Etiology

Stress and Stimulus Modifications of Disease Severity in the Wart Disease of Potato

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ABSTRACT

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Sprouted potato tubers were inoculated with *Synchytrium endobioticum* in soil and aqueous suspension. During subsequent growth, watering was adjusted to develop stress or nonstress conditions. Among 30 weekly trials of nonstressed plants, fresh wart tissue mass was generally about 1.5% of fresh green plant top mass irrespective of variations in individual weights. When, however, wart tissue mass exceeded 1.5% of fresh green plant top mass, wart weights were observed to increase and associated green top weights to decrease. Under moisture stress conditions, both types of tissue demonstrated weight decrease. Healthy plants were little affected by the changes in levels of moisture stress. Plants grown in earthworm-infested soil showed increase in top green weight but no change in wart tissue weight. Results indicate that plant vigor was affected by the fungus, and that wart tissue appeared to grow at the expense of the host.

Additional key words: potato wart disease, wart index.

Wart disease of potato, caused by the economically important pathogen *Synchytrium endobioticum* (Schilb.) Perc., was observed in Newfoundland at the turn of the present century. The disease was also found on mainland North America in Pennsylvania, Maryland, and West Virginia in the 1920s, notwithstanding True's (16) warning in 1910 on its likely introduction. The United States was declared free of the disease in 1974 (2), but it remains widespread in Newfoundland (8).

A U.K. Board of Agriculture & Fisheries extension leaflet (1) states, "The vigour of the plant is not affected in the early stages, and it has been observed that diseased plants frequently grow larger ... than those which are free of the disease." The obligate intracellular fungus is maintained, at St. John's, on greenhouse-grown potato (*Solanum tuberosum* L.) plants. We have often observed that heavily infected plants appeared to have reduced top growth. There is a lack of information concerning the role of host vigor in influencing symptom expression in potato wart disease (8). Such information is important for determining the effects of treatments on infected plants and in developing an understanding of host-pathogen interactions. The studies reported here were conducted to demonstrate a relationship between symptom expression and host vigor in the absence of gross limiting factors and the influence of moisture stress as a limiting factor on symptom expression.

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MATERIALS AND METHODS

Disease development in the greenhouse under favorable conditions. Two weeks before inoculation, tubers of potato cultivar Arran Victory, which is susceptible to potato wart pathotype 2 (13), were removed from cold (2 C) storage and allowed to germinate at about 20 C and 80% relative humidity in low diffuse light in a controlled environment room (7). To each tuber was attached a 1-cm cube of freshly harvested wart tissue. Each cube was speared on a toothpick inserted into the rose end of a tuber adjacent to sprouts. Ten tubers were planted 15 cm deep in a soil bench and grown for 8 wk in 30 weekly replications. To optimize infection, plants were irrigated heavily during the second week of growth, but irrigated lightly in the first and remaining 6 wk (6). The soil was fertilized with controlled-release 14-14-14 Osmocote (Marman

U.S.A., Inc., Tampa, FL) at 0.5 kg/m^2; soil temperature at the 15-cm depth was maintained at 16 ± 3 C with a subterranean cooling unit (9); daylight was supplemented with fluorescent lighting to give a 14-hr photoperiod.

Disease development in moisture-stressed plants. Three kinds of experiments were performed to evaluate disease development under moisture stress regimes. In the first experiment (experiment A), six groups of 15 tubers each were inoculated with fresh wart tissue and planted, during July and August, in greenhouse soil benches. Plants were grown as previously described except that at 6 wk before harvest two groups were irrigated to field capacity (FC) every 84 hr, and two groups to FC every 168 hr.

In the second experiment (experiment B), 18 germinated tubers were planted in soil benches in three double rows, three tubers per row. Tubers in each odd-numbered row were inoculated with fresh wart tissue; after the initial 2-wk irrigation regime, each double row was irrigated to FC either every 24, 84, or 168 hr for the remaining 6 wk. This experiment was repeated six times from August to November.

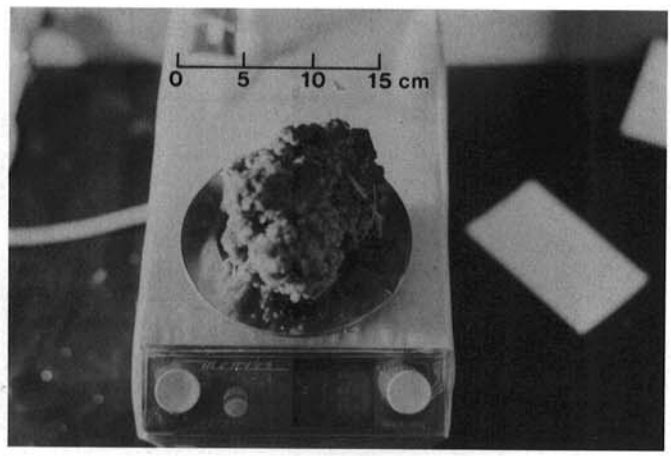

Fig. 1. A 55.5-g potato wart gall separated from the parent plant.

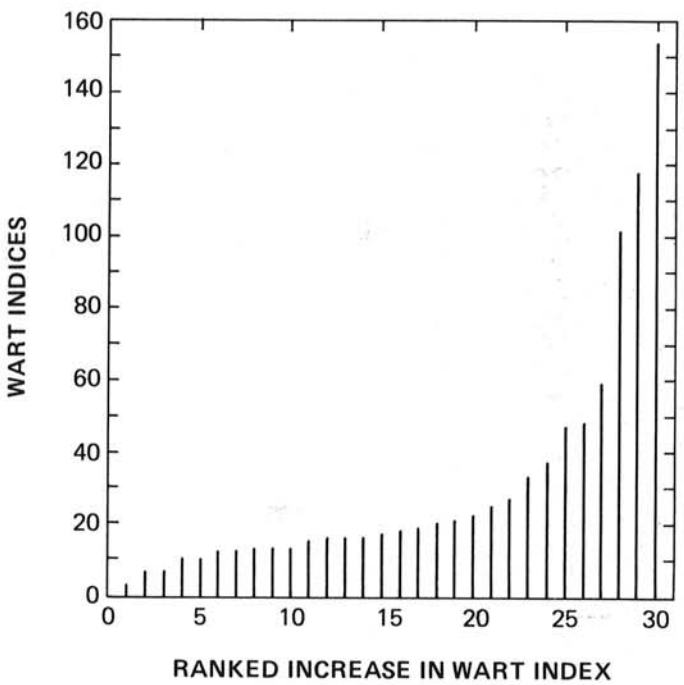

Fig. 2. Ranked increase in potato wart index (WI) of thirty weekly trials in which potatoes were inoculated with *Synchytrium endobioticum*. WI = (weight of wart tissue mass [g])/(weight of green top mass [kg]). Each value is the average of ten plants.

In the third experiment (experiment C), 15 germinated tubers were immersion-inoculated with fresh wart tissue for 4 hr after the manner of Zakopal and Spitzova (17). Each tuber was planted in an 18-cm-diameter × 32-cm-tall ceramic drainable pot containing perlite:peat moss (2:1, v/v) mix fertilized with 14-14-14 Osmocote at 0.5 kg/m^2. The pots were divided into three groups, irrigated daily to FC for the first 3 wk, and then irrigated (by group) to FC every 24 hr, 84 hr, or 168 hr until harvest. The pots were kept in the controlled environment room at 80% relative humidity, 20 C, 10,000 lux, and a 14-hr photoperiod.

Disease development in stimulated plants. Since it was discovered that growth of infected plants was depressed by moisture stress, an attempt was made to examine infected plants under a stimulus regime. Therefore, two 60 cm (length) × 35 cm (width) × 25 cm (height) boxes were filled with greenhouse soil into which was incorporated Osmocote and crumbled wart proliferations (yielding about 2,000 propagules per gram of soil). One box was infested with clean 7.5- to 10-cm-long earthworms, about eight worms per liter of soil, freshly dug from wart-free soil. Three germinated tubers were planted in each box, irrigated to FC for the first 2 wk and then lightly watered for the next 6 wk, illuminated with a 14-hr photoperiod, and kept in an aerial temperature of 18 ± 3 C. Two repetitions were carried out in the greenhouse in May and August.

Evaluation of plant growth and disease development. Data collected in all experiments included percent infection of each group of plants, weight of wart tissue (Fig. 1), fresh green top weight per plant, and for the earthworm experiment the number and weight of tubers per plant. The ratio, weight of wart to weight of fresh green top growth, was calculated for each group of plants and is referred to as the "wart index" (WI).

RESULTS

Disease development in nonstressed plants. Variations in WI that resulted from thirty weekly plantings in the absence of gross limiting factors are presented as a ranked series (Fig. 2). Ranking produced an exponential-type series. Most variations lay within the expected range, but some values occurred as outliers far beyond that range.

Note that the WI is a composite value of two parameters—fresh tissue weight of wart and plant top growth. This index is useful for

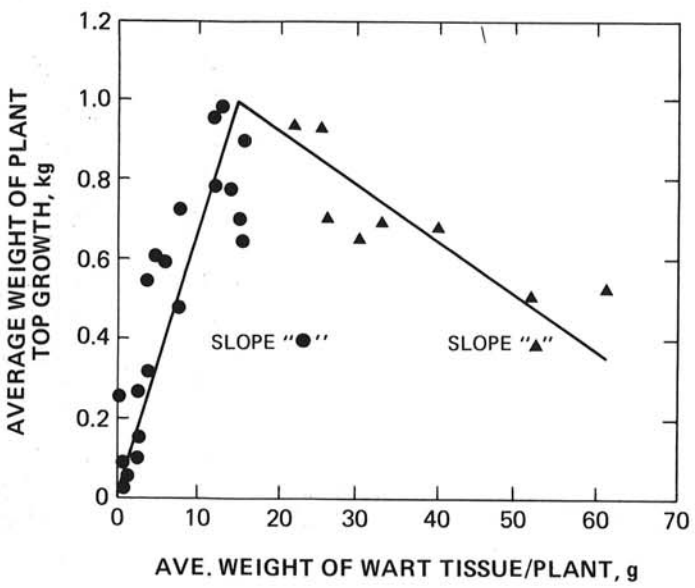

Fig. 3. Relation between weight of potato wart tissue and weight of top growth of potato plants infected with *Synchytrium endobioticum*. Slopes are derived from linear regressions of all wart weight values equal to (●) and greater than (△) 1.5% of top growth weight, respectively. The value of each locus is the average of ten plants. Two data points coincide (x/y, 3/0.1 and 26/0.7) making a total of 30 data points.

comparisons made in our pathogenesis studies on wart disease. Considerable variation occurred in the present study in the values of wart mass (1–60 g per plant) and fresh green top weight (50–900 g). The results show, first, that under conditions conducive to maximum symptom expression in greenhouse soils, wart tissue appears to increase in mass as plant mass increases. This is illustrated in slope generated by the solid circles in Fig. 3 where wart tissue mass approximated about 1.5% of total top green weight for 20/30 data points. Second, when the wart mass exceeded this value, increasing amounts of wart were associated with decreasing amounts of plant top growth. This aspect is also clearly illustrated in slope generated by the solid triangles in Fig. 3.

Disease development in stressed plants. Values for greenhouse-grown and growth room-grown plants are displayed in Fig. 4. Among WI values (not displayed in Fig. 4), generally the WI at 24 hr is greater than the WI at 84 hr and is equal to the WI at 168 hr but no significant differences were found by using Duncan's multiple range test, $P = 0.05$. It is evident, however, that less plant and wart tissue mass were produced as stress increased; notwithstanding a slight exception with wart in ceramic pots in the 84-hr watering regime, the reduced productions were parallel. Among healthy but stressed plants (Fig. 4B) little change in top weight occurred. The results strongly suggest that infection adversely influenced the vigor of the plants under stress.

Percent infection varied somewhat with moisture stress level. Infection at the highest level of stress was always the lowest.

Disease development in presence of earthworms. In earthworm-infested soil and without gross limiting factors, the WI in the control exceeded WI in presence of earthworms. The weights of wart tissue were essentially the same for plants in both the control soil and earthworm-infested soil in each trial; 100% infection occurred throughout. The difference in WI, thus, lay in the increased plant mass (and tuber weight) in the presence of earthworms, and here the values were considerably higher than those in the control boxes (plant top growth weight 1.48:1.00; tuber weight, 1.86:1.00).

DISCUSSION

Results of this study indicate that the vigor of potato plants was substantially affected by *S. endobioticum*. Does wart tissue proliferate at the expense of the host? Under nonstress conditions the WI remained the same until a certain size of wart mass was reached. With increased mass plant vigor appeared to diminish, consequently reducing fresh green top mass.

Tuber weight data, except in the earthworm experiment in which the objective was to test for stimulation of plant growth, are not included because tuber production varies with the season (9) and including those data would have confounded the pathogenesis picture. Therefore, "top weight" was used as the infection parameter for monitoring seasonal changes.

Infections were found at stem bases. This indicated the likelihood of early infection. Watering regimes were optimized in the first 2 wk of growth to encourage zoospore movement, and conditions for all inoculations were essentially similar. The effects of stress induction, therefore, probably occurred during the later infection period, in which phase wart tissue was already developing hypertrophically. It is worth noting, nevertheless, that infection was least at the highest stress level, and this suggests that some reinfection through secondary cycles may have occurred in the more frequently watered plants. It is possible that stress negatively influenced infection in ways not yet known.

The increase in wart mass and associated lower plant tissue weight bears out the observation that heavily warted plants appear to lack vigor and are stunted. Under the environmentally imposed stress, healthy plants remained vigorous. As stress increased,

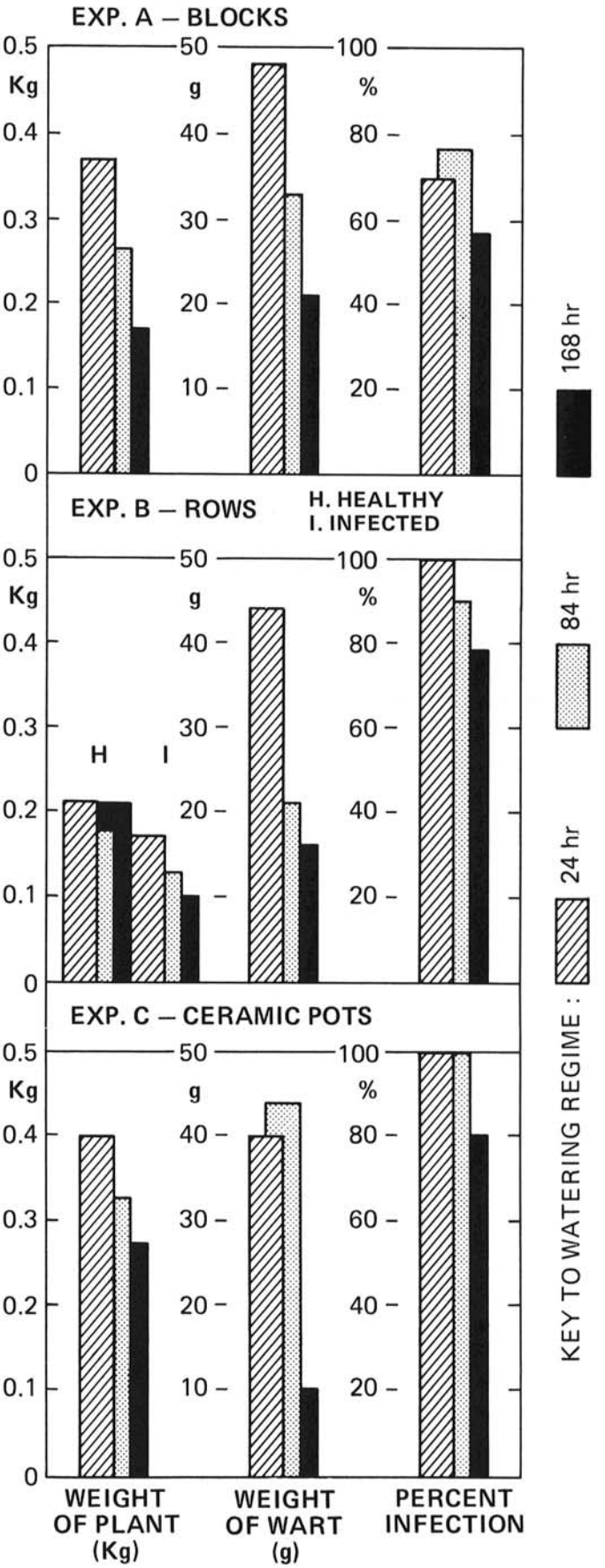

Fig. 4. Comparisons of weights of plant tops and wart tissue masses, and percent infection of potato plants infected with *Synchytrium endobioticum* after subjection to three levels of water stress. Stress induced by irrigating to field capacity every 24, 84, or 168 hr. **A,** Single stress level applied to one block of plants at a time. **B,** Simultaneous application of stress levels to plants in rows. **C,** Stresses applied to plants grown singly in pots in a growth room. H = healthy and I = infected.

infected plants were affected adversely and increasingly by the stress regimes. This suggests that growth activities in the wart tissue acted to impose stress in the host.

If infection produced stress that diminished plant vigor, then it would be expected that environmentally induced stress would further diminish vigor. Thus, stimulation of host vigor ought to have offset the debilitating effects of infection. We were reluctant to use fertilization (beyond that of the Osmocote) because modification of the ionic strength of the solutes in the soil water in only one box would jeopardize the nature of the experiment. Field potato soil is generally rich in earthworms, and although the direct effect of earthworms on plant growth is an unsettled question (15), earthworm activity is viewed favorably as a soil-building activity beneficial to soil structure and aeration, and hence, also to plant growth (4). The growth stimulus resulting from their use in this study suggests that increased plant vigor did offset the observed effects of infection on plant vigor. Field soils may contain up to 100 propagules of *S. endobioticum* per gram. Thus, the quantity of sporangia in this experiment was ample.

In discussing the anatomy of wart tissue, Artschwager (3) points out that the tissue appears to be adapted primarily for conduction, not support. He cites the lack of typical fibers and pitted vessels, and the fact that xylem vessels remain cellulosic for long periods. He also notes that conducting tissue advances independently close to the periphery of the wart tissue, thus emphasizing the necessity for rapid movement of water and dissolved substances. Reingard and Pashkar (14) demonstrated the localization of auxin-type growth substances in the peripheral zone of intense wart growth and suggested that wart tissue formation requires enhanced mobility of growth stimulants. In Lipsits' (11) results, pathic processes in potato wart disease are related positively to host vigor.

Hampson (5) and Mitchell and Rice (12) demonstrated, respectively, the movement of ^{14}C-labeled assimilates to rusted (*Uromyces phaseoli*) pinto bean tissue, clubroot (*Plasmodiophora brassicae*)-affected tissue of cabbage, and potato wart diseased tissue (although lacking the direct internal evidence provided above) also appears to act as a nutrient and metabolic sink.

Not only is *S. endobioticum* parasitic in the cells of potato tissue, but the tissue itself appears as though it were parasitic on the host in the manner that Klein and Link (10) state of crown gall disease, that the tumor "robs" the host of its food reserves. It is concluded that infection of potato by *S. endobioticum* allows a continued, rapid, and unregulated proliferation of wart mass which acts as an open sink for plant nutrients. The consequences of the rapid growth activity of wart tissue would be to increase the wart tissue surface area, predispose the plant to further infection through enhanced secondary disease cycle development, and so enhance the reproductive potential of the fungal population. Thus, fertile wart-infested soil would increase the chances for inoculum buildup and hence increase the probability of pathotype development.

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