application, amount of inoculum present and disease severity are encountered. Methods of evaluating the efficacy of copper fungicides under controlled conditions have been developed for *S. oleagina* (López Doncel et al., 2000) and *P.s. savastanoi* (Teviotdale and Krueger, 2004) which eliminate the constraints mentioned above. This methodology has shown a consistent protective effect of copper compounds against both pathogens, without significant differences among products at the same dosage rate of copper (Viruega et al., 2002; Teviotdale and Krueger, 2004). The methodology of evaluation under controlled conditions is being developed for anthracnose caused by *Colletotrichum spp.* (Trapero and Roca, 2004).

Artificial inoculation of detached leaves and olive plants with *S. oleagina* has established a relationship between the amount of copper on the leaves and the incidence of the disease (Marchal, 2002; Roca et al., 2006) which could be useful to determine the necessity of copper applications in olive orchards. A post-infection effect of copper products has been observed up to 7 days after inoculation which is probably related to the penetration of copper into the cuticle of the leaves where the pathogen lives, or to the induction of systemic resistance against the pathogen.

Persistence on the leaves

Besides the inherent inhibitory effect of Cu^{2+} , the efficacy of copper products under field conditions depends on their retention on the leaf surface when it rains. Few studies have been conducted on the effect of rain on copper retention in the field. Soriano and Porrás (1994) developed a method based on the reaction between rubeanic acid and copper which produces a black precipitate. However,

the intensity of this reaction appears to be related to the solubility of the copper compound more than to the dose of copper (Marchal et al., 2003). Currently, persistence is evaluated by dipping leaves in 0.1N hydrochloric acid and analyzing the copper concentration in the resultant solution by atomic absorption spectrophotometry. Using this technique on copper-sprayed leaves from olive trees and olive cuttings, significant differences have been observed among products. These differences were not related to the kind of copper-salt (hydroxide, oxide, oxychloride, sulphate) or rate of application. They were related to the commercial product, indicating that adjuvants are an important part of the efficacy of the fungicide under field conditions.

Secondary effects

The copper ion Cu^{2+} is toxic to plant cells and this is the basis for the use of relatively insoluble or fixed copper fungicides that release very low levels of Cu^{2+} which are adequate for fungicidal activity, but not high enough to adversely affect the host plant cells (Campillo, 1998). In olive trees, copper induces a selective leaf drop of diseased leaves which reduces the availability of inoculum (Bonifacio, 1964; Ramos, 1973). When diseased olive plants were sprayed with pure copper sulphate and commercial copper products, copper sulphate showed a significant selective defoliant effect (Roca et al., 2006). A copper application is recommended during the spring when young leaves are most susceptible to infection by *S. oleagina*, and this may coincide with flowering. Studies carried out on olive trees and olive cuttings sprayed with copper products during the flowering period have not shown any adverse effect on the number of viable flowers or on the number of fruits per shoot (Roca et al., 2006).

The use of copper products has increased the total copper concentration in vineyard soils (Pietrzak and McPhail, 2004), although no studies have been carried out in olive orchards. Copper has a harmful effect on microbial populations in soil (Merrington et al., 2002). However, worms are able to avoid copper-contaminated soils (Van Zwieten et al., 2004). In aquatic environments, copper has a notable effect on plankton and indirectly on the whole ecosystem (De Oliveira-Filho et al., 2004). Copper is considered a low oral toxicity element for humans (Campillo, 1998).

Induced resistance

To combat invasion by microbial pathogens and insects, plants can activate a systemic response that establishes an enhanced defensive capacity in tissues distant from the site of primary attack. This systemically induced response is called systemic acquired resistance (SAR) (Corné et al., 2004). Several organic or inorganic chemical substances are able to induce this kind of response in plants and some of them are commercially available and used against plant pathogens (Kessman et al., 1994; Hammerschmidt and Kuc, 1995; Kuc, 2001). Copper fungicides and other chemical substances have been shown to induce SAR in olive plants inoculated with *S. oleagina* (Roca et al., 2006). The protective effect shown by some antagonistic microbes isolated from olive leaves could be related to SAR (Viruega et al., 2002; Segura, 2003). Defence genes involved in the response against infection by *S. oleagina* and which respond to some inducer molecules have been identified (Benítez et al., 2005). Experiments using olive plants are being carried out to investigate this phenomenon and its use in an integrated disease management system. ■

REFERENCES

- Aldebis, H.K. and Trapero, A. 2002 *Influencia de la variedad y técnicas de cultivo sobre las principales enfermedades del olivar*. Jornadas de Investigación y Transferencia de Tecnología al Sector Oleícola. 20-21 noviembre, Córdoba.
- Alvarado, M. and Benito, J. 1975 *Consideraciones sobre el momento de lucha contra el Repilo del olivo (Cycloconium oleaginum Cast.) en la provincia de Sevilla (España)*. II Seminario Oleícola Internacional, Córdoba. 12 pp.
- Avila, A. 2005 *Etiología y Epidemiología de Pseudocercospora cladosporioides, agente del Emplomado del olivo*. Tesis doctoral. ETSIAM Univ. de Córdoba.
- Benítez, Y., Botella, M.A., Trapero, A., Alsalimiya, M., Caballero, J.L., Dorado, G. and Muñoz-Blanco, J. 2005 *Molecular analysis of the interaction between Olea europaea and the biotrophic fungus Spilocaea oleagina*. Mol. Plant Pathol. 6: 425-438.
- Bertolini, E., Penyalver, R., García, A., Olmos, A., Quesada, J.M., Cambra, M. and López, M.M. 2003 *Highly sensitive detection of Pseudomonas savastanoi pv. savastanoi in asymptomatic olive plants by nested-PCR in a single closed tube*. J. Microbiol. Methods 52: 261-266.
- Bohórquez, J.M. 1997 *Efecto de la nutrición del olivo sobre el Repilo causado por Spilocaea oleagina*. Trabajo Profesional Fin de Carrera, ETSIAM, Universidad de Córdoba. 164 pp.
- Bonifacio, A. 1964 *Confronto tra alcuni anticrittogamici sulla azione defogliante delle foglie di olivo*. Notiz. Malta. Piante 68: 79-84.
- Campillo, R. 1998 *Los productos cúpricos en la olivicultura actual*. Phytoma España 102: 159-167.
- Corné, M.J. Pieterse and Van Loon, L.C. 2004 *NPR1: the spider in the web of induced resistance signalling pathways*. Curr. Opin. Plant Biol. 7: 456-464.
- González-Lamothe, R., Segura, R., Trapero, A., Baldón, L., Botella, M. A. and Valpuesta, V. 2002 *Phylogeny of the fungus Spilocaea oleagina, the causal agent of peacock leaf spot in olive*. FEMS Microbiology Letters, 210: 149-155.
- Hammerschmidt, R. and Kuc, J. 1995 *Induced resistance to disease in plants*. Kluwer Academic Publishers, Dordrecht, The Netherlands. 182 pp.
- Kessman, H., Staub, T., Hofman, C., Maetzke, T., Herzog, J., Ward, E., Uknesh, S. and Ryals, J. 1994 *Induction of systemic acquired resistance in plants by chemicals*. Annu. Rev. Phytopathol. 32: 439-459.
- Kuc, J. 2001. *Concepts and direction of induced systemic resistance in plants and its application*. Eur. J. Plant Pathol. 107: 7-12.
- López Doncel, L.M., Viruega, J.R., Zarco, A. and Trapero, A. 2000 *Respuesta del olivo a la inoculación con Spilocaea oleagina, agente del Repilo*. Boletín de Sanidad Vegetal 26: 349-363. Ministerio de Agricultura, Pesca y Alimentación.
- Marchal, F. 2002 *Evaluación de la persistencia de fungicidas cúpricos en hojas de olivo*. Trabajo Profesional Fin de Carrera, ETSIAM, Univ. de Córdoba. 177 pp.
- Marchal, F., Alcántara, E., Roca, L.F., Boned, J. and Trapero, A. 2003 *Evaluación de la persistencia de fungicidas cúpricos en hoja de olivo*. Vida Rural 176: 52-56.
- Martín, M.P., y García-Figueres, F. and Trapero, A. 2002 *Iniciadores específicos para detectar las especies de Colletotrichum causantes de la antracnosis de los olivos*. Bol. San. Veg. Plagas 28: 43-50.
- Merrington, G., Rogers, S.L. and Van Zwieten, L. 2002 *The potential impact of long-term copper fungicide usage on soil microbial biomass and microbial activity in avocado orchards*. Aust. J. Soil Res. 40: 749-759.
- Oliveira-Filho, E.C., de Matos, R. and Roma, F.J. 2004 *Comparative study on the susceptibility of fresh water species to copper-based pesticides*. Chemosphere 56: 369-374.
- Pietrzack, U. and McPhail, D.C. 2004 *Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia*. Geoderma 122: 151-166.
- Quesada, J.M., Peñalver, R., García, A., Salcedo, C.I., Climent, F. and López, M.M. 2004 *Evolución de las poblaciones epifitas de Pseudomonas savastanoi pv. savastanoi en olivo: efecto de tratamientos químicos de control sobre la dinámica poblacional*. XI Congreso de la Sociedad Española de Fitopatología. 14-18 octubre, Almería.
- Ramos, 1973 *Ensayos de la lucha contra el repilo del olivo en Granada. V. Acción defoliante de algunos fungicidas*. Bol. Infor. Plagas 111: 45-51.
- Roca, L.F., Zamri, A., Alsalimiya, A. and Trapero, A. 2006 *Resistencia sistémica adquirida en olivo al Repilo causado por Spilocaea oleagina*. XIII Congreso de la Sociedad Española de Fitopatología. Murcia.
- Sánchez Pacheco, N. 1999 *Evaluación in vitro de fungicidas contra Spilocaea oleagina, agente del Repilo del olivo*. Trabajo de Investigación Fin de Carrera, ETSIAM, Univ. de Córdoba, Córdoba. 151 pp.
- Segura, R. 2003 *Evaluación de microorganismos antagonistas para el control del Repilo y de la Antracnosis del olivo*. Tesis Doctoral, ETSIAM, Universidad de Córdoba. 331 pp.
- Soriano, M.L. and Porras, A. 1994 *Métodos de evaluación de la distribución y persistencia de fungicidas cúpricos en hoja de olivo*. Cuadernos de Fitopatología I: 115-121; II: 168-182.
- Teviotdale, B.L. and Krueger, B. 1998 *Olive knot control and assessment of copper resistance in Pseudomonas savastanoi*. KAC Plant Protection Quarterly 8: 1-4.
- Teviotdale, B.L. and Krueger, B. 2004 *Effects of timing of copper sprays, defoliation, rainfall, and inoculum concentration on incidence of olive knot disease*. Plant Dis. 88: 131-135.
- Tjamos, E.C., Graniti, A., Smith, I.M. and Lamberti, F., eds. 1993 *Conference on olive diseases*. EPPO Bulletin 23: 365-550.
- Trapero, A. and Blanco, M.A. 2004 *Enfermedades*. In: *El Cultivo del olivo*. Barranco, D., Fernández Escobar, R., Rallo, L., eds. Junta de Andalucía y Mundi-Prensa. Madrid, pp 517-664.
- Trapero, A. and Roca, L.F. 2004 *Bases epidemiológicas para el control integrado de los "Repilos" del olivo*. Phytoma España 164: 130-137.
- Van Zwieten, L., Rust, J., Kingston, T., Merrington, G. and Morris, S. 2004 *Influence of copper fungicide residues on occurrence of earthworms in avocado orchard soils*. Sci. Total Environ. 329: 29-41.
- Viruega, J.R. and Trapero, A. 1999 *Epidemiology of leaf spot of olive trees caused by Spilocaea oleagina in Southern Spain*. Acta Hort. 474: 531-534.
- Viruega, J.R., Trapero, A. and Morno, S. 2002 *Efficacy of Kresoxim-methyl against olive leaf spot caused by Spilocaea oleagina*. Acta Hort. 586: 801-804.