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Conference Report

50th 'Deutsche Pflanzenschutztagung', September 23-26, 1996, Münster, Germany

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GENERAL REMARKS

From 23. - 26. September 1996 the Biologische Bundesanstalt für Land- und Forstwirtschaft held the 50th 'Deutsche Pflanzenschutztagung' (German Plant Protection Conference) in Münster with more than 1000 participants. Approximately 350 presentations were classified into different specialities which were discussed in five parallel sessions. In addition, a poster session with 250 posters also included the culture and pesticide protection of the tobacco plant. The subjects treated in the various sessions were very broad. Besides the increasing importance of natural plant protection agents, modern biotechnological and genetic methods used for pest control and analysis of pest organisms in cultivars were a major discussion topic. Manufacturers of agrochemicals used the opportunity to present their newly developed products.

ORAL PRESENTATIONS

A.v. TIEDEMANN (Rostock University), who had been awarded with the Julius-Kühn-Preis for his contributions in the field of ozone damage in plants, presented a contribution entitled 'Disease predisposition by ozone - implications for agriculture and phytopathological research'. High ozone levels can cause severe cell damage to various plants. Multiple investigations which can contribute to explain biochemical mechanisms occurring in the case of ozone damage have been carried out with ozone sensitive (*Bel W 3*) and ozone resistant (*Bel B*) tobacco types. Thereby it became apparent, that ozone almost exclusively enters the plant through the stomata of the plants and generates different reactive oxygen species such as superoxide, hydrogen peroxide and OH-radicals in the cytosol. This leads to senescence induction and premature cell necrosis. In order to counter this oxidative stress, plants have developed highly efficient enzymatic and nonenzymatic protection mechanisms. Observations reveal that in the case of pest infection (e.g., *Botrytis cinerea*), the fungus must attack the plant - like ozone - via an oxidative pathway. Meanwhile attempts have been made to induce the defence and detoxification systems of the plants through moderate oxidative stress, in order to protect the plant from infection by pathogens. In the case of *Botrytis cinerea* it could be established that vines and horse beans pretreated with moderate ozone concentrations became more resistant. The decrease of susceptibility is dose dependent.
R. RINGEL (Cyanamid Agrar GmbH) presented the properties of the fungicide dimethomorph as a measure to combat fungicide pathogens in viticulture. Dimethomorph which is also discussed as a possible agent against blue mold in tobacco has systemic properties with protective, curative and antisporulant effects. The concentrations necessary range between 1-10 ppm and are very low. Thirty minutes after treatment with Forum (trade name) the fungicide cell walls enter a process of decomposition. The effect of treatment lasts approximately 14

days. The LD_{50} = 3900 mg/kg (rat, p.o.).
Gene technology methods can be used to indicate the parental grade of pathogenic fungicides. The characterization and grading of fungi by the internal transcribed spacer-restriction fragment length polymorphisme (ITS-RFLP) and random amplified polymorphic DNA (RAPD) was presented by O. HERING (Biologische Bundesanstalt für Land- und Forstwirtschaft). RAPD-examinations identified a total of 31 sample groups of which 23 species and *F. solani* with 5 specialized forms and 3 varieties were found.
J. BENDIEK (Robert-Koch-Institute, Berlin) presented details referring to the release of transgenic plants in Germany and the EU. In the European Community, a total of 31 field trials with transgenic tobacco exist. In Germany only one release occurred. A total of 42 applications for the release of transgenic plants have been permitted in Germany. The majority of field trails are made with herbicide-tolerant plant species (Germany: 39; EU: 442). In 1996, 13 field trials with transgenic plants have been prevented by opponents to genetic engineering.
K. SCHMUTZLER and H. BEESTERMÖLLER (Agr Evo GmbH) also presented their experience on trials with transgenic plants in Germany. In 1993 Agr Evo GmbH made the decision to increase the yield in cultivated plants with the help of genetic engeneering. The first attempt was the registration of glufosinate- (trade name: Basta) tolerant maize, rape and sugar beet. The glufosinate tolerance was achieved by the introduction of a phosphinotricin-N-acetyltransferase (PAT)-gene, which enables the plant to block a toxic ammonia enrichment in the tissue by acetylation of glufosinate. According to SCHMUTZLER and BEESTERMÖLLER, special measures have to be undertaken before and after the application to the Robert-Koch-Institute in Berlin for approval of field trials. These include providing information on field trials with genetically engineered plants to public authorities and the public before application is made to the Ministry and Plant Protection Agency. A treaty must be made with the farmer and information provided to the local mayor, planters association and the general public. After the application, information sessions for the public and interested circles, trial guidances and information of the local press have to be organized. Despite this careful planning, Agr Evo GmbH had to accept that 8 out of 15 trial cultures had been destroyed. It seems that the tendency for sabotage of transgenic field trials is increasing in Germany.
H. KESSMANN (CIBA-GEIGY AG) presented the molecular basics of systemically activated resistance (SAR) in plants. According to this, plants which have been infected locally with a virulent or avirulent microorganism develop a systemic, unspecific and long lasting resistance against fungi, bacteria and viruses, the so-called SAR. Salicylic acid is a central component of the natural SAR signal chain, which can lead to an accumulation of 'Pathogenesis-related-proteins' (PRP). It is known, that some PRPs play an essential role in the defence of pathogens. Meanwhile chemical activators have been discovered, e.g., isonicotinic acid (INA) or benzothiadiazol (BTH; trade name: Bion), which act as functional analogues to salicylic acid and may also cause SAR. An application of these activating substances will only begin to be fully effective after a specific time-elapse (Bion: 3 days), so that in the case of a strong pathogenic infestation the application of conventional plant protection agents is recommended. It should be emphasized, that the chemical activators INA and BTH have no direct pesticide effects but develop their efficacy by triggering the defence systems of the plant. These compounds are therefore registered as 'plant reinforcing agents' and are authorized in Germany. Theoretically they can also be applied for tobacco treatment.
L.F. FECKER (Biologische Bundesanstalt für Land- und Forstwirtschaft) presented the expression of coat protein specific single chain antibody fragments (scFv) in different cell compartments of *Nicotiana benthamiana* for the generation of resistance against beet necrotic yellow vein virus (BNYVV). Starting from cell lines which form monoclonal antibodies against the BNYVV coating protein, scFv-coded DNA fragments were cloned in *E. coli* and expressed. For the genetic expression in plants, these sequences together with additional sequences which are genetically engineered with a plant specific signal peptide were put under the control of the CaMV 35S-promotor and were transferred with the help of *A. tumefaciens* in *Nicotiana benthamiana*. It became obvious, that these genetically modified 'constructs' induced the production of larger amounts of scFv-protein. The progeny of these transgenic plants was partly resistant against the BNYVV.
Several presentations dealt with the efficacy of the insecticide effects of the components of the tropical Neem-tree (*Azadirachta indica*). C.P. ZEBITZ (Hohenheim University) and H. VOGT (Biologische Bundesanstalt für Land- und Forstwirtschaft) presented the possible use of Neem Azal-T/S for fruit culture. At a concentration of 30 g azadirachtin A/ha, the substance showed positive effects against numerous parasites of apple cultures. H. KLEEBERG (Trifolio-M GmbH) reported the efficiency and degradability of Neem Azal-T/S, which has been submitted for registration for application in fruit culture, forestry and potato cultivation by Trifolio-M GmbH in Germany. After uptake of Neem Azal-T/S the effects observed in insects are numerous (stopping of feeding and increasing inactivity of the larvae, occurrence of moulting obstruction and infertility).

In a poster presentation, R. TROß and B. RUCH (Trifolio-M GmbH) et al. reported on the degradation process of azadirachtin A, the main component of Neem Azal-T/S. The compound is degradable in the course of a few days only in plants, soil and water. In water solutions the degradation rate is strongly related to pH; at pH = 4 the half life is 10 days. According to B. Ruch, Neem Azal-T/S produced by Trifolio-M GmbH is almost odourless, in contrast to other already commercialized Neem products.

Meanwhile, efforts are being undertaken to extract the insecticide components of *Azadirachta indica* and particularly azadirachtin from cell cultures of the Neem plant (A. Wewetzer, Humboldt-University Berlin). For this means, Neem callus tissue was cultivated on different substrates (McCown, MS, Nitsch and Nitsch, White's medium) with different carbon sources (sucrose, maltose) and the azadirachtin yield was measured by HPLC. It was determined that the azadirachtin concentration in the cell cultures is strongly influenced by the substrate medium and the carbon source used. The highest yields (ppm-level) were obtained on White's medium with a concentration of 15 g/l sucrose. Nevertheless the results have shown that for industrial production of Neem-components by cell culture techniques, an increase in the yields is necessary.

Azoxystrobin (M. Konradt; ZENECA Agro), a strobilurin, detected in the fungus *Strobilurus tenacellus* is another new substance with interesting fungicide effects. Azoxystrobin obstructs the mitochondrial respiration of fungi by inhibiting the transport of electrons between cytochrome b and c_1 and obtains a wide-range fungicide spectrum against various *ascomycete*, *basidiomycete*, *deuteromycete* and *oomycete* pathogens. In addition, the product has low toxicity ($LD_{50} > 5000$ mg/kg; rat, p.o.) in mammalians and a low persistence in the environment. Azoxystrobin is under worldwide testing in 50 of the most important cultures of cereals, rice, cotton, tomato, sugar beet, rape, etc. It still has to be proven if the product can also be used as a fungicide in tobacco culture.